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Ice Storm Damage to Virginia Coastal Plain Forests during the Christmas 1998 Ice Storm

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ABSTRACT

On December 23-25, 1998, a major ice storm struck southeastern Virginia. The storm-deposited glaze ice felled trees and limbs, causing a power outage and highway blockage. Between February and April, 1999, we recorded occurrence, severity, and type of damage to trees over 2.5 cm dbh in nine mostly gently sloping plots in Matoaka Woods at the College of William and Mary. Frequency and severity of damage varied with species and with size of trees. Canopy damage occurred in 75% of large *Fagus grandifolia* trees, but in only 6% of small *Sassafras albidum* stems. As a group, small (2.5 to 15 cm dbh) trees were less likely to be damaged than large (15 cm dbh) trees, but about as likely to be severely damaged. Damage type also varied among the species and size. Despite severe damage to public utilities, damage within the forest was not great. Since few trees lost their entire crown, canopy gap sizes were small, and it not clear that much change in forest composition will result from this storm. However, increased density of ground litter will contribute to greater mineral release, and this plus small gaps may promote growth of already present seedlings and saplings.

INTRODUCTION

On December 23, 24, and 25, 1998, a major ice storm affected southeastern Virginia. Precipitation in the form of sleet and freezing rain accumulated to 1-3 cm of ice across the region, with Williamsburg reporting 3 cm of precipitation for the three-day period. In the City of Williamsburg and surrounding counties, 400,000 customers lost power for three to ten days following the storm. Many roads, including portions of Interstate 64 near Lightfoot, VA, were rendered impassable by fallen branches and trees (NCDC 1998a,b). The storm's impact on the community was certainly severe, and much of the infrastructure damage was caused by ice-felled branches and trees along roadsides and on forest margins.

Based on the degree of damage readily observable from the roads, we felt that this storm presented an ideal opportunity to determine the effects of ice accumulation on local forests. The great damage to roadside and forest margin trees, however, was due to their peculiar location. Without adjacent vegetation of comparable height to support their accumulated weight in ice, and with either asymmetric or fuller crowns due to lack of competition for light, individuals in the open would likely be more susceptible to damage than those in the forest. Nevertheless, preliminary investigation of our potential study sites indicated that, although the damage within the forest was not as heavy as on its margins, it did appear significant enough to provide data for a meaningful study on the dominant tree species of the area.

We surmised that the College Woods (also called Matoaka Woods), a forested area owned by the College of William and Mary, was an ideal place for a small-scale investigation into the susceptibility to ice of several major tree species on the Coastal Plain of Virginia. Matoaka Woods is made up of a variety of small, homogenous stands dominated by canopy species such as tulip poplar (*Liriodendron tulipifera*), oaks (*Quercus* spp.), beech (*Fagus grandifolia*), and loblolly pine (*Pinus taeda*). The mosaic pattern of the woods (farmed and forested patches were abandoned or last timbered at various times for various reasons) has allowed for a diversity of species, and also has ensured equal representation of a broad spectrum of size classes. In this study, our primary goal was to survey the amount and type of damage to each of the more abundant tree species in Matoaka Woods. Of secondary interest was the comparison of damage among different size individuals of the same species.

METHODS

Our field survey was conducted in the Matoaka Woods of the College of William and Mary between February 3 and April 7, 1999. No further forest-ravaging natural phenomena occurred between the end of the Christmas storm and the completion of our survey. Sampling sites were chosen based on the constituent species and apparent age of the dominant individuals: younger and older stands dominated by oak species, tulip poplar, loblolly pine, and beech were sought out with the hopes of comparing damage between different aged canopy trees of the same species or genus, as well as among the different species. The sampling sites were widely spread throughout the woods.

We chose to follow Seischab et al. (1993) in our methodology. We marked a 20x40-meter plot at each sampling site. Each of these was broken into four 10x20 meter subplots for ease in sampling. In each subplot, trees larger than 2.5 cm dbh were identified by species and were placed in one of two size categories: between 2.5 and 15 cm dbh and over 15 cm dbh. In general, trees in the smaller size class were subcanopy, and those in the larger size class were in the canopy. Though we took measures to avoid bias toward areas likely to be heavily damaged (such as steep slopes above ravines; Warrillow and Mou 1999), beech-dominated stands could not be found in the more level portions of the woods. Thus, in order to sample beech, it was necessary to place two plots on slopes. Effects on the results due to this difference in topography will be discussed later.

Each tree surveyed was placed in a damage class between 0 and 7 based on percent canopy loss due to ice damage. A rating of 0 corresponded to no perceptible damage, 1 to ≤ 5 % canopy loss, 2 to 6-10% canopy loss, 3 to 11-25% canopy loss, 4 to 26-50% canopy loss, 5 to 51-75% canopy loss, and 6 to 76-99% canopy loss. A rating of 7 was given where damage was so severe that mortality was likely. Though we quantified canopy damage as an estimate of percent of canopy lost, the accuracy of our estimates was necessarily subject to error, for we were not able to observe the leafed out canopies of deciduous trees, nor had we previously documented canopy sizes for any of the trees surveyed. However, every effort was made to be consistent.

We recorded the nature of the damage to each tree, noting whether each damaged tree was uprooted (symbolized by o- in the tables), had its main stem broken (symbolized by / \), had its main stem bent or bowed (Ç), had one or more branches completely broken from the tree (o), had one or more branches broken but still attached to the tree (Λ). We also noted whether the damage, of whatever type, was direct (as a result of ice accumulation on the tree in question) or secondary (a result of ice-laden branches,

TABLE 1. Field data for individuals ≥ 15 cm dbh. See text for description of damage classes and types.

Species	Sample size	Damage class 0	1	2	3	4	5	6	7	Damage type o -	/\	Ç	Λ	o	s
Pinus taeda	53	29	2	7	3	3	1	2	6	-	7	2	1	12	-
Liriodendron tulipifera	47	27	5	6	2	3	2	2	-	-	-	-	-	15	-
Quercus alba	35	21	6	1	3	3	1	-	-	-	-	-	5	4	-
Fagus grandifolia	28	7	5	4	4	3	4	1	-	-	1	-	4	16	1
Oxydendron arboreum	23	13	4	-	4	1	-	1	-	-	1	2	-	5	2
Liquidambar styraciflua	15	12	1	-	-	-	1	1	-	-	-	-	-	2	-
Quercus velutina	11	6	2	2	1	-	-	-	-	-	-	-	2	1	-
Acer rubrum	9	4	-	-	2	1	-	2	-	-	1	-	2	4	-
Carya glabra	8	6	-	-	-	1	1	-	-	-	-	-	2	-	2
Quercus falcata	6	3	1	1	1	-	-	-	-	-	-	-	-	2	-
Nyssa sylvatica	5	3	-	-	2	-	-	-	-	-	-	-	1	1	-
Quercus rubra	4	4	-	-	-	-	-	-	-	-	-	-	-	-	-
Ilex opaca	3	1	2	-	-	-	-	-	-	-	-	-	1	-	-
Quercus coccinea	3	2	-	-	1	-	-	-	-	-	-	-	-	1	-
Cornus florida	2	2	-	-	-	-	-	-	-	-	-	-	-	-	-
Carya tomentosa	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Fraxinius americana	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Prunus serotina	1	-	-	-	-	-	-	-	1	-	1	-	-	-	-

canopies, or entire trees falling on individuals below). Recently fallen live branches ≥ 2.5 cm at the broken base (butt end) found in the plots were tallied by species and size; any above 10 cm diameter at the base were further noted. We did not attempt to quantify the deadwood since it was impossible to distinguish dead material felled by this storm from that previously on the ground.

By performing our investigation in the winter and early spring immediately following the ice storm, we were able to easily determine the most recent open wounds and fallen branches, for the infection and decay dependent on warm temperatures had not begun. We also avoided the possibility of additional damage from other natural disasters (such as windstorms, including the hurricane that struck the study area the following summer). Because no new growth had begun on bent or wounded stems, we could distinguish fresh bending from older bending or breaking, since trees previously damaged had redirected their foliage or sprouted new stems during the last growth season. The lack of intervening foliage in the understory made it easier to examine damage to canopy trees, but, as mentioned previously, percent canopy loss was harder to estimate accurately without foliage.

RESULTS

We found no significant differences in damage between older stands and younger stands with the same dominant species. Because of this finding, descriptions of individual plots have not been included, and all data from each species have been merged to reflect interspecific differences and differences between the canopy and understory classes. The amount and type of damage incurred by the 27 species we encountered during our survey is shown in Tables 1 (individuals ≥ 15 cm dbh) and 2 (individuals < 15 cm dbh).

TABLE 2. Field data for individuals < 15cm dbh. See text for description of damage classes and types.

	Sample	Damage class								Damage type					
Species	size	0	1	2	3	4	5	6	7	o-	/\	Ç	Λ	o	s
Liriodendron tulipifera	146	109	12	5	1	1	4	4	9	-	12	9	3	5	8
Cornus florida	132	106	8	2	6	5	-	2	3	-	-	8	1	02	12
Acer rubrum	75	46	6	5	6	2	2	5	3	-	4	6	10	4	5
Oxydendron arboreum	54	27	4	4	4	2	-	6	7	3	2	11	4	4	12
Ilex opaca	49	31	3	4	5	5	1	-	-	-	-	7	3	6	8
Liquidambar styraciflua	44	36	3	1	-	2	-	1	1	-	1	4	1	1	3
Fagus grandifolia	40	36	3	-	-	-	-	1	-	-	1	2	-	-	1
Nyssa sylvatica	37	31	3	2	1	-	-	-	-	-	-	-	2	1	-
Sassafras albidum	17	16	-	-	-	-	1	-	-	-	-	-	-	1	-
Carya glabra	14	9	2	-	1	1	-	-	1	-	1	2	-	-	2
Quercus alba	6	5	1	-	-	-	-	-	-	-	-	-	-	-	-
Pinus taeda	5	1	-	-	2	-	-	-	2	-	2	2	-	-	-
Carya tomentosa	3	3	-	-	-	-	-	-	-	-	-	-	-	-	-
Castanea dentata	2	2	-	-	-	-	-	-	-	-	-	-	-	-	-
Cercis canadensis	5	3	1	1	-	-	-	-	-	-	-	1	1	1	1
Quercus velutina	5	4	1	-	-	-	-	-	-	-	-	-	-	-	-
Juniperus virginiana	4	2	2	-	-	-	-	-	-	-	-	1	-	-	-
Vitis rotundifolia	2	2	-	-	-	-	-	-	-	-	-	-	-	-	-
Diospyros virginiana	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Kalmia latifolia	1	-	-	-	1	-	-	-	-	-	-	-	1	-	-
Quercus rubra	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Vaccinium corymbosm	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Viburnum nudum	1	-	1	-	-	-	-	-	-	-	-	1	-	-	1

Tables 3 through 6 show the occurrence and severity of damage to the most abundant species in Matoaka Woods. When the Chi square test was applied to these data, only the outlying values (those furthest apart) proved statistically different. That is, in Table 3 the degree to which *Fagus grandifolia* was affected by the storm was significantly different only from that of *Liquidambar styraciflua* of the same size—there were no statistically significant differences among the other species. When the results for occurrence and severity of damage to all small trees combined versus all large trees combined were compared (Table 7), statistically significant differences between size classes were achieved. Of the 908 trees surveyed, 32% were damaged by this ice storm, and 12% were severely damaged (placed in damage class 4 or above, which means they have lost 25% of their crown). Smaller trees were less likely to be damaged than larger trees, but when damaged, smaller trees were about as likely to incur severe damage as larger trees. Table 8 shows the results from our tally of fresh fallen branches

DISCUSSION

Although it was impossible to gather quantitative data on damage in open areas or on forest margins (the necessity of quick cleanup to restore community infrastructure prevented it), our final results suggest that the severity of roadside damage greatly overrepresents the damage from the storm as a whole. Compared to other studies of ice storm damage to forests, the occurrence and severity of damage in Matoaka Woods

TABLE 3. Frequency of damage in large trees (dbh ≥ 15 cm). Species with sample size < 10 are not included.

Species	Sample size	Number damaged	% damaged
Fagus grandifolia	28	21	75
Pinus taeda	53	24	45
Quercus velutina	11	5	45
Oxydendron arboreum	23	10	43
Liriodendron tulipifera	47	20	43
Quercus alba	35	14	40
Liquidambar styraciflua	15	3	20

TABLE 4. Frequency of ice damage in small trees (dbh < 15 cm). Species with sample size < 10 are not included.

Species	Sample size	Number damaged	% damaged
Oxydendron arboreum	54	27	50
Acer rubrum	75	29	39
Ilex opaca	49	18	37
Carya glabra	14	5	36
Liriodendron tulipifera	146	36	25
Cornus florida	132	26	20
Liquidambar styraciflua	44	8	18
Nyssa sylvatica	37	6	16
Fagus grandifolia	40	4	10
Sassafras albidum	17	1	6

places the December 1998 storm among the less destructive of these reported in published studies. Whitney and Johnson (1984) documented that 46% of all trees surveyed after a southwestern Virginia glaze storm were severely damaged. In another study following an Ohio icing event, 15.5% of trees surveyed were severely damaged (Boerner et al., 1988). Our results indicate that 8% of stems were severely affected by this storm. That is not to say that the storm was not severe from a Coastal Plain perspective, for we found no previous study describing glaze damage in this area.

The results show that throughout the area surveyed, large trees were more frequently damaged than small ones. Given the lack of foliage in the canopy and the duration of the precipitation during this storm, it is likely that in hardwood stands subcanopy stems were exposed to icing on the same order as larger trees. In addition, the occurrence of secondary damage (that caused by the falling limb or crown of a neighbor, usually a canopy tree) was much greater in the smaller size class than the larger. Note Tables 1 and 2: small trees suffered 53 instances of secondary damage vs. 5 such cases large trees. Thus, except for stands dominated by evergreens, one would expect that the absence of canopy shelter and the likelihood of secondary damage would make subcanopy trees even more susceptible to storm damage. As this was not the case, some other factors must account for the smaller trees' resistance.

TABLE 5. Severity of damage in large trees (dbh ≥ 15 cm).

Species (over 15 cm dbh)	Number damaged (all damage classes)	Number severely damaged (damage class 4 or above)	% of all trees with severe damage	% of damaged trees with severe damage
Pinus taeda	24	12	23	50
Fagus grandifolia	21	8	29	38
Liriodendron tulipifera	20	7	15	35
Liquidambar styraciflua	3	2	13	33
Quercus alba	14	4	11	29
Oxydendron arboreum	10	2	9	20
Quercus velutina	5	0	0	0

TABLE 6. Severity of damage to small trees (dbh < 15 cm).

Species (under 15 cm dbh)	Number damaged (all damage classes)	Number severely damaged (damage class 4 or above)	% of all trees with severe damage	% of damaged trees with severe damage
Sassafras albidum	1	1	6	100
Oxydendron arboreum	27	15	28	56
Liriodendron tulipifera	36	18	12	50
Liquidambar styraciflua	8	4	9	50
Acer rubrum	29	12	16	41
Carya glabra	5	2	14	40
Cornus florida	26	10	8	38
Ilex opaca	18	6	12	33
Fagus grandifolia	4	1	3	25
Nyssa sylvatica	6	0	0	0

Many small trees suffering from secondary damage were simply bowed over, some with their crowns forced all the way to the ground, but they were rarely broken. Accordingly, we observed 54 events of bending in small trees and only 4 in large trees (Tables 1 and 2). Whitney and Johnson (1984) and Boerner et al. (1988) have also noted the relative elasticity of younger wood as a factor in glaze damage susceptibility. Therefore, it is likely that in the month interim between the melting of the ice and the beginning of our investigation, small trees temporarily bent under the weight of the ice had already straightened themselves due to their youthful resilience.

Among the various species, several deserve attention due to their exceptional tendency toward, or resistance to, damage. While individuals in the large tree category were generally canopy trees, one case deserves special attention. Our initial survey of damage seemed to indicate that tulip poplar would be among the most susceptible, but results show that it falls near the mean value for occurrence of damage in the large tree category. One possible explanation for our initial impression of high damage to tulip poplar is that the usual type of damage to large trees of this species was loss of complete

TABLE 7. Frequency and severity of damage in all trees surveyed.

	Total number	Number damaged	% damaged	Number severely damaged (damage class 4 or above)	% severely damaged
Over 15 cm dbh	264	119	45	44	16
Under 15 cm dbh	644	173	27	71	11
Combined (all sizes)	908	292	32	115	12

TABLE 8. Branches over 2.5 cm in diameter at butt end found in plots.

Species	Number of storm-felled branches over 2.5 cm diameter butt end	Number of those branches over 10 cm diameter butt end	Total number of damaged trees	Felled branches/ damaged trees
Liriodendron tulipifera	154	7	56	2.8
Pinus taeda	42	14	28	1.5
Fagus grandifolia	36	8	25	1.4
Pinus virginiana	6	1	6	1.0
Quercus velutina	5	1	6	0.83
Quercus alba	10	4	15	0.67
Acer rubrum	13	0	34	0.38
Oxydendron arboreum	9	0	37	0.24

branches (Table 2), resulting in a high abundance of broken tulip poplar branches on the ground (Table 8). Another fact that directly affects our data is that in our older pine stand, there were a large number of tulip poplar stems just over 15 cm dbh growing beneath the canopy. It is likely that the full canopy of the evergreens shielded these tulip poplars from an otherwise destructive ice load, and thus skewed the damage results for the large size class of tulip poplar.

The large value for percent of *Fagus grandifolia* stems damaged requires explanation. While most of the dominant species in Matoaka Woods could be represented in plots with little or no overall slope, American beech occurred as the dominant canopy species only on the steep slopes of drainage ravines. Trees located on slopes often have asymmetrical crowns which, when laden with ice, become unbalanced and more susceptible to breakage than similar trees with uniform crowns. These observations are echoed by Boerner et al. (1988), Bruederle and Stearns (1985), Seischab et al. (1993), Warrillow and Mou (1999), and Rhoades (1999). The few smaller American beech individuals located in plots dominated by other species were normally unbroken, and though their branches were typically bowed, we could not confidently attribute that bowing to the December icing event. On the other hand, the residential neighborhoods in the Williamsburg Area with the most severe infrastructure damage were neighborhoods carved out of a beech-rich forest some 40 years ago, and fallen beech branches were a major cause of the damage. Thus, we can't rule out the possibility that older beech trees are particularly susceptible to ice storm damage. We did not observe frequent uprooting of beech or any other species in the beech-dominated slope plots, however, in contrast to observations of frequent uprooting on steep slopes during ice storms in mountainous areas of Virginia (Warrillow and Mou 1999; R. W. Rhoades 1999 and pers. comm.).

We observed that Virginia and loblolly pines were literally wiped out on roadsides throughout the storm's path. When standing alone, growth patterns of loblolly and Virginia pine create a top-heavy tree. When loaded with ice, these species tend to lose their entire canopies. Especially in Virginia pine, this was usually by snapping of the main trunk two to four meters above the ground, rather than by uprooting (in contrast to the findings of Warrillow and Mou (1999) in western Virginia). The roadside condition of asymmetrical canopies and the increased surface area presented by their needles makes evergreens particularly susceptible to primary ice damage. Boerner et al. (1988) also observed high instance of crown loss in evergreens, which they attributed to the accumulation of ice on needles. Total crown loss was less prevalent in the forest due to the support offered by neighboring trees, but we did witness several cases of 100 % canopy loss from the pines in our plots (see also Buttrick, 1922). Whitney and Johnson (1984) observed, as we did, that both pine species and tulip poplar were often severely damaged. Their inability to sprout adventitious stems makes severe damage particularly destructive for pines; three-fourths of the severely damaged Virginia pine stems surveyed in Whitney and Johnson's study were dead after two years, but only 5 percent of severely damaged tulip poplar stems had perished.

Fallen branches in plots make up a third subset of data. The quantity of branches found on the ground for a given species supports earlier conclusions about frequency and type of damage. For instance, Tables 1 and 2 indicate that *Liriodendron tulipifera* was prone to lose branches or whole crowns (in the case of smaller stems) by clean break. Table 9 supports this tendency in tulip poplar, as most of the ground litter tallied could be attributed to this species.

Although not recorded, the number of already dead branches that fell during the storm was apparently extremely high, especially in oak-dominated areas. We had no quantitative records of pre-storm ground deadwood in our study area, but in a forest elsewhere in the county, H. Sahli and S. Ware observed a several-fold increase in amount of litter from already dead branches on the ground between October 1998, before the storm, and February 1999, after the storm. After severe ice storms the large increase in ground layer biomass from recently broken branches is usually regarded as increasing the threat of forest fires in the following summer. Though less than the biomass from newly broken branches, the contribution to litter of already dead wood brought to the ground by the storm should not be overlooked. Further, this dead wood is already in a state of partial decay, and may provide quicker flush of minerals to the soil than newly broken branches. The mineral flush from the increased amount of decaying gound litter (both already dead and newly broken) in combination with storm-induced openings in the canopy will probably lead to a thickening of the understory in the more damaged locations in the forest.

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Trachelomonas spp. and Other Euglenophyceae Taxa in a Southeastern Virginia Lake

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ABSTRACT

Trachelomonas species from Lake Kilby, a reservoir lake in southeastern Virginia are described with supportive electron micrographs . The most abundant *Trachelomonas* species were *T. hispida* and *T. volvocina.* Other members of the Euglenophyceae occurring in this lake are identified.

INTRODUCTION

Within freshwater phytoplankton communities, representatives of the genus *Trachelomonas* Ehrenberg (phylum Euglenophyta, class Euglenophyceae) are often common and abundant. These microscopic, unicellular, and flagellated cells are encased in a lorica (test) whose characteristic surface morphology (among other factors), provides significant information for their identification. Representative *Trachelomonas* species that were identified in Lake Kilby, a relatively small reservoir lake in southeastern Virginia, are described with representative scanning electron microscope prints. A listing of other species within the Euglenophyceae category that were present in Lake Kilby are also given.

Lake Kilby is located in Suffolk, Virginia (76°38′W, 36°42′40″N) and its waters serve as a regional water supply source. Typical of other reservoirs established within an impounded watershed segment, the lake has an elongated and generally narrow basin, mostly shallow, with a maximum depth of approximately 6-7 meters, and a surface area of 90 ha (Norman, 1985). Seasonal stratification occurs in the deeper regions, with submerged tree stumps found throughout its more shallow sections. During periods of low water level, additional water from the Potomac aquifer will be pumped into the lake (Schafran and Scully, 1994). This pumping is most common beginning in September and continuing into late fall. When this occurs the oxygen level in the lake decreases, with the total phosphorus concentrations and alkalinity increasing. In addition, as a control measure to reduce the occurrence of algal blooms, copper sulfate is often added to the lake surface between March and November.

METHODS

Water samples (500 ml) were taken from the upper 1 meter depth at 4 stations that were placed along the length of the lake. Collections were made once in June and twice in July 1996. The samples were collected with a Kemmerer sampler and preserved with Lugol's solution. A modified Utermöhl method was followed where the water sample was passed through a series of settling and siphoning steps (3) to provide a 40 ml concentrate of the original water sample (Marshall and Alden, 1990). Fractions of this concentrate (based on the density of the plankton and suspended solids present) were placed in settling chambers and examined at both 315x and 500x magnification using a Zeiss (inverted) plankton microscope. Taxon concentrations were determined from this analysis. Representative water samples were then prepared for examination using a Cambridge Stereoscan model S-100 scanning electron microscope. This report emphasizes only the euglenophycean species identified in the sample analysis. Water quality variables were determined by personnel from the City of Portsmouth. Identification references included Huber-Pestalozzi (1955), Conforti and Nudelman (1994), Couté and Iltis (1981), Couté and Thérézien (1985), and Wolowski (1998).

RESULTS

The year 1996 may be considered a "wet" year with the highest annual rainfall for this area recorded over the last decade at 65.8 inches, with a monthly mean of 5.49". Subsequently this resulted in approximately 56 million gallons of water pumped into the lake from the Potomac aquifer for 1996, which is considerably less than what would be added during a "dry" year, as occurred in 1997 when this region had 42.7 inches of rainfall and the amount of pumping reached 181 million gallons. For the two months (June-July 1996) when water samples were taken, the pH ranged from 5.7 to 6.2. The surface oxygen was from 3.1 to5.9 mg L^{-1}, orthophosphates 0.06 to 0.09 mg L^{-1}, nitrates 0.07 to 0.1 mg L^{-1}, and nitrites remained at 0.02 mg L^{-1} . The water's surface temperature ranged from 20.1 to 25.3 °C. The lake is considered eutrophic. During this period cell concentrations for the total *Trachelomonas* spp. at the four stations ranged from 2-183 x 10^3 cells L^{-1} in June and 29-108 x 10^3 cell L^{-1} in July. These concentrations for the *Euglena* spp. ranged from 2-11 x 10^3 cells L^{-1} in June and 2-15 x 10^3 cell L^{-1} in July, and for *Phacus* spp. ranged from 6-63 x 10^3 cells L^{-1} in June and 2-88 x 10^3 cell L^{-1} in July.

The genus Trachelomonas:

Following mainly Huber-Pestalozzi (1955): The genus *Trachelomonas* is represented by unicellular, free swimming cells, enclosed in a lorica, usually spherical, oval, or spindle shaped. The cell surface may be smooth, rough, pitted, and possess small or large size punctae. The cell surface may be with, or without spines; and when present the spines may vary in size, thickness, and location among different species. The flagellar pore may have an annular ring, or possess a collar that would vary in its shape, length, and the presence of spines. These cells are common in shallow lakes, ponds, and swamp waters.

Trachelomonas acanthophora Stokes

Lorica spindle-form, having an ellipsoidal central area with ends extended. The anterior end contains a tubular collar, ending terminally with the rim of the collar possessing a ring of spines (6-8) diverted outwardly. The collar length is around 18 microns. The caudal end is more narrow, but slightly conical, tubular, with the terminus containing a ring of spines (5-6) diverted outwardly. The surface of the central body contains punctae, with thick pointed spines (3-5 microns long). Spines also on the caudal extension, with some but fewer spines on the lower part of the collar. Variability in shape and size of central cell body. Similar to *T. Dastuguei* Balech. See Huber-Pestalozzi (1955). Approximately 37-65 X 20-25 microns in size, yellow brown in color. Figure 1.

Trachelomonas acanthostoma Stokes emend. DeFlandre

Represented by a sub-spherical or broadly ellipsoidal lorica, having a smooth surface, containing fine punctae. May be without a collar, or with a low collar, with the flagellar pore surrounded by 1 or 2 rings of short spines. 26-31 x 22-27 microns in size. See Huber-Pestalozzi (1955) and Wolowski (1998). Color reddish brown. Figure 2.

Trachelomonas alisoviana Skvortzov

Spherical to slightly ellipsoidal lorica, with the entire surface pitted, and having a flagellar pore surrounded by a distinct and raised annular rim. Flagellar pore is 1.5- 2.0 microns. Size 15-20 microns. See Couté and Thérézien (1985). Figure 3.

Trachelomonas armata v. *Steinii* Lemmermann emend. Deflandre

Possesses an egg-shaped lorica, with a fairly smooth surface, containing minute punctae. The posterior end is slightly broader. The collar is represented by a low elevated rim containing a circle of spines. The spines are concentrated at both ends of the cell. Small straight spines (2 microns) at the anterior end. A cluster of long, curved, thick spines (8-12 microns) are at the posterior end. Similar to *T. armata* v. *longispina* (Playf.) Deflandre illustrated in Prescott 1956), and *T. armata* v. *longa* Deflandre. Also see Huber-Pestalozzi (1955), Couté and Thérézien (1985). Size, 35-40 x 25-30 microns. Yellow brown in color. Figure 4.

Trachelomonas globularis v. Boyeri (Palmer) Conrad

The lorica has a spherical shape, with short conical-shaped spines scattered over the surface, which are inter-spaced by punctae. Golden brown in color. Size 9-14 microns. See Huber-Pestalozzi (1955). Figure 5.

Trachelomonas hispida (Perty) Stein

The lorica is ellipsoidal, with short spines and punctae over the entire surface of the cell. The collar is either absent, or it is only slightly developed. A very common species. Light brown to reddish brown in color. 19-25 x 15-19 microns in size. See Huber-Pestalozzi (1955). Figure 6.

Trachelomonas hispida v. *coronata* Lemmermann

Possesses an elongated ellipsoidal shape lorica, with a surface covered with short spines. There is a slight development of a collar, with the collar rim encircled by row of short spines. 22-30 x 8-15 microns in size. A common species, with variable ranges in size. Dark brown in color. Refer to Couté and Iltis (1981), Huber-Pestalozzi (1955), and Wolowski (1998). Figure 7.

Trachelomonas intermedia Dangeard

Has a lorica that is sub-spherical to broad ellipsoidal in shape, with a collar having a low, but distinct rim, but is not raised. The cell surface is rough, dense, and contains punctae. The cell is dark brown in color. 15-22 microns. (See Couté and Thérézien, p.114, Plate 11, figs.1-3, 1985; Huber-Pestalozzi, 1955).

Trachelomonas Raciborskii Woloszynska

Lorica ellipsoid in shape, with no developed collar. Distinct, sparsely distributed spines, not dense, mostly concentrated at polar ends of cell. Surface area contains punctae. Size 30-34 x 25-28 microns. Dark brown in color. See Couté and Thérézien (1985), Huber-Pestalozzi (1955). Figure 8.

Trachelomonas similis Swirenko

Ellipsoidal shaped lorica, coarse rough surface, with punctae scattered over surface, but no spines. Possesses a thick, coarse collar, extending (4-6 microns), bent from the vertical. Golden brown in color. 24-29 x 16-20 microns in size. See Wolowski (1998). Figure 9.

Trachelomonas superba Swirenko emend. Deflandre

Lorica ellipsoidal, with small punctae over surface. Different length conical-shaped spines over the surface, being longer at the polar ends. Possesses a low collar encircled by ring of spines. Reddish brown in color. Size 30-36 x 23-28 microns. See Couté and Thérézien (1985). Figure 10.

Trachelomonas volvocina Ehrenberg

Lorica globular, spherical shaped, with a surface that is generally smooth, lacking spines. The collar is either lacking, or the collar is slightly developed. With no collar, flagellar pore is surrounded by slightly raised annular region. Very common. Diameter 7-28 microns, reddish brown to dark brown color. See Huber-Pestalozzi (1955). Figure 11.

Another taxon, listed as *Trachelomonas* sp., was noted only once in a July sample, but it could not be identified to species. It is spherical in shape, possessing a definite pole to pole pattern of raised ribs, having a longitudinal type pattern, somewhat similar to *T. Stokesiana* Palmer (Huber-Pestalozzi, 1955), but with greater conformity in the pattern of rib development. Dark brown in color, cell diameter 18-25 microns.

Other Euglenophyceae:

Thirty-three euglenophycean species were recorded for Lake Kilby from these samples (Table 1). These included 13 *Trachelomonas*, 10 *Phacus*, 9 *Euglena*, and 1 *Lepocinclis* sp. The more abundant and common representatives within these genera were *Euglena deses*, *E. mutabilis*, *Phacus caudatus*, *P. longicauda*, *P. monilatus*, *P. suecicus*, *Trachelomonas hispida*, *T. hispida* v. *coronata*, and *T. volvocina*. *Phacus monilatus* produced an extensive bloom in July, with cell concentrations at 74.6×10^3 l^{-1}. The other members of these groups were not abundant and were only intermittently found at the different stations during the months of collection. These results compare closely with species reported by Woodson and Seaburg (1968) in Lake Chesdin, another reservoir lake in Virginia. They reported 42 species, including 17 *Trachelomonas*, 11 *Phacus*, 10 *Euglena*, and 4 *Lepocinclis* in a year-long study. In addition, Marshall and Burchardt (1998) also found the Euglenophyceae well-represented in the tidal-freshwater region of the James River (Virginia), where they identified 2 *Trachelomonas*, 4 *Phacus*, 7 *Euglena*, and 2 *Strombomonas* species.

SUMMARY

Lake Kilby is a shallow, reservoir lake, that is subject to seasonal manipulation regarding water that is removed for regional usage and that which is pumped into the lake from a sub-surface aquifer. In addition to precipitation, normal surface flow and drainage enters its watershed and the lake. This includes the passage of water from surrounding swamps. A total of 33 euglenophycean species were identified in Lake Kilby during a sampling period in June and July 1996. These species include a diverse representation of taxa within the genera of *Euglena*, *Phacus*, and *Trachelomonas*. From this group, twelve *Trachelomonas* species are described in detail. The most common species within this category were *T. hispida, T. hispida* v. *coronata,* and *T. volvocina*. The other *Trachelomonas* species were less abundant, and not consistently recorded at all the sampling sites. Several species were rare. The genus *Phacus* was well-represented, with *P. monilatus* attaining bloom concentrations, whereas, *Euglena* spp. were less abundant. The examination of the *Trachelomonas* cells with scanning

electron microscopy noted some range of variation in the outer cell morphology and dimensions of several species. Such phenotypic variations may be expected due to the different sets of daily or monthly environmental factors that would influence the development of these assemblages.

ACKNOWLEDGEMENT

Appreciation is given to the Virginia Department of Game and Inland Fisheries who supported components of this study, to Mitchell Norman who assisted in the phytoplankton collections in Lake Kilby, and to David Seaborn for the SEM preparation and operation.

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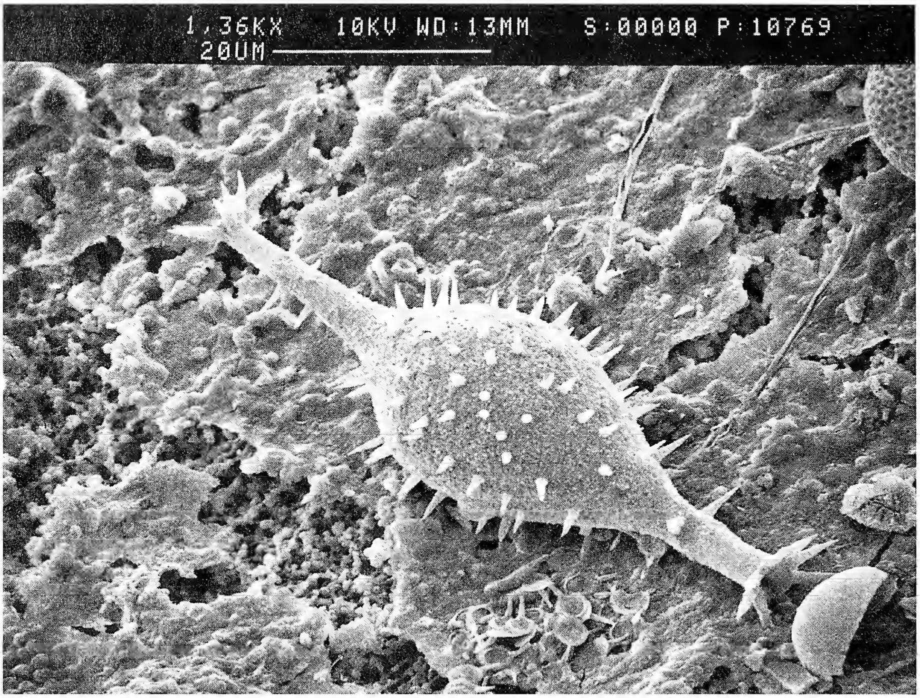

FIGURE 1. *Trachelomonas acanthophora* Stokes

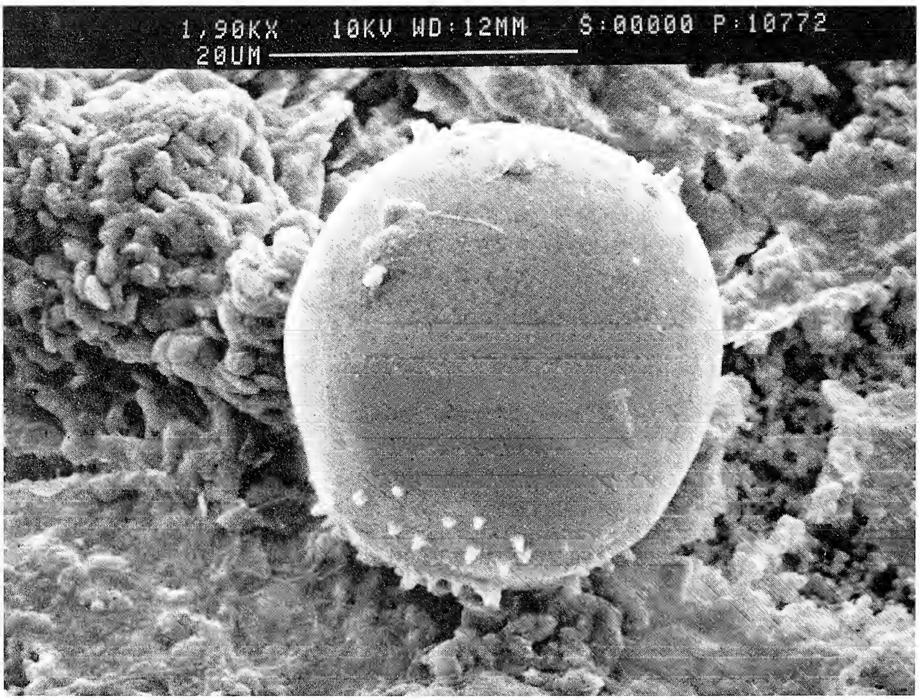

FIGURE 2. *Trachelomonas acanthostoma* Stokes emend. Deflandre

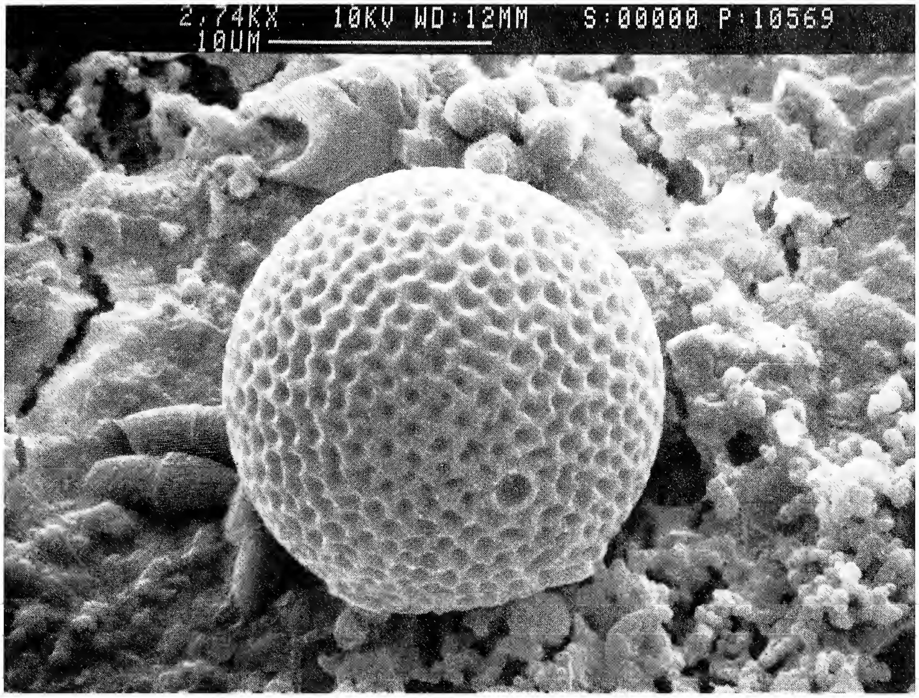

FIGURE 3. *Trachelomonas alisoviana* Skvortzov

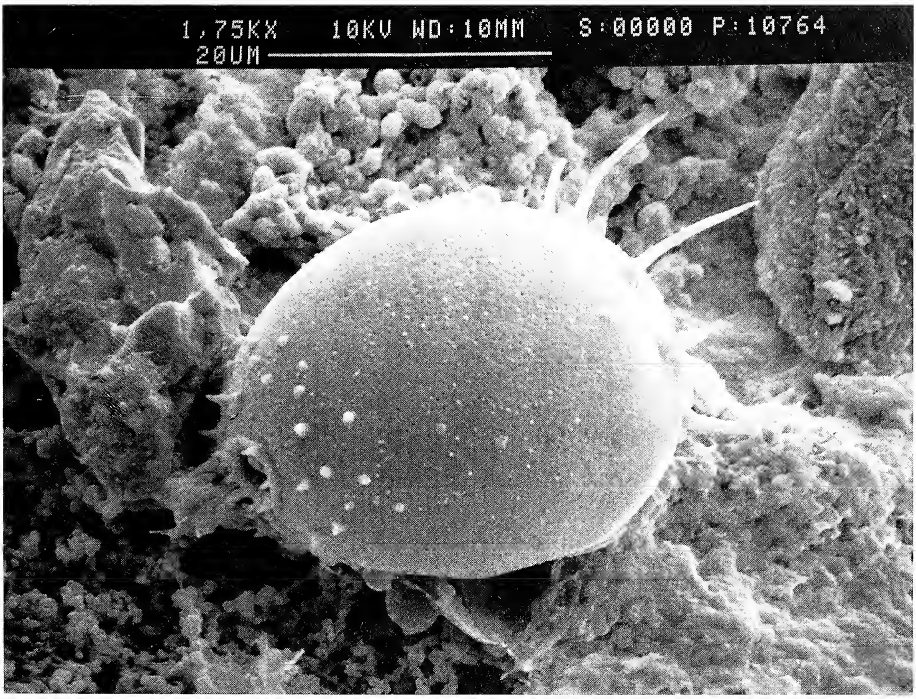

FIGURE 4. *Trachelomonas armata* v. *Steinii* Lemmermann emend. Deflandre

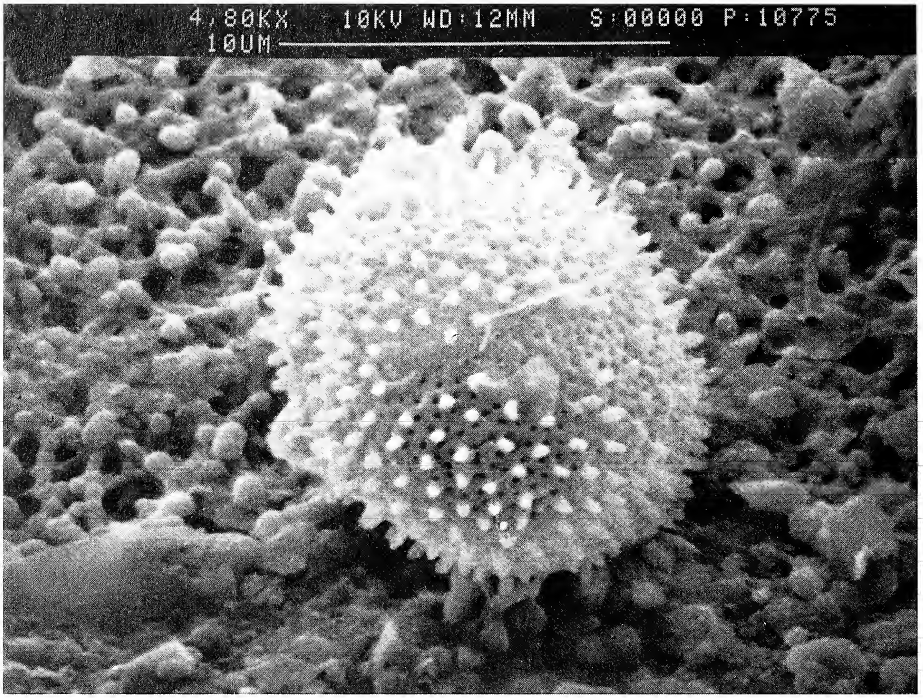

FIGURE 5. *Trachelomonas globularis* v. *Boyeri* (Palmer) Conrad

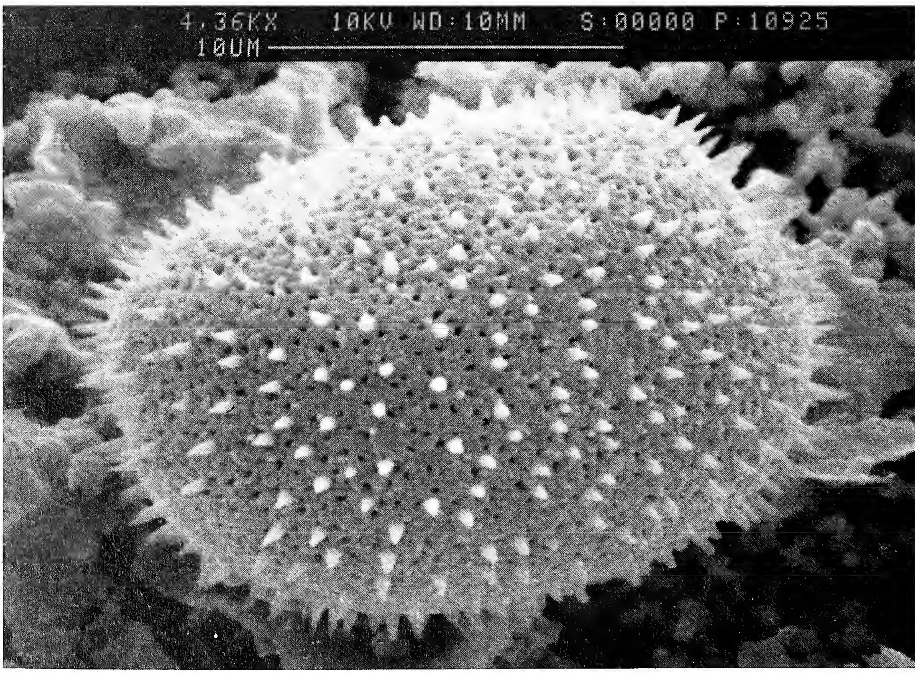

FIGURE 6. *Trachelomonas hispida* (Perty) Stein

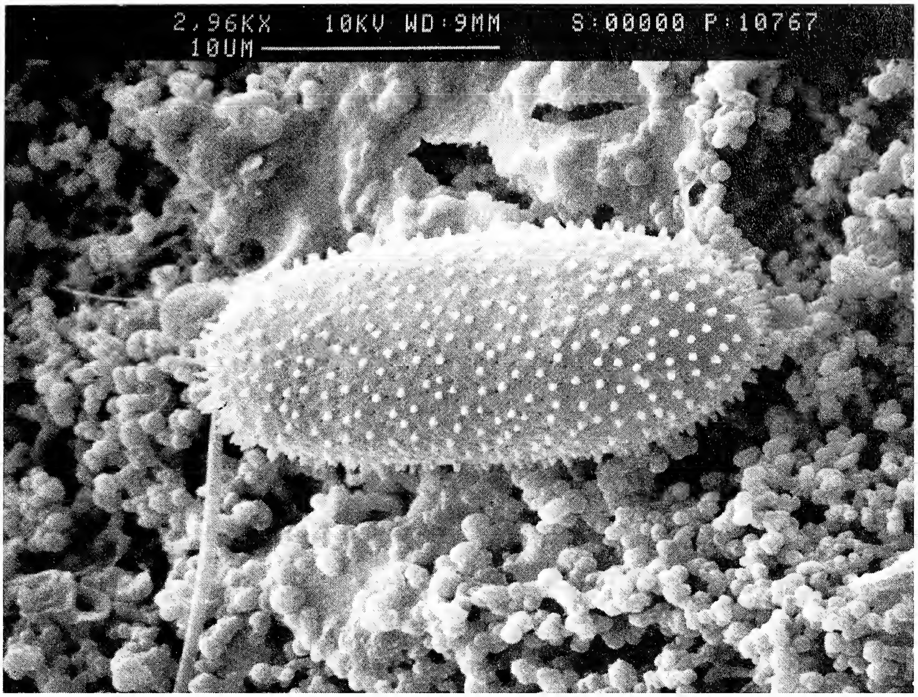

FIGURE 7. *Trachelomonas hispida* v. *coronata* Lemmermann

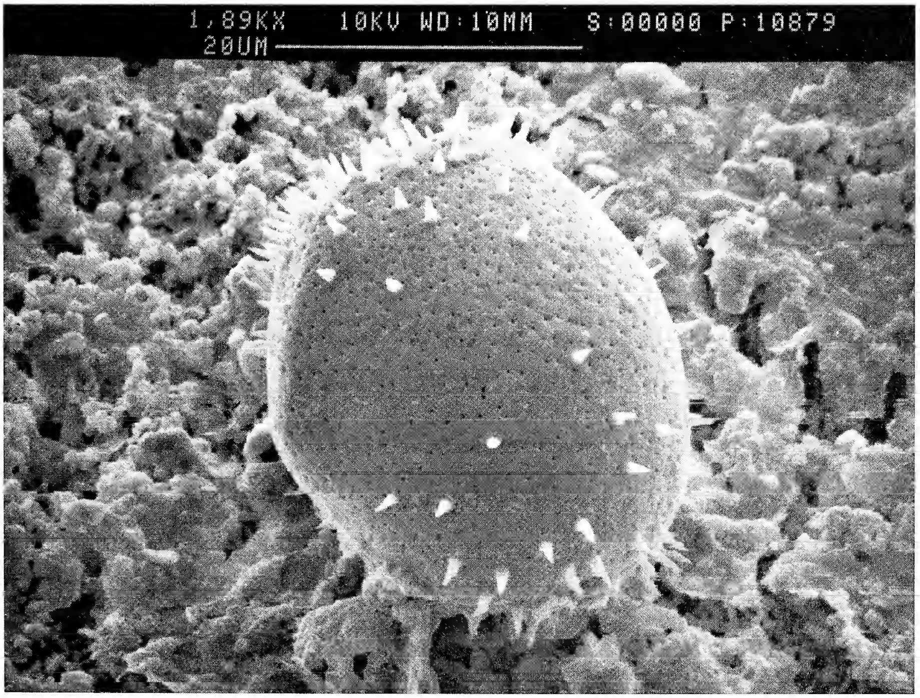

FIGURE 8. *Trachelomonas Raciborskii Woloszynska*

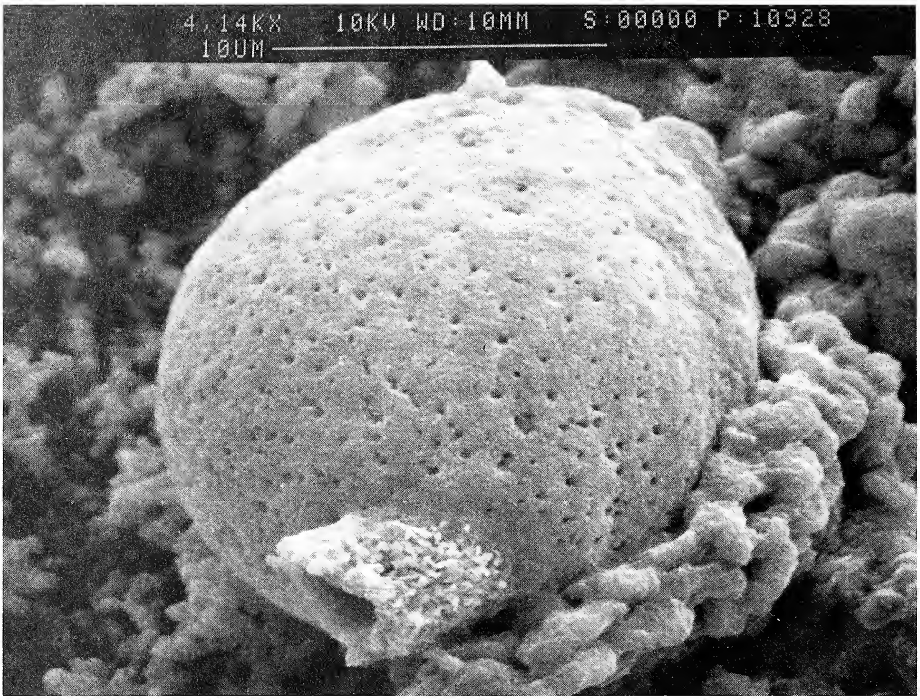

FIGURE 9. *Trachelomonas similis* Swirenko

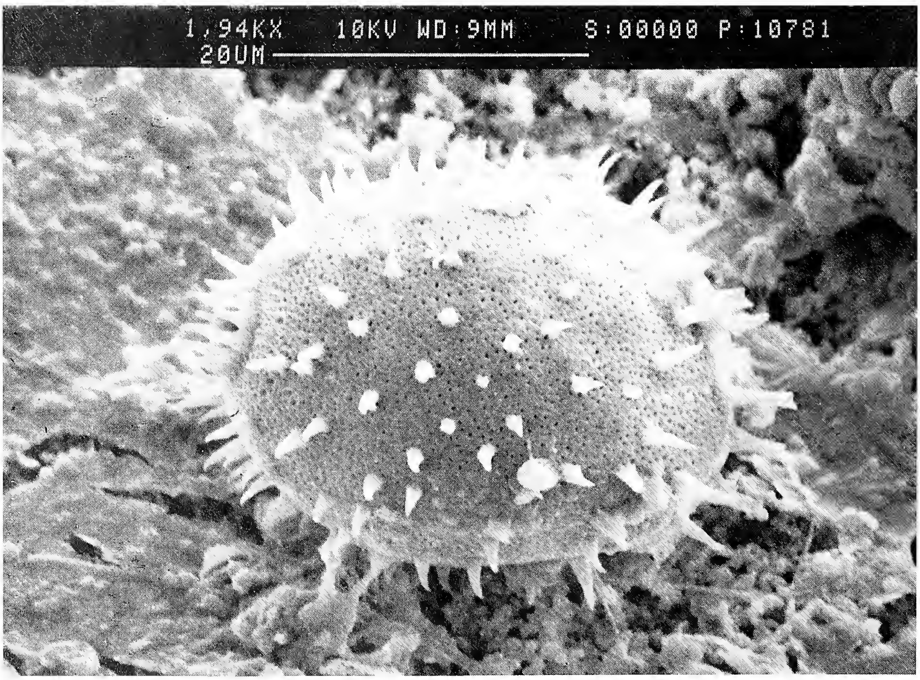

FIGURE 10. *Trachelomonas superba* Swirenko emend. Deflandre

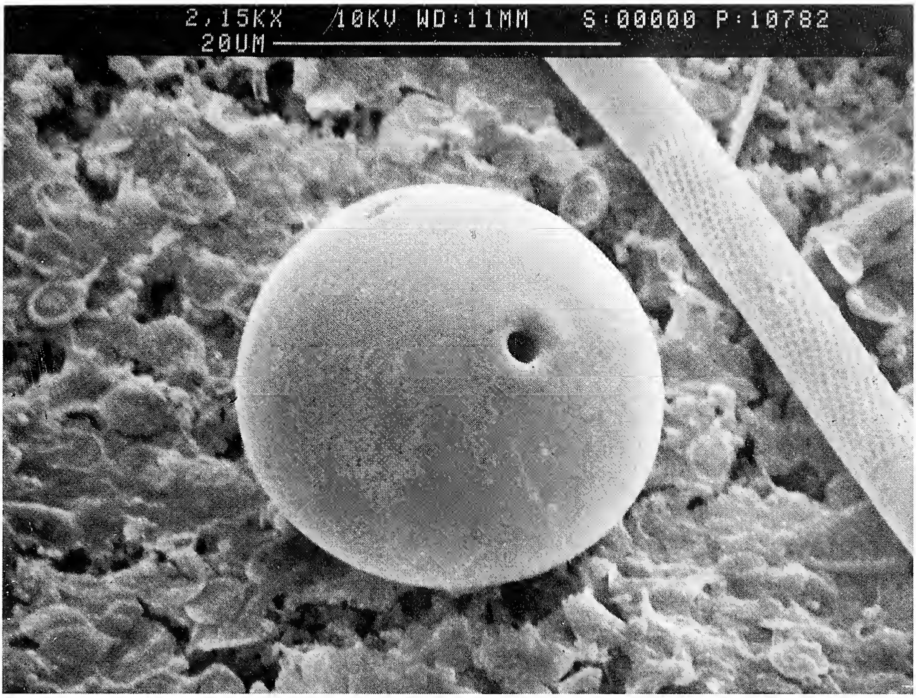

FIGURE 11. *Trachelomonas volvocina Ehrenberg*

TABLE 1. Species within the Euglenophyceae observed in Lake Kilby, June and July 1996.

Euglena acus Ehrenberg
Euglena caudata Hübner
Euglena deses Ehrenberg
Euglena Ehrenbergii Klebs
Euglena mutabilis Schmitz
Euglena oxyuris Schmarda
Euglena proxima Dangeard
Euglena spirogyra Ehrenberg
Euglena sp.
Lepocinclis ovum (Ehrenberg) Lemmermann
Phacus acuminatus Stokes
Phacus caudatus Hübner
Phacus helicoides Pochman
Phacus Lemmermannii (Swirenko) Skvortzov
Phacus longicauda (Ehrenberg) Dujardin
Phacus monilatus Stokes
P*hacus obicularis* Hübner
Phacus suecicus Lemmermann
Phacus tortus (Lemmermann) Skvortzov
Phacus undulatus (Skvortzov)
Trachelomonas acanthophora Stokes
Trachelomonas acanthostoma Stokes emend. Deflandre
Trachelomonas alisoviana Skvortzov
Trachelomonas armata v. *Steinii* Lemmermann emend. Deflandre
Trachelomonas globularis v. *Boyeri* (Palmer) Conrad
Trachelomonas hispida (Perty) Stein
Trachelomonas hispida v. *coronata* Lemmermann
Trachelomonas intermedia Dangeard
Trachelomonas raciborskii Woloszynska
Trachelomonas similis Swirenko
Trachelomonas sp.
Trachelomonas superba Swirenko emend. Deflandre
Trachelomonas volvocina Ehrenberg

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Comparison of Spawning and Non-spawning Substrates in Nests of Species of *Exoglossum* and *Nocomis* (Actinopterygii: Cyprinidae)

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ABSTRACT

Percent composition of pebble size classes from spawning and non-spawning substrates of nests were used to test the hypothesis that distribution of pebble sizes is random in nests of *Exoglossum laurae*, *Exoglossum maxillingua*, *Nocomis leptocephalus*, *Nocomis micropogon*, *Nocomis platyrhynchus*, and *Nocomis raneyi* in Virginia. In nests of the two species of *Exoglossum*, spawning areas (i.e., upstream bases of nests) contain significantly greater amounts of the 6.0 mm size class, and significantly smaller amounts of the 2.5 mm size class of stones than do non-spawning areas. Spawning areas (i.e., pits) in nests of *N. leptocephalus* contain significantly more 6.0 mm pebbles than non-spawning areas, whereas spawning areas (i.e., troughs) in nests of *N. micropogon*, *N. platyrhynchus*, and *N. raneyi* are composed of significantly more 6.0 and 11.3 mm pebbles than non-spawning areas. In all *Nocomis* species, there was significant selection against the largest (23.0 mm) size class of pebbles in spawning areas. Male *Exoglossum* and *Nocomis* expend significant amounts of time reorganizing substrate material in spawning areas of their nests before and during spawning. Reorganizing pebbles results in relatively uniform sizes of substrate material at spawning areas in nests of each species. We propose that selection of 6.0 and 11.3 mm size classes for spawning areas is related to spawning behaviors and enhancement of survival of eggs and larvae in nests. Sizes larger than 11.3 mm interfere with spawning behaviors, and sizes smaller than 6.0 mm form a compacted substrate, which can crush eggs and larvae during trough or pit reshaping, and impede water flow, and consequently, aeration of eggs and larvae.

INTRODUCTION

Reighard (1943), Lobb and Orth (1988), Maurakis et al. (1991a,b; 1992a; 1998), and Vives (1990) present qualitative and quantitative information of overall composition of pebble nests constructed by males in species of *Exoglossum* (*E. laurae* and *E. maxillingua*), and *Nocomis* (*N. biguttatus, N. leptocephalus, N. micropogon, N. platyrhynchus*, and *N. raneyi*). However, no attention is given to composition of substrates at specific sites in nests where spawning occurs. In *Exoglossum* and *Nocomis*, a completed nest is typically a dome-shaped mound of pebbles (Van Duzer, 1939; Lachner, 1952; Maurakis, 1991b), and the spawning act is restricted to a specific site that accounts for only a small portion of the total nest substrate. In *Exoglossum*, the spawning act occurs at the upstream base of the nest (Van Duzer, 1939; Maurakis, 1991b). In *Nocomis*, spawning occurs either in a pit (*N. leptocephalus*, Maurakis et al., 1991a, Sabaj et al., 2000) or in the upstream half of a trough (*N. micropogon*, *N. platyrhynchus*, *N. raneyi*; Maurakis et al., 1991a, Maurakis, 1998; Sabaj et al., 2000) located on the upstream slope of the nest. Direct observations and reviews of video recordings of nest construction and breeding behaviors of males in *N. platyrhynchus* and *N. raneyi* in Virginia by Maurakis (1998) reveal that considerable time is spent by males in reorganizing substrate material where spawning occurs, and that apparent sizes of substrate material are smaller than those in non-spawning areas of nests.

Objectives of our study are to analyze percent composition of size classes of pebbles from spawning and non-spawning substrates to test whether the distribution of pebble sizes is random in nests of *E. laurae*, *E. maxillingua*, *N. leptocephalus*, *N. micropogon*, *N. platyrhynchus*, and *N. raneyi* in Virginia.

MATERIALS EXAMINED

The state, drainage, collection number (EGM=Eugene G. Maurakis), locality, collection date, and number of nests in parenthesis for *Exoglossum* and *Nocomis* species are:

Exoglossum laurae. Virginia: New, EGM-VA-439 B, Craig Co., Clemmons farm, Sinking Cr., St. Rt. 42, 28 May 1999, (9).

Exoglossum maxillingua. Virginia: James, EGM-VA-438, Craig Co., Johns Cr., Co. Rt. 658 at Maggie, 13 May 1999, (6); EGM-VA-440, Craig Co., Johns Cr., Co. Rt. 632, 0.3 km upstream of Maggie, 28 May 1999, (6); EGM-VA-443, Rockbridge Co., South R., Co. Rt. 608 Bridge, 3 km S of Vesuvius, 29 May 1999, (2).

Nocomis leptocephalus. Virginia: Rappahannock, EGM-VA-427, Faquier Co., Thumb Run, Co. Rt. 688, 29 May 1998, (2); James, EGM-VA-428, Albemarle Co., Stockton Cr., Co. Rt. 691, 50 m. from US 250, 3.2 km W of Yanay Mills, 3 June 1998, (5); EGM-VA-429, Albemarle Co., confluence of Mechum R. and Lickinghole Cr., US Rts. 240 and 250 Jct., 3 June 1998, (2); EGM-VA-438, Craig Co., Johns Cr., Co. Rt. 658 at Maggie, 13 May 1999, (1); EGM-VA-445, Nelsen Co., Rockfish R., Co. Rt. 612, 1 mi S. of St. Rt. 151, 29 May, 1999, (3); New, EGM-VA-437, Craig Co., Clemmons farm, Sinking Cr., St. Rt. 42, 13 May 1999, (6); EGM-VA-439 B, Craig Co., Clemmons farm, Sinking Cr., St. Rt. 42, 28 May 1999, (2).

Nocomis micropogon. Virginia: Potomac, EGM-VA-426, Loudon Co., Catoctin Cr., Co. Rt. 664 at Taylorsville, 27 May 1998, (6); James, EGM-VA-444, Albemarle Co., confluence of Mechum R. and Lickinghole Cr. at US Rts. 240 and 250 Jct., 29 May 1999, (2); EGM-VA-445, Nelsen Co., Rockfish R., Co. Rt. 612, 1.6 km S of St. Rt. 151, 29 May 1999, (1).

Nocomis platyrhynchus. Virginia: New, EGM-VA-416, Montgomery-Floyd Co. Line, Little R., St. Rt. 8 Brigde and 1 km upstream on dirt road, 16 May 1998, (2); EGM-VA-417, Montgomery Co., Little R. at Co. Rt. 693 and 613 Jct., E of Snowville about 8 km W of Riner, 16 May 1998, (2); EGM-VA-439 A, Montgomery Co., Little R. at Jct. Co. Rt. 693 and 613, about 8 km W of Riner, 13 May 1999 (4); EGM-VA-441, Montgomery-Floyd Co. Line, Little R., St. Rt. 8, under bridge and along Little Camp Rd, 28 May 1999, (4).

Nocomis raneyi. Virginia: James, EGM-VA-424, Rockbridge Co., Maury R. at US Rt. 60 at Ben Salem wayside, 23 May 1998, (4); EGM-VA-442, Rockbridge Co., Maury R. at US Rt. 60 at Ben Salem wayside, 28 May 1999, (7).

MATERIALS AND METHODS

Pebble samples of spawning and non-spawning substrates in nests of each species were collected in a 1-liter plastic beaker. Spawning and non-spawning substrate samples were air-dried and sifted through five custom-built wire sieves. Mesh sizes of sieves (23.0, 11.3, 6.0, 2.5, and 0.8 mm) were determined by commercially available prefabricated screen sizes and provide a more detailed account of nest composition than standard sieve samplers described by Hynes (1970). Material (<0.8 mm) that sifted through the smallest size mesh was collected in a pan. Weights of materials in each sieve size and pan were used to calculate the percentage of material per mesh and pan size. Percentages of size classes (based on weights) were used in electivity indices (Ivlev, 1961) to calculate the relative proportion of each pebble size class in spawning areas and non-spawning areas of nests. The equation $E = (n - p) / (n + p)$ (where E = pebble size selection, n = the percentage of a particular pebble size in the spawning area of the nest, and p = the percentage of a particular pebble size in the non-spawning area of the nest) was used to determine if selection of pebble size for the spawning area was nonrandom. Electivity index values range from 1 to − 1. Values closer to one indicate a greater selection of a particular pebble size. Percentages and electivity values were transformed to arcsin equivalents. Differences in average percentages of each size class of stones between spawning and non-spawning areas in nests of each species were tested with a *t*-test (SAS, 1996). Differences in average percentages and those of electivity values among pebble size classes of spawning and non-spawning areas in nests of each species were determined with a General Linear Model and Duncan's Multiple Range Test (SAS, 1996).

RESULTS

Exoglossum species:

At spawning substrates, percentages of 6.0 and 2.5 mm size classes ($\bar{x}$=73.91 and 21.3, respectively) in nests of *E. laurae*, and those of 6.0 and 2.5 mm size classes ($\bar{x}$=73.61 and 21.87, respectively) in nests of *E. maxillingua* were significantly greater than those of other size classes in each species (Table 1). In non-spawning substrates, percentages of 6.0 and 2.5 mm size classes of pebbles in nests of *E. laurae* ($\bar{x}$=49.48 and 40.26, respectively), and those for *E. maxillingua* ($\bar{x}$=59.79 and 34.99, respectively) were significantly higher than averages of other size classes (Table 2).

t-test values:

In nests of each species, the average amount of 6.0 mm stones in spawning areas was significantly greater than that in non-spawning areas (Table 3). Conversely, the amount of 2.5 mm stones in spawning areas was significantly lower than that in non-spawning areas in nests of each species (Table 3).

Electivity values:

Only the 6.0 mm size class of stones had positive electivity values at spawning substrates in nests of each species of *Exoglossum* (Table 4).

TABLE 1. Average percentage of material by size class in spawning areas of nests of *Exoglossum laurae, Exoglossum maxillingua, Nocomis leptocephalus, Nocomis micropogon, Nocomis platyrhynchus,* and *Nocomis raneyi*. Underscored means do not differ significantly.

Species	% spawning nest area size class (mm)					
E. laurae	23.0	<0.8	0.8	11.3	2.5	6.0
$\bar{x}$	0.00	0.33	0.63	3.81	21.30	73.91
F=126.68; p=0.0001; df=5						
E. maxillingua	23.0	<0.8	0.8	11.3	2.5	6.0
$\bar{x}$	0.00	0.10	0.27	3.17	22.87	73.61
F=718.02; p=0.0001; df=5						
N. leptocephalus	<0.8	0.8	2.5	23.0	6.0	11.3
$\bar{x}$	0.05	0.07	2.85	4.80	28.47	63.76
F=214.62; p=0.0001; df=5						
N. micropogon	<0.8	0.8	2.5	23.0	6.0	11.3
$\bar{x}$	0.13	0.15	0.67	8.57	12.03	78.46
F=168.21; p=0.0001; df=5						
N. platyrhynchus	<0.8	0.8	2.5	6.0	23.0	11.3
$\bar{x}$	0.03	0.04	0.18	7.94	22.94	68.84
F=87.69; p=0.0001; df=5						
N. raneyi	<0.8	0.8	2.5	6.0	23.0	11.3
$\bar{x}$	0.04	0.10	0.69	14.29	15.16	69.68
F=139.90; p=0.0001; df=5						

Nocomis species:

In spawning areas, percentages of the 11.3 mm size class were significantly higher than those of other size classes in nests of all species of *Nocomis* (Table 1). Although lower in proportion to the 11.3 mm size class, other size classes were significantly higher at spawning areas in nests of all species of *Nocomis*: 6.0 mm in *N. leptocephalus*; 6.0 and 23.0 mm in *N. micropogon*; 23.0 mm in *N. platyrhynchus*; and 23.0 and 6.0 mm in *N. raneyi* (Table 1). In non-spawning areas, percentages of the 11.3 and 23.0 mm size classes were significantly higher than those of other size classes in nests of each species of *Nocomis* (Table 2).

t-test values:

In *N. leptocephalus*, amounts of both 6.0 and 2.5 mm stones in spawning areas were significantly greater than those in non-spawning areas (Table 3). Conversely, amount of 23.0 mm size class of stones in spawning areas was significantly lower than that of the same size class in non-spawning areas (Table 3). In *N. micropogon, N. platyrhynchus,* and *N. raneyi,* amounts of 11.3 and 6.0 mm stones in spawning areas of nests of each species were significantly greater than those of the same size classes at non-spawning areas. As in *N. leptocephalus*, amounts of 23.0 mm size class of stones at

TABLE 2. Average percentage of material by size class in non-spawning areas of nests of *Exoglossum laurae, Exoglossum maxillingua, Nocomis leptocephalus, Nocomis micropogon, Nocomis platyrhynchus,* and *Nocomis raneyi.* Underscored means do not differ significantly.

Species	% non-spawning nest area size class (mm)					
E. laurae	23.0	<0.8	0.8	11.3	2.5	6.0
$\bar{x}$	0.00	0.37	1.43	8.47	40.26	49.48
F=49.43; p=0.0001; df=5						
E. maxillingua	23.0	<0.8	0.8	11.3	2.5	6.0
$\bar{x}$	0.00	0.54	1.73	2.94	34.99	59.79
F=406.65; p=0.0001; df=5						
N. leptocephalus	0.8	<0.8	2.5	6.0	23.0	11.3
$\bar{x}$	0.06	0.06	0.95	12.36	19.90	68.55
F=128.22 p=0.0001; df=5						
N. micropogon	0.8	<0.8	2.5	6.0	23.0	11.3
$\bar{x}$	0.13	0.14	0.35	5.84	34.92	58.62
F=37.27; p=0.0001; df=5						
N. platyrhynchus	0.8	<0.8	2.5	6.0	11.3	23.0
$\bar{x}$	0.06	0.09	0.43	2.47	31.68	65.28
F=124.24; p=0.0001; df=5						
N. raneyi	<0.8	0.8	2.5	6.0	11.3	23.0
$\bar{x}$	0.08	0.08	0.17	2.86	38.40	58.44
F=85.19; p=0.0001; df=5						

spawning areas were significantly lower than those of the same size class at non-spawning areas in nests of *N. micropogon, N. platyrhynchus,* and *N. raneyi* (Table 3).

Electivity Values:

In *N. leptocephalus*, average electivity values for 6.0 ($\bar{x}$ = 0.41)and 2.5 ($\bar{x}$ = 0.35) mm size classes were significantly higher than those of all other size classes ($\bar{x}$ range = −0.60 to 0.02) (Table 4). In *N. micropogon*, average electivity values for 6.0 ($\bar{x}$ = 0.52) and 2.5 ($\bar{x}$ = 0.39) mm size classes were significantly greater than those of other size classes ($\bar{x}$ range=−0.75to −0.14) with one exception: they did not differ from that of 11.3 mm size class ($\bar{x}$ = 0.16) (Table 4). In *N. platyrhynchus*, average electivity values for 11.3 mm size class ($\bar{x}$ = 0.40) and 6.0 mm size class ($\bar{x}$ = 0.39) were significantly higher than those of all other size classes ($\bar{x}$ range=−0.57to −0.18) with one exception: they did not differ from that of 2.5 mm size class ($\bar{x}$ = 0.08) (Table 4). In *N. raneyi*, average electivity values for 6.0 ($\bar{x}$ = 0.64), 2.5 ($\bar{x}$ = 0.38), and 11.3 ($\bar{x}$ = 0.31) mm size classes were significantly different from other size classes ($\bar{x}$ range = −0.62 to −0.12) (Table 4).

TABLE 3. Probability of substrate amount by size class in spawning areas greater than that in non-spawning areas in nests of *Exoglossum laurae, Exoglossum maxillingua, Nocomis leptocephalus, Nocomis micropogon, Nocomis platyrhynchus*, and *Nocomis raneyi*.

Species	Size class (mm)	Mean	Std Error	T	Prob>|T|
E. laurae	23.0	0	0		
	11.3	-4.6555556	3.4267782	-1.3585810	0.2113
	6.0	24.4333333	3.3591914	7.2735759	0.0001
	2.5	-18.9555556	5.6464126	-3.3570971	0.0100
	0.8	-0.8066667	0.3665757	-2.2005457	0.0589
	<0.8	-0.0400000	0.0874960	-0.4571636	0.6597
E. maxillingua	23.0	0	0	.	.
	11.3	0.2357143	0.9997036	0.2357842	0.8173
	6.0	13.8214286	1.9150411	7.2173014	0.0001
	2.5	-12.1142857	2.2246337	-5.4455192	0.0001
	0.8	-1.4600000	0.2303771	-6.3374344	0.0001
	< 0.8	-0.3721429	0.0932159	-3.9922673	0.0015
N. leptocephalus	23.0	-15.0952381	3.5144157	-4.2952340	0.0004
	11.3	-4.7952381	3.6559116	-1.3116395	0.2045
	6.0	16.1047619	2.3179165	6.9479474	0.0001
	2.5	1.9014286	0.8170837	2.3270914	0.0306
	0.8	0.0076190	0.0169720	0.4489178	0.6583
	<0.8	-0.0142857	0.0119835	-1.1921105	0.2472
N. micropogon	23.0	-26.3555556	6.0544087	-4.3531180	0.0024
	11.3	19.8333333	6.3812007	3.1080880	0.0145
	6.0	6.1888889	1.7459035	3.5448058	0.0076
	2.5	0.3177778	0.2232884	1.4231718	0.1925
	0.8	0.0200000	0.0457044	0.4375950	0.6733
	<0.8	-0.0122222	0.0438044	-0.2790180	0.7873
N. platyrhynchus	23.0	-42.3333333	3.9072453	-10.8345728	0.0001
	11.3	37.1583333	2.7921822	13.3079901	0.0001
	6.0	5.4750000	1.8429688	2.9707502	0.0127
	2.5	-0.2391667	0.3215221	-0.7438577	0.4726
	0.8	-0.0208333	0.0178995	-1.1639071	0.2691
	<0.8	-0.0600000	0.0378193	-1.5864896	0.1409
N. raneyi	23.0	-43.2818182	4.0513481	10.6833126	0.0001
	11.3	31.2818182	5.4995011	5.6881193	0.0002
	6.0	11.4272727	2.9427906	3.8831416	0.0030
	2.5	.5163636	0.2665342	1.9373258	0.0814
	0.8	0.0209091	0.0445473	0.4693682	0.6489
	<0.8	-0.0381818	0.0277265	-1.3770862	0.1985

TABLE 4. Average electivity value per size class of pebbles of spawning areas in nests of *Exoglossum laurae, Exoglossum maxillingua, Nocomis leptocephalus, Nocomis micropogon, Nocomis platyrhynchus,* and *Nocomis raneyi*. Underscored means do not differ significantly.

Species	Electivity per pebble size class (mm)					
E. laurae	23.0	0.8	11.3	2.5	<0.8	6.0
$\bar{x}$	-0.99	-0.49	-0.37	-0.31	-0.16	0.20
F=8.69; p=0.0001; df=5						
E. maxillingua	23.0	0.8	<0.8	2.5	11.3	6.0
$\bar{x}$	0.99	-0.71	0.54	-0.21	-0.11	0.11
F=25.50; p=0.0001; df=5						
N. leptocephalus	23.0	<0.8	11.3	0.8	2.5	6.0
$\bar{x}$	-0.60	-0.15	-0.03	0.02	0.35	0.41
F=14.06; p=0.0001; df=5						
N. micropogon	23.0	<0.8	0.8	11.3	2.5	6.0
$\bar{x}$	-0.75	-0.45	-0.14	0.16	0.39	0.52
F=10.97; p=0.0001; df=5						
N. platyrhynchus	23.0	<0.8	0.8	2.5	6.0	11.3
$\bar{x}$	-0.57	-0.42	-0.18	0.08	0.39	0.40
F=10.37; p=0.0001; df=5						
N. raneyi	23.0	<0.8	0.8	11.3	2.5	6.0
$\bar{x}$	-0.62	-0.28	-0.12	0.31	0.38	0.64
F=11.79; p=0.0001; df=5						

DISCUSSION

We reject the hypothesis that pebbles are distributed randomly between spawning and non-spawning areas in nests constructed by males in *E. laurae*, *E. maxillingua*, *N. leptocephalus*, *N. micropogon*, *N. platyrhynchus*, and *N. raneyi*. In nests of the two species of *Exoglossum*, spawning areas (i.e., upstream bases of nests) contain significantly greater amounts of 6.0 mm size class of stones than non-spawning areas. In contrast, significantly smaller amounts of 2.5 mm size pebbles were present in their spawning areas. Similarly, spawning areas (i.e., pits) in nests of *N. leptocephalus* contain more 6.0 mm pebbles than non-spawning areas, whereas spawning areas (i.e., troughs) in nests of *N. micropogon*, *N. platyrhynchus*, and *N. raneyi* are composed of significantly more 6.0 and 11.3 mm pebbles than non-spawning areas. In all *Nocomis* species, there was significant selection against the largest (23.0 mm) size class of pebbles in spawning areas. Male *Exoglossum* and *Nocomis* expend significant amounts of time reshaping and reorganizing substrate material in spawning areas of

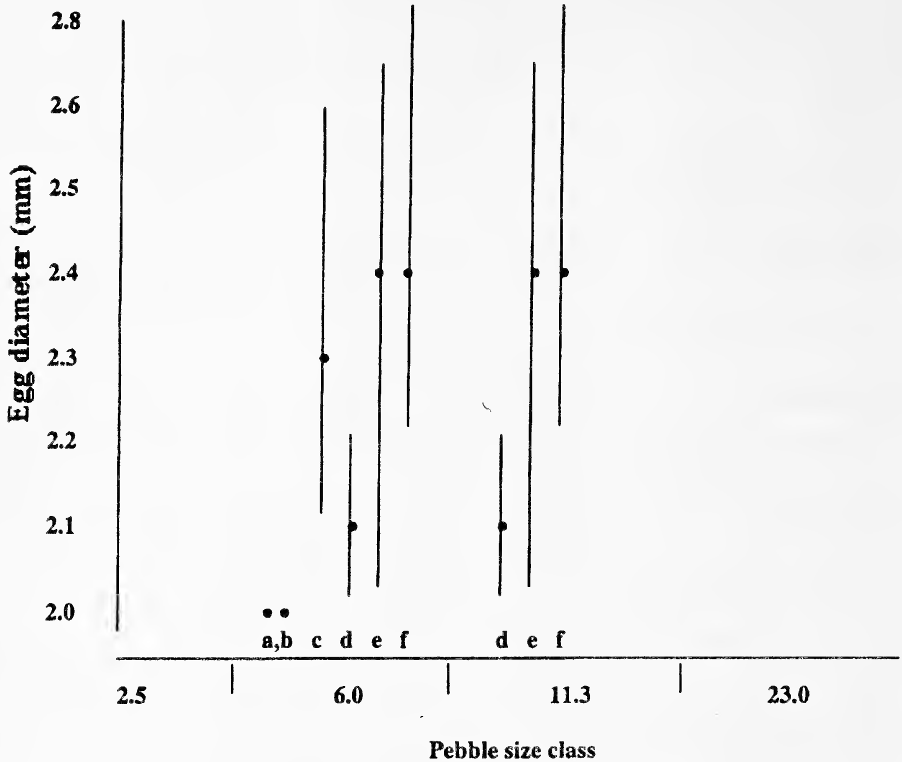

FIGURE 1. Distribution of egg diameter per size class of pebbles by species of *Exoglossum* and *Nocomis*. Key: a=*E. laurae* (Raney, 1939); b=*E. maxillingua* (Van Duzer, 1939); c=*N. leptocephalus* (pers. obs., EGM-NC-211) d=*N. platyrhynchus* (Zorman and Maurakis, 2000) e= *N. micropogon* (Fish, 1932; Cooper, 1980; Buynak and Mohr, 1980); f=*N. raneyi* (Maurakis et al., 1992b).

their nests before and during spawning (Raney, 1939; Van Duzer, 1939; Sabaj, 1992; Maurakis et al., 1991b). Reorganizing pebbles results in relatively uniform sizes of substrate material in spawning areas of nests of each species. We propose that uniform composition of substrates in spawning areas is related to spawning behaviors and enhancement of survival of eggs and larvae in nests. In *Exoglossum* and *Nocomis*, a successful spawn is the end result of a precise sequence of male-female interactions (i.e., approach, alignment, run, clasp) coordinated in part by the topography of the pebble nest (Sabaj, 1992; Maurakis, 1998; Sabaj et al., 2000). By reshaping and reorganizing the spawning substrate, the male removes obstructions (e.g. stones >11.3 mm size class) that may interfere with the sequence of spawning behaviors.

Size uniformity of pebbles and resultant interstices in spawning substrates also may afford conditions that prevent crushing and smothering of buried eggs and post-hatch larvae. Egg diameters of all species range from 2.0-2.8 mm and spawning substrates range from 6.0 – 11.3 mm. (Figure 1). Spawning substrates do not contain extremely small gravel (2.5 mm) and sand ($\leq$ 0.8 mm). These smaller sizes form compacted substrate material, which can crush eggs and larvae during trough or pit reshaping, and impede water flow, and consequently, aeration of eggs and larvae.

Sabaj (1992) and Maurakis et al. (1992a) stated nest-building male *Exoglossum* and *Nocomis* cause reduced water current velocities for egg deposition by physically modifying an area of streambed, a result of their nest building activity. We concur with their findings, and stipulate that in modifying the substrate, males are selective in which sizes of stones they deposit in spawning areas before and during spawning.

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Environmental effects on yield and agronomic traits of common bean (*Phaseolus vulgaris L.*)

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ABSTRACT

Common bean (*Phaseolus vulgaris* L.) demand is increasing with an alarming rate around the world, especially in Latin America, Africa, and Asia. Therefore, increased bean yield per hectare is the best way to meet the world demand rather than expansion of area under cultivation. The objectives of this experiment were to determine the genotypic variations for green bean and dry seed yield and magnitude of genotype x environment interaction effects on yield and yield components of common bean. Thirteen genotypes were planted during the 1992, 1994, and 1995 growing seasons. Genotypes were evaluated for green pod and seed yield and yield components at R7 and R9 growth stages. Years differed significantly for all recorded parameters at both R7 and R9 stages. Genotypes and genotype x year interaction were also differed significantly for most measured parameters at both stages. The genotype Eagle showed the highest green pod yield, while Branco and Blue Ridge ranked second and third, respectively when averaged over the three years. Number of pods plant^{-1}, hundred pod weight and pod length were positively and significantly correlated with green pod yield. Number of pods plant^{-1} showed the highest correlation ($r = 0.61^{**}$) with green pod yield. All the recorded parameters were positively significantly correlated with dry seed yield. Plant height was negatively correlated with seed size, number of seeds plant^{-1} and seed weight plant^{-1}. Number of pods plant^{-1} was positively correlated ($r = 0.51^{**}$) and seed size exhibited highest correlation value ($r = 0.48^{**}$) with seed yield. Seed size and number of pods plant^{-1} can be effectively used for indirect selection of green pod yield and dry seed yield of common bean.

Key words: Common bean, *Phaseolus vulgaris*, yield, growth stage.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is the principal source of dietary protein, vitamins and minerals for more than 500 million people in Latin America and Africa (FAO, 1991; CIAT, 1992). Common bean is grown for its green leaves, green pods, and green and/or dry seeds. Dry leaves, threshed pods, and stalks are fed to animals and used as fuel for cooking, especially in developing countries of Africa and Asia (Sirbelnagel et al., 1991). Beans are a major horticultural crop in the USA, where crop

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efficiency in acquiring and using water and nutrients is of interest as a component of sustainable agriculture (Jonathan and Beem, 1993).

Bean production has to be increased by about 42% in Latin America and 75% in Africa to satisfy the expected demand (Janssen, 1989). The projected current demand in China alone is 4.3 million tons, an increase of 22.8% compared to 1989 where the demand was 3.5 million tons (World Bank, 1989). Bean production in the developing countries is often on marginal land, and few developing countries have significant reserves of arable land that can be exploited to bean cultivation. Increased yield per hectare is the best way to meet the world demand rather than expansion of area under cultivation (Xiaolong Yan et al., 1995).

Average bean yields in most developing countries are <20% of the yield potential (CIAT, 1991), which indicates that substantial improvement in bean could be realized by increasing yields per unit land area. Increase in productivity can be attributed to genetic gains, greater use of production inputs, better agronomic practices, and more favorable growing environments. It is the combination of all these factors which provide maximum yield per unit of cropped land.

The purpose of most crop breeding programs is to increase and/or stabilize the harvestable yield per unit of land area grown at a production cost which maximizes returns to growers (Sirbelnagel et al., 1991). The primary objective of many plant breeders is the development of genotypes that are consistently high yielding over a range of environments. Genotype x environment interactions can hinder progress from selection by masking genotypic effects (Comstock and Moll, 1963). Genotype x environment interactions are often described as inconsistent differences among genotypes from one environment to another. The inconsistency could be due the difference in responses of the same set of genes to different environments and the expression of different sets of genes in different environments (Falconer, 1952; Robertson, 1959; Yang and Baker, 1991).

Yield of common bean has bean negatively associated with seed size (White and Gonzalez, 1990; White et al., 1992). However, positive relationships where reported between soybean, *Glycine max* (L.) Merr. seed size and yield (LeRoy et al., 1991; Tinius et al., 1991), and in favabean, *Vicia faba* L. (Dantuma et al., 1983). On the other hand, Mebrahtu et. al. (1991) reported that number of seeds plant^{-1} and number of pods plant^{-1} were the most important components of bean seed yield. The objectives of this study were to determine: a) the genotypic variations for green bean and dry seed yield and b) the magnitude of genotype x environment interaction effects on yield and yield components of common bean.

MATERIALS AND METHODS

Thirteen common bean genotypes were planted in four-row plots arranged in randomized complete block design with four replications during 1992-1995. However, no substantial yield was harvested in 1993, therefore, results of the other three years are only reported. The experiments were conducted on Abell Sandy loam (fine loamy mixed, Theramic Aquatic Haplidults) soil, at Randolph Research Farm of VSU, Petersburg, Virginia. Each four-row plot was 3 m long, with 75 cm spacing between rows. A seeding rate of 23 seeds m^{-1} of row was used. Following soil test recommendations, 200 kg ha^{-1} P and K fertilizer was applied to the soil. Trifluralin herbicide was incorporated into the soil prior to planting at the recommended rate.

TABLE 1. Combined mean square analysis of variance for green pod and seed yields and yield components harvested at green pod stage (R7) and dry seed growth stage (R9).

Source of Variance	dF	GPY (Kg ha^{-1})	SY (Kg ha^{-1})	$NGPP^{-1}$	HPW (g)	HSW (g)	SWP^{-1} (g)	GPL (cm)	DPL (cm)	Plant Height
Year (Y)	2	1715^{**}	59.4^{**}	703.2^{**}	616893^{**}	514^{**}	1125^{**}	18.5^{*}	59.7^{**}	530^{**}
Rep (Y)	9	144	0.9	62.5	16572	9.3	58.7	2.5	2.7	35.7
Genotype (G)	12	82^{*}	2.1^{ns}	79.5^{**}	28036^{**}	44.0^{ns}	81.9^{ns}	11.6^{**}	7.2^{**}	132^{ns}
Y X G	24	75^{*}	1.4^{**}	32.5^{*}	10579^{*}	22.1^{**}	473^{**}	1.9^{*}	1.1^{**}	54.8^{**}
Error	108	45	0.6	18.1	6094	5.0	15.8	1.5	1.9	23.4

*, ** Significant at 0.5 and 0.01 probability levels, respectively.
ns = not significant.
GPY = Green pod yield, SY = seed yield, NGPP = Number of green pods/plant; HPW = Hundred pod weight; SWP = Seed weight per plant; GPL = Green pod length; DPL = Dry pod length

Genotypes were evaluated at green pod (R7) and dry seed (R9) stages for various agronomic parameters (Robert Hall 1991). One meter of each of the two center rows was harvested at both stages. At R7, the harvested materials were put into plastic bags and brought to the laboratory. Five plants from each above ground harvested plot were randomly selected and, plant height, number of pods $plant^{-1}$, hundred pod weight, pod length and green pod yield were recorded as described by Nienhius and Singh (1985). The pods from each harvested plot were pulled manually and the weight was recorded as green pod yield. At R9 stage, number of pods $plant^{-1}$, pod length, number of seeds $plant^{-1}$, and hundred seed weight, were recorded from the sampled five plants and seed yield was recorded after threshing the remaining harvested materials. Data were analyzed by Statistical Analysis System (SAS for Microsoft Windows, Release 6.1, 1994) as randomized complete block design. After testing the homogeneity of error variances, combined data over years were analyzed. Genotypes were considered as fixed effects and years as random effects. Least significant differences (LSD) at 5 % probability level were used to compare means.

RESULTS AND DISCUSSION

Green Pod Stage (R7):

The analysis of variance indicated significant year effect for all parameters measured, suggesting that multiple year testing is required to make appropriate selection from the tested parameters (Table 1). Significant genotypic effects also were observed for all measured parameters at R7 stage with the exception of plant height. These results suggest that genetic variability exists among the genotypes to make selection and improvement through hybridization. Furthermore, genotype x year interactions were significantly different for all the parameters. These significant interactions indicated that the performance of genotypes differed from one year (growing season) to another.

The overall genotypic green pod yield mean was 19,528 kg ha^{-1}, and ranged from 14,821 kg ha^{-1} for VB90-3 to 22,768 kg ha^{-1} for Eagle followed by Branco and Blue Ridge. Due to the weather that prevailed during most of the early growing stages in 1994, the mean green pod yield of the best performers was reduced by 32-62% (Table 2). Blue Ridge had the highest 100 green pod weight when averaged across the years

TABLE 2. Mean green pod and dry seed yield (kg ha^{-1}) of common bean genotypes during three growing seasons at Randolph Research Station, Petersburg, Virginia

Genotypes	YEAR 1992	1994	1995	Grand Mean	YEAR 1992	1994	1995	Grand Mean
	Green Pod				Dry Seed			
Eagle	22063	15844	30398	22768	1190	3954	901	1839
Wrangler	26740	11078	24195	20671	2229	3711	1289	2410
Hialeah	24616	10980	20748	18782	2355	4159	907	2474
Prosperity	26254	15280	22485	21339	2685	2328	406	1806
Mustang	21757	13493	21077	18776	2240	2095	851	1695
Blue Ridge	22550	21130	23753	22478	1545	4008	1307	2286
Acclaim	26103	10399	22941	19814	2116	3097	1616	2276
Delta	18700	12845	16478	16008	1584	3062	1228	1958
Branco	29267	9433	29407	22702	2067	2894	1269	2076
Strike	24167	8940	28643	20583	1997	3071	810	1956
Roman II	26633	10157	19750	18847	1704	2046	1217	1655
Pinto III	11257	18370	19189	16272	1950	5072	2300	3107
VB90-3	15410	9714	19339	14821	3059	3939	1593	2864
Grand Mean	22732	12897	22945	19528	2056	3354	1207	1527
LSD (0.05)	12076	10907	7461	10148	1275	1310	741	625

followed by Roman II and Delta (Table 3). However, in the individual years, the 100 pod weight of Roman II which was ranked the highest in 1992 was reduced by 28% in 1994. Similarly, Wrangler which ranked third in 1992 was reduced by 16% in 1994. The cultivar Prosperity had the longest green pod length when averaged across years (Table 4). Green pod length ranged from 14.61cm for Prosperity to 11.02 cm for breeding line VB90-3. Prosperity ranked first in 1992 and 1995, and second in 1994. The early dry season of 1994 did not affect significantly the green pod length.

The cultivar Blue Ridge ranked the highest in number of pods plant^{-1} followed by Acclaim and Eagle when averaged across the years (Table 3). In individual years, Eagle, Prosperity and Blue Ridge were ranked first, second and third, respectively in 1992. However, number of pods plant^{-1} was reduced by 54%, 49% and 34%, respectively in 1994. Blue Ridge ranked among the highest in green pod yield, hundred-pod weight, green pod length, and number of pods plant^{-1} in overall genotypic ranking.

The simple linear correlations at green pod stage for the genotypes are reported in Table 5a. Among the parameters measured, number of pods plant^{-1}, hundred pod weight, and pod length were positively and significantly correlated to green pod yield. Number of pods plant^{-1} showed the highest correlation (r = 0.61**) with green pod yield. Plant height was negatively correlated to hundred-pod weight, which was the parameter second to number of pods plant^{-1} correlated to green pod yield. This suggests that even though plant height was not directly and negatively correlated to green pod yield, it will negatively affect hundred-pod weight, which is directly correlated to green pod yield. Plant height was also negatively correlated to seed size and pod length.

TABLE 3. Mean of hundred-green pod weight (HPW) and mean number of pods plant^{-1} (NGPP^{-1}) of common bean genotypes during three growing seasons and harvested at green pod stage (R7) at Randolph Research Station, Petersburg, Virginia

Genotypes	YEAR 1992	1994	1995	Grand Mean	YEAR 1992	1994	1995	Grand Mean
	HPW (g)				NGPP^{-1}			
Eagle	437	498	607	514	19.70	9.05	16.60	15.12
Wrangler	492	412	706	537	17.75	8.10	10.90	12.25
Hialeah	470	533	646	549	16.95	9.00	16.60	13.96
Prosperity	471	468	616	519	19.45	9.95	15.75	14.18
Mustang	407	458	535	467	16.00	9.80	16.25	14.02
Blue Ridge	498	499	769	589	19.45	12.90	19.75	17.37
Acclaim	433	422	730	535	17.05	8.90	19.20	15.05
Delta	477	494	731	567	12.80	8.15	9.25	10.07
Branco	461	472	548	493	14.55	9.40	20.90	14.95
Strike	406	346	559	437	18.20	7.00	18.40	14.53
Roman II	588	422	728	579	16.00	8.40	8.90	11.10
Pinto III	437	384	630	484	9.20	11.35	13.25	9.32
VB90-3	370	437	553	453	8.15	12.05	8.50	9.33
Grand Mean	457	451	643	517	15.79	9.04	14.94	13.26
LSD (0.05)	102	135	117	118	5.5	7.0	7.2	6.6

Dry Seed Stage (R9):

Mean square analysis of variance for dry seed yield and yield components at dry seed stage (R9) are shown in Table 1. The genotypes showed the highest mean dry seed yield in 1994 compared to the other two years (Table 2). The high seed yield of 1994 could be due to compensatory growth after plants received adequate moisture later in the growing season. However, 1995 was the lowest in dry seed yield among the three years. The overall genotypic dry seed yield was 1,527 kg ha^{-1}, and ranged from 1,655 for Roman II to 3,107 for Pinto III. The genotype Pinto III had the highest dry seed yield followed by VB90-3 and Hialeah, while Roman II had the lowest when averaged over the three years. The breeding line VB90-3 which yielded lowest in green pod yield ranked second highest in dry seed yield over the years. In 1992, VB90-3 ranked first in dry seed yield, this yield was decreased by 48% in 1995. Similarly, Prosperity and Hialeah, which ranked second and third in 1992, their yields were reduced also by 85% and 61%, respectively. The overall genotypic number of pods plant^{-1} was 11.1, and ranged from 13.6 for Hialeah to 7.6 for Roman II. Hialeah was ranked first in 1992 followed by Strike and Eagle. While in the succeeding years, the number of pods plant^{-1} was significantly low (Table 3). The overall genotypic mean hundred seed weight mean was 23.6 grams, and ranged from 27.5 for Roman II to 19.6 for Branco (Table 6). Roman II had the highest hundred-seed weight followed by Pinto III and VB90-3. Averaged across the years, VB90-3 had the highest number of seeds plant^{-1} followed by Acclaim and Strike. (Table 4). VB90-3 ranked first in 1992 and 1994 and second in 1995 for number of seeds plant^{-1}. The three top dry seed yielding genotypes Pinto111, VB90-3 and Hialeah, where among the lowest yielders in green pod yield.

TABLE 4. Mean green pod length (GPL) and number of dry seeds plant^{-1} (NDSP^{-1}) of common bean genotypes during three growing seasons at Randolph Research Station, Petersburg, Virginia

Genotypes	YEAR 1992 GPL (cm)	1994	1995	Grand Mean	YEAR 1992 NDSP^{-1}	1994	1995	Grand Mean
Eagle	12.37	12.97	13.82	13.06	17.9	58.5	44.6	40.3
Wrangler	12.02	12.52	13.50	12.68	33.4	59.2	58.1	50.3
Hialeah	13.30	14.45	14.13	13.96	35.3	48.1	72.5	52.0
Prosperity	14.81	14.37	14.64	14.61	40.3	34.1	34.5	36.3
Mustang	11.96	11.95	14.12	12.68	33.6	42.6	74.6	50.3
Blue Ridge	12.82	12.75	13.84	13.14	23.1	40.9	78.5	47.5
Acclaim	11.38	13.22	13.03	12.55	31.7	54.6	79.1	55.2
Delta	13.23	12.15	13.99	13.13	23.8	39.8	48.5	37.4
Branco	12.45	11.29	13.22	12.32	31.0	39.9	72.8	47.9
Strike	12.26	12.35	13.69	12.77	22.5	51.6	84.7	52.9
Roman II	11.70	10.32	12.51	11.51	25.5	22.1	32.5	26.7
Pinto III	10.49	12.15	11.84	11.49	29.2	56.2	53.5	46.3
VB90-3	9.80	12.05	11.20	11.02	45.9	91.0	83.5	73.5
Grand Mean	12.20	12.50	13.35	12.68	30.2	49.1	62.9	47.4
LSD (0.05)	1.35	2.24	1.75	1.78	19.5	22.6	29.8	13.0

Similarly, Pinto III and VB90-3 ranked the lowest in R7 growth stage for hundred green pod weight, number of pods plant^{-1} and pod length (Tables 3, and 4). This suggests that different genotypes are to be selected for R7 and R9 productions. This idea was supported by Spearman's Rank Correlation between R7 and R9 which was negative but not significant, indicating that breeders have to select and breed for individual components separately.

The simple linear correlations at dry seed stage for the genotypes are reported in Table 5b. Most of the parameters recorded were positively and significantly correlated with dry seed yield. Plant height was the only parameter that was negatively correlated with seed size and number of seeds plant^{-1}. Among the parameters measured, number of pods plant^{-1} and seed size showed the highest correlations with seed yield. Similar correlations were observed between number of pods plant $^{-1}$, and seed size with green pod yield (Table 5a). However, White and Gonzalez (1990) and White et. al. (1992) reported negative association of seed size with common bean yield. The negative relationship of seed size and yield potential of common bean runs counter to the observation that, within a given seed lot, plants originating from larger seeds perform better than plants originating from small seed in common bean (Clark and Beck, 1968; Sangakkara, 1989). This suggests that some genetic factor associated with large seed size, rather than large seed size itself, may be responsible for the lower yield of large seeded cultivars. Secondly, yield differences associated with seed size in common bean may be an artifact of differences in regions of domestication between large and small-seeded cultivars. Genotypes originated from the Andean highlands are predominantly large seeded, while genotypes of Meso-American background are predominantly small seeded and are grown mainly in warmer environments (Laing et al., 1984;

TABLE 5a. Simple linear correlation coefficient analysis among five agronomic traits, of bean genotypes harvested at green pod growth stage (R7).

Agronomic traits	Yield (kg ha^{-1})	Height (cm)	Pods plant^{-1} Number	100 pod weight (g)	Pod length (cm)
Yield	1.0	0.02	0.61**	0.40**	0.29**
Height		1.0	-0.10	-0.25**	-0.14
Pods plant^{-1}			1.0	0.23**	0.25**
100 pod weight				1.0	0.42**
Pod length					1.0

*, ** Correlation coefficient significantly different at 0.05 and 0.01 probability levels, respectively.

TABLE 5b. Simple linear coefficient analysis among four agronomic traits, of bean genotypes harvested at seed dry seed stage.

Agronomic traits	Seed yield (kg ha^{-1})	Pods plant^{-1} Number	100 seed weight (g)	Seeds plant^{-1}
Yield	1.0	0.51**	0.48**	0.18*
Pods plant^{-1}		1.0	-0.24**	0.58**
100 seed weight			1.0	0.05
Seeds plant^{-1}				1.0

*, ** Correlation coefficient significantly different at 0.05 and 0.01 probability levels, respectively.

Singh et al., 1991). Thus, the observed differences in yield between large and small-seeded genotypes may be a function of cultivar adaptation associated with region of domestication. White and Gonzalez (1990) offered an alternative explanation, that cultivar seed size is correlated with cell size in the seed and in the rest of the plant, and that cell size may have an important influence on growth and yield. Similar to soybean as reported by Clark and Beck (1968) and Sangakkara (1989), our data show that there is a strong positive correlation between seed size and dry seed yield in the genotypes tested in Virginia.

SUMMARY AND CONCLUSIONS

Significant differences were observed for all the recorded parameters at both green pod and dry seed growth stages. Genotypes x year interactions differed significantly for all parameters at R7 stage, and genotypes also differed for all parameters measured except for plant height. However, genotypes differed significantly for only number of seeds plant^{-1} and pod length at R9 stage. The overall genotypic green pod yield was 19,528 kg ha^{-1} and ranged from 14,821 kg ha^{-1} for VB90-3 to 22,768 kg ha^{-1} for Eagle, while dry seed yield genotypic mean was 1,527 kg ha^{-1}, and ranged from 1,655 for Roman II to 3,107 for Pinto III. The genotypes Eagle and Pinto III had the highest green pod and dry seed yields respectively. Blue Ridge ranked the highest in green pod yield, hundred pod weight, green pod length, and number of pods plant^{-1} over the years. The genotypes had the highest green pod yield in 1995 and the highest dry seed yield in 1994 compared to the other two years. The three top dry seed yielding genotypes Pinto III, VB90-3 and Hialeah, where among the lowest yielders in green pod yield. Similarly, Pinto III and VB90-3 ranked the lowest in R7 growth stage for hundred green pod weight, pod length and number of pods plant^{-1}. This suggests that different genotypes are to be selected for green pod (R7) and dry seed yield (R9) productions.

Among the parameters measured, number of pods plant^{-1}, hundred-pod weight and pod length were positively and significantly correlated with green pod yield. Number of pods plant^{-1} showed the highest correlation (r = 0.61**) with green pod yield. All the recorded parameters were positively and significantly correlated with dry seed yield. Number of pods plant^{-1} (r = - 0.51**) and seed size (r = 0.48**) showed the highest correlations with dry seed yield among the parameters measured at R9 stage. Similar results were observed when genotypes were harvested at green pod stage. However, our results contradict to the results of White and Gonzalez (1990) and White et al. (1992) who reported negative association between seed size and common bean yield. Our data suggested that the two parameters, seed size and number of pod plant^{-1} can be used effectively for indirect selection of green pod and seed yield in common bean.

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A New Error Analysis for Brun's Constant

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ABSTRACT

Enumeration of the twin primes, and the sum of their reciprocals, is extended to 3×10^{15}, yielding the count $\pi_2(3\times10^{15}) = 3310517800844$. A more accurate estimate is obtained for Brun's constant, $B_2 = 1.90216\,05823 \pm 0.00000\,00008$. Error analysis is presented to support the contention that this estimate produces a 95 % confidence interval for B_2. In addition, published values of the count $\pi(x)$ of primes, obtained previously by indirect means, are verified by direct count to $x = 3 \times 10^{15}$.

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INTRODUCTION

The set $K_2 = \{(3,5),(5,7),(11,13),(17,19),\ldots\}$ of twin prime pairs $(q, q+2)$ has been studied by many investigators, including Glaisher (1878), Brun (1919), Hardy and Littlewood (1923), Sutton (1937), Selmer (1942), Sexton (1954), Lehmer (1957), Fröberg (1961), Gruenberger and Armerding (1965), Weintraub (1973), Bohman (1973), Shanks and Wrench (1974), Brent (1975), and Nicely (1995).

The present study results from the continuation of a project initiated in 1993, with results to 10^{14} previously published in (Nicely, 1995). A detailed description of the general problem, the computational methods employed, and the incidental discovery of the Pentium® FDIV flaw may be found there, with additional details given in (Nicely, 1999); only a brief summary will be included here.

The prime numbers themselves continue to retain most of their secrets, but still less is known about the twin primes. A matter as fundamental as the infinitude of K_2 remains undecided—the famous "twin prime conjecture." Nonetheless, Brun (1919) proved that in any event the sum of the reciprocals,

$$(1)\qquad B_2 = \left(\frac{1}{3}+\frac{1}{5}\right)+\left(\frac{1}{5}+\frac{1}{7}\right)+\left(\frac{1}{11}+\frac{1}{13}\right)+\left(\frac{1}{17}+\frac{1}{19}\right)+\cdots,$$

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is convergent, in contrast to the known divergence of the sum of the reciprocals of all the primes (Brun actually omitted the first term in parentheses, which of course does not affect the convergence). The limit of this sum, styled Brun's sum or Brun's constant, is often denoted as simply B, but henceforth the author will use B_2. In this instance, as in a number of others noted in this paper, identifiers have been changed from those in (Brent, 1975) and (Nicely, 1995), in anticipation of the need for analogous symbols to be used in the study of prime constellations other than the twins.

The twin prime conjecture is a consequence of a much stronger result, an asymptotic relationship conjectured by Hardy and Littlewood (1923, pp. 42-44):

$$\pi_2(x) \sim L_2(x) = 2c_2 \int_2^x \frac{dt}{\ln^2 t} \quad , \tag{2}$$

where $\pi_2(x)$ represents the count of twin prime pairs $(q, q+2)$ such that $q \leq x$, and c_2 denotes the "twin-prime constant," computed to 42D by Wrench (1961),

$$c_2 = 0.66016\ 18158\ 46869\ 57392\ 78121\ 10014\ 55577\ 84326\ 23\ldots \quad . \tag{3}$$

The validity of the conjecture (2), often titled the Hardy-Littlewood approximation, is central to the estimation of Brun's constant and the error bounds in this paper. The Hardy-Littlewood approximation is itself a consequence of the yet more general "prime k-tuples conjecture," also set forth in their 1923 work. See Riesel (1994, pp. 60–83) for an illuminating exposition of these concepts.

Although (1) is convergent, the monotonically increasing partial sums approach the limit with agonizing slowness; summing the first thousand million reciprocals is still insufficient to bring us within five percent of the estimated value of the limit. However, assuming the validity of the Hardy-Littlewood approximation (2), a first-order extrapolation was derived by Fröberg (1961) and further studied by Brent (1975),

$$B_2 = S_2(x) + \frac{4c_2}{\ln x} + O\left(\frac{1}{\sqrt{x}\ \ln x}\right) \quad , \tag{4}$$

with an accelerated rate of convergence $O(\sqrt{x})$ faster than (1). Here $S_2(x)$ is the partial sum

$$S_2(x) = \sum_{q \leq x} \left(\frac{1}{q} + \frac{1}{q+2}\right) \quad . \tag{5}$$

of the reciprocals of all the twin prime pairs $(q, q+2)$ for which $q \leq x$. Note that $S_2(x)$ is written as $B(x)$ in (Brent, 1975) and (Nicely, 1995). The first-order extrapolation of $S_2(x)$ to approximate B_2 consists of the first two terms of the right hand side of (4); this was indicated as $B^*(x)$ in (Brent, 1975) and (Nicely, 1995), but we write it here as $F_2(x)$:

$$F_2(x) = S_2(x) + \frac{4c_2}{\ln x} \quad . \tag{6}$$

The final term in (4) is the author's conjectured error or remainder term, inspired by Brent's (1975) probabilistic analysis. As discussed by Shanks and Wrench (1974, p. 298), no effective second-order extrapolation is known.

COMPUTATIONAL TECHNIQUE

These calculations were carried out as part of a more comprehensive project, including in addition the tabulation of prime gaps and other prime constellations. Computations began in 1993 and have since proceeded almost without interruption, although several months' work was lost early on due to the Pentium® FDIV flaw. The calculations were distributed asynchronously across several (varying from a few to more than two dozen) personal computers, using Intel® processors (mostly classic Pentiums®), extended DOS and Windows™ operating systems, and code written in C. The algorithm employed the classic sieve of Eratosthenes to carry out an exhaustive generation and enumeration of the primes. To guard against errors, all calculations were performed in duplicate on separate systems; in addition, the count $\pi(x)$ of primes was maintained and checked periodically against known values, such as those published by Riesel (1994, pp. 380–383). The values obtained for the count $\pi_2(x)$ of twin prime pairs agreed to 10^{11} with those of Brent (1975, with addendum), and to 10^{14} with those of Kutrib and Richstein (1996). Excluding software bugs and the Pentium® FDIV flaw, approximately forty-nine instances of machine errors were detected and corrected, most apparently the result of transient bit errors in memory (DRAM) chips. One of these instances contained at least 364 individual errors.

As mentioned previously, additional details regarding the computational technique, and the Pentium® FDIV affair, are available in (Nicely, 1995, 1999), and also at the author's URL.

COMPUTATIONAL RESULTS

Table 1 contains a brief summary of the computational results, including the counts $\pi_2(x)$ of twin prime pairs; the values of the discrepancy, denoted here by $\delta_2(x)$, between $\pi_2(x)$ and the Hardy-Littlewood approximation:

$$\delta_2(x) = L_2(x) - \pi_2(x) \quad ; \tag{7}$$

the partial sums $S_2(x)$ of the reciprocals of the twins; and the first-order extrapolations $F_2(x)$ of $S_2(x)$ to the limit, according to (6), members of a sequence believed to be converging to Brun's constant B_2. Note that the discrepancy $\delta_2(x)$ was written in (Brent, 1975) and (Nicely, 1995) as $r_3(x)$; Brent also rounded this value to the nearest integer.

Table 1 includes results previously published for powers of ten to 10^{14}, in addition to new results from the present study at additional increments of 10^{14}, ending with the results for the present upper bound of computation, $x_0 = 3 \times 10^{15}$. Updated and more extensive versions of Table 1 are being maintained at the author's URL. Also available there are equally extensive

TABLE 1. Counts of twin prime pairs and estimates of Brun's constant.

x	$\pi_2(x)$	$\delta_2(x)$	$S_2(x)$	$F_2(x)$
10^1	2	2.84	0.8761904761904761905	2.0230090113326
10^2	8	5.54	1.3309903657190867570	1.9043996332901
10^3	35	10.80	1.5180324635595909885	1.9003053086070
10^4	205	9.21	1.6168935574322006462	1.9035981912177
10^5	1224	24.71	1.6727995848277415480	1.9021632918562
10^6	8169	79.03	1.7107769308042211063	1.9019133533279
10^7	58980	−226.18	1.7383570439172709388	1.9021882632233
10^8	440312	55.79	1.7588156210679749679	1.9021679379607
10^9	3424506	802.16	1.7747359576385368007	1.9021602393210
10^{10}	27412679	−1262.47	1.7874785027192415475	1.9021603562335
10^{11}	224376048	−7183.32	1.7979043109551191615	1.9021605414226
10^{12}	1870585220	−25353.18	1.8065924191758825917	1.9021606304377
10^{13}	15834664872	−66566.94	1.8139437606846070596	1.9021605710802
10^{14}	135780321665	−56770.51	1.8202449681302705289	1.9021605777833
2.0×10^{14}	259858400254	−286596.19	1.8219692563019236634	1.9021605806674
3.0×10^{14}	380041003032	−386165.49	1.8229446574498899187	1.9021605813179
4.0×10^{14}	497794845572	−687458.42	1.8236224494488219106	1.9021605828234
5.0×10^{14}	613790177314	−495402.94	1.8241402488570614635	1.9021605819011
6.0×10^{14}	728412916123	−399030.90	1.8245582810368460212	1.9021605816028
7.0×10^{14}	841912734248	−330271.47	1.8249082431039834264	1.9021605813540
8.0×10^{14}	954464283498	−207253.20	1.8252088524969516994	1.9021605810407
9.0×10^{14}	1066196920739	−459168.78	1.8254720744000806297	1.9021605816527
10^{15}	1177209242304	−750443.32	1.8257060132402797152	1.9021605822498
1.1×10^{15}	1287579137984	−732612.87	1.8259164099409972759	1.9021605822159
1.2×10^{15}	1397370335220	−761338.54	1.8261074785718993129	1.9021605822802
1.3×10^{15}	1506635099560	−762644.45	1.8262824008978027694	1.9021605822837
1.4×10^{15}	1615417411648	−785068.05	1.8264436378766369280	1.9021605823288
1.5×10^{15}	1723754585354	−761213.67	1.8265931311402050729	1.9021605823084
1.6×10^{15}	1831678961614	−851925.37	1.8267324395006005931	1.9021605824283
1.7×10^{15}	1939218595600	−1129122.83	1.8268628327687977085	1.9021605827604
1.8×10^{15}	2046397121805	−678331.73	1.8269853577548725890	1.9021605822393
1.9×10^{15}	2153237307407	−562823.58	1.8271008903959923363	1.9021605821153
2.0×10^{15}	2259758303674	−612652.24	1.8272101680098151140	1.9021605821628
2.1×10^{15}	2365977242191	−653062.89	1.8273138179643056714	1.9021605822014
2.2×10^{15}	2471909670028	−643465.53	1.8274123785364204712	1.9021605821937
2.3×10^{15}	2577569863563	−750111.35	1.8275063150448871463	1.9021605822851
2.4×10^{15}	2682970233099	−552427.29	1.8275960317894826243	1.9021605821145
2.5×10^{15}	2788122612616	−168258.89	1.8276818830618905359	1.9021605818032
2.6×10^{15}	2893038573759	−430246.96	1.8277641812367275962	1.9021605820124
2.7×10^{15}	2997726948096	−292107.29	1.8278432012390461693	1.9021605819106
2.8×10^{15}	3102197972961	−876051.32	1.8279191890118998763	1.9021605823359
2.9×10^{15}	3206458423771	−521046.38	1.8279923621701145073	1.9021605820865
3.0×10^{15}	3310517800844	−897422.15	1.8280629180352850193	1.9021605823404

tables of the values of $\pi(x)$ recorded in this project, at much finer granularity than those commonly available for arguments exceeding 10^{11}. Indeed, the direct enumeration of the primes has been extended to a new upper bound by this project, culminating in the value $\pi(3 \times 10^{15}) = 86688602810119$. This result, as well as a number of other previously published values (Riesel, 1994, pp. 380–382) which were known only through indirect calculations, is now confirmed by direct count in the present study.

BRUN'S CONSTANT AND THE ERROR ANALYSIS

The first-order extrapolation $F_2(x_0) = F_2(3 \times 10^{15})$ is believed to yield the most accurate value known to date for Brun's constant,

$$B_2 = 1.90216\,05823 \pm 0.00000\,00008 \tag{8}$$

The error estimate is believed to define a 95 % confidence interval for the value of B_2. I have no rigorous analytical proof of this assertion regarding the error estimate; rather, it is an inference from the analysis (presented below) of the available numerical data. The notion of a "95 % confidence interval" is to be interpreted as follows. Based on the available numerical data, the author believes that whenever the technique used for this error analysis is applied to a sufficiently numerous sample of distinct integers $x > 1$, Brun's constant B_2 will lie between $F_2(x) - E_2(x)$ and $F_2(x) + E_2(x)$ for at least 95 % of the integers in the sample. Here $E_2(x)$ is the error bound function stated in (11) below; the error estimate given in (8) is a special case of this error bound function, namely $E_2(x_0)$. More precisely, given any set Z_1 of distinct integers $x > 1$, there will always exist a superset Z_2 of distinct integers $x > 1$, $Z_1 \subseteq Z_2$, such that $F_2(x) - E_2(x) \leq B_2 \leq F_2(x) + E_2(x)$ for at least 95 % of the integers in Z_2.

The algorithm for obtaining and validating this error bound function will now be explained. Discussion and justification of certain details of the procedure will be deferred until a later point in this paper.

(A) A set S of sample test points is chosen from the available numerical data; this set should be a reasonably large subset of all the available data points, avoiding any known bias in the associated values of S_2 or F_2. Indeed, S might be chosen as the entire set T of all recorded data points, up to and including the current upper bound $x_0 = 3 \times 10^{15}$ of computation; there are 300081 points in T, consisting of the lattice $(10^{10})(10^{10})(x_0)$ together with the "decade values" $x = k \cdot 10^n$ $(k = 1 \ldots 9,\ \ n = 1 \ldots 9)$. However, the calculations to be carried out in the error analysis then become excessive. We choose instead for S the lattice $(10^{12})(10^{12})(x_0)$, consisting of 3000 equally spaced data points, extending to the current upper limit of computation, the increment being one (U. S.) trillion.

(B) For each $x \in S$, we obtain an error bound on $F_2(x)$, presumably representing a 95 % confidence interval, by determining the value of a parameter

$K_{95}(x)$ such that, for at least 95 % of the points in the set $U = \{t : t \in T, t \leq x/2\}$,

$$|F_2(x) - F_2(t)| < \frac{K_{95}(x)}{\sqrt{t} \cdot \ln t} \; . \tag{9}$$

Here the form of the "scaling factor" in the denominator is inferred from the remainder term conjectured in (4). The data points $t > x/2$ are excluded from U to minimize any artificial reduction in the error estimate resulting from the implicit bias of $F_2(t)$ toward $F_2(x)$ as $t \to x$.

(C) We now reason as follows. Since for each $x \in S$, at least 95 % of the (relevant) preceding extrapolations $F_2(t)$, $t \in U$, agree with $F_2(x)$ within the bound in (9), we assume that this property will remain valid for arbitrarily large values of x as well. We now interchange x and t in (9), as well as the order of the resulting terms on the left hand side, and take the limit as $t \to +\infty$.

$$|F_2(x) - \lim_{t \to +\infty} F_2(t)| \leq \frac{\lim_{t \to +\infty} K_{95}(t)}{\sqrt{x} \cdot \ln x} \; . \tag{10}$$

The numerical evidence indicates that the positive function $K_{95}(x)$ is either roughly constant, or exhibits an overall decreasing trend masked by small scale variations (see Table 2). Thus we can obtain an approximate upper bound on the error by using $K_{95}(x)$ in place of the (unknown) limit of $K_{95}(t)$ in (10). This produces the desired error bound function $E_2(x)$:

$$|F_2(x) - B_2| \leq E_2(x) = \frac{K_{95}(x)}{\sqrt{x} \cdot \ln x} \; . \tag{11}$$

Determination of the error bound at any specific x then becomes a matter of calculating $K_{95}(x)$ and substituting into (11).

Analysis of the data yields the value $K_{95}(x_0) = 1.380$. Substitution into (11) then gives

$$E_2(x_0) = \frac{1.380}{\sqrt{x_0} \cdot \ln x_0} \approx 0.00000\,00007\,06989 \; . \tag{12}$$

Rounding up produces the error estimate stated in (8).

VALIDATION OF THE ERROR ANALYSIS

Since the *ad hoc* error analysis algorithm described and employed clearly lacks a rigorous analytical foundation, additional examination of the empirical evidence was undertaken, in an effort to find supporting evidence, or lack thereof.

The validation process consisted of comparing the confidence intervals obtained for B_2 at each x in the "lower half" $S' = \{x : x \in S, x \leq x_0/2\}$ of S (the values near x_0 being excluded for reasons similar to those given for set U) with the (presumably) best value obtained at x_0. Simply put, the issue is this: what percentage of the confidence intervals obtained for each $x \in S'$ actually contain the best known point estimate for B_2, given in (8) (and to

TABLE 2. Performance data for the error analysis algorithm.

$x/10^{12}$	$K_{95}(x)$	$E_2(x) \times 10^{10}$	Success %
1	2.074	750.61	100.00
10	2.218	234.32	80.00
100	1.758	54.53	94.00
200	1.602	34.40	83.50
300	1.582	27.40	89.00
400	2.047	30.44	91.75
500	1.688	22.30	93.40
600	1.564	18.76	94.50
700	1.451	16.04	94.86
800	1.320	13.60	95.25
900	1.487	14.39	94.89
1000	1.658	15.18	95.40
1100	1.623	14.13	95.82
1200	1.626	13.52	96.17
1300	1.608	12.82	96.46
1400	1.606	12.31	96.71
1500	1.584	11.70	96.93
1600	1.612	11.51	97.12
1700	1.747	12.08	97.29
1800	1.511	10.14	97.44
1900	1.455	9.49	97.58
2000	1.454	9.23	97.70
2100	1.450	8.97	97.81
2200	1.434	8.65	97.91
2300	1.449	8.54	98.00
2400	1.381	7.96	98.08
2500	1.269	7.16	98.16
2600	1.321	7.30	98.23
2700	1.277	6.92	98.30
2800	1.399	7.43	98.36
2900	1.307	6.82	98.41
3000	1.380	7.07	98.47

greater precision, if not accuracy, in the last entry of Table 1)? For example, applying our error analysis technique to the data for $x \leq 10^{14}$ yields $K_{95}(10^{14}) = 1.758$, and substitution into (11) then produces the confidence interval $B_2 = 1.90216\ 05777\ 83 \pm 0.00000\ 00054\ 53$. Since our best estimate for B_2 lies within this interval, we consider the error estimate algorithm to be a success at $x = 10^{14}$. On the other hand, applying the algorithm to the data for $x \leq x_1 = 8.13 \times 10^{14}$, we obtain $K_{95}(x_1) = 1.306$, with the resulting confidence interval $B_2 = 1.90216\ 05809\ 53 \ \pm\ 0.00000\ 00013\ 34$, which constitutes a failure.

A survey of all the points $x \in S'$ reveals that 96.93 % (1454 of 1500) produce confidence intervals containing our current best point estimate for B_2. These calculations, briefly summarized in Table 2, also show the trends in the values of $K_{95}(x)$, $E_2(x)$, and the cumulative percentage of successful (in the sense described above) error estimates generated by the algorithm. The available data thus indicates that our algorithm has been successful (actually performing beyond expectation) in producing valid 95 % confidence intervals for the estimates of B_2. Therefore we anticipate that the error bounds thus obtained for larger values of x, including our current upper bound of computation $x_0 = 3 \times 10^{15}$, will also yield valid 95 % confidence intervals for the value of B_2.

CRITIQUE AND FURTHER REMARKS

The results of additional analysis of the data, conducted in order to address various weaknesses of the error analysis algorithm described, are now summarized.

• Further reduction of the "cutoff" fraction for the selection of sample points in sets U and S' (for example, restricting these sets to the smallest quarter, rather than the smaller half of the eligible values) had no significant effect on the results. Of course, if the restriction is relaxed or eliminated, the effect is to artificially inflate the success percentage of the algorithm. This may be observed in the entries of the last column of Table 2, for values of $x > 1.5 \times 10^{15}$.

• Increasing the density of the sample sets S and S' in T (for example, reducing the increment to 10^{11} rather than 10^{12}) had no significant effect on the results.

• Replacing the presumed best estimate $F_2(x_0)$ for B_2 by another value within the specified confidence interval (8) (both endpoint values were tested) had no significant effect on the conclusions.

• Replacing Brent's (1975) scaling factor $\sqrt{x} \cdot \ln x$ (corresponding to the denominator of the remainder term in (4)) with other plausible possibilities had no significant effect on the results. Among the candidates checked were $\sqrt{x} \cdot \ln x \cdot \ln\ln x$, $\sqrt{x} \cdot (\ln x)^2$, $\sqrt{x} \cdot \ln x \cdot (\ln\ln x)^2$, $\sqrt{x} \cdot \ln x \cdot \ln\ln x \cdot \ln\ln\ln x$, and $\sqrt{x}$. Results produced by each of these scaling factors are summarized in Table 3; note that the values for K_{95} and the error are calculated at $x = x_0 = 3 \times 10^{15}$, while the success percentages are evaluated at $x = x_0/2 = 1.5 \times 10^{15}$, as in our principal error analysis; furthermore, the values of the error are in units of 10^{-10}. The available numerical data is seen to be insufficient to either confirm or reject the error term conjectured by the author in (4), or any of the alternatives. On the other hand, since the use of these alternatives had little impact on the final results of the error analysis, the validity of the algorithm

TABLE 3. Impact of various scaling factors on the error analysis.

Scaling factor	K_{95}	Error	Success %
$\sqrt{x} \cdot \ln x$	1.380	7.07	96.93
$\sqrt{x} \cdot \ln x \cdot \ln\ln x$	4.809	6.89	96.27
$\sqrt{x} \cdot (\ln x)^2$	45.40	6.53	94.80
$\sqrt{x} \cdot \ln x \cdot (\ln\ln x)^2$	16.80	6.74	95.67
$\sqrt{x} \cdot \ln x \cdot \ln\ln x \cdot \ln\ln\ln x$	6.016	6.77	95.87
$\sqrt{x}$	0.043	7.83	99.00

appears to be relatively insensitive to the precise nature of the scaling factor (remainder term). Let it be noted that one could make a case, based on the results in Table 3, for a more agressive error estimate of 0.00000 00006 58 in (8); the author prefers the more conservative value previously stated.

• Other analysis techniques were investigated as well, but none was found superior to the one described. Efforts to use weighted or unweighted data averaging or smoothing, or linear regression techniques, in an attempt to obtain a more accurate value of Brun's constant, have not met with success. Harmonic analysis and fast Fourier transforms have been suggested by various colleagues as promising techniques for analysis of the data, but I have not pursued this avenue. I will attempt to post enough of the raw data at my URL so that other investigators may experiment with their own techniques; perhaps some other method will indeed be more successful than my own in producing a more accurate extrapolation, or a superior error bound.

• The error bound formula $E_2(x)$ in (11) is a generalization of that obtained by Brent (1975). As a consequence of a quite different line of reasoning, Brent arrived at the constant 3.5 in place of $K_{95}(x)$, and believed this to produce an 88 % confidence interval for his estimate for B_2. It now appears that Brent's error estimate was quite conservative. On the other hand, the error bound obtained in (Nicely, 1995) was specifically designed to represent one computed standard deviation at 10^{14}, and the present estimate for B_2 differs from that value by more than two of those standard deviations. As pointed out above, the present technique, when applied to the portion of the data for $x \leq 10^{14}$, produces a 95 % confidence interval containing the current best estimate for B_2 (and even containing the entire current best confidence interval); since a 95 % confidence interval corresponds to about ± 1.96 standard deviations (for a normal distribution), that result implies $\sigma(10^{14}) = 0.00000\ 00028$, a more conservative value than the estimate of 0.00000 00021 arrived at (using a different approach) in (Nicely, 1995).

• As the upper bound x_2 of computation for $\pi_2(x)$ and $S_2(x)$ is extended, corresponding error estimates can be obtained by analyzing the new totality of data to determine $K_{95}(x_2)$, according to part (B) of the error analysis algorithm, then substituting into (11). Note that there is no need to recompute $K_{95}(x)$ for any x other than the value for which a new error bound is desired; computation of $K_{95}(x)$ over the entire sample set was carried out only to explain and validate the algorithm. Indeed, based on the variation exhibited by $K_{95}(x)$ in Table 2, a rough error estimate could be obtained by simply using $K_{95}(x_2) = K_{95}(x_0) = 1.380$; or if a quite conservative value is desired, use $K_{95}(x_2) = 2$.

• Finally, it must be emphasized that both the value of B_2 and the associated error estimate obtained in this paper are entirely dependent on the validity of the Hardy-Littlewood approximation (2). All the numerical evidence to date strongly supports this conjecture, but one must maintain some informed skepticism; after all, the numerical evidence to the current level of computations also supports the famous conjecture that $\mathrm{Li}(x) > \pi(x)$, eventually disproved by Littlewood (1914) himself. Absent a major theoretical breakthrough, it will be difficult indeed to improve significantly on either the estimate or error bound herein presented for Brun's constant. As Shanks and Wrench (1974, p. 299) noted, the calculation of B_2 to eight or nine decimals is (was) extremely difficult—or at least computationally intensive—and twenty decimals of precision remains as remote now as it was then. Equation (11) indicates that computations may have to be extended to 10^{17} just to settle the tenth decimal place, and twenty decimals would require calculations out to perhaps 10^{36} —a figure far exceeding the total number of machine cycles available in the cumulative projected lifetimes of all the CPUs currently on our planet.

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Isolation and Fusion of Cotton Protoplasts

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ABSTRACT

Protoplasts were isolated from five species of *Gossypium*. Protoplast yield and viability were affected by incubation conditions, osmolarity, purification procedures, and cell source. Using an optimized procedure, highly viable protoplasts were isolated from cell suspensions, callus cultures, and leaf tissue of *G. hirsutum*, *G. arboreum*, *G. klotzschianum*, *G. harknessii*, and *G. herbaceum*. Protoplasts of *G. harknessii* were enucleated and successfully fused with protoplasts of *G. hirsutum*.

INTRODUCTION

Modification of plants through tissue culture methods, such as somatic cell fusion has the potential of providing immediate benefits to agriculture (Evans and Sharp, 1986), because genetic characteristics can be transferred by somatic cell fusion without the necessity of isolation and identification of the genes responsible for the trait. Many aspects of plant improvement through somatic cell hybridization have been reviewed previously (Evans and Flick, 1983; Schieder, 1982).

An important plant breeding tool is male sterility. Through sexual crosses, Meyer (1975) demonstrated that *Gossypium harknessii* Brandegee cytoplasm in the nuclear background of *G. hirsutum* L. resulted in plants with CMS. Production of these cotton plants required extensive backcrossing, and the seed set was limited.

Although the genetic basis for cytoplasmic male sterility (CMS) is not understood (Boeshore et al., 1985; Jigeng and Yi-mong, 1983; Levings and Pring, 1979), this trait has been transferred to a male fertile plant through protoplast fusion (Zelcer et al., 1978) and subsequent hybrid regeneration. The fusion of *G. harknessii* cytoplasts with *G. hirsutum* protoplasts should also produce *G. hirsutum* plants exhibiting CMS. As a first step in this process, a technique to rapidly obtain good yields of highly-viable protoplasts suitable for protoplast fusion was developed. Protoplast yields and viability exceeded other published accounts (Bhojwani et al., 1977; El-Shihy and Evans, 1983; Finer and Smith, 1982; Firoozabady and DeBoer, 1986; Khasanov and Butenko, 1979). Additionally, cytoplasts were prepared and fused with these protoplasts.

MATERIALS AND METHODS

Plant Material

Cotton plants *G. hirsutum* L. var. Coker 310, Stoneville 213, and Paymaster 145, were grown in potting soil in an environmentally-controlled growth chamber which

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was programmed for 12 h of light with a temperature of 30 C and 12 h of darkness at 20 C. Plants were watered as necessary and fertilized with Osmocote slow-release fertilizer. Young, rapidly-expanding leaves were selected as source leaves for protoplast isolation.

Callus cultures of *G. hirsutum* L., *G. harknessii* Brandg., *G. arboreum* L., *G. herbaceum* L., and *G. klotzschianum* Anderss. were maintained on a medium consisting of: macronutrients and micronutrients of Murashige and Skoog (1962) (MS salts); 2 mg/L NAA; 1 mg/L 2-iP; 30 g/L glucose; a vitamin mixture consisting of 1 mg/L thiamine-HCl; 0.5 mg/L pyridoxine-HCl; 0.5 mg/L nicotinic acid; and 100 mg/L *myo*-inositol (Smith et al., 1977). Media were sterilized by autoclaving for 15 min at 121° C. Prior to autoclaving and addition of agar, the pH was adjusted to 5.7-5.8 and medium was solidified with 0.8% Difco Bacto agar. Cultures were subcultured at monthly intervals.

Suspension cultures of *G. hirsutum* and *G. harknessii* were maintained in a medium described above with the following modifications: NAA reduced to 1 mg/L; BAP, 0.1 mg/L substituted for 2-iP; agar was omitted. Suspension cultures were maintained at a 16:8 h photoperiod (60 rpm). Cultures were supplied with fresh medium weekly and serially subcultured every 3 weeks.

Protoplast isolation

The general isolation procedure was developed using cotton cell suspension cultures of *G. hirsutum*. Basal isolation medium consisted of macronutrients of MS salt (Murashige and Skoog, 1962), 5 mM MES (2-(N-morpholino)ethanesulfonic acid), 0.7 M mannitol, 5% (w/v) Cellulysin cellulose and 1% (w/v) Macerase pectinase at a pH of 5.7. Effect of osmotic potential on protoplast isolation and viability was determined by reduction of mannitol to 0.5 M and 0.3 M. Effect of macronutrient composition on protoplast isolation and viability was determined by testing full and half strength MS macronutrients and full strength macronutrients from Gamborg's (1975) medium. Effects of enzyme concentration and length of incubation were tested by reduction of the enzyme concentration to 1/2 and by increasing the incubation period from 5 h to 24 h. Isolated protoplasts were purified either by filtration through a nylon mesh with 100 mm pores, by centrifugation at 125 RCF for 6 min layered over a 20% (w/v) sucrose solution, or by a combination of filtration and centrifugation treatments.

Prior to incubation in the isolation medium, cells from the suspension culture were plasmolyzed in a solution identical to the respective isolation medium without enzymes for approximately 30 min. Plasmolyzed tissue was incubated in protoplast isolation medium for 5 h at 28° C in a water bath reciprocal shaker at 40 CPM. Protoplasts used for enucleation experiments were also isolated using this procedure.

Cell counts were made using a haemocytometer. Cell viability was measured using the Evans' blue dye exclusion technique (Onyia et al., 1984). Protoplasts of the other species were obtained from callus cultures and young plant leaves using the procedure developed for cell suspension cultures.

Cytoplast Preparation and Protoplast Fusion

G. harknessii protoplasts were enucleated by centrifugation at 31,000 RCF for 1 h on an iso-osmotic step gradient (Lorz and Potrykus, 1980). Enucleation of protoplasts to form cytoplasts was verified using epi-fluorescence microscopy (Zeiss) following

TABLE 1. Yield and viability of protoplasts isolated in media of various osmotic strength after 5 hours incubation in protoplast isolation medium.

Mannitol (M)	Water Potential of Medium (MPa)	Yield (No/ml PCV[a])	Viability (%)
0.3	-0.97	3.2×10^5	96.7
0.5	-1.48	2.7×10^5	95.7
0.7	-2.07	1.9×10^5	91.9

[a] PCV =packed cell volume

incubation of protoplasts and cytoplasts for at least 1 h in DAPI (4,6-diamidino-2-phenylindole) at 1 mg/mL.

Protoplasts of *G. hirsutum* were mixed with an excess of *G. harknessii* cytoplasts (approximately 2:1 cytoplasts:protoplasts). A red pigmented cell line of *G. hirsutum* was used in some experiments to provide a visual marker for interspecific fusion. Protoplasts and cytoplasts were fused using the procedure by Evans (1983), modified by substitution of 0.5 M glucose for sorbitol in the enzyme wash solution. Fusion was promoted by a 50% (w/v) PEG (mw 6000) solution (Evans, 1983). The PEG fusing solution was eluted with either a glycine buffer (50 mM glycine, 50 mM $CaCl_2 \cdot 2H_20$, 0.3 M glucose, pH 10.5) followed by a wash with culture media, or by a Tris buffer (5 mM Tris , 50 mM $CaCl_2 \cdot 2H_20$, 0.3 M glucose, pH 7.0) followed by a wash with culture media, or eluted by washing with culture media alone (pH 5.7-5.8).

Protoplasts and fusion products were cultured in various media based on Murashige and Skoog's (1962), Gamborg's (1975) or Kao and Michayluk's (1975) formulations. Liquid culture, agar or agarose-solidified media and nurse cultures were all used in an attempt to promote growth of protoplasts and fusion products.

RESULTS

Protoplasts of high viability were obtained from all the *Gossypium* species examined and from leaf, as well as callus and suspension cultures, using this procedure. The highest yield and viability were obtained using the isolation medium with 0.3 M mannitol (Table 1). As the medium osmotic strength was increased, protoplast yield and viability decreased. Isolation medium containing 0.3 M mannitol was therefore selected for subsequent trials.

Isolation medium macronutrient content had no effect on yield (Table 2). Similar results were obtained when macro-salts of MS were at full- or half-strength. Yield and viability were only slightly decreased by substitution of Gamborg's macronutrients. MS macronutrients at full-strength were chosen for routine use.

Reduction of enzyme concentration by half had no effect on viability over a 5 h period but did decrease protoplast yield (Table 3). An increase in incubation period from 5 h to 24 h resulted in decreased total yield and decreased viability. Loss of viability was slightly greater at the lower concentration of enzymes (Table 3). A 5 h incubation period using 5% Cellulysin and 1% Macerase was selected as the standard procedure.

Several purification procedures were compared for their effect on protoplast yield and viability (Table 4). Filtration of the protoplast suspension through a nylon mesh

TABLE 2. Effect on protoplast yield of various macronutrient formulations in the protoplast isolation medium. Protoplasts were isolated from suspension cultures of *G. hirsutum*.

Macronutrient Formulation	Strength	Yield (No/ml PCV)	Viability (%)
Murashige & Skoog	1X	4.4×10^5	96.2
Murashige & Skoog	0.5X	4.5×10^5	94.0
Gamborg	1X	4.3×10^5	91.7

TABLE 3. Effect of enzyme concentration and incubation period on yield and viability of protoplasts.

Cellulysin (% w/v)	Macerace (% w/v)	Incubation (hrs)	Yield (No/ml PCV)	Viability (%)
5.0	1.0	5	3.1×10^5	100
2.5	0.5	5	5.2×10^4	100
5.0	1.0	24	1.0×10^5	93.2
2.5	0.5	24	1.4×10^4	89.8

TABLE 4. Effect of protoplast yield and viability of several purification procedures by filtration, centrifugation, or their combination.

Purification Method	Recovery % of Protoplasts (%)	Number of Protoplasts/ mL PCV Yield	Initial Viability (%)	Viability After Purification (%)
Filtration, 100 mM Mesh	100	4.0×10^6	92.3	82.1
Floatation over Sucrose	68	1.7×10^6	88.4	77.8
Filtration and Floatation	26	9.0×10^5	86.9	85.7

with 100 mm pores, allowed protoplasts, cell fragments and cells with partially-digested walls to pass through resulting in an impure population of protoplasts. In contrast, purification by centrifugation was superior. Cell clumps and cell fragments sedimented into the sucrose layer while intact protoplasts floated above the sucrose in the suspension medium. Both purification by filtration and by centrifugation led to about a 10% decrease in viability. Purification by centrifugation gave approximately two-thirds the yield of filtration. When filtration and centrifugation procedures were combined, recovery was reduced more than would be expected by a simple additive effect of the two procedures.

The optimized procedure was used to isolate protoplasts from five different species of *Gossypium* (Table 5). Yields ranged from 10^5-10^7 protoplasts per g dw of tissue.

TABLE 5. Yield and viability of protoplasts isolated from various cotton species and tissue sources.

Species	Material	Yield (No/gdw)	Viability (%)
G. hirsutum	leaf	1.1×10^{7}	93.1
	callus	6.6×10^{6}	67.9
	suspension	1.4×10^{8}	89.5
G. harknessii	callus	4.8×10^{7}	85.1
	suspension	1.1×10^{8}	81.8
G. arboreum	callus	3.3×10^{5}	76.0
G. herbaceum	callus	8.6×10^{7}	85.3
G. klotzschianum	callus	5.4×10^{7}	84.8

Yields were higher for suspension cultured cells of *G. hirsutum* and *G. harknessii* than from callus of the respective species. Leaves of *G. hirsutum* yielded more protoplasts than did callus, but less protoplasts than did suspension cultures. All isolated protoplasts had good viability (Table 5). Protoplast yield from callus varied with the friability of the callus tissue. Callus of *G. arboreum* and *G. hirsutum* was very nodular and compact which resulted in lowered protoplast yields. However, callus of *G. herbaceum*, *G. klotzschianum* and *G. harknessii* was friable and soft and yields were accordingly greater.

Protoplast to cytoplast fusion was initiated using a 50% (w/v) PEG fusion solution. The PEG solution caused protoplast adhesion to occur, but there was virtually no fusion until the PEG solution was eluted with high pH glycine buffer. Following elution, 50% of all visible objects were still single protoplasts that had not fused. Additionally, 38% of the viewed objects were multicellular fusion products. Only 20% of the objects were formed by adhesion of only two protoplasts. An additional 2% were bicellular fusion products in which the fusion event had clearly occurred at the time of evaluation.

However, when PEG solution was eluted with Tris buffer (pH 7.0) or with culture media (pH 5.8) there was virtually no fusion of adhering protoplasts. Clearly the higher pH of the glycine buffer was effective in stimulating fusion of protoplasts and cytoplasts. Furthermore, by increasing the ratio of cytoplasts to protoplasts from 1:1 to 2:1, the excess of cytoplasts increased the number of protoplast-cytoplast fusion products (4-24%).

DISCUSSION

Genetic modification of cotton through protoplast methods requires a procedure for isolating adequate numbers of viable protoplasts. We systematically evaluated isolation parameters and developed a system with widespread applicability to cotton species for the production of protoplasts suitable for fusion experiments.

In past studies of cotton protoplast isolation (El-Shihy and Evans, 1983; Finer and Smith, 1982; Firoozabady and DeBoer, 1986), enzyme concentration and incubation periods were evaluated, but concentrations were varied for a single, fixed time interval, or incubation period was varied, for one concentration of the enzymes. Results of these earlier studies indicate that low enzyme concentration for prolonged periods give best protoplast yield and viability (Potrykus and Shillito, 1986). Our results indicated that a higher concentration of enzyme used for a shorter period of time produced a greater yield of protoplasts with better viability. No benefits were derived from prolonging incubation periods, instead, viability and yield decreased.

Protoplast purification by filtration is the most commonly used technique (Evans, 1983). In our experiments, cotton protoplasts were isolated in greater numbers with greater viability with the flotation method. However, filtration did not remove ruptured cells, cell fragments, or cells with incompletely digested cell walls. The protoplast population that was recovered from flotation was extremely pure.

Changes in macronutrient composition in the isolation medium had almost no effect on yield, but had a slight effect on viability. This may have reflected differences in osmolality of the solutions. During purification, protoplasts should be maintained in solutions of similar osmotic pressure (Potrykus and Shillito, 1986). Therefore, it may be best to select macronutrients for isolation media with the final culture medium in mind. Macronutrient composition is probably of little overall consequence if the incubation and purification time is sufficiently short.

The largest component of osmotic pressure in our solution was mannitol. Mannitol is a commonly employed osmoticum and has been used in cotton protoplast isolation in the range of 0.4 M (El-Shihy and Evans, 1983; Firoozabady and DeBoer, 1986) to 0.7 M (Finer and Smith, 1982). Khasanov and Butenko (1979) tested mannitol concentrations over the range of 0.3-0.9 M and concluded that 0.5 M was optimum for yield. However, they did not evaluate protoplast viability. We observed a reduction in viability after only 5 h. It is likely that these differences would be more pronounced after an extended incubation period when the cells would have been exposed to the water stress of the high concentration mannitol solution for a longer period of time. The effects of water stress should be more widely considered, especially in procedures with extended incubation periods.

Our method of protoplast isolation and purification has wide applicability with cotton tissue sources and species. We isolated highly-viable protoplasts from five species of cotton and from leaf tissues as well as callus and suspension cultures. Suspension cultures and young, rapidly expending leaves from mature plants are good sources for the isolation of plant protoplasts. Khasanov and Butenko (1979) were unable to isolate protoplasts from cotton leaves, but could isolate protoplasts from cotyledons. Others have isolated protoplasts from cotton cotyledons (El-Shihy and Evans, 1983; Firoozabady and DeBoer, 1986), young leaves (Firoozabady and DeBoer, 1986) and callus cultures (Bhojwani, et al., 1977; Finer and Smith, 1982). In addition to evaluating leaves and callus, we extended the trials to include suspension cultures and found that cell suspensions invariably produced the highest yields of protoplasts.

Our overall procedure results in a high yield of protoplasts with good viability. Furthermore, the procedure is relatively quick compared to other published procedures and is advantageous for use in fusion experiments. Chemical fusion procedures are harsh. Successful fusion and subsequent hybrid cell growth will be favored if the protoplasts are initially viable.

We were able to demonstrate fusion not only of protoplasts, but also of protoplasts with cytoplasts using a standard fusion procedure (Evans 1983). Protoplast-cytoplast fusion products were obtained in 4-24% of all fusion events. Although numerous methods have been used for indirect selection of fusion products such as complementation (Carlson et al., 1972; Glimelius et al., 1978; Melchers and Labib, 1974) or inactivation (Medgyesy et al., 1980; Zelcer et al., 1978). We used a pigmented cell line to allow immediate visual scoring of fusion events.

Cytoplasmically-determined traits have been transferred when organelles were left in their native milieu inside an enucleated protoplast (Maliga et al., 1982) or in a nuclear-inactivated protoplast (Zelcer et al., 1978). To demonstrate the potential for such a system in cotton, we enucleated protoplasts using a published procedure (Lorz and Potrykus, 1980) to form cytoplasts.

Protoplast fusion was not promoted by PEG alone, as reported by Kao and Michayluk (1974), but required a high pH treatment as described by Keller and Melchers (1973). Elution of the PEG with a neutral buffer or with slightly acid culture medium did not promote fusion. For cotton protoplasts, it seems that a high pH elution step is essential for good fusion.

Regeneration of cotton plants from protoplasts has seemed intractable in the past (Bhojwani et al., 1977; El-Shihy and Evans, 1983; Finer and Smith, 1982; Firoozabady and DeBoer, 1986; Khasanov and Butenko, 1979) with protoplast cultures not growing well despite numerous approaches. However, for one cotton cultivar, plants have been regenerated from callus that developed from protoplasts (Peeters et al., 1994). We have taken the next step in demonstrating the potential for development of new cotton lines through protoplast-cytoplast fusions. Genetic modification by protoplast-protoplast or protoplast-cytoplast fusion may lead to agronomically-useful cotton hybrids.

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PROCEEDINGS

ABSTRACTS OF PAPERS, 79th Annual Meeting of the Virginia Academy of Science, May 22-25, 2001,James Madison University, Harrisonburg, Virginia

Aeronautical and Aerospace Sciences

NUMERICAL STUDY ON SUPERSONIC MIXING. T. M. Abdel-Salam, S. N. Tiwari & T.O. Mohieldin, Department of Mechanical Engineering, Old Dominion University, Norfolk, VA 23529. In recent years, considerable fundamental research has been conducted in response to the increased interest in the development of scramjet propulsion systems. Significant amount of experimental and numerical research have been directed towards injectors design that must produce rapid mixing and combustion of the fuel and air. Numerical results for a three-dimensional compressible flowfield around a swept ramp fuel injector are presented. The main goal of this research is to address the capability of an existing CFD code to simulate dual-mode mixing and combustion in scramjet engine. This investigation has focused on comparing the numerical results with the existing experimental results obtained by researchers at the University of Virginia. Experimental data was obtained on the tunnel center-plane and three-sectional planes downstream of the ramp. There is very good agreement in jet penetration, spreading, shape and peak concentration. Overall, the code applied to the specific problem shows an excellent agreement with the experimental data. Comparison of the non reaction and reaction results is also presented. The use of unstructured grid in the code highly reduced the CPU time required to model the current problem.

AERODYNAMIC CHARACTERISTICS OF AN AIRPLANE CONFIGURATION WITH VARIOUS WING AND TAIL ARRANGEMENTS. M.Leroy Spearman ,Systems Analysis Branch, NASA-Langley Research Center, Hampton, VA 23681 & Jill C. Harper, Industrial and Systems Engineering Dept, Va. Polytechnic Inst. & State Univ., Blacksburg, VA 24061. A review has been made of some wind-tunnel data for the purpose of assessing the aerodynamic behavior of airplane designs having various wing and tail arrangements. The basic design consists of a body with a swept wing and a swept horizontal tail. The wing and the tail could each be mounted in a high, mid, or low position on the body. The horizontal tail could also be located at three longitudinal positions aft of the wing. Thus a matrix of 27 wing-tail positions was available and these were tested over a Mach number range from 0.50 to 4.63. The purpose of the investigation was to illustrate the effects of interference flow fields on the aerodynamic behavior as affected by the geometric design and the flight attitude. In general, the results indicate that coplanar wing-tail arrangements are reasonably well behaved whereas some nonplanar arrangements result in undesirable nonlinearities. In addition, there are some arrangements in which the flow fields may be favorably exploited.

HOPF- BIFURCATION OF A 2-D SUPERSONIC AEROELASTIC SYSTEM FEATURING CUBIC PHYSICAL AND AERODYNAMICAL NONLINEARITIES. Piergiovanni Marzocca [1], Liviu Librescu[1], & Gianfranco Chiocchia[2], [1] Engineering Science and Mechanics Dept., Va.Polytechnic Inst.& State Univ., Blacksburg, VA 24061 & [2] Dipartmento di Ingegneria Aeronautica e Spaziale, Politecnico di Torino, 10129 Italy. The next generation of aerospace vehicles may feature increasing structural flexibility , operate in severe environmental conditions and have greater maneuverability than current one's. To achieve such requirements, an exploitation of the load carrying capacity of the structure and the capability that is provided should be used. A better knowledge of the factors contributing to the occurrence of the flutter instability boundary, and an understanding of the factors determining the character of the flutter boundary, (benign or catastrophic), is required. Techniques enabling one to convert a catastrophic flutter into a benign flutter are needed. This investigation examines the catastrophic and benign character of the flutter instability boundary of 2-D lifting surfaces in a supersonic flow field. To address the problem, the method based on the Liapunov First Quanity is used to study the bifurcational behavior of the aeroelastic system near the flutter boundary. The study should enhance the reliability of aeroelastic analysis and design criteria for aircraft.

SHOT PEENING - THE OBSCURE PROCESS. Abdelrahman M. Rabie. , ISAT Dept., James Madison Univ.,Harrisonburg VA 22807. Shot peening is a cold-working process used to improve the fatigue life and corrosion resistance of metallic components. The performance of the peened components is governed by a number of inter-related parameters; one of the influential parameter is peening coverage. In manufacturing, the coverage should be monitored to ensure the consistency of the peening process as well as the quality of the peened components. Therefore, on-line methods, based on objective criteria, to evaluate and monitor coverage are progressively introduced to replace current off-line, time consuming, subjective methods. Several advancements in sensory, measurements, and computer technologies have contributed to the introduction of such new methodologies. The presentation covers description of the peening process and its applications, process parameters, and methodologies used in coverage control.

A BRIEF SURVEY OF SOME HISTORICAL AIRPLANE CONFIGURATION TYPES. M. Leroy Spearman, Systems Analysis Branch, NASA-Langley Research Center, Hampton, VA 23681. Over the years airplane designs have appeared in many shapes and forms. How should an airplane look and what must be considered in the conceptual design? Fundamentally an airplane is designed to support itself at specified flight conditions with a specified payload. The lift required for sustained flight could be provided with only a lifting wing and this has given rise to many flying-wing designs. However, some considerations other than sustained flight have lead to a variety of other design features. Requirements for stability, control and maneuverability have lead to the addition of tail surfaces behind the wing (aft tail) or in front of the wing (canard). Special requirements for the accommodation of passengers and cargo have lead to various body arrangements. Thus the large variety of airplane configurations that have appeared have been dictated by different and often conflicting requirements that include performance, mission, and safety.

Agriculture, Forestry and Aquaculture Science

A COMPARISON OF HAND-HARVESTED AND MACHINE-HARVESTED BUTTER BEANS. Carl E. Niedziela Jr. & Christopher D. Mullins, Virginia Cooperative Extension, Virginia State University, Petersburg, VA 23806. Butter bean or baby lima bean (*Phaseolus lunatus* L.) production is limited in Virginia due to the availability and cost of labor for harvesting. This study was conducted to determine the effect of harvest method on the yield of commercial butter bean production. On 26 May 2000, a 0.44 ha field on a private farm near Skippers, Va. was planted with 'Thorogreen' butter beans. The field was laid-out in a randomized complete block with four replications and two treatments (hand-harvested and machine-harvested). Each 3.6 m x 136 m plot was two rows wide. Border rows were left on each side of the field. The hand-harvested plots were picked twice (9-10Aug. and 17Aug.). The machine-harvested plots were harvested once (16Aug) using a Pixall BH100 single-row bean harvester. Means were separated using least significant difference. There were no differences between the first hand-harvest and the machine harvest. However, the fresh pod yield was higher for the two hand-harvests (4959 kg/ha) than a single machine-harvest (2013 kg/ha). The shelled bean yield was higher for two hand-harvests (1313 kg/ha) than a single machine-harvest (606 kg/ha). The shelled weight per 15 kg of beans was equal for the two treatments.

ENVIRONMENT-FRIENDLY PRODUCTION OF CANTALOUPE AND SWEET CORN. Ronald A. Bowen and Harbans L. Bhardwaj, Agricultural Research Station, Virginia State University, Petersburg, VA 23806. In order to reduce/eliminate use of inorganic nitrogen fertilizers, we conducted two separate field experiments during each of 1999 and 2000 to evaluate the potential of winter cover crops, especially lupin, to meet nitrogen needs of cantaloupe and sweet corn. The treatments consisted of lupin, hairy vetch, Austrian winter pea (AWP), recommended rate of nitrogen fertilizer, and a control. The cantaloupe and sweet corn were grown following winter production of cover crops in a randomized complete block design with four replications. During 1999, the cantaloupe yields were 53.6, 45.0, 23.1, 13.0, and 5.6 MT/ha whereas the sweet corn yields were 8.5, 5.6, 3.1, 1.5, and 0.7 MT/ha, respectively following lupin, hairy vetch, AWP, 110 kg N/ha, and unfertilized control. During 2000, the cantaloupe yields were 27.8, 26.3, 8.6, 5.8, and 2.2 MT/ha whereas fresh sweet corn ear yields were 5.2, 3.9, 4.0, 4.8, and 1.2 MT/ha, respectively following lupin, hairy vetch, Austrian winter pea, control fertilized with 110 kg N/ha, and unfertilized control. These results demonstrate that lupin can be an excellent winter cover crop for meeting nitrogen needs and environment-friendly production of cantaloupe and sweet corn.

PROSPECTS OF WHITE LUPIN AS A WINTER GRAIN CROP IN VIRGINIA. Harbans L. Bhardwaj, Agricultural Research Station, Virginia State University, Petersburg, VA 23806. White lupin (*Lupinus albus* L.) has the potential to be an alternative winter grain crop. Lupin can provide nutritious food/feed grains and also reduce input costs and environmental pollution related to use of N fertilizers. Lupin seeds (32-38% protein) are suitable for on-farm utilization since high temperature cooking to neutralize anti-nutritional factors is not needed. The New Crops Program of Virginia State University has evaluated lupin production in Virginia for the past several years. These efforts have identified the need for winter hardy and high yielding cultivars, characterization of alkaloid content in lupin seed and its relationship with productivity, and strategies for management of anthracnose. Experiments with French cultivars have indicated that seed yield of up to 6547 kg/ha with a range from 3777 to 6547 is attainable. The cold weather during 2000-2001 season has helped us identify winter hardy germplasm received from USDA-ARS and Auburn University. Preliminary research with alkaloids has indicated that "Bitter" lupins (high alkaloid content) have an advantage over "Sweet" lupins (alkaloid-free or low alkaloid content). Considerable variation exists among lupin germplasm for development of high yielding sweet lupin cultivars. Prospects of developing lupin as a grain crop are good.

UNDERSTORY VEGETATION PATTERNS IN SHELTERWOOD STANDS. Heather K. Douglas & Mary E. Lehman, Dept. of Natural Sciences, Longwood College, Farmville, VA 23909. The forest management technique of shelterwood cutting results in increased sunlight penetration to the understory. This study assessed understory vegetation diversity and species composition patterns relative to time since shelterwood cutting disturbance. Percent coverage was estimated for all plant species found in randomly selected plots in a control site and in sites that had been shelterwood cut 2, 3, or 6 years ago. Species richness and diversity declined significantly with age since shelterwood cutting. Some species were commonly found throughout all sites, regardless of age since shelterwood disturbance (e.g., *Quercus* spp., *Acer rubrum*). Some species that dominated 2- and 3-year sites declined in importance or were not found in the 6-year site and the control site (e.g., *Liriodendron tulipifera, Andropogon virginicus, Rhus glabra*).

PERFORMANCE, HEALTH AND IMMUNE STATUS OF BEEF CALVES FED PROTEIN AND ENERGY SUPPLEMENTS DURING THE BACKGROUNDING PERIOD. R. J. Austin, J. P. Fontenot, W. S. Swecker, R. K. Shanklin, J. Fike & A. M. Shank, Department of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA. 24060. Newly received or newly weaned calves are highly susceptible to the incidence of bovine respiratory disease. Four backgrounding trials were conducted to examine the effects of protein and energy supplements to stressed calves consuming grass hay and stockpiled forages. Supplements included different levels of protein and energy. In all trials, supplemented cattle gained faster than unsupplemented cattle by d 42 ($P<0.05$). Daily gains were higher ($P<0.03$) for steers supplemented with corn compared to a 15% protein supplement. Steers supplemented at 0.5% body weight (BW) had higher gains than steers supplemented at 1.0% BW ($P<0.05$). During week 1, steers grazing fescue had higher daily gains than those grazing fescue-alfalfa ($P<0.05$). Blood glutathione peroxidase levels were not affected by supplements fed, but generally increased over time. In all trials, no consistent differences were detected in morbidity, number of animals treated or serum mineral concentrations due to treatment.

THE EFFECT OF AMOUNT AND FORM OF PHOSPHORUS AND PHYTASE SUPPLEMENTATION ON PHOSPHORUS UTILIZATION BY RUMINANTS. R. K. Shanklin, J.P. Fontenot, J.S. Radcliffe, R.J. Austin, & J.P. Rice, Department of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0306. Two metabolism trials were conducted to investigate the effects of the chemical form of phosphorus (P) and phytase supplementation. Eighteen wether lambs (average BW = 23 kg) were allotted to six diets for each of two metabolism trials. The diets were: 1) low P alone, or supplemented with 2) inorganic P, 3) phytic acid, 4) phytic acid and phytase, 5) cottonseed meal or 6) cottonseed meal and phytase. Apparent absorption of P was lowest ($P<0.005$) for the low P treatment compared to the other treatments. Ruminal fluid P was higher ($P<0.05$) for lambs fed supplemental P. Within supplementation treatments, ruminal fluid P was higher ($P<0.05$) for lambs fed organic P than for those fed inorganic P. Cottonseed meal resulted in higher ($P<0.001$) ruminal fluid P than phytic acid. Addition of phytase to the diets with organic P resulted in more P in the ruminal fluid ($P<0.04$) and tended to increse ($P<0.12$) serum P. There was a decrease ($P<0.003$) in serum P associated with the low P treatment.

TEACHING WATER QUALITY: VIRGINIA STATE UNIVERSITY'S YOUTH AQUACULTURE PROGRAM. Albert O. Reid & Brian L. Nerrie, Cooperative Extension, Virginia State University, Petersburg, VA 23806. Virginia State University's Youth Aquaculture Program (YAP) has met and exceeded its objectives. It was originally designed in 1991 to increase student awareness of farming in the aquatic environment. Educational programs have been provided to more than 500 schools in the commonwealth of Virginia. These programs included aquaculture farm visits, in-school presentations, field days, and educational displays. YAP has expanded to include student directed aquaculture production in a number of school systems. Water quality is one of the most important areas of study for students. Eight high schools with production systems were selected in which aquaculture is used as an educational tool to expose students to water quality issues. A four-step educational program was utilized. The steps include: pretest, observation, hands-on work, and post-test. Parameters explored were: temperature, dissolved oxygen, pH, chlorine/chloramines, ammonia, alkalinity, and hardness.

SMALL SCALE POND SPAWNING OF KOI CARP. Scott H. Newton, Cooperative Extension, Virginia State University, Petersburg, Va 23806. Koi carp, *Cyprinus carpio,* are colorful ornamental fish used mainly in small pools, which includes water gardens and aquatic pools in theme parks, zoos, and hotels where these fish may be easily viewed. They are highly prized by collectors worldwide. Four-year-old adult fish averaging 2.5 pounds each were stocked into three small ponds (1.8 ac) in May, 2000 to produce fingerling fish for small farm sales. Spawning mats were placed in the ponds in corner areas. The only management practice was water level maintenance until the spring, 2001. Ponds were drained and fish harvested during April, 2001 to recover the adults and one-year-old fingerlings. All adults were recovered for 100 percent survival. Approximately 1000 fingerlings were recovered from the three ponds for an average of 330 fish per pond that could be marketed. This experiment demonstrates another opportunity for aquaculture product production on a small farm scale to diversify market potentials.

RAINBOW TROUT CAGE CULTURE: SETTLEABLE SOLIDS COLLECTOR. Brian L. Nerrie & Albert O. Reid. Cooperative Extension, Virginia State University, Petersburg, VA 23806. Environmental impacts of agriculture, including aquaculture, are being watched by the media, the public and by government regulatory agencies. Minimizing negative effects on the environment is one objective of the aquaculture industry, which strives for sustainability. Cage culture is an alternative enterprise that brings underutilized water bodies into production. Rainbow trout are double-cropped during the winter season in cages from which warm-water catfish or hybrid striped bass have been harvested. Sinking feed, used to limit competition by birds, may be used, but results in feed loss through the cage bottom. Nutrient inputs derived from cage culture (unconsumed feed, feces, organic debris) can be reduced utilizing a settleable solids collector. The cost, design and construction of a portable settleable solids collector for cage culture are described. Implications of excess nutrient effluent from cage culture operations are discussed for Virginia and international operations.

FUNGAL AND BACTERIAL EPIZOOTIC IN BROWN BULLHEAD (*AMEIURUS NEBULOSUS*) FROM AN URBAN FISHERY. Stan Webb[1], Greg Garman[2], Steve McIninich[2], David Crosby[3], [1]Dept. Of Biology, [2]Center for Environmental Studies, VCU, and [3]Cooperative Extension, PO Box 9081, VSU, Petersburg, VA 23806. During April 1999, an urban fishery, Swan Lake in Bryd Park, Richmond, had a fish kill involving over 300 brown bullheads. VCU personnel investigated this epizootic. External clinical signs showed bullheads had light to heavy infestation of leeches and lesions involving a *Saprolegnia* sp. Internal pathology showed livers were discolored with focal hemorrhagic areas. *Aeromonas hydrophila* and *Plesiomonas shigelloides* were recovered from external lesions, liver, and gall bladder. The pathology of external lesions was a necrotizing dermatitis into the underlying muscles with focal areas of necrosis and invasive bacteria. Additionally, pathology revealed that bullheads were infected with metacercria called yellow grub. The cause of the epizootic was not determined by the investigation. However, since leeches used in modern medicine act as vectors for *Aeromonas* infections in humans, leeches may have been the primary vector causing *Aeromonas* and *Plesiomonas* septicemia in these brown bullheads.

FISH HEALTH STATUS IN VIRGINIA AQUACULTURE INDUSTRY: AN UPDATE. David Crosby, Cooperative Extension, PO Box 9081, VSU, Petersburg, VA 23806. In order to assist the emerging aquaculture industry in Virginia with fish disease problems and health issues, a Fish Health Diagnostic Laboratory was established at Virginia State University in 1993. Since, the laboratory has processed over 280 cases from Virginia fish producers. Fish health problems in Virginia's emerging aquaculture industry have varied with producer expertise, culture systems, and species. During 1996 to 1999, the laboratory processed 39 inspections and routine checks, mainly with tilapia. Results of these inspections found *Ichthyobodo* on tilapia approximately 50% of the time. No particular disease group dominated as a health problem for fish producers in Virginia. Parasites constituted about 30% to 40% of diagnostic casework from 1993 to 1999. The ciliated protozoan, *Trichodina,* was the most commonly found parasite on fish being raised in cages. Several significant fish diseases (Enteric septicemia of catfish, proliferate gill disease, and *Streptococcus)* have been identified in Virginia. The principal fish health issue among Virginia trout producers is fish health certification for interstate shipment.

COMPARISON OF RAINBOW, BROOK, AND GOLDEN TROUT IN CAGE CULTURE. Scott H. Newton, Cooperative Extension, Virginia State University, Petersburg, Va 23806. Research has been conducted over the past 16 years at VSU on winter cage culture of trout in non-traditional areas in Virginia. The 2000 - 2001 winter season was the first to compare production of the three primary Virginia freshwater trout. Survival of rainbow (92%) and golden (91%) trout was significantly better than brook trout (67%). Growth rates of all fish were similar at approximately 250% for the period. Production aspects of rainbow and golden trout were essentially the same, as expected, because the golden trout is a color variation of the rainbow trout. Fish size at stocking, feeding frequency and weather conditions are the primary variables affecting fish size at harvest in the spring.

ENVIRONMENTAL EFFECTS ON OIL, POLYUNSATURATED FATTY ACID, AND SOME PHYTOCHEMICALS IN VEGETABLE SOYBEAN. Ali Mohamed, Agricultural Research Station, VSU, Petersburg, Va 23806; Mentreddy Rao, Fort Valley State University, Fort Valley, GA 31030 & Mebrahtu, Agricultural Research Station, VSU. To introduce a new high protein vegetable to the American diet and to diversify the soybean use, soybean research programs at VSU and FVSU were aimed at developing vegetable soybean cultivars that would adapt to different US environments. Vegetable soybeans (*Glycine max* [L.] Merr.) are those which are harvested at R6 - R7 growth stage while the pod is still green and the seeds have developed to fill about 80-90% of the pod width. Twenty vegetable soybean genotypes from Virginia and fourteen from Georgia were analyzed for protein, oil, phytate, tocopherols, sterols, trypsin inhibitor activities, and unsaturated fatty acid profile. Mean protein, oil, and phytate were 7.6, 7.3, and 0.0164 g/100g fresh seeds, respectively. The mean total tocopherol was 309.24mg/g Freeze-dried seeds. d-tocopherol (127.59 mg/g) was higher than a and g-tocopherol (97.53 and 84.13, respectively). Variations in total sterol and sterol patterns were also found. The overall means were 234.8, 44.6, and 45.6 mg/g freeze-dried seeds for b-sitosterol, stigmasterol and campesterol, respectively.

PHYSIOLOGICAL EFFECTS OF SOYBEAN PHYTOCHEMICALS AND ITS POSSIBLE ROLE INCONTROLLING GLUCOSE LEVEL IN DIABETIC RATS. Ali Mohamed, ARS, VSU, Petersburg, Va 23806 Sam Bhathena, BNHRC/ARS/USDA, Beltsville, MD; Manuel Velasquez, Medical Center/George Washington University, Washington DC. & Ali Abdel Aziz, ARS, VSU. Soybeans are nutrient-dense, a high-quality source of protein, fiber-rich and vitamins. Soybeans are unique among legumes because of their high isoflavones contents. Protective and therapeutic benefits of soybean and its phytochemicals intakes have been documented. Soy bean has been reported to have protective effects on chronic diseases such as atherosclerosis, diabetes, and osteoporosis which are known to be influenced by hormonal related factors. Feeding studies with soybean showed a significant effect on the level of insulin, ACTH, testosterone, triidiodothyrornine (T3) and thyroxine (T4). Soy diet lowered plasma ACTH compared to casein fed rats. Rats fed soy protein had lower testosterone but higher T3 and T4 levels compared to casein fed rats. The data showed that long term feeding of soybean affects hormones that are involved in lipid and carbohydrate metabolism and cellular growth which may partly contribute to the long term protective effects of soy meal on chronic diseases.

PRELIMINARY PROTEIN/LIPID REQUIREMENTS AND OVERVIEW OF STATUS OF SUMMER FLOUNDER (*Paralichthys dentatus*) RESEARCH IN VIRGINIA. M. H. Schwarz[1], T.G. Gaylord[1], R.W. Cool[1], G. Davitt[1], M.L Jahncke[1], and S.R. Craig[2], [1]VSAREC 102 S. King Street, Hampton VA, 23666, and [2]College of Veterinary Medicine, VPI&SU. Limited data on nutritional requirements of summer flounder grown in recirculating aquaculture systems (RAS) is a serious constraint to development of summer flounder as a viable aquaculture species. In one study, dietary lipid levels were tested ranging from 8 to 12%, and while not statistically validated due to a high degree of variability within replicates, the 8 percent lipid level demonstrated improved growth and survival. Dietary lipid level was significant at ($P<0.003$) with respect to lipid levels in finray muscle and inversely to liver lipid content. In a protein study 35, 40, 45, 50, 55, and 60% dietary protein was investigated. Fish in the study were compromised with a bacterial pathogen resulting in termination of the study by week eight, however at ($P<0.05$) mortalities in replicates fed 35, 40, and 45% protein were significantly higher than 55 and 60% protein diets. The 50% protein diets was not significantly different at ($P<0.05$) to either the lower or higher protein diets.

THERMAL OPTIMA FOR THE PRODUCTION OF SUMMER FLOUNDER (*Paralichthys dentatus*). R. W. Cool[1], T. G. Gaylord[1], M. H. Schwarz[1], M. L. Jahncke[1], and S. R. Craig[2], Virginia Seafood Agricultural Research and Extension Center, 102 S. King Street, Hampton, VA 23669 and [2]VPI&SU, Blacksburg, VA. 24061. The objective of this study was to evaluate the environmental temperature most suitable to culture juvenile summer flounder. Nine 38-l aquaria, in replicates of three (19, 24, 29^0 C), were stocked with ten juvenile flounder per tank (9.5 g/fish). Fish were fed to satiation once daily and amounts of feed were quantified. At the termination of the 10-week trial, fish were weighed individually and three fish per aquarium were dissected for intraperitoneal fat weight, muscle weight (divided into ordinal white muscle and finray muscle) and liver weight. The remaining fish were pooled for whole body composition analysis. Muscle and liver were processed for protein and lipid determination. Analyses was performed using SAS and differences were deemed significant at $P<0.1$. Growth in the 29^{0}C tanks was significantly greater than the 19^{0}C, but not significantly greater than the 24^{0}C tanks. Condition indices found only the finray muscle in the 29^{0}C tanks to be significantly higher than the 19^0 and 24^{0}C tanks. Lipid content in the 29^{0}C tanks was significantly higher than the 19^0 and 24^{0}C tanks.

WHAT DO GOOD BUGS EAT WHEN THEY RUN OUT OF BAD BUGS? M. Kraemer, R. Grayson, and J. McConnell. P.O. Box 9061, Virginia State University, Petersburg, VA 23806.
Greenhouse vegetable production is largely dependent on biological control because of a lack of effective pesticides registered for greenhouse vegetable use and increasing pesticide resistance found in major pest species. Costs to establish and maintain natural enemies can be substantial. When host populations decline the natural enemy often declines rapidly as food resources become scarce. The result is a rapid resurgence of the pest populations and additional natural enemies must be ordered. We investigated the potential use of dill flowers as a supplimental food for parasitoids (*Encarsia formosa, Eretmocerus californicus*) of *Bemesia* type whiteflies, a major greenhouse pest. Umbels of dill flowers were excised and placed in plexiglass cages, with stems in either water with or without systemic pesticides (dimethoate, malathion). After 3 days 98 to 100 percent of the parasitoids in the systemic treatments were dead (18% to 32% were found on the flowers) whereas less than 20% of the control had died. This indicated that the parasitoids had feed on dill nectar. Parasitoid use of dill nectar was confirmed using Energy Dispersive X-ray Analysis (EDXA) of insects caged with excised dill flowers in rubidium laced water. EDXA was shown to be sensitive enough to use this technique for the smallest of insects.

COMPATIBILITY, YIELD AND QUALITY OF ‘MATUA’ PRAIRIEGRASS GROWN WITH LEGUMES. J. F. Guay[1], A. O. Abaye[1], P. R. Peterson[2], and J. P. Fontenot[1], [1]Va. Polytechnic Inst. & State Univ., Blacksburg, VA 24061, and [2]University of Minnesota, St. Paul, MN 55108.
‘Grasslands Matua’, *Bromus willdenowii (Kunth),* is a cool-season, perennial, high quality, high producing bromegrass variety. In the spring of 1998, alfalfa (*Medicago sativa*), red clover (*Trifolium pratense)*, ladino clover (*Trifolium repens*), and annual lespedeza (*Lespedeza stipulacea*) were drilled into an existing matua stand. Botanical composition, quality, and yield were estimated. No significance differences due to the legume treatments were found for 1998 harvests. During the 1999 growing season, ladino clover and red clover frequently comprised over 50 percent of the plots and significantly decreased the amount of matua in the stands ($p<0.05$), indicating a possible incompatibility. In 1999, ladino clover and red clover increased total yield ($p<0.05$). The greatest improvement in quality also occurred in the ladino clover and red clover treatments during the 1999 growing season, with a 10 to 20 percent decrease in neutral detergent fiber ($p<0.05$). Although legumes increased the total yield and the quality of the forage mixture, ladino clover and red clover out competed matua at the seeding rates used.

Archaeology

SETTLEMENT PATTERNS ON VIRGINIA'S APPALACHIAN PLATEAU: TOTO, I DON'T THINK WE'RE IN KANSAS OR EVEN RICHMOND ANYMORE. Michael B. Barber, USDA-Forest Service, 5162 Valleypointe Parkway, Roanoke, VA 24015. The typical settlement models developed for the Middle Atlantic region are reviewed and modified through the additions of bedrock geology, tool function, lithic type, site setting, and social organization. The over-generalizing of 1970s is confounded by collected data and used to create lower level expectations. The Appalachian Plateau of Virginia is used to pragmatically test the applicability of the new model. While the model has the flexibility to provide "fit" for the physiographic region, site types such as quarries and quarry reduction stations are absent.

COMMUNITY-BASED ARCHAEOLOGY AND HISTORY AT ITS BEST: THE ELK RUN ANGLICAN CHURCH RESEARCH PROGRAM. John T. Eddins, Cultural Resource Division, The Louis Berger Group, 1819 H. Street N.W., Suite 900, Washington, D.C. 20006. The Elk Run Anglican Church site (44FQ179) research program is a community run history and archaeological research program in southern Fauquier County, Virginia. Elk Run was the first Anglican Church established in the mid-18th century in the frontier region that would later become Fauquier County. The church was abandoned shortly after 1800. After more than a century and a half of absence from the public consciousness, the Elk Run Church has become a renewed focus of community activity and interest. A local preservation committee has initiated a broad program of historical and archaeological research, based on community volunteer effort. The archaeological research has thus far resulted in the exposure of a symmetrical cruciform church foundation and one burial in an associated cemetery. Individual interests, education, entertainment, and community history have all been served through the volunteer program.

ARCHAEOLOGY AND ARCHITECTURE AT THE "FIELDING LEWIS STORE," FREDERICKSBURG, VIRGINIA. Michael Klein and Emily Lindtveit, Center for Historic Preservation, Mary Washington College, Fredericksburg, Va. Architectural analysis and archaeological fieldwork were conducted at the two-and-one-half-story Georgian structure generally referred to as the "Fielding Lewis Store," located in Fredericksburg, Virginia. Independent analytical methods produced evidence for a circa 1750 construction date and a circa 1808 renovation of the building. These dates: 1) place the storehouse among the earliest brick structures in Fredericksburg; and 2) suggest that the renovation of the building followed the devastating 1807 fire that destroyed a significant portion of downtown Fredericksburg.

ANALYSIS OF FAUNAL REMAINS FROM THE KITCHEN FEATURE, MOUNT PLEASANT SITE, ORANGE COUNTY, VIRGINIA. Laurel D. Miller, Dept. of Soc. and Anth., James Madison Univ., Harrisonburg, Va. 22807. Preliminary analysis of the faunal remains from the kitchen feature at the Mount Pleasant Site, Montpelier, Orange County, suggests that the Madison family slaves occupied this site. The faunal remains were analyzed according to species and element using authoritative manuals and comparative skeletal materials at James Madison University. The findings from this site were then compared to the Kingsmill Plantation Slave Quarter site in Williamsburg and the Storehouse and Dry Well at Monticello. Of the identifiable pieces in the assemblage, the majority of the remains were identified as the domestic pig, *S. scrofa*, with a small amount of large mammal, small mammal, bird, fish and reptile included. The body parts represented are consistent with the findings at the Kingsmill Quarter site and the Storehouse site, both representative of the typical slave diet in the Virginia Piedmont and Coastal Plain regions.

THE COLLECTING AREA AS THE UNIT OF ANALYSIS: THOUGHTS ON THE LANDSCAPE APPROACH AND NON-SITE SPECIFIC ARCHAEOLOGY. Carole L. Nash, Dept. of Soc. And Anth., James Madison Univ., Harrisonburg, Va. 22807. An analysis of Virginia Department of Historic Resources Archaeological Site Inventory Forms for Madison County, Virginia demonstrates great variation in the understanding of what constitutes an archaeological site. Based on field checks, 70% of the forms for sites on private land actually document collecting areas: poorly defined areas of cultural activity that, if surveyed according to current standards, would be better characterized as overlapping or spatially discrete, multiple occupations. This paper presents the results of the Madison County study and considers an archaeological practice based on the collecting area as the unit of analysis. Examples of this "non-site archaeology," which incorporates a larger-scale landscape approach, are given.

THE ANALYSIS OF A GUILFORD-AGED CHIPPING CLUSTER FROM 44GY18. George A. Tolley. U.S.D.A. Forest Service, , 5162 Valleypointe Parkway, Roanoke, VA 24015. During the last two weeks of July, 2000 the George Washington and Jefferson National Forests sponsored a joint Archeological Society of Virginia Certification Field School and Passport in Time Program at the Fairwoods Livery Site (44GY18) in Grayson County, Virginia. This is the second field school the forests has sponsored to investigate the prehistoric use of Mount Rogers rhyolite at this site. This paper will address the results from the analysis of a Guilford- aged chipping cluster that was discovered during this latter field school. This chipping cluster, unique to the site at this time, contained two Guilford projectile points with more than 100 pieces of rhyolite debitage, flakes, and core fragments. The analysis of this material will be concentrated on identifying the variety of rhyolite pieces that formed this chipping cluster, any tools or utilized flakes within the cluster, and identify if possible particular knapping techniques utilized by the Guilford occupants.

ANALYSIS OF LITHIC MATERIAL FOUND IN UNIT N1287.5 W2.5 OF THE FAIRWOOD LIVERY SITE (44GY18). William H. Weddle, III, Dept. of Soc. and Anth., Radford University, Radford, VA 24142. 3,858 artifacts were excavated from the 2.5' square unit at this site in Grayson County, Virginia. 3,777 of these artifacts are debitage associated with lithic reduction. 97.9% of the debitage was produced from porphyritic rhyolite, 1.3% is chert and the remaining debitage consists of chalcedony/chert, chalcedony, quartz, crystal quartz, jasper and greenstone. 70 tools were excavated, of which 70% are cutting tools. Most of the tools were also produced from porphyritic rhyolite. Diagnostic artifacts excavated include three complete projectile points and one small grit tempered pottery sherd. The oldest projectile point excavated is a Kirk point of light blue rhyolite dating to 9450 to 8850 BP (Early Archaic). The Guilford point dates to approximately 4950 BP (Middle Archaic). The Brewerton point dates to 4850 to 3650 BP (Late Archaic). The pottery sherd indicates Woodland period use of the site. The site appears to have been used for lithic reduction. However, the presence of the cutting tools indicates that the site had other uses as well. The same types of artifacts were found in all levels, indicating that the site was used for the same or similar functions from the Early Archaic through the Woodland periods.

AN ANAYLSIS OF A RECENTLY EXCAVATED GLASS ASSEMBLAGE FROM THE MOUNT PLEASANT SITE, ORANGE COUNTY, VIRGINIA. Megan B. Veness, Dept. of Soc. and Anth., James Madison Univ., Harrisonburg, Va 22807. Mount Pleasant, themid-18th century boyhood home of President James Madison Jr., has been under excavation by Montpelier archaeology staff and James Madison University archaeological field schools for the past six years. Over 1300 pieces of glass were excavated from two features (a root cellar and a kitchen) during the 2000 field school. The amount, variety and total vessel count is important for determining the activities represented in the features, as well as the general date of deposition. The predominant glass type was wine bottle glass; a minimum of ten vessels were cross-mended and four different groups were identified. The bottles in each group exhibited different characteristics such in color, size and shape and were classified according to the typology developed by Noel-Hume.

Astronomy, Mathematics and Physics

A MODEL OF ONE-DIMENSIONAL COUPLED HARMONIC OSCILLATORS AND APPLICATIONS. Robert Knapik, Rick Massaro, & G.R. Taylor, Physics Dept., James Madison Univ., Harrisonburg, VA 22807. In certain crystal lattice structures, vibrations of atoms oscillate under a linear restoring force in approximately one dimension. These systems can be modeled by using weakly coupled, one-dimensional harmonic oscillators. A physical model, which consisted of gliders linked by springs moving on an air track, was analyzed in order to investigate these coupled spring-mass systems. This system was limited to the size of the track so a computer model was developed to extend the experiment. Methods and techniques from classical mechanics, linear algebra and Fourier analysis were used to develop the computer model and to further analyze the physical model. The equations of motion of a system of n oscillating masses can be solved through numerical techniques. The results from the experiments show that the computational analysis of the n-mass system is a reliable way of finding the normal mode frequencies. This analysis can be extended to investigate the properties of solids whose atomic behaviors can be simplified to this one-dimensional model.

TEMPERATURE DEPENDENT ELECTRICAL PROPERTIES IN TRANSPARENT CONDUCTING OXIDE THIN FILMS. Timothy J. Nagle[1], Gerald R. Taylor, Jr[1] David Lawrence[2], & Geoff Stenger[2], Department of Physics[1], ISAT[2], James Madison Univ, Harrisonburg, Va. 22807. Transparent conductive thin films are in high demand due to their potential for use in liquid crystal displays, photovoltaic cells, space applications, and other electro-optical devices. Transparent ITO thin films were deposited on glass substrates by varying techniques. Such properties of the films as electrical resistivity, Hall coefficient, Hall mobility, and carrier concentration will be discussed, in addition to a summary of the experimental techniques employed. The temperature dependence of electrical properties was studied from 77K to room temperature. Also discussed are such surface properties as grain size and microstructure as measured using atomic force microscopy (AFM) and scanning tunneling microscopy (STM).

LIGHT SOURCES IN TIME AND ENERGY CALIBRATION. Jason Mace & Kevin Giovanetti, Physics Dept., James Madison Univ., Harrisonburg, VA 22807. Key issues in measuring the lifetime of a Muon to one ppm will be presented. A brief overview of the μlan detector and the Muon lifetime experiment at Paul Scherrer Institute will be provided as background. Problems typically encountered in time calibration, including systematic effects will be covered. An initial design of the calibration system will be shown along with possible light sources.

PARALLEL-PROCESSING N-BODY ORBIT COMPUTATION. Justin Lacy & Joseph W. Rudmin, Physics Dept., James Madison Univ., Harrisonburg, VA 22807. An ephemeris of the solar system was generated using Maclaurin Series polynomials on three different computer sys-tems, for the purpose of a speed comparison. In all three systems, the algorithm used to generate the polynomials was the Parker-Sochacki modification of the Picard iteration, which is well-suited for parallel processing. The first system was a nine-computer Beowulf-type parallel pro-cessing system, consisting of 500 MHz PC's connected together with serial Ethernet cards. The code was written in High-performance Fortran, and included the motions and all of the gravita-tional interactions of the sun and nine planets. In the second system, the code was written in For-tran 77 operating on a 600 MHz PC with a 32-bit Unix operating system. In the third system, the code was written using a compiled Basic (Power Basic) operating on a single 600 Mhz PC with the Dos 16-bit operating system. The two single-computer systems were roughly equal speed, whereas the parallel processing system was about 70 times slower, probably due to limitations of the coaxial serial communication lines.

SCIENTIFIC APPLICATIONS FOR DATABASES. William M. Quarles and Kevin L. Giovanetti, Dept. of Phys., James Madison Univ., Harrisonburg, VA 22807-0001. There are two main types of databases, flat and relational. Flat databases are simple lists or tables, do not require specialized software, but cannot store much information. Relational databases establish relationship between several flat databases, and are very powerful tools for storing a large amount of information with a complicated organization. Open source software and its advantages are introduced. Oracle, an expensive commercial relational database program, is being quickly displaced in use MySQL, a faster and free open source program. PHP is an open source server-side scripting language for creating dynamic webpages, and has intrinsic functions for interfacing with many other programs, including MySQL and Apache Web Server. Many web developers believe that Apache, PHP, and MySQL are the ultimate combination for creating a website. Finally, three applied databases using MySQL are shown: the membership database for the CLAS Collaboration at Jefferson Lab; the calibration database for Hall B of Jefferson Lab; and NAIS, PostDoc, and ScienceDesk at NASA. The Giovanetti Team's work in databases included installing MySQL, creating the membership database, and investigating interfacing using ODBC, Perl, PHP 4.0, and Java. (Supported by the National Science Foundation).

THE AEROACOUSTICS OF EXTERNAL-COANDA WASTE GAS FLARES. Caroline Smith, Dept. of Mathematics, James Madison Univ., Harrisonburg, VA 22807. (Invited paper) The problem of noise disturbance emanating from gas flares that are used by the oil industry will serve as an example of how mathematics can be used to solve real problems. Methods for modeling the noise emitted by plane and curved wall jets, and how these techniques were applied to external-coanda waste-gas flares of the type commonly used in the petrochemical industry will be given. These models in conjunction with measurements on real and simulated flares lead to the development of novel new flare nozzles which considerably reduced the turbulent mixing noise associated with such flares.

ANALOG COMPUTER SIMULATION OF NONLINEAR LASER DYNAMICS. Nicholas L. Silverman, Steven M. Klotz, & David W. Sukow, Department of Physics and Engineering, Washington and Lee University, Lexington, VA 24450-0303. We study the nonlinear dynamical behavior of a semiconductor laser subject to delayed opto-electronic feedback. We perform this study by designing a simple electronic circuit whose behavior is governed by the same delay-differential equations that govern the laser system. This circuit analog approach offers important experimental advantages compared with the full opto-electronic laser system, since it operates at much lower frequencies and has easily tunable parameters. Furthermore, the circuit can be investigated systematically using automated data acquisition and control of system parameters. The dynamics we observe are in good agreement with numerical and theoretical predictions. One interesting modification of the idealized circuit is the addition of a low-pass filter into the delayed feedback loop; this models the unavoidable frequency dependence inherent in the real laser system. Contrary to some current hypotheses, we find that such filtering does not affect the fundamental oscillation frequency of the system. The only apparent effect is to shift the global dynamics in a manner that reflects the reduced overall feedback strength as a result of filtering.

CALCULATING THE ATTENUATION OF SOUND IN A BUBBLY LIQUID USING THE KRAMER-KRONIG RELATIONS. Stanley A. Cheyne, Walter C. McDermott, & Patrick J. Martin, Department of Physics and Astronomy, Hampden-Sydney College, Hampden-Sydney, VA 23943. The attenuation of sound is calculated using the Kramer-Kronig Relations. The Kramer-Kronig Relations make it possible to calculate attenuation as a function of frequency if the phase speed is known. Experimental and theoretical phase speed data is used to calculate attenuation and compared to theory [K.W.Commander and A. Prosperetti, "Linear pressure waves in bubbly liquids: Comparison between theory and experiment," J. Acoust. Soc. Am. **85**, 732-746 (1989)]. Attenuation is strong near the individual bubble resonance making it difficult to make direct measurements. Converting experimentally determined phase speed data makes it possible to obtain attenuation data indirectly. PACS number: 43.35 Bf.

IMMEDIATE CREATING AN ACTIVE LEARNING CLASSROOM--USE OF STUDENT POLLING FOR FEEDBACK. William W. McNairy, Dept. of Physics, Duke University, Durham, NC 27708. In the Spring of 2001 at Duke a system of infrared remote control devices was installed for use in the Introductory Physics courses. Student evaluations of these courses in past have consistently ranked the lecture element low in value compared with other components of the course. Instructors in the lecture (70-150 students) also had difficulty in eliciting responses to polling questions, whether they were of a conceptual or computational nature. We have installed a response system which uses individual IR transmitters similar to TV/VCR remote controls. Students are polled on questions with up to 10 responses and can indicate three levels of confidence for their response. Projection of the computer interface screen shows students that their responses have been recorded. Results of the poll can then be immediately displayed on a bar graph. This flexible system then allows for re-polling after a discussion period which allows for 'peer instruction' or after a live demonstration of the principle being explored. Initial response of students has been very universal and enthusiastic.

TIDES IN THE IONOSPHERE MEASURED USING LOW FREQUENCY B FIELDS. Michael J. Wallace Hampden-Sydney Col., Hampden-Sydney,VA 23943-0726, & John P. Wallace, Casting Analysis, Weyers Cave, VA 24486. Measuring the magnetic fields in the N-S and E-W directions at the earth's surface in the frequency range from 1 Hertz to 1290 Hertz over periods of months allows the detections of phenomenon in and above the earth-ionospheric cavity. Using a narrow frequency band measuring technique we were able to reject anthropogenic noise and isolate long-term periodic data from the incoherent noise generated in above the surface the earth due to lightening, geomagnetic storms and other unidentified sources.

ATMOSPHERIC CARBON AND THE FUTURE OF THE RADIOCARBON DATING METHOD. Ross T. Thomas, Dept. of Physics, & Troy J. Siemers, Dept. of Mathematics, Virginia Military Inst., Lexington, VA 24450. Many branches of science rely upon the radiocarbon dating technique as a tool for determining the age of organic samples. Knowledge of historical concentrations of atmospheric carbon over time is critical in making accurate age determinations with the technique. Since the beginning of the industrial revolution humans have substantially increased the amount of carbon in the atmosphere. These increases cause ambiguities in the results of radiocarbon dating for young samples. Increased coal utilization and even greater amounts of atmospheric carbon over the next 20 years will make the situation much worse. Corrections may only be possible if atmospheric carbon levels in the future are assessed on a regional basis around the world.

A SIMPLE DEMONSTRATION OF FLUID FLOW AS RELATED TO ARTERIAL DISEASE. Richard. B. Minnix, Dept. of Physics, Virginia Military Inst., Lexington, VA 24450. The rate at which a fluid can be pumped through a cylindrical tube depends upon several factors: the pressure gradient, the viscosity of the liquid, and the radius of the tube. A simple apparatus designed to keep the first two factors constant consists of two soda straws (2.5 mm and 5.0 mm inside diameter) of the same length attached via rubber stoppers at the same depth on opposite sides of a gallon plastic milk jug used as a common reservoir. While results do not agree with Poiseuille's Law, the measured volume flow rate is shown to depend dramatically upon the radius of the tube.

Biology

ANABOLIC AND CATABOLIC PATHWAYS FOR MAINTAINING PHOSPHATIDYLCHOLINE HOMEOSTASIS. R. Trisdorfer, S. E. Barbour & A. Kapur, Dept. of Microbiology and Immunology, Virginia Commonwealth University. Phosphatidylcholine (PC) is the most abundant phospholipid in mammalian cell membranes. Maintenance of PC mass is critical to cell survival. Cells treated with exogenous enzymes that disrupt this balance rapidly activate enzymes that will reestablish homeostasis. In the Mc Ardle-RH7777 (MCA) rat hepatocytes, addition of an anabolic pathway through transfection of a vector for the synthetic enzyme phosphatidylethanolamine N-methyltransferase (PEMT) causes no change in PC mass. My data indicate that these transfected cells also show a decrease in one of their degradative enzymes, calcium-independent phospholipase A2 (iPLA2) as a possible reaction to the decrease in other synthetic enzymes in these cells. In Chinese Hamster Ovary (CHO) cells treatment with the catabolic enzyme secreted phospholipase A2 (sPLA2) causes an increase in PC synthesis thereby stabilizing PC mass. My data indicate that CTP:phosphocholine cytidylyltransferase (CT), an important synthetic enzyme, is induced to translocate to the membrane and thus become active when this treatment occurs. These findings further reinforce the importance of PC homeostasis in mammalian cells and the interrelatedness of the metabolic pathways involved.

INVOLVEMENT OF BLOOD CLOTTING IN PREGNANCY LOSS INDUCED BY LIPOPOLYSACCHARIDE INJECTION IN CD-1 MICE. Jory C. Enzler[1], Carolyn M. Conway[2], & Arthur F. Conway[1], [1]Dept. of Biology, Randolph-Macon College, and [2]Dept. of Biology, Virginia Commonwealth University. Pregnant CD-1 mice were injected in a tail vein with 5mg of *E. coli* lipopolysaccharide (LPS) in phosphate-buffered saline (PBS) on the ninth day of gestation and then sacrificed 6 or 12 hours after injection. Reproductive tracts were frozen at -50 Celsius and sectioned at 20 micrometers. Control mice were injected with PBS or left uninjected. LPS injection caused a significant decrease in maternal body weight, a significant increase in total endogenous peroxidase staining in the trophoblastic giant cell layer at the maternal-embryonic interface (indicating increased tissue destruction in that location), and an increased accumulation of granulocytes in the decidua below the mesometrium. These changes are consistent with the initial stages of LPS-induced pregnancy loss. Immunostaining for fibrinogen (probably reacting primarily with polymerized fibrin under our conditions) was not significantly altered by LPS treatment in any of the locations sampled in implantation sites, so these results are not consistent with the involvement of the terminal steps of blood coagulation in the early changes leading to LPS-induced pregnancy loss.

BRIEF ELECTRICAL STIMULATION OF THE FOOT OF THE NEONATAL RAT INDUCES A PERMANENT INCREASE IN ADULT PAIN SENSITIVITY. Maria LaPlante, Andrea Gocke & Corey Cleland, Department of Biology, James Madison University, Harrisonburg, VA 22980. Inflammatory injury in neonatal rats can lead to long-lasting increases in adult pain sensitivity. However, it is unknown whether the pain messages that impinge on the spinal cord or the peripheral inflammation are responsible. Consequently, we developed a model in which electrical stimulation of the paw is used to mimic painful sensory input, but without associated tissue damage. Anesthetized neonatal rat pups received electrical stimulation of the left paw at varying developmental stages, stimulation intensities and frequencies. After reaching adulthood (56 days), their thermal pain sensitivity was tested. The results revealed that the electrically stimulated left paw was more sensitive to pain than the right paw, supporting the hypothesis that activation of the nerve fibers at an early age without tissue damage causes long-term effects on the pain sensitivity of rats. In addition, maximal increases in pain sensitivity were found at P-5 and at intensities greater than 2.5 mA, consistent with activation of C fibers. Variation in frequency of stimulation did not appear to alter the increased pain sensitivity. (Supported by NIH and the Jeffress Foundation).

ANALYSIS OF THE *TRANSPARENT TESTA* MUTANTS OF *ARABIDOPSIS THALIANA* FOR A SUBSTRATE REGULATED FEEDBACK MECHANISM OF ENZYME EXPRESSION. Michelle M. Barthet & Brenda S. J. Winkel, Dept. of Biology, Viginia Tech, Blacksburg, VA 24061. The *transparent testa* (*tt*) loci identify genes of flavonoid metabolism in *Arabidopsis*. Although the flavonoid pathway itself has been extensively studied, many questions remain regarding the regulation of its enzymes. A previous investigation of the *tt* mutants uncovered a possible feedback mechanism in the flavonoid pathway. We hypothesize that this mechanism may be activated by the flavonoid intermediate, naringenin chalcone, or by flavonol glycosides. This feedback mechanism appears to elevate levels of the flavonoid enzymes, chalcone synthase and flavanone 3-hydroxylase, in at least two mutant lines, *tt3* and *tt5*. Chalcone isomerase appears to be overexpressed in the *tt6* mutant line. New evidence from analysis of mRNA and protein indicates that feedback control operates at different points of gene expression for each of these enzymes. Feeding experiments are being performed to test whether these effects can be replicated in wild type *Arabidopsis* plants and to determine the specific intermediates or end-products involved. (Supported by: Sigma Xi Grant-in-Aid of Research and the Graduate Research Development Project [GRDP], Va. Polytechnic Inst. & State Univ.).

MITOCHONDRIA-ENDOPLASMIC RETICULUM SIGNALLING IN APOPTOTIC NEURONAL DEATH IN AN Al-INDUCED NEURODEGENERATION MODEL. Othman Ghribi,[1] David A. DeWitt,[2] Michael S. Forbes[1], Mary M. Herman,[3] John Savory[1], [1]Dept. of Pathology, University of Virginia, Charlottesville, VA [2]Dept. of Biology and Chemistry, Liberty University, Lynchburg, VA. [3]IRP, NIMH, NIH. Neurodegenerative diseases, including Alzheimer's disease, are characterized by a progressive and selective loss of neurons which may involve apoptosis. Intracisternal administration of aluminum maltolate to New Zealand white rabbits results in many of the histologic and biochemical changes associated with Alzheimer's disease. Aluminum maltolate induced the release of cytochrome c from the mitochondria which is a key event triggering apoptosis. Co-administration of cyclosporin A prevented this release. Previous work has also implicated the ER in regulating apoptosis, either independently of or in concert with mitochondria. Members of the *Bcl-2* family of proteins have been shown to either inhibit apoptosis, as is the case with Bcl-2, or promote it, in the case of Bax. The treatment of young adult rabbits induced both cytochrome *c* translocation from brain mitochondria to cytosol, and caspase-3 activation. Furthermore, these effects are accompanied by a decrease in Bcl-2 and an increase in Bax in immunoblots of ER extracts. Supported by the Department of Defense.

HOMOLOGY MODELING OF THE *tt4* MUTANTS IN *ARABIDOPSIS THALIANA*. C. D. Dana[1], D. R. Bevan[2] & B. Winkel-Shirley[1] [1]Depts. of Biol. and [2]Biochem., Virginia Tech, Blacksburg, VA 24061. Chalcone synthase (CHS) catalyzes the first committed step in flavonoid biosynthesis, a major pathway of plant secondary metabolism. An allelic series for the Arabidopsis CHS locus, *tt4*, has been characterized at the gene, protein, and end product levels by Saslowsky and co-workers. The mutations identified in each of these alleles were also located on a homology model of the wild type Arabidopsis CHS protein, which is based on the crystal structure of CHS from *Medicago sativa* published by Ferrer and co-workers. We have extended this analysis, refining the original model and generating structures for the mutant proteins, in order to determine how mutations may affect protein structure and function. The *tt4*(UV113) allele contains a mutation that causes a disruption of enzymatic activity while maintaining a stable protein. In contrast, the *tt4*(38G1R) mutation results in a truncated protein that is both inactive and unstable. Interestingly, two temperature sensitive alleles, *tt4*(UV01) and *tt4*(UV25), exhibit significant conformational differences when modeled at different temperatures. These computational approaches are providing new insights into how residues outside the CHS active site may impact enzyme activity, function, and stability.

ALUMINUM INDUCED APOPTOSIS *IN VITRO* AS A MODEL OF ALZHEIMER'S DISEASE. Douglas W. Strand[1], Othman Ghribi,[2] Nena Fox[3], Kathleen J.S. Griffioen[1], Michael S. Forbes[2], John Savory[2], & David A. DeWitt[1], [1]Dept. of Biology and Chemistry, Liberty University, Lynchburg, VA [2]Dept. of Pathology, and [3]Dept. of Microbiology, University of Virginia, Charlottesville, VA. The fundamental mechanism for cell loss in Alzheimer's disease is currently unknown although recent evidence suggests a possible role for apoptosis. In previous studies, intracisternal aluminum (Al) administration to aged rabbits has yielded many biochemical and pathological similarities to Alzheimer's disease and implicates apoptosis. To better understand Al induced neurodegeneration, we have developed an in vitro model using human neuroblastoma (NT2) cells. Al was shown to lead to substantial cell death within 24 hours of incubation. Nuclear fragmentation and condensation suggestive of apoptosis was observed as early as 3 hours and increased substantially through 24 hours. Detection of cytochrome c provided evidence that Al induced cell death occurred through apoptosis since Al treated cells had reduced mitochondrial immunoreactivity. We propose that this *in vitro* model, with Al as a trigger, may be used to test theories of neurodegeneration as well as screen potential therapeutic agents. Supported by the Jeffress Memorial Trust.

NEURAL MECHANISMS UNDERLYING HYPERALGESIA IN THE RAT. Jason Freund & Corey Cleland, Department of Biology, James Madison University, Harrisonburg, VA 22980. Inflammation causes increased pain sensitivity, known as hyperalgesia. There is substantial indirect evidence that the underlying changes occur in the peripheral nervous system, spinal cord and the brain, however direct estimates of the individual functional contributions are lacking. We have developed a model to directly examine the magnitude of spinal cord contributions to hyperalgesia in Freund's Adjuvant-treated rats. Rats were inflamed by injection of Freund's adjuvant (100%, 30 ml) into the left hindpaw. The following day, rats were anesthetized and spinalized and EMG electrodes were placed bilaterally around flexor muscle in both hindlimbs. The following day, electrical stimulation was delivered to the surface of the foot to evoke withdrawal reflexes whose magnitude was quantified by EMG. Our results show that unilateral inflammation unexpectedly depressed the spinal component of the flexion withdrawal reflex bilaterally. This is in contrast to a net increase in reflex strength in response to natural stimulation, suggesting that there is a large peripheral sensitization that exceeds the central de-sensitization. (Supported by NIH and the Jeffress Foundation).

CONTINUED ASSESSMENT OF COTTON STAINER RECOVERY FROM HURRICANE IMPACT. Harold J. Grau, Dept. of Biol., Chem., & Env. Sci., Christopher Newport Univ., Newport News, VA. 23606. Cotton stainers (*Dysdercus* sp.) are pan-tropical hemipterous insects that feed primarily on Malvaceous plants. Several distinct populations of *D. andreae* are found on St. Thomas, USVI. In September of 1995, and again in July of 1996, the island suffered direct hits by tropical hurricanes. As might be expected, the populations of *D. andreae* exhibited severe reductions in densities and distribution, being totally eliminated from several locations. Data collected in July 2000 show that the Hull Bay population had made significant recovery in terms of densities and habitat utilization, although they were still below pre-hurricane levels; average body size measures were considerably smaller than in the two years immediately after the storms. Two other populations had returned to pre-storm average body sizes, but their population densities and habitat utilization had decreased from that of 1998, dramatically so in the case of the Tutu Beach population. Dry conditions that preceded data collection may have contributed to the low numbers of insects during that time. Recovery from the storms appears to be complete for those populations that survived the initial impact. (additional information is available at http://users.cnu.edu/~hgrau/)

BOTTLENOSE DOLPHIN UTILIZATION OF THE ELIZABETH RIVER, VIRGINIA. Kevin M. Foss & James R. Reed, Dept. of Biology, Chemistry and Environmental Science, Christopher Newport University, Newport News, VA 23606. As the Elizabeth River is both toxin laden and extensively used by commercial, military and pleasure craft, it presents a unique and previously unstudied habitat for Bottlenose dolphins (*Tursiops truncatus*). Using standard protocols for dolphin research, 43 cruises were made over the course of three years and data was recorded on behavior, location and number and animals were recorded on film. The most common behavior observed was feeding. An annual pattern of usage of the river began in May, with a peak in August and no activity from November to April. A diurnal pattern of movement appeared to be used, with no effects seen due to tidal state or weather. Spatially, the dolphins used the main branch of the river most frequently, but with common use of the Lafayette River, a tributary. Using the photo ID data, 125 individuals have been identified using the Elizabeth River during the summer of 2000. A Caughley recapture estimate was used to derive a total population estimate of 216. Habituation to small boat traffic has been noted, as has reactions such as bunching and extended diving near larger vessels. A solitary dolphin overwintering for the past two winters and seen begging has been found dead from propeller strike.

REPRODUCTIVE ECOLOGY OF THE EASTERN BOX TURTLE (*TERRAPENE CAROLINA CAROLINA*) IN A MIXED OAK-PINE WOODLAND IN THE CENTRAL VIRGINIA PIEDMONT. Gordon L.Wilson, Dept. of Biol., George Mason Univ., Fairfax, VA. 22030-4444. A three year ecological study focusing on the reproduction of the eastern box turtle began in the fall of 1999 and will continue through the summer of 2002. Twelve females were radio-tracked through the summer of 2000, weighed at each capture, and X-rayed two to three times during June and July to determine if they were gravid. If gravid, clutch size, egg size, and egg weight were obtained. Mean clutch size was 3.2 (n = 5). Mean egg length and width were 36.4 mm and 21.1 mm, respectively (n = 4). Mean egg weight was 10.3 g (n = 4). Mitchell believed that throughout Virginia, box turtles laid a single clutch of eggs per year. However, one female (#60) laid two clutches in the summer of 2000. Her first clutch of three eggs was laid in mid-June and her second clutch of two eggs was laid in mid-July. Population data on this population was also gathered during this study. Home ranges of the twelve females were calculated using the bivariate normal method. The mean home range size was 7.9 ha (S.D.= 9.6). The population estimate of the 11 ha study site was 279 turtles using the Peterson Index. Population density was 25 per ha. The F:M sex ratio was 1:1.5 for the 96 turtles whose sex was determined.

METABOLISM OF EXOGENOUS BIOGENIC AMINES IN TOAD-EATING AND NON-TOAD-EATING SNAKES. Lauren E. Laitala & John Temple, Dept. of Biol., Mary Washington College, Fredericksburg, VA 22401. The Florida banded water snake (*Nerodia fasciata*) is one of a few species of snakes that includes toads (Genus *Bufo*) in their diet and can tolerate toad toxin. When fed to non-toad eating snakes, toad toxin induces extensive cardiac arrhythmia, ventricular fibrillation and eventual lethal cardiac and muscular tetany. Serotonin is one component of toad toxin that is known to affect heart rate, vasoconstriction and blood pressure in vertebrates. The goal of this research was to address the hypothesis that toad-eating water snakes (*N. fasciata*) would have a greater ability to break down orally-administered serotonin than non-toad-eating rat snakes (*Elaphe obsoleta*). Following an oral dosage of serotonin (0.00052mg/g body weight), blood was collected hourly for six hours and the serotonin and metabolite levels were analyzed by HPLC. Results indicate that there is no significant difference in serotonin clearance from the blood between the two snake species at the dose and times tested. (Supported by Sigma Xi Grant-in Aid of Research and the Mary Washington College DuPont Summer Science Program).

IN VITRO STUDY OF MOUSE MAMMARY TUMOR VIRUS. Dheeraj K. Goswami & Lynn O. Lewis, Dept. Biological Sciences, Mary Washington College, Fredericksburg, VA 22401. Mouse mammary tumor viruses have been linked to cancer in mice since the 1930's. However, only recently have there been data that have linked the viruses to breast cancer in humans. One of the difficulties in the study of this connection is that researchers have been unable to produce cancer cells in the tissue culture of mouse mammary cells. The purpose of this study is to try to produce cancer cells in tissue culture using a variety of hormones and chemicals. Mouse mammary cells with and without viruses were used to compare and contrast the effects of the hormones and chemicals on their growth and structure. Numerous trials were conducted with a combination of progesterone, estrogen and the carcinogen 2,4-dimethylbenzanthracine. A control of 300 cells per 75 cm^2 flask was used with their growth being recorded after five 12-hour intervals (60 hours total). The effects of the hormones with the carcinogen were recorded at various points over a month-long span. The data showed that while the hormones and carcinogens did have an impact on the structure and growth of the cells, they were apparently not able to produce any cancer cells in the tissue culture.

THE EFFECTS OF VINCRISTINE AND PACLITAXEL ON BCL-2 LEVELS IN ME-180 AND CAOV-3 CARCINOMA CELLS. Karyn Havas & Rosemary Barra, Dept. of Biol. Sciences, Mary Washington College, Fredericksburg, VA 22401. Bcl-2 is a protein that defines a new class of oncogenes. Its primary role in a healthy cell is to regulate the occurrence of apoptosis, or programmed cell death. Many haemopoeitic cancers are characterized by a mutation affecting Bcl-2 expression. The over expression of this gene increases the ability of a cell to survive, even under adverse conditions and in the presence of DNA damage. The goal of this investigation was to determine the effects of two antineoplastic, antimicrotubular drugs, paclitaxel and vincristine, on the levels of Bcl-2 in ovarian and cervical carcinoma cells. Initial studies were performed using the MTT cytotoxicity assay to determine the LD_{50} of each of the drugs. An immunoblot procedure was carried out following treatment of cultured cells with the drugs at concentrations below the LD_{50} value and a Bcl-2 ELISA assay was performed to quantify the Bcl-2 levels. The results indicate that expression of Bcl-2 increases following treatment with vincristine and paclitaxel. It was noted that Bax, a proapoptotic protein, and various dimers were formed. It was concluded that the two drugs stress the cells causing an increase in Bcl-2 levels. These results suggest that Bcl-2 may play a role in decreasing the effectiveness of the drugs.

THE EFFECTS OF METHYL-b-CYCLODEXTRIN AND LOVASTATIN ON EMBRYONIC AXIAL TORSION AND ORGAN PLACEMENT IN CHICKENS. Tamera Y. Sandrof, Douglas H. Shedd, Department of Biology, & Ann M. Fabirkiewicz, Department of Chemistry, Randolph-Macon Woman's College. The *sonic hedgehog* (*shh*) gene produces a signaling molecule, Sonic hedgehog protein (Shh), exclusively on the left side of the primitive streak in the avian embryo. Shh undergoes autoproteolysis and binds cholesterol onto its newly formed carboxyl terminus. The new molecule indirectly activates the signaling for the dextral rotation of both the primary axis and the heart. Chick embryos were treated with methyl-b-cyclodextrin and lovastatin to reduce the level of cholesterol and inhibit the Shh signaling process. The treatment did not produce a statistically significant effect on torsion or heart looping. With greater numbers, however, the effects on heart looping may have been statistically significant.

MOLECULAR REGULATION OF VITAMIN D ACTIVATION: INVOLVEMENT OF THE RENAL VITAMIN D RECEPTOR (VDR) DOWN REGULATION. A Bajwa & MJ Beckman, Virginia Commonwealth University, Richmond, VA, USA. The synthesis of 1,25-$(OH)_2D_3$ is regulated by dietary calcium, by PTH to increase 1α-OHase and decrease 24-hydroxylase and by 1,25-$(OH)_2D_3$ which regulates its own synthesis through a VDR dependent negative feedback effect on both 1α-OHase and preproparathyroid hormone production. This study examines the molecular mechanism of renal vitamin D activation induced by hypocalcemia, and tests the hypothesis that the activation process is the result of negative feedback regulation blockade at the transcriptional level. Differential display RT-PCR was carried out using RNA isolated from the 2 groups that demonstrated extremes in regulation of 1a-OHase gene expressions, the -Ca+D group and the +Ca+D/1,25$(OH)_2D_3$ group. A panel of 8 different combinations of anchor and arbitrary primers produced 7 gene products that correlate with hypocalcemia and 6 gene products that correlated with hypercalcemia. Authentic differentially regulated gene products in the two samples will be confirmed by the real time RT-PCR method. It is expected that this study will result in identification of two sets of gene products that participate in either increased 1a-OHase gene expression or that participate in 1a-OHase gene suppression.

Biomedical and General Engineering

THERMODYNAMIC ANALYSIS OF A SOLAR ASSISTED HEAT PUMP. Ahmed AL-Mogbel, Francis B. Gorozabel, Sushil K. Chaturvedi, College Of Engineering and Technology, Old Dominion University, Norfolk, VA 23529. A direct expansion solar-assisted heat pumped is analyzed for domestic hot water applications. The heat pump employs a solar collector panel that also acts as the evaporator for the heat pump cycle. The system employs refrigerant R-134a that is expanded directly into the solar panel where it is evaporated by solar energy. The thermal performance of the system is evaluated for three refrigerants namely R-12, R-22, R-134a, by employing the first and second law of thermodynamics. The system parameters, including the collector area and the compressor RPM, are chosen so that the evaporator to ambient temperature difference is in a narrow range of -1 °C to 14 °C depending the solar radiation intensity in the collector plane. Results show that for R-134a, the coefficient of performance varies from 3.3 to 4.88 for winter like conditions. A comparison of results obtained for R-12 with R-134a shows that the system performance is degraded by about 5% when R-134a is used as the refrigerant. For parameters considered in this study the second law efficiency ranges from 0.32 to 0.45 depending on the refrigerant and the level of incident solar radiation.

GEOMETRIC PARAMETERS EFFECTING FINITE ELEMENT ANALYSIS OF INDENTATION OF ARTICULAR CARTILAGE. Corrie E. Spoon & Jennifer S. Wayne, Orthopaedic Research Laboratory, Depts. of Biomedical Engineering and Orthopaedic Surgery, Virginia Commonwealth University, Richmond, VA 23298. Indentation tests are commonly used to determine the mechanical properties of articular cartilage. This investigation sought to evaluate the effect of changing geometric parameters on the properties determined from creep indentation. Finite element analysis (FEA) was used to simulate the indentation of normal and repair cartilage with varying ratios of indenter radius to cartilage height (a/h=0.5,1.5) and cartilage radius to indenter radius (r/a=2, 5). The vertical displacement of the cartilage under the indenter from FEA was curve fit to the biphasic theory to determine the aggregate modulus, permeability, and Poisson's ratio. The curve fit properties were compared to determine the effect of altering the geometric parameters. The effects of geometric changes were independent of cartilage properties (repair vs. normal). Aggregate modulus was not greatly affected by the geometric changes studied. Permeability was affected by changes in indenter and cartilage lengths for a/h=0.5. Indentation experiments of cartilage with a/h=1.5 are not affected by r/a for values of 2 and 5. Experimental setups with a/h=0.5 should have r/a values greater than 2.

DEVELOPMENT OF TDI NANOSCALE STRUCTURES. D. Pestov, N. Levit & G. Tepper, Department of Chemical Engineering Virginia Commonwealth University, Richmond, VA 23284. In this work we combine a spray on technique known as Rapid Expansion of Supercritical Solution (RESS) and gas phase crosslinking techniques to develop a new nanoscale composite material. By RESS we obtained about 1 micron diameter particles of poly(dimethylsiloxane), bis(12-hydroxystearate) terminated. These particles were then exposed to 2,4-toluyne diisocyanate (TDI) saturated vapor at room temperature. After this reaction a composite material was obtained. It consists of cross-linked initial siloxane and a new nanoscale needle like TDI polymer. It is found that the TDI polymer needles initiate from surface imperfections. Scanning Electron and Atomic Force microscopy was used for imaging. Based on FT-IR data and microscope-imaging, a scheme for the TDI polymerization reaction is proposed. The composite material has a high surface/volume ratio and potential for use in sensor applications. This work was funded by the EPA, National Center for Environmental Research and Quality Assurance.

HEALTH CARE THROUGH INTERNET. Shan Lu & Ding Y. Fei, Department of Biomedical Engineering, Virginia Commonwealth Univ., Richmond, VA 23298. A real time health care system was developed for out-of-hospital patients. It takes advantage of Internet and wireless communication technology. Two portable devices (PE504 and Vitalpoll) and certain software were developed to implement this system. PE504 is a microcontroller-based device which can collect bio-signals and transfer them to Vitalpoll. It has 4 independent channels suitable for ECG, heart rate, blood pressure and body temperature. The sampling rate is up to 40kHz and the resolution is 12 bit. Vitalpoll will collect and store medical data coming from the patient and finally transfer them to an internet access point (home PC or mobile handhold system) by using Bluetooth wireless technology. It has RS232, USB and PCMCIA interface so that it can be connected to various medical devices. DSP was also used to process the signal. System analysis and management software is developed to pace, monitor and control the Vitalpoll and PE504. The software is running from a Windows 2000 server located in the hospital monitor center. The physicians in the center can monitor the real time medical signal of any patients in the network by their IP addresses. All the patients' records are stored in the database that can be accessed by certain physicians. This project is funded by Department of Biomedical Engineering, VCU.

MECHANICAL CHARACTERISTICS OF A TENDON/SUTURE INTERFACE. John R. Owen[1], Timothy J. Marqueen[1], Charles L. McDowell[1], Jennifer S. Wayne[1,2], & Thomas P. Loughran[1], Orthopaedic Research Laboratory, [1]Depts. of Orthopaedic Surgery and [2]Biomedical Engineering, Virginia Commonwealth University, Richmond, VA 23298. Extensive tendon healing research has been performed over the years. Characteristic changes in repair site tensile strength during healing have been defined, as well as, the tendon's intrinsic ability to heal, and the efficacy of various suture patterns. Yet, gapping of tendon ends during healing continues to be a significant problem. All research to date has focused on the tendon/tendon interface. None have investigated the separate role of the tendon/suture interface in the healing process. This study investigates the role of this one factor. Without cutting the tendon, sutures were placed in the deep flexor tendons of forty chickens. The animals were euthanized at nine time points ranging from immediately after surgery to four weeks later. Suture pullout tests were performed and revealed a significant decrease in strength during the first six days following surgery and a recovery of strength during the third and fourth weeks. These results support the hypothesis that the suture/tendon interface plays a significant role in the tendon healing process. This study was funded by a grant from Virginia Commonwealth University's A.D. Williams Foundation.

CHANGES IN LOWER EXTREMITY PERFORMANCE DURING A DYNAMIC, HIGH-INTENSITY EXERCISE. Michael L. Madigan, Dept. of Biomedical Engineering & Peter E. Pidcoe, Dept. of Physical Therapy, Virginia Commonwealth University, Richmond, VA 23298. The purpose of this study was to investigate the effects of neuromuscular fatigue on lower extremity landing biomechanics. Ground reaction force and kinematic data were collected from 12 healthy male subjects during the performance of a fatiguing, single-leg landing protocol. Joint torque and power estimates were calculated using a 3-D link-segment model and an inverse dynamic analysis technique. As subjects fatigued, frontal plane torque at the hip decreased resulting in less frontal plane work being performed at the hip. This suggests more frontal plane movement was transmitted though the pelvis, exposing the lumbar spine to higher stresses. Sagittal plane torque decreased at the knee and ankle, and increased at the hip. This redistribution of torques resulted in less work, or energy absorption, being performed at the knee and ankle. As a result, this decrease in energy absorption may expose the passive musculoskeletal structures within the knee and ankle to higher stresses.

THE EFFECT OF SURFACE PREPARATION ON CARRIER LIFTIMES IN DETECTOR GRADE CdZnTe. R.F. Kessick & G. Tepper, Department of Chemical Engineering, Virginia Commonwealth University, VA 23284. The spectroscopic performance of cadmium zinc telluride (CZT) room temperature radiation detectors is currently limited by both bulk and surface imperfections introduced during the growth, harvesting and fabrication of these devices. Bulk imperfections have been relatively well studied and are known to trap charge and reduce detector performance. Surface imperfections including mechanical damage or adsorbed chemical species are known to trap charge or increase leakage current, but it has proven difficult to characterize the electronic properties of CZT surfaces. It is desirable to characterize the electronic properties of CZT surfaces to understand the effects of processing treatments such as mechanical polishing, chemical etching or passivation. Here it is shown that contactless thermally stimulated lifetime measurements using a pulsed laser microwave cavity perturbation method can provide important information on electronic decay on the semiconductor surface. Carrier lifetimes were measured as a function of surface roughness and chemical etching, the surface effects were resolved by analyzing distinct features in the electronic decay profiles generated by the contactless method.

A MODIFIED TIME-COST TRADE-OFF MODEL FOR MANUFACTURING APPLICATIONS. Hisham M. AbdelSalam & Han P. Bao, Dept. of Mech. Engr., Old Dominion Univ., KDH 238, Norfolk, VA 23529. In addition to scheduling projects, project managers are frequently confronted with the problem of having to reduce the scheduled project total completion time (indicated by the "Critical Path Method" network analysis) to meet a pre-specified deadline. This problem, project crashing or Time-Cost Trade-Off, has been formulated and solved using several methods, starting with enumerative methods and ending with artificial intelligence. Despite the fact that the widespread use of CPM technique was mainly achieved by, and for, construction industry applications, one can state that CPM is equally suitable for planning any one-time projects involved in the manufacturing industry. In this paper, a modified time-cost trade-off model is being presented and implemented. While the demonstration is based on a relatively small network, the potential for application to huge projects will be the key finding of this paper.

MECHANICAL FUNCTION PREDICTED BY MRI PARAMETERS IN CARTILAGE. K. J. Shields, K. A. Kraft[1], J. S. Wayne, D.G.Disler[2], J. R. Owen, & C. Yin, Orthopaedic Research Laboratory, Departments of Biomedical Engineering and Orthopaedic Surgery, and [1]Department of Radiology, Virginia Commonwealth University, Richmond, VA 23298 and [2]Commonwealth Radiology, Richmond, VA 23298. Healthy articular cartilage functions to facilitate lubrication and stress distribution in diarthroidal joints. Degeneration of cartilage, such as in osteoarthritis, results in deterioration of these functions as damage occurs in the collagen/proteoglycan network of cartilage. Correlating changes in composition with mechanical function through non-invasive techniques such as MRI is valuable. The current study examined the effects of proteoglycan depletion and collagen depletion on the biomechanical properties of modulus and permeability and correlated the results with MRI parameters. Samples of cartilage from porcine patellae were evaluated biomechanically and through MRI. The samples were then enzymatically treated to deplete a portion of the proteoglycan component or the collagen component. The biomechanical and MRI characteristics were then re-evaluated. Results indicate alterations to the biochemical composition yielded differences in the biomechanical properties and the MRI parameters. This study was funded by Virginia's Commonwealth Health Research Board (CHRB).

USING SMART RADIOS TO BOOKMARK BROADCAST CONTENT. Alen Docef, Virginia Commonwealth University, Richmond, VA 23284 & Bruce R. MacAlister, Punchee, Inc., 1805 Grove Avenue, Richmond, VA 23220. The Punchee Wireless Interactive Radio is a service that provides radio advertisers and audiences with a one-button solution to the problem of advertisement bookmarking. Subscribers to the service can react to selected commercials by pushing a button on an Information Appliance. The system uses broadcast bookmarks to record and store subscriber selections. Punchee.com then allows subscribers to explore the branded products via the Internet. The interactive radio system must be able to recognize the audio material being bookmarked, via audio watermarking. Two watermarking algorithms have been implemented for the Punchee system: an in-house algorithm, and the commercial Bluespike algorithm. The algorithms have been evaluated comparatively for reliability, latency, and robustness. The in-house watermarking algorithm has a smaller latency, while the Bluespike algorithm is more robust. Both algorithms are robust enough to withstand digital/analog/digital conversion and transmission via analog FM modulation. A demonstration system was developed for the complete Punchee system: user device, monitoring station, and central database server. The project also developed embedded logic in a "smart" car radio that, on user's spoken command, bookmarks the broadcast and transmits it to the server.

Botany

CHESTNUT BLIGT: AMERICAS WORST SILVAN TRADGEDY. Eric P. Hogan & Gary J. Griffin, Dept. of Plant Path. Phys. and Weed Science., Va Polytechnic Inst. & State Univ., Blacksburg, Va 24061. The American chestnut (*Castanea dentata*) was for many years regarded as one of the most economically important hardwoods in the US. The tree provided rot resistant lumber, chestnuts for food, and tannins for leather. In the early 1900's the chestnut blight fungus (Cryphonectria parasitica) was introduced into the United States and Europe. The blight destroyed nearly all the canopy American chestnuts and reduced the tree to an under-story shrub. The fungus invades the tree through a wound in the bark and produces a canker, killing tissue as it expands. Once the fungus has girdled the tree and invaded the vascular cambium the tree will die. On the surface of the cankers, fruiting bodies produce sexual and asexual spores, which cause spread. In Europe, infected trees have recovered naturally from the blight. This is primarily the result of the spread of naturally occurring hypovirulent (= low virulence) isolates of the fungus. Hypovirulent strains exist in the U.S. as well, but the limited spread of hypovirulent strains and high susceptibility of American chestnut has prevented natural recovery. Hope for the future involves further study of hypovirulence, breeding programs with American and Asian blight resistance and forest management strategies.

SPATIAL PATTERN OF WHITE HYPOVIRULENT ISOLATES OF *CRYPHONECTRIA PARASITICA* ON GRAFTED AMERICAN CHESTNUT TREES. Eric P. Hogan & Gary J. Griffin, Dept. of Plant Path. Phys. and Weed Science., Va Polytechnic Inst. & State Univ., Blacksburg, Va 24061. In 1980 a clearcut plot of grafted American chestnut trees (*Castanea dentata*) was established in Lesesne State Forest, Virginia. In 1982-83 naturally formed blight cankers, within a zone ranging from the ground to 183cm on the grafted trees, were inoculated with a mixture of four European (white), and six pigmented, hypovirulent strains of the chestnut blight fungus (*Cryphonectria parasitica*). After 14 years it was determined that the white strains had spread throughout three of the inoculated grafts, which have low levels of blight damage. Natural blight cankers were sampled from these three trees to determine the spatial pattern of the white isolates and white vc groups among cankers on the grafted American chestnut trees. Forty-eight vc groups were identified among the 110 white isolates collected. Using a double matrix statistical test described by Milgroom et al., the pattern of white vc groups among the three grafts was found to be aggregated ($P=0.019$), whereas the pattern of white isolates was found to be random ($P=0.325$). These findings suggest that aggregation of white vc groups and random distribution of white isolates are favorable to biological control of chestnut blight.

THE SEASONAL DISTRIBUTION OF AUTOTROPHIC PICOPLANKTON IN THE CHESAPEAKE BAY. Bonnie Brown[1] and Harold G. Marshall[2]. [1]Dept. Oceanography, Earth, and Atmospheric Science., [2]Dept. Biological Sciences, Old Dominion University, Norfolk, Va., 23529-0266. Water samples from 7 lower Chesapeake Bay stations were monitored monthly with water samples taken above and below the pycnocline, and then processed for epifluorescence microscopic examination. Data from a ten year period is presented. The abundance and distribution of autotrophic picoplankton in the Chesapeake Bay indicated a single summer pulse characterized this community at all the stations. The maxima occurred usually during July or August, with minimum abundance occurring in winter. The onset and duration of the summer maximum often varied under the influence of water flow in the system, but was generally initiated by June, peaking in either July or August, then declining into winter. Abundance generally increased with rising water temperature, and decreased with declining water temperatures. Summer pulses were at 10^6 to 10^8 cells/ml, with lowest winter concentrations at approximately 10^4 cells/ml. Cell abundance was generally higher above the pycnocline, except during colder periods of winter. Supported by Va. Dept. Environmental Quality.

MONITORING VIRGINIA ESTUARIES FOR THE TOXIC DINOFLAGELLATE *PFIESTERIA PISCICIDA* AND *PFIESTERIA*-LIKE DINOFLAGELLATES. Todd Stem and Harold G. Marshall, Dept. Biological Sciences, Old Dominion University, Norfolk, Va., 23529-0266. For the past 3 years we have been monitoring Virginia estuaries for *Pfiesteria* spp. and *Pfiesteria*-like organisms (PLO) as one component of the Virginia Task Force on Pfiesteria to determine their presence, and degree of representation in these waters. Our results indicate PLO are ubiquitous in Virginia estuaries and the Chesapeake Bay. Monthly or twice monthly samples were examined each year from over 100 stations between June and November. *Gyrodinium* spp., *Cryptoperidiniopsis* spp., and *Gymnodinium* spp. were the more abundant PLO. Greatest concentrations were in shallow waters of 10-20 ppt salinity, oxygen at >6 mg/l, and temperatures >20 degrees C. Highest concentrations reached 49,000 cells/ml, and located along the Virginia inlets of the Potomac River, and lowest at ocean shoreline estuaries. No toxic *Pfiesteria* strains were detected. In 2000, 72.6% of the water samples contained PLO, with 8.0% of these having concentrations over 200 cells/ml. Associated studies in our laboratory involve SEM validations of species, cell cultures of clones of these species. Our results are confirmed with other established external labs for verification of results. Supported by the Virginia Dept. of Health, Va. Dept. of Environmental Quality, and Center for Disease Control and Prevention.

PHYTOPLANKTON COMPOSITION IN VIRGINIA ESTUARIES. Jennifer McNally , Todd Stem, Bonnie Brown, and Harold G. Marshall, Dept. Biological Sciences, Old Dominion University, Norfolk, Va., 23529-0266. The Chesapeake Bay phytoplankton populations are dominated by a diatom flora that produces a peak spring bloom followed by lesser seasonal peaks in summer and fall.

Long term monitoring indicates significant variability in the onset and duration of these growth patterns. Cyanobacteria and dinoflagellates share summer bloom dominance, with the major components decreasing into winter months. Less favorable water quality conditions (high nutrients, reduced light availability) are favored by cyanobacteria, dinoflagellates, and picoplankton; with more favorable conditions (lower nutrient levels, increased light availability) dominated by a diatom flora. Sporadic dinoflagellate blooms begin in early spring and continue into early fall. The presence of cyanobacteria within the system is common, with at least 14 potential toxin producers (dinoflagellates and diatoms) identified within the estuary. Increased nutrient loadings would promote shifts in the floral balance from a diatom based system to one less favorable as a food and oxygen source, and increase the abundance and distribution of cyanobacteria, dinoflagellates, and possibly any introduced exotic species in this estuary. Supported by the Virginia Department of Environmental Quality.

OSMOTIC TOLERANCE IN *SAINTPAULIA IONANTHA*. Michael H. Renfroe, Dept. of Biology, James Madison University, Harrisonburg, VA 22807. Cryopreservation of African violet (*Saintpaulia ionantha* Wendl.) offers the potential for long-term germplasm preservation. Successful cryopreservation relies on removal of intracellular water. Such water removal subjects the plant tissue to water stress. Therefore, investigation of osmotic tolerance is an essential step to successful cryopreservation. A recent approach to cryopreservation involves encapsulation and dehydration of excised shoot tips, followed by freezing in liquid nitrogen. African violet shoot tips subjected to this procedure did not survive the treatment. Experimental investigations showed that dehydration in a culture medium supplemented with 0.75 M sucrose was the critical step producing the tissue injury. Additional experiments were conducted to investigate the conditioning of shoot tips to osmotic stress. Shoot tips were serially transferred to media of increased osmolarity, then to a growth medium on which they were observed for recovery and growth. Shoots on medium supplemented with 0.75 M sucrose recovered as well as did shoots on a medium with 0.525 M sucrose, but neither of these groups grew as well as did shoots on medium with 0.3 M sucrose. However, shoots that were serially conditioned to osmotic stress grew better than shoots without the conditioning.

SCANNING ELECTRON MICROSCOPY EXAMINATION OF 150 YEAR OLD HERBARIUM SPECIMENS OF CYANOPROCARYOTE TAXA ORIGINALLY PREPARED BY L. RABENHORST. Lubomira Burchardt [1] and Harold G. Marshall[2], [1]Department of Hydrobiology, Adam Mickiewicz University, Poznan, Poland, [2]Dept. Biological Sciences, Old Dominion University, Norfolk, Va., 23529-0266. During the mid-1800's, L. Rabenhorst made extensive collections of algae throughout Europe, and one of his herbarium collections is located at Adam Mickiewicz University. From this collection samples from 47 dried cyanoprocaryota herbarium specimens were prepared for examined with scanning electron microscopy and 40 of these provided various levels of external morphological traits. Cell walls, filament details and dimensions, and remnants of gelatinous sheaths were observed, with sufficient phenotypic characteristics presented for many of these taxa to be used for comparative identification with currently used taxonomic keys. Species within *Scytonema, Cryptococcus, Nostoc, Gloeocapsa, Tolypothrix, Microcystis, Oscillatoria, Phormidium* are among those that provided vivid details. This study indicates SEM can be used in the examination of the external morphology in some of these dried algal specimens. Although this usage may be limited, it represents another approach that may be applied in the examination of many of these herbarium algal taxa, and have value when used for comparisons to similar species in current collections.

POPULATION GENETICS OF TWO VIRGINIA MALLOWS (*ILIAMNA COREI* AND *I. REMOTA)* USING ISSRS. Tracey A. B. Slotta and Duncan M. Porter, Biology Department, Virginia Tech, Blacksburg, VA. Two species in *Iliamna* Greene, *I. corei* and *I. remota*, have been questioned as to their designation as separate species. *Iliamna* remota, the Kankakee Mallow, is found in several disjunct populations in eastern Illinois, Indiana, and western Virginia. It is speculated that *I. remota* was introduced into Virginia during the early 1900s via human activity and railways. *Iliamna corei*, the Peters Mountain Mallow, is known from one population located in southwest Virginia on Peters Mountain. Currently, their populations do not overlap and their habitats differ greatly. Both *I. corei* and *I. remota* are classified as endangered at the state level and *I. corei* is federally listed as well. Conservation efforts are underway to preserve populations in both species. Inter-simple sequence repeats (ISSRs) were used to investigate genetic similarities of the two species. ISSRs have been shown to be informative in species and population studies and for estimating genetic diversity of rare and endangered plant species. Ten primers were used for 37 individuals representing *I. corei* and *I. remota*. For *I. remota*, four Virginia, one Indiana, and four Illinois populations were sampled. *Iliamna* corei representatives were obtained from the natural population on Peters Mountain and the research garden at Virginia Tech. The data were analyzed using cluster analysis and by UPGMA and neighbor-joining analysis. The results correlate with the geological distribution of the species and suggest multiple introduction events of *I. remota* to Virginia.

POLLINATION BIOLOGY OF THE GALÁPAGOS ENDEMIC *TOURNEFORTIA RUFO-SERICEA* HOOK. F. (BORAGINACEAE). Conley K. McMullen, Dept. of Biol., James Madison Univ., Harrisonburg, Va. 22807. *Tournefortia rufo-sericea* Hook. f. (Boraginaceae) is an endemic shrub that inhabits the moist uplands of several islands in the Galápagos archipelago. Research on its reproductive ecology, which included visitor observations, bagging experiments, and nectar studies, was conducted with an emphasis on elucidating the importance of diurnal versus nocturnal pollination. Ants were found to be the most common visitor to the small, white flowers of this species, and they were the only diurnal visitors. Ants, beetles, moths, and crickets made visits at night. Diurnal insect activity proved to be greater than nocturnal insect activity. However, the flowers of this species produce little pollen and virtually no nectar, and the insects do not appear to be important pollen vectors. Bagging experiments indicate that *T. rufo-sericea* is highly autogamous, and there is no significant difference in fruit set between flowers exposed to pollinators during the day and those exposed at night. These results support the hypothesis that the plant species able to establish themselves early in the history of the islands were those that possessed upon arrival, or subsequently evolved, the ability to self-pollinate. The initial absence of insect pollinators in the archipelago selected for this mode of reproduction.

A PRELIMINARY PHYLOGENETIC STUDY OF *ALTERNANTHERA FORSSK.* (AMARANTHACEAE) IN THE GALAPAGOS ISLANDS. Jennifer A. Clevinger, Conley K. McMullen, Curtis C. Clevinger & Steven M. Bernacki, Dept. of Biology, James Madison University, Harrisonburg, VA 22801. Fourteen species of *Alternanthera* Forssk. (Amaranthaceae) are known to inhabit the Galápagos Islands. Six species are endemic, five are natives and three are introduced weeds. The majority of these species are thought to have originated in the western (Andean) region of South America, and arrived in Galápagos via long-distance dispersal by birds. This study is using chloroplast and nuclear DNA markers to construct a phylogeny of the Galápagos members of *Alternanthera*. The phylogeny will be used to: 1) determine the number of colonization events needed to account for all members of the genus within the archipelago; 2) identify mainland sister group(s); 3) demonstrate the role of adaptive radiation in this process; and 4) prepare a taxonomic revision of Galápagos members of *Alternanthera*. Preliminary data from the internal transcribed spacer (ITS) region of the nuclear ribosomal repeat and the chloroplast non-coding regions between the *trnT* and *trnF* genes suggest that at least two colonization events must be hypothesized to account for the endemic species found on the Galápagos Islands.

WHAT DO GENES TELL US ABOUT EVOLUTION OF FLOWERING PLANTS. Khidir W. Hilu[1] and Thomas Borsch [2]. Department of Biology. [1]Virginia Polytechnic Institute and State University, Blacksburg, VA. [2]Botanisches Institute und Botanischer Garten, University of Bonn, Germany. Evolution and early divergence of flowering plants have been a disputable matter. Different hypotheses have been proposed on the identities of most basal lineages and the subsequent diversification. Recent molecular studies have resulted in radical changes of earlier concepts on angiosperm evolution. However, some inconsistencies exist among recent phylogenies, particularly for the basal lineages. Combining data sets HAS improved the picture particularly when large numbers of genes are included, but dominance of individual genes in a combined data set is a consideration. Our work in this area using the spacers and the trnL intron of the plastid region trnT-trnL HAS resolved a phylogeny congruent with that obtained from the 5-gene, 3-genome phylogeny. At the base of the phylogeny, Amborella represents the earliest diverging angiosperm, followed by Nymphaeales and an Austrobaileya-Illicium-Schisandra clade. Both eudicots and monocots are monophyletic. The dicot Ceratophyllum appears in close affinity with monocots. The congruence between phylogenies based on the combined data sets and the trnT-trnF-region implies that a fast evolving noncoding region can provide valuable historical information useful in deep level phylogenies.

A MODEL STUDY OF DISPARITIES IN GENE DIFFERENTIATION OF THE PLASTID AND NUCLEAR GENOMES OF THE PEANUT PLANT. Melillo, D. Monroe, and K.W. Hilu.Department of Biology. Virginia Tech, VA 24060. The cultivated peanut, *Arachis hypogaea*, belongs to *Arachis* section *Arachis* (Fabaceae, legumes). Within section *Arachis* there are about 29 species including the crop. Section *Arachis* consists of three different genomes (A, B and D), and two tetraploid species (*A. hypogaea* and *A. monticola*). The peanut crop is thought to be an allopolyploid derived from *A. monticola*, which most likely evolved from two of the wild species within section *Arachis*. The objective of this project is to examine the genetic relationships between the crop and species of section Arachis using sequences from the nuclear ITS and the plastid trnT-F regions. ITS show no variability among species. The trnA-trnB region of trnT-F show indels and nucleotide substations that resolved two clades demonstrating genetic relationships among genomes and wild and cultivated species. However, additional information from the trnC-trnF region is needed for better resolution.

THE NATION'S CHAMPION AMERICAN ELM IS SICK. R. Jay Stipes. Virginia Tech, Blacksburg, VA 24061-0331, Karen B. Stipes, Blacksburg High School, Blacksburg, VA 24060 and Chad Husby, Ohio State University, Columbus, OH 43210. The nation's largest American elm (*Ulmus americana* L.), located near Traverse City, MI, has contracted Dutch elm disease (DED) caused by the fungal pathogen, *Ophiostoma novo-ulmi*. (=*Ceratocystis ulmi*). The mammoth, multi-stemmed specimen estimated to be 300-400 yrs. Old exhibits a height of ca. 112ft (= ca 10 stories), a circumference of ca. 23.5 ft. and a branch spread of ca. 115 ft. We observed extensive and generalized symptoms in July, 2000, and the pathogen was successfully isolated from biopsied stem and foliar tissues on standard laboratory agar media. The tree is located in a very high disease hazard site, surrounded by hundreds of diseased and dying elms. Successful therapeutic intervention of DED with systemic fungitoxicants in advanced stages (more than ca. 20% crown symptom expression) of systemic infection is rarely successful. We deem the prognosis to be disappointing even if some disease remission results from massive fungicide administration. Such enormous botanical tragedies as this underscore the vital needs for public education and awareness, of scouting for diseased trees, of epidemiological data and overall and most of all for the *prevention* of disease.

CHARACTERIZATION OF MICROSATELLITE LOCI FROM FLOWERING DOGWOOD (*Cornus florida*). Liles, J. S. and P. R. Cabe, Washington and Lee University. We cloned, sequenced, and designed primers for nine unique dinucleotide microsatellite loci from the flowering dogwood (Cornus florida). Each primer set was individually optimized for annealing temperature and Mg++ concentration. Primers were tagged fluorescently, and PCR products from each primer pair were generated from eighteen individuals; fragments were run on ABI 377 automated sequencers for allele identification. Of the nine loci, one was monomorphic, and the remaining eight showed high levels of variation, with 7-13 alleles per locus and observed heterozygosities between 0.67 and 1.0. These markers will be ideal for studies of population structure and dispersal of pollen and seeds.

GROWTH, MORTALITY AND RECRUITMENT IN AN OAK FOREST IN SOUTHWESTERN VIRGINIA. Richard W. Rhoades, 611 Rose Ave., Blacksburg, VA 24060. Permanent plots in an Appalachian oak forest dominated by *Quercus prinus*, *Q. alba*, and *Acer rubrum* were sampled in 1994 and every 2 years until 2000 to determine growth of trees and saplings as well as changes in density of all classes of vegetation. Growth was determined by repeated dbh measurement. Mortality was determined by differences in density over the six-year period corrected for percent recruitment. A 56% increase in sapling density was significant, but a 6% increase in high seedlings and a 25% decrease in low seedlings were not significant. Radial growth varied from 3.7 mm/yr for Q. alba to 1.8 mm/yr for *Nyssa sylvatica*. Sapling growth was much lower, 0.6 – 1.6 mm/yr. Measures of growth fell within the range reported in other studies. There were significant differences among species of saplings, but not of trees. It is the conclusion of this study that the stand, a maturing, second-growth forest, is in a state of flux. Increases in saplings of white pine and red maple will probably result in dominance by these species with fewer oaks in about twenty years.

INTERACTIONS OF ALLELOPATHIC AND NUTRIENT STRESSES. Timothy D. Marshall & Mary E. Lehman, Dept. of Biol., Longwood College, Farmville, Va. 23909. In nature, plants seldom have ideal growing conditions; they often experience stress from a variety of sources. While most research focuses on these stresses individually, it is more realistic to look at the interaction of stresses. The interaction of two common stresses, allelopathy and nutrient stress, was examined in a nutrient culture system, using cucumber (*Cucumis sativus*) as a bioassay species and ferulic acid as a representative allelochemical. Exposure to a severe nutrient deficiency stress (1/64 X Hoagland's solution) reduced the ability of cucumber seedlings to recover from subsequent ferulic acid stress. When the treatment order was reversed, chronic exposure to low concentrations of ferulic acid (0.1, 0.2, 0.4 mM) had little influence on the effects of subsequent nutrient stress. The nutrient treatment was the major determinant of growth irrespective of previous allelochemical exposure. This study suggests that allelopathic and nutrient stresses may interact, with nutrient stress predominating.

PFIESTERIA TOXIN STABILITY AND RESTORATION OF TOXICITY FROM ALGAL-GROWN CULTURES. Brian Dyer, Andrew Gordon, Harold Marshall, and Robert Knight. Dept. of Biological Sciences, Old Dominion University, Norfolk, VA 23529-0266. The ichthyotoxicity of the dinoflagellate *Pfiesteria piscicida* has been successfully demonstrated and maintained in our laboratory using the standard tilapia bioassay. Numerous preliminary experiments have been performed and some of the results are given below. Our goal in this research is to isolate, purify and identify the *Pfiesteria* toxin(s). Using modified bioassay procedures we have maintained *P. piscicida* toxicity for more than 1 year. Toxicity was restored when an algal-grown culture (6 weeks) was re-exposed to fish. Fresh filtrates of toxic *Pfiesteria* aquarium waters were compared to control filtrates. While filtrates were toxic, they showed a significant reduction in toxicity to fish over a 48 hour period when compared to *Pfiesteria*-containing toxic waters. Frozen filtrates, up to one month, showed no appreciable reduction in toxicity when compared to fresh filtrates. Filtrate toxicity was retained after storage for 48 hours at room temperature. These results indicate that the component of toxicity observed in tilapia bioassay is associated with vegetative *Pfiesteria* cells rather than dissolved toxin only. Supported by Va. Dept. Health, Va. Dept. Environmental Quality and the Center for Disease and Prevention.

COMPARISON OF PHYTOPLANKTON SUCCESSION IN A FARM POND AND A STORM WATER RETENTION POND LOCATED IN A COMMERCIAL SHOPPING AREA. Bethany J. Gobeille, Geoffrey J. Wiedenmayer, & Stephen W. Fuller Department of Biology, Mary Washington College, Fredericksburg, Va. 22401. Growth and succession of phytoplankton are affected by a number of factors, including temperature and nutrient levels, as is described by the Plankton Ecology Group (PEG) model (Sommer, 1989). Two ponds in Fredericksburg, Virginia were the focus of this study, which took place from September 2000 to April 2001. Pond A is located at Hazelwild Farm in a rural area of Fredericksburg, while pond B is located in Central Park, a highly commercialized area. The environmental settings of pond B seemed to indicate a higher level of pollution exposure than pond A; however, no noticeable difference in the number or diversity of phytoplankton classes was noted. Chemical nutrient concentrations in pond A consistently had much higher levels of nitrogen and silica than did pond B, while phosphorus levels in both ponds were similar. Additionally, the phytoplankton succession in both ponds loosely correlated with the succession pattern predicted by the PEG model.

ANALYSIS OF NATURAL HYBRIDIZATION BETWEEN *RHODODENDRON PERICLYMENOIDES* AND *R. ATLANTICUM*: I. FLORAL CHARACTERS. Monica Harris and Bruce L. King, Dept. of Biol., Randolph-Macon College, Ashland, VA 23005. Ten floral characters and discriminant analysis were used to compare populations of *R. periclymenoides* and *R. atlanticum* and to examine putative hybrid populations. Most of the floral characters provided some discrimination of populations within species but the greatest discrimination was between species. Discriminant analysis provided no evidence of hybridization in one putative hybrid population. In another putative hybrid population the variation pattern shown by discriminant analysis was interpreted as evidence of bidirectional introgression supporting the conclusions drawn by King (2000) from micromolecular evidence and feeding patterns of a monophagous leaf beetle. One discriminant function discriminated among *R. periclymenoides*, *R. atlanticum*, and the hybrid population. The intermediacy of putative hybrids was demonstrated in the second discriminant function.

THE FLORA OF VIRGINIA PROJECT. J. Christopher Ludwig, Department of Conservation and Recreation Division of Natural Heritage, 217 Governor Street, 3rd floor, Richmond, Virginia 23219. The Flora of Virginia Project has been initiated to produce a manual to the 3700+ vascular plant taxa in Virginia. At the minimum, the manual will include keys, descriptions, habitat, range maps, and illustrations of the Virginia taxa. Additional information such as wetland status, chromosome numbers, heritage rarity ranks, photographs, and economic uses may be included as appendices, either within the manual or through electronic media (web, attached compact disc, etc.). The manual will provide a tool for plant identification and study for use by broadest of professional and avocational users from academia, government, industry and the public. It will assimilate and build on the rich tradition of botanical exploration of Virginia culminating in works such as the 1739 *Flora Virginica* by John Clayton. The latest genetic-based information on evolutionary relationships will be incorporated into the work along with the best traditional taxonomic approaches. The manual will increase interest in the appreciation and conservation of Virginia's diverse and unique botanical heritage. The completion is scheduled for December 2007.

Chemistry

REACTIONS BETWEEN CHLOROCARBONS, HYDROCARBONS, AND CuO, C.R. Vestal, H.M. Sturgill, & T.C. DeVore, Department of Chemistry, James Madison University, 22807. Flow kinetics were as used to investigate the reaction between 2,4-pentanedione, methanol, or tetrachloromethane and copper (II) oxide. The reaction with 2,4-pentanedione produced CO_2, Cu_2O, Cu, 2-propanone, ketene and traces of acetic acid at temperatures above 550 K. Water may also have been produced, but this product has not been identified with certainty. Once initiated, the reaction rate increased rapidly before decreasing as the CuO surface was depleted. This is consistent with a branching chain mechanism similar to the mechanisms often found in combustion processes. Methanol also reacted rapidly above 550 K to largely produce CO_2 and Cu. Tetrachloromethane did not react nearly as readily. Phosgene and $CuCl_x$ (x=1,2) were the main products produced during this reaction. The reaction kinetics have been determined for each of these reactions and a possible reaction mechanism has been developed for the CuO/ 2,4- pentanedione reaction.

MICELLAR CATALYSIS OF NITRIC OXIDE DISSOCIATION FROM ZWITTERIONIC DIAZENIUMDIOLATES. Stacy E. Price, Patricia Lorenzo & Keith M. Davies, Dept. of Chemistry, George Mason University, Fairfax, VA 22030. Diazeniumdiolates of the structure $R_1R_2N[N(O)NO]^-$, are of pharmacological interest since they spontaneously dissociate into nitric oxide (NO) in aqueous solution. We studied the dissociation rates of four zwitterionic diazeniumdiolates (R_1=R_2=$CH_2CH_2NH_3^+$), (R_1=R_2=$CH_2CH_2CH_2NH_3^+$), (a spermine derivative), and (R_1=$CH_2CH_2CH_2NH_3^+$, R_2=$CH_2CH_2CH_3$) in phosphate buffer solutions in the presence of SDS micelles. All reactions were SDS catalyzed and conform to a pseudo-phase kinetic model with the substrate partitioned between the aqueous and micellar phases. The magnitude of the binding constants shows good correlation with the number of quaternary nitrogen sites in the substrate, consistent with a coulombic interaction between the protonated amino groups of the diazeniumdiolate and the negatively-charge micellar surface. The rate constant for the micellar-bound substrate showed a five-fold increase in reaction rate over that in the aqueous phase. The rate enhancement is believed to be due to an elevated hydrogen-ion concentration in the Stern layer surrounding the micelle. Adding hydrophobic salts, $Et_4N^+Cl^-$, inhibits the catalysis by displacing H^+ from the Stern layer.

SYNTHESES OF HIGHLY FUNCTIONAL NORBORNENE DERIVATIVES AND SUBSEQUENT RING OPENING METATHESIS POLYMERIZATION USING RUTHENIUM CATALYSTS. James H. Wynne[1,2], Christopher T. Lloyd[1], Steven E. Bullock[2] & Robert F. Cozzens,[1,2] [1]Dept. of Chemistry, GMU and [2]Chemistry Division, Naval Research Laboratory, Code 6120, Washington, DC 20375. There is a recent demand for novel monomers to produce block polymers possessing electrostrictive or conductive properties. Due to the ring opening metathesis polymerization mechanism of which they undergo, norbornene and its subsequent derivatives are the substrates of choice. We report the synthesis of a series of highly functional norbornene monomers. Formation of the Diels-Alder adduct of cyclopentadiene and a variety of dienophiles afford novel intermediates possessing the norbornene substrate. Subsequent alterations to the previously synthesized adducts afford even more derivatives containing functionalities such as alkyl groups, bulky protecting groups as well as chelated metal atoms such as gold. The synthesis, characterization, function and design will be discussed.

ART AND CHEMISTRY: COMMON INTERSECTIONS. William H. K. Wightman,[1] & James P. Wightman,[2] [1]School of Art and Art History, James Madison University, Harrisonburg, Virginia 22807 and [2]Department of Chemistry, Virginia Polytechnic Institute & State University, Blacksburg, Virginia 24061. A number of demonstrations are used to illustrate principles discussed in general chemistry including the ignition of hydrogen balloons, precipitation reactions, universal indicators, and flame emission. These demonstrations add a sense of relevance and immediacy to the lecture. Presentation and discussion of the demonstrations is traditionally done not unexpectedly in terms of chemical concepts and vocabulary. However, from a visual arts perspective, chemical demonstrations can be rich in the vocabulary and processes of that field as well. This study examines the connectivity between chemical demonstrations and select elements/processes unique to the visual arts, proposes that an aesthetic sensibility surrounded and subsequently shaped the chemical demonstrations conducted by Michael Faraday, and establishes the pedagogical relationship between science and art instruction as guided by aesthetic considerations.

SYNTHESIS OF SIDEROPHORES: 4-HYDROXY-2ALKYLQUINOLINES. Sean R. Donohue & Wayne M. Stalick, Department of Chemistry, George Mason University, Fairfax, VA, 22030. A study of iron transport in P*seudomonas aeruginosa* led to the isolation of a class of iron chelators associated with the cytoplasmic membrane of iron rich cells. These siderophores were isolated in extremely small amounts, but spectroscopic identification suggested that the two most prevalent constituents were 4-hydroxy-2-nonylquinoline and 4-hydroxy-2-heptylquinoline. Synthesis of the postulated quinolines and comparison of the synthesized product to the biological extracts will provide confirmation of structure. The initial approach at synthesis involved blocking the hydroxy group followed by side chain elongation of commercially available 4-hydroxy-2-methylquinoline(**1**). Blocking the hydroxy group was a problem because the quinoline exists primarily as its keto tautomer and is unreactive. The second approach involved reacting (**1**) with two equivalents of base to form a dianion, which upon reaction with one equivalent of alkyl halide should permit elongation of the methyl substituent. A variety of changes in the base, solvents, and electrophiles used have, thus far, been unsuccessful in producing the product in high yields.

SYNTHESIS OF SIDEROPHORES: 8-HYDROXY-2-ALKYLQUINOLINES. Habib Ziayee & Wayne M. Stalick, Department of Chemistry, George Mason University, Fairfax, VA, 22030. In iron-rich environments an association between an iron chelator and the cytoplasmic membrane of *Pseudomonas aeruginosa* allows for the transportation and metabolism of iron. These siderophores were isolated in extremely small amounts and were thought to be 8-hydroxy-2-alkylquinolines. Siderophores are compounds that facilitate iron uptake by organisms and can be either membrane-bound or secreted. The 8-hydroxy-2-alkylquinolines are well known iron chelators that bind iron via the oxygen and nitrogen as a bidentate ligand. The initial approach at synthesis involved blocking the hydroxy group followed by side chain elongation of the commercially available 8-hydroxy-2-methylquinoline. Experimentally, blocking the 8-hydroxy group occurred through the reaction of the starting material with K_2CO_3 and CH_3I in acetone. This method provided relatively good yields. The second step involved reacting the 8-methoxy-2-alkylquinoline product with base to form an anion, which upon reaction with 1-iodohexane gave the corresponding carbon chain elongation. The final product has yet to be isolated, however, GC results show its presence, in the case of the 8-methoxy-2-heptylquinaldine.

THE SYNTHESIS OF ANALOGS OF 2-AMINOETHOXYDIPHENYL BORATE (APB) AS POTENTILA MODULATORS OF STORE-OPERATED CALCIUM CHANNELS (SOCC) IN HUMAN PLATEKETS. Sally Elliott & Roy L. Williams, Dept. of Chem./.Biochem., Old Dominion Univ., Norfolk, Va. 23529-0126. APB has been described in the literature as a membrane permeable agent capable of modulating calcium entry into human platelets and thus inhibiting platelet aggregation. Such compounds may be considered of value with regard to potential cardiovascular dysfunction. This laboratory has undertaken the synthesis of several novel analogs of APB, which will help define or characterize the possible APB receptor on the SOCC. These new compounds were also designed to test the potential of APB to exist in a five membered ring. Diphenyl boronic anhydride has been condensed with N.N-dimethyl ethanolamine and 2-amino-1-phenylethanol to give two new APB analogs, which have been characterized by infrared, and nuclear magnetic resonance spectroscopy (nmr). Specific nmr data of APB and the dimethyl analog would suggest that APB does indeed exist in a five membered ring form. Methylation of this dimethyl analog leads to a rapid degradation of this compound and the release of choline methiodide.

POTENTIAL ENTACTOGENIC ANALOGS OF INDOLE AMINES. Richard Seitz & Roy L. Williams, Dept. of Chem./Biochem.,Old Dominion Univ., Norfolk, Va. 23529-0126. The methylene dioxyphenethylamine known as Ecstasy has been characterized as an entactogen, or a drug that can apparently enhance one's empathy level but it has recently become an highly acceptable street drug. The mechanism of action of this unique drug appears to be associated with modulation of the serotonin neurochemistry in the CNS. Earlier studies from this laboratory described the synthesis of two new potential entactogens (AS1 and AS2) using methylene dioxyphenyl propanone and ammonia. These two compounds were found to exhibit a level of activity in mice which was interesting but difficult to describe or compare to known sedative/hypnotics or analgesics. We have now elected to synthesize serotonin or typtamine analogs in an effort to hopefully study the structure-activity-relationship (SAR) of this class of drugs. Methylene dioxyphenyl propanone was condensed with tryptamine in methanol at room temperature at a pH of 6 and the intermediate imine was then reduced with sodium cyanoborohydride in situ. The free base of this tryptamine analog of Ecstasy was isolated in 45 % yield and characterized with ir, nmr and elemental analysis. We have not carried out any biological evaluations of this compound to-date.

PHYTOESTROGENS IN CRANBERRY JUICE: POTENTIAL HEALTH BENEFITS. Laura Fauntleroy & Roy L. Williams, Dept. of Chem./Biochem., Old Dominion Univ., Norfolk, Va. 23529-0126. The consumption of cranberry juice as a means of treating or preventing urinary tract infections (UTIs) in women is a well established paradigm and is a widely accepted folk remedy for this particular dysfunction. The mechanism of action associated with cranberry juice consumption and UTIs has not been elucidated to date. Several possible mechanisms have been proposed but discarded over the years. We have recently detected the presence of several important phytestrogens in cranberry juice including the isoflavonoids genistein and daidzein. We have also detected relatively low levels of a unique stilbene compound known as trans-resveratrol (TR). Other researchers have shown that genistein can induce apoptosis, or scheduled cell death, in certain cancer cell lines. We have recently shown that TR is also capable of inducing apoptosis in human prostate cancer cells *in vitro*. This ability to induce apoptosis raises an interesting question with regard to a possible new mode of action of cranberry juice and the treatment and prevention of UTIs in women. We will describe our method of extraction and analysis of cranberry juice using high-pressure liquid chromatography (HPLC) and discuss the possible involvement of these phytoestrogens in combating UTIs.

PHTOCHEMISTRY OF BIOLOGICALLY ACTIVE CIS-TRANS STILBENES. Paul Kazas & Roy L. Williams. Dept. of Chem./Biochem., Old Dominion Univ., Norfolk, Va. 23529-0126. Over the past several years many researchers have investigated the biological properties of the stilbene based phytochemical known as trans-resveratrol (TR). This compound exhibits a remarkable profile of positive health effects as a potent antioxidant, an anti-cancer agent and an agent capable of inhibiting human platelet aggregation as well as inducing apoptosis in human cancer cells *in vitro*. TR is observed to undergo rapid but not complete photolytic isomerization to the less stable cis form. This cis isomer has also been shown to be quite active biologically and is found in a variety of plant extracts. We have studied this photolysis in an effort to obtain samples of the cis isomer for further biological testing. This paper will describe the photolysis of TR and several analogs of TR including the triacetyl and trimethoxy derivatives, which have been synthesized in this laboratory. Trimethoxy TR appears to undergo a rapid trans to cis conversion while the triacetyl derivative in inactive. We will also describe the photolysis of a tetra hydroxy analog of TR known as piceatannol. Other studies in this laboratory have shown that wine contains low levels of these stilbene moieties which is of interest to the current wine and health issue.

SYNTHESIS AND CHARACTERIZATION OF CONTROLLED SIZE ACCEPTOR POLYMER BLOCKS FOR POTENTIAL PHOTO-ELECTRONIC APPLICATIONS. Yiqing Wang,[1] Zhen Fan,[1] Charls Taft,[1], & Sam Sun,[1,2] [1]Center for Materials Research and [2]Department of Chemistry, Norfolk State University, Norfolk, VA 23504. The design, synthesis and characterization of an electron deficient acceptor conjugated polymer block are described. The conjugated acceptor copolymer block is a part of an novel nano phase separated D-B-A block copolymer system composed of conjugated donor block -nonconjugated bridge - conjugated acceptor block. This novel block copolymer system is expected to enhance the opto-electronic energy conversion efficiencies. Two key functional monomers: *[4-(Diethoxy-phosphorylmethyl)-5-(2-ethyl-hexane-1-sulfonyl)-2-(2-ethyl-hexyloxy)-benzyl]-phosphonic acid diethyl ester* and *[4-(Diethoxy-phosphorylmethyl)-5-(2-ethyl-hexane-1-sulfonyl)-2-(2-ethyl-hexyloxy)-benzyl]-phosphonic acid diethyl ester* have been synthesized and characterized. They are used in the ongoing work of building different sized acceptor copolymer block. The experimental procedure and characterization are described.

SYNTHESIS AND CHARACTERIZATION OF COMPLEXES OF Co(II), Ni(II), Cu(II) AND Zn(II) WITH AMIC ACID LIGANDS AS MOLECULAR MODELS FOR METAL-DOPED POLYIMIDES. Dennis Thekkudan, D.L. Polo, L.M. Vallarino & J.W. Williams (CHEM 406L), Department of Chemistry, Virginia Commonwealth University, Richmond, VA 23284-2006. This work is part of an ongoing class project that investigates the coordinating ability of the amic acid sites of polyimides through a study of the metal complexes of representative monomeric amic acid models. The ligands, N-(4-chlorophenyl)phthalamic acid (H-ClNPPA) and N-(3-methyl-phenyl)phthalamic acid (H-mMeNPPA), were synthesized by condensation of phthalic anhydride with the appropriate substituted aniline. They were then reacted with the metal acetates to yield complexes of the general formula $ML_2\times(\text{solvent})_n$, where n = 0.5 – 1.5 and the solvent is acetic acid, water and/or methanol. The complexes of Co(II), Ni(II) and Zn(II) had identical IR spectra and solubility patterns. On the basis of the d-d electronic spectra of the Co(II) and Ni(II) species, and of the ^{1}H NMR spectrum of the Zn(II) species, these complexes were assigned an octahedral coordination geometry with the amic acid anions acting as bidentate chelating ligands *via* the carboxylate groups. The less soluble Cu(II) complex, which had a somewhat different IR spectrum, was instead assigned a dimeric or polymeric structure with bridging carboxylates.

CHARACTERIZATION OF MOUSE GUANINE-7-METHYLTRANSFERASE MRNA. Keith E. Newbrough & Thomas O. Sitz, Dept. of Biochemistry, Virginia Tech, Blacksburg, VA 24061. The guanine-7-methylation of the cap structure in eucaryotic mRNA is essential for ribosome binding and translation. Recently the cDNA sequence for the human guanine-7-methyltransferase has been determined. The mRNA for this important enzyme is 6,203 nucleotides long. It has an unusually long 3'-untranslated region, 4,576 nucleotides in length. This mRNA codes for a protein 476 amino acids long. We wish to determine the length of the mRNA for this methyltransferase in mice by northern blot analysis. Initial experiments with a 40 nucleotide long DNA probe we unsuccessful. In addition, when random primer labeling of the complete human cDNA sequence was used as a probe of both human and mouse mRNA, no bands were observed. The truncated coding region of the human cDNA sequence that is highly conserved, was subcloned into a transcription vector, pSP73. This will be used to make an RNA probe to determine the size of the mouse methyltransferase mRNA. Genomic analysis of mouse ESTs have allowed us to determine the sequence and coding region of the mouse guanine-7-methyltransferase mRNA. It appears to be 2,035 nucleotides long with a 3'-untranslated region only 550 nucleotides long, substantially smaller then the human mRNA.

ACTIVE SITE LABELING OF GUANINE-7-METHYLTRANSFERASE. Amber R. Bonham & Thomas O. Sitz, Dept. of Biochemistry, Virginia Tech, Blacksburg, VA 24061. The guanine-7-methlytransferase (GMT) enzyme modifies the 5'-cap structure found in eucaryotic mRNAs. Without the methylation the mRNA does not function, i.e. the mRNA is not translated into protein. We have been able to label the active site of this enzyme by cross-linking ^{32}P-labeled RNA to the GMT with short wavelength UV light. We wanted to determine if non-capped RNA and a short single strand of DNA would also bind to the enzyme. We used a DNA oligo 18 deoxynucleotides long (18mer) and an RNA oligo 11 nucleotides in length (11mer). Both of these nucleic acids bound and were cross-linked to the GMT enzyme. We normally label the 3'-end of nucleic acids with the enzyme RNA ligase and ^{32}P-cytidine bis phosphate (*pCp). This enzyme would not transfer the *pCp to DNA but did label the RNA well. The 5'-ends of the 11mer RNA and the 18mer DNA were labeled with the enzyme polynucleotide kinase and g-^{32}P-ATP. To determine if the radioactive cross-linking was specific, the cap analog GpppG was added to reactions. The cap analog inhibited the binding of the capped 6mer RNA and 11mer RNA by about 50% and the binding of the 18mer DNA by about 30%. Therefore, this GMT enzyme does bind non-capped RNA and single stranded DNA.

LUMINESCENT pH SENSORS: FACTORS AFFECTING RESPONSE. Bernadette A. Higgins & Benjamin A. DeGraff, Dept. of Chem. MSC 7701, James Madison University, Harrisonburg, VA 22807. A new area within chemical sensor design is pH sensing because of the desire to measure pH remotely. A pH change can be detected by changes in the luminescence, which will depend on the molecule's protonation stste. In this research, a pH sensitive 1,10- phenanthroline derivative was synthesized and complexed with ruthenium (II) or rhenium (I) and other ligands to make various complexes. The pH sensitivity of the complexes was characterized using absorbance and emission spectra and lifetime measurements at various pH levels. The pH dependence of the complexes was modeled using a simple two species model for the equilibrium between the protonated and deprotonated state of the complex. The pH sensitivity was evaluated using this model to determine the protonation state at various pH levels. These complexes exhibit pH dependent photophysical properties including changes in pH can alter the emission brightness, color, and lifetime.

THE REACTIONS BETWEEN CCl_4 AND V_2O_X (X = 3, 4, AND 5). J. Sumner, D. Linnestaedt, & T.C. DeVore, Department of Chemistry MSC 7701, James Madison University, 22807. The kinetics of the reactions between tetrachloromethane (CCl_4) and the vanadium (III, IV, V) oxides at temperatures between 400 K and 900 K were investigated using a flow reactor coupled to a Fourier Transform Infrared Spectrometer. The principal gas phase products produced initially were phosgene and $VOCl_3$. X-ray diffraction of the solid residue from the reaction showed that reduced vanadium oxides and non-volatile vanadium oxychlorides were also produced during this reaction. All of the reactions had similar activation energies (~ 85 kJ/ mol) and steric factors (~$5 * 10^7$ min^{-1}), suggesting that they followed a similar mechanism. A mechanism similar to that presented by Klabunde (Environ. Sci. Technol. 1994, *28*, 1243) was developed. The enthalpies of reaction calculated for the steps in this mechanism indicate that the transfer of chlorine atoms is the rate-limiting step in this process.

APPLICATIONS OF FOURIER TRANSFORM-INFRARED (FT-IR) MICROSCOPY TO FORENSICS. Jenny M. Oran, Donna S. Amenta, & Thomas N. Gallaher, Department of Chemistry MSC 7701, James Madison University, Harrisonburg, VA 22807. Fourier Transform - Infrared (FT-IR) Microscopy is a useful tool in the forensic analysis of trace evidence (i.e. hairs, fibers, powders, etc.). It requires little sample preparation and causes minimal sample destruction. These aspects are important in forensic science especially when there is a limited amount of evidence collected from a crime scene. The purpose of this research is to demonstrate the power of FT-IR Microscopy and Attenuated Total Reflectance, in identifying powders and fibers that can be found at a crime scene.

A MOLYBDENUM COMPLEX CONTAINING A CROWN ETHER PROTOTYPE. Katherine E. Norton, Cristina Angelo, D. S. Amenta, J. W. Gilje, & A.D. Morton Department of Chemistry, James Madison University, MSC 7701 Harrisonburg, VA 22807. The purpose of this research is to synthesize, characterize, and study the reactivity of a transition metal complex whose ligand contains a crown ether. First, model compounds $Mo(CO)_3Cp(CH_2)_3C_6H_3$ (**1**) and $Mo(CO)_3Cp(CH_2)_3C_6H_3(OCH_3)_2$ (**2**) were synthesized and an x-ray crystal structure was obtained for **1**. Molecular modeling calculations were performed on **1** and found to be in good agreement with the x-ray data. Further calculations on **2** and a crown ether analogue **3** demonstrated conformations very similar to that of **1**. However, insertion of a sodium cation into the crown ether cavity resulted in a conformational change, which brought the sodium cation into proximity with a carbonyl group in **3**. Compound **2** was converted into an acyl complex by carbonyl insertion mediated by the addition of a phosphine ligand. Reaction rate studies of **2** with triphenylphosphine were monitored by ^{31}P and ^{1}H NMR. Work is progressing toward the preparation of **3**, where the influence of cation inclusion within the crown ether moiety on the rate of migratory insertion will be assessed.

Computer Science

(No Abstracts Submitted)

Education

(No Abstracts Submitted)

Environmental Science

SOLID PHASE MICROEXTRACTION OF PESTICIDES IN THE SOUTH FORK HOLSTON RIVER. L.J. Hainsworth, B.K. Patton & S. Turner, Emory & Henry College, Emory, VA. The South Fork Holston River is a relatively clean body of water. The watershed encompasses approximately 135,000 acres, most of which is woodland. Christmas tree farming has, over the past several years, become a major agricultural industry in the headwaters of the South Fork Holston. Since Christmas tree farming often involves significant pesticide use, farms have the potential to negatively impact aquatic ecosystems in the watershed into which they drain. This project was designed to evaluate the levels of several target pesticides in the river. Samples were collected at 2 stations along Whitetop Laurel Creek using solid-phase microextraction field samplers. The samplers were placed directly in the stream for 30 minutes, then analyzed by GC/MS (Shimadzu QP5000). The resulting mass spectra were identified using NIST AMDIS peak deconvolution and spectral identification software. No target compounds were discovered in any of the samples analyzed. The project will continue through May 2002.

PROPAGATION OF JUVENILE MUSSELS AT A NATIONAL FISH HATCHERY. A. K. Mummert, Newcomb T.J., & Neves R.J., Dept. of Fisheries and Wildlife Science & Cherry D. S., Dept. of Biology, Va. Polytechnic Inst. & S.U., Blacksburg, Va 24061. Captive propagation is often recommended as a recovery strategy for declining freshwater mussel populations. In partnership with U.S. Fish & Wildlife Service, attempts to establish suitable culture conditions at White Sulphur Springs N.F.H. are ongoing. Bioassays with juvenile mussels were undertaken to assess whether ammonia levels at the facility are suitable for juvenile survival. Additionally, bioassay results can aid in evaluating the extent to which environmental ammonia levels may contribute to mussel declines. In this study, juveniles of 2 species of freshwater mussels, *Lampsilis fasciola* and *Villosa iris*, were exposed to 5 concentrations of ammonium chloride over a 96 h test period, in static-renewal conditions. Trials were run at 12° C and 20° C, and mortality and water chemistry parameters were monitored at 24 h intervals. The 96 h LC_{50} values were calculated using the Trimmed Spearman Karber method. Substantial differences in sensitivity between the 2 temperatures were not observed for either species. However, differences in tolerance were exhibited by the 2 species, with *L. fasciola* being more tolerant of unionized ammonia than *V. iris* (mean 96 h LC_{50}'s of 0.24 and 0.11 mg/L NH_3-N, respectively). The LC_{50}'s of these 2 species are comparable to values reported for organisms typically used to set water quality standards. Based on documented levels of ammonia in the aquatic environment from anthropogenic sources, ammonia may limit freshwater mussel populations at affected sites.

OVULATION RATES IN COLORADO MULE DEER. S.D. Aksamit & P.F. Scanlon, Dept. Fisheries and Wildlife Sci., Virginia Tech., Blacksburg, VA 24061. Reproductive tracts were recovered from female mule deer (*Odocoileus hemionus*) following hunts at the U.S. Air Force Academy, Colorado Springs, CO which were held during the months of October, November and December. Ovulation was judged by the presence of corpora lutea in the ovaries. Numbers and sites of ovulations were recorded and ages of individual does were determined from tooth characteristics. No does ovulated prior to November and only 3 does killed in November had ovulated. Numbers of ovulations ranged from 1 to 4; the modal value was 2. In aggregate, 52.2% of ovulations were recorded in right ovaries. No fawns had ovulated. Ovulation rates tended to increase with age. Mean numbers of ovulations by age group were as follows: 1.4 for 1.5 y.o.does (n=5);1.9 for 2.5 y.o. does (n=18); 1.7 for 4.5 y.o. does (n=3);2.0 for 5.5 y.o. does (n= 12); and 2.0 for 6.5 y.o. does (n=14).

REPRODUCTIVE ORGAN WEIGHTS AND SPERMATOZOAN NUMBERS IN COLORADO MULE DEER. R. M. Brooks, Jr. & P. F. Scanlon. Dept. Fisheries and Wildlife Sci., Virginia Tech., Blacksburg, VA 24061. Reproductive organs of male mule deer, *Odocoileus hemionus*, recovered following hunts at the U.S. Air Force Academy, Colorado Springs, CO, were dissected. Weights of testes and epididymides were taken and spermatozoan numbers in testes and epididymides were determined. Data were related to month of death: deer were obtained in October, November, and December. Testes and epididymides weights increased with age through all age-groups, fawn to 3.5+y.o. Testes weights peaked in October and were declining in December. Epididymal weights were at a peak in December. Testicular spermatozoan numbers were at a peak in October and declined thereafter. Epididymal spermatozoan numbers were at a peak in November and declined dramatically in December. Changes in organ weights and in spermatozoan numbers are consistent with a build-up of spermatogenesis in preparation for a well-synchronized breeding season in late November and use of spermatozoa at that time. [Project supported by Minority Academic Opportunities Program, Virginia Tech]

STRENGTH MEASUREMENTS IN MULE DEER ANTLER TINES IN RELATION TO FLUORIDE CONTENT. T.E. Doggett, P.F. Scanlon, & J.H. Wilson. Depts. Fisheries and Wildlife Sciences and Biological Systems Engineering, Virginia Tech, Blacksburg VA 24061. Antlers of mule deer at the US Air Force Academy, Colorado Springs, CO were observed to have a high rate of breakages and they were found to have high concentrations of fluorides in teeth, antlers, and bones. Antlers were recovered from Colorado mule deer following hunting activities. Antler tines were designated Tine 1 through Tine 5 beginning with the brow tine (Tine1) and proceeding outward on the antler. Segments 18.8mm in width were sawn, using a saw with 2 blades in parallel, from all tines except brow tines. Antler segments were subjected to compression tests to failure using a SINTECH Universal MTS machine and the following measurements were generated: peak stress, energy to peak load, and modulus of elasticity. Fluoride concentrations in antler tines were determined using an ORION ion selective electrode. Clear relationships between strength measurements and fluoride concentrations were difficult to establish. However, Tine-5 was more resistant to compression than other tines probably due to a higher modulus of elasticity, which may be due to later growth of this tine. [Project supported by Minority Academic Opportunities Program, Virginia Tech]

PATTERNS IN DEER-VEHICLE COLLISIONS IN TWO VIRGINIA URBAN AREAS. C.R. Faustino & P.F. Scanlon, Dept. Fisheries and Wildlife Sci., Virginia Tech., Blacksburg, VA 24061. Records of deer-vehicle collisions in Lynchburg, VA and Blacksburg, VA were examined to determine patterns useful in management of deer in relation to urban and suburban traffic. Data were available for the intervals 1987 to 1998 for Lynchburg and for 1990 to1998 for Blacksburg. Total numbers of deer-vehicle collisions increased annually in Lynchburg until a management program was instituted in 1993 and the declined steadily thereafter. Deer-vehicle collisions continued to increase in Blacksburg until 1997. While the trends in deer-vehicle collisions were increasing such accidents represented an increased percentage of all accidents. In both locations frequency of deer-vehicle collisions was worst in November and during the late evenings and before midnight. Deer-vehicle collisions occurred most frequently when people were commuting home from work under conditions of darkness. Reductions in accidents resulted when the Lynchburg deer management program was implemented. A major portion of that program consisted of deer removal. Educational programs informing commuters of when the risks of deer-vehicle collisions are greatest together with information on deer-collision avoidance strategies could prove beneficial as a component of a comprehensive urban deer management program. [Project supported by Minority Academic Opportunities Program, Virginia Tech]

DIFFERENCES IN GASTRO-INTESTINAL ANATOMY AMONG SMALL ANIMAL SPECIES. C.R. Faustino & P.F. Scanlon, Dept. Fisheries and Wildlife Sci., Virginia Tech., Blacksburg, VA 24061. Gastro-intestinal (GI) tracts were examined in 12 species to compare dimensions of GI tract components and to relate tract dimensions to food habits. The most conspicuous difference among species was in the presence or absence of the cecum and in the relative (i.e. % of GI tract length) size of the cecum. In general, GI tracts were shorter in carnivorous species (*Canis latrans*, *Vulpes fulva*, and *Blarina brevicauda*) and the cecum was small or absent. The GI tract of and omnivore, *Didelphis virginiana*, was relatively short and the cecum was 4.1% of the GI tract length. The relative length of the cecum (i.e. % of GI tract length) in 3 squirrel species (*Tamias striatus*, *Sciurus carolinensus*, and *Glaucomys volans*) ranged from 3.2 to 5.1%. In the seed eating species, *Peromyscus leucopus*, relative cecum length was 6.4%. In 3 herbivorous species (*Microtus pennsylvaticus*, *M. pinetorum*, and *Zapus hudsonius*) relative cecum length ranged from 12 to 16%. Herbivorous species had the longest GI tracts. [Supported by Pratt Animal Nutrition Foundation].

STRENGTH MEASUREMENTS OF MULE DEER BONES IN RELATION TO FLUORIDE CONCENTRATIONS. C. R. Hutchison, P. F. Scanlon, J. H. Wilson & L. M. Borrero-Yu. Depts. Fisheries and Wildlife Sci., and Biological Systems Engineering, Virginia Tech, Blacksburg VA 24061. A problem of frequently broken antlers in the mule deer population at the U.S. Air Force Academy, Colorado Springs, CO was associated with elevated fluoride concentrations in hard tissues (teeth, bones and antlers). This report concerns an attempt to relate measures of bone strengths to fluoride concentrations. Intact cannon (i.e. fused metacarpals) bones of mule deer were dissected free of tissue and subjected to three-point bending tests on an MTS Syntech instrument which yielded 3 data outputs (stress, energy and modulus of elasticity). These outputs were related to age and sex of deer and to fluoride contents of bones. There were no clear relationship between bone strength measurements and fluoride concentrations. Bones of males absorbed significantly more energy prior to breaking than did those of females. They were also bigger. There were no clear relationship between bone strength measurements and age. Female values for modulus of elasticity were significantly higher than those for males indicating that females had stiffer, more brittle bones.[Supported by Pratt Animal Nutrition Foundation].

MAMMAL COLLISIONS WITH U.S. AIR FORCE AIRCRAFT, 1987-1996. C. M. Lensch, C. A. Tedrow, & P. F. Scanlon. Dept. Fisheries and Wildlife Sci., Virginia Tech., Blacksburg, VA 24061. Problems with aircraft and birds are well known but mammals also adversely impact aircraft operations. The record system for bird-aircraft strikes also records incidents involving mammals. The records for the period 1987 to 1996 were examined for mammal-related incidents. Data were examined by species involved and by time and place of incidents. Bats were involved in the majority of incidents. Fifteen species of bats were identified in mammal-aircraft strikes. A majority of bats were not identified. Bats were involved in strikes in nine countries in addition to the United States. Other species involved in mamma-aircraft collisions included: two species of deer (white-tailed and black-tailed), coyotes, dogs, a cat, chipmunks, jackrabbits, and unspecified rabbits. Practically all collisions occurred at night or at dawn or dusk. Bat species present difficult management problems as they are nocturnal and insectivorous. Large mammal species, such as deer can be excluded from airfield areas.

STRENGTH MEASUREMENTS IN WING BONES OF NORTHERN BOBWHITES IN RELATION TO FLUORIDE CONTENT. C.D. Ponds, P.F. Scanlon, & J.H. Wilson. Depts. Fisheries and Wildlife Sciences, and Biological Systems Engineering, Virginia Tech, Blacksburg VA 24061. Relationships of fluoride concentration in bones of Northern Bobwhites, *Colinus virginianus*, to measures of bone strength were determined in housed and wild Bobwhites. Wings of Bobwhites were obtained from hunters and radii and ulnae were dissected free. From a sample of housed Bobwhites radii, ulnae, and tibiae were dissected. Bones were tested using a Sintech Universal MTS in 3 point bending tests to failure to determine peak stress and peak energy to stress. Fluoride concentrations were determined in radii using an ion selective electrode (ORION). Fluoride concentrations in radii were twice as high in housed bobwhites as in comparably aged wild bobwhites. Energy absorbed prior to breakage by radii was higher in housed bobwhites though the stress (which depends in part on bone cross-sectional area) was comparable in both groups. Access to fluoridated water in captivity seemed to increase fluoride content of bones while influencing select bone strength characteristics. [Project supported by Minority Academic Opportunities Program, Virginia Tech]

CARUNCLE NUMBERS IN UTERI OF RED DEER (*Cervus elaphus*). P. A. Strickland, P. F. Scanlon, & R. Kelly. Dept. Fisheries and Wildlife Sci., Virginia Tech., Blacksburg, VA 24061. The placenta in deer species is classified as "cotyledonary". This type of placenta is common to ruminants. Cotyledonary placentas are characterized as having specific attachment sites "caruncles" within the uterus. Numbers of caruncles vary considerably among ruminants and in several species not all caruncles are attached during pregnancy. Reproductive organs of red deer from New Zealand farms and from Norwegian hunting grounds were dissected and numbers of caruncles were determined. Numbers of caruncles ranged from 4 to 12 with a modal value of 8. The overall mean was 8.2. Caruncles were equally distributed among the uterine horns. Red deer have more caruncles than white-tailed or mule deer, less than moose, and considerably less than cattle, sheep, or goats. [Project supported by Minority Academic Opportunities Program, Virginia Tech]

FOOD PASSAGE RATES IN THREE BIRD SPECIES. R.E. Stultz, R.B. Hiller & P.F. Scanlon. Dept. Fisheries & Wildlife Sci. Virginia Tech., Blacksburg, VA 24061. Passage rates of food in wild birds relate to the potential for spread of viable biological entities through feces. Feeding chromic oxide and its recovery in feces allows estimation of passage rates. Rates of passage of food through the gastro-intestinal tracts of 3 bird species were studied. Five individuals of each of the following species were used: Canada geese, *Branta canadensis*; Japanese quail, *Coturnix coturnix japonica*; and Northern bobwhite, *Colinus virginianus*. All were housed individually and were fed *ad libitum* after a capsule with a known amount of chromic oxide was force-fed to each. Feces were recovered hourly over 24 hours. Chromic oxide was measured in individual feces samples and food passage rates determined. In Canada Geese initial chromic oxide recovery was at 2 hours post consumption, peaked at 4-5 hours, and declined through 10 hours to negligible amounts. In Japanese quail initial chromic oxide recovery was at 1 hour, peaked at 2-3 hours, and declined to negligible amounts beyond 8 hours. In Northern bobwhites initial recovery was at 1 hour, peak recovery was at 3-4 hours, and recovery declined to negligible amounts after 9 hours. Food moves rapidly (12-24 hours) through all 3 species. Considerable potential exists for Canada geese to transport botanical entities by geese given their mobility.

STRENGTH MEASUREMENTS OF RIBS OF MULE DEER. J. H. Wilson, P.F. Scanlon, T.W. Pettit, L. M. Borrero-Yu, & E. J. Wilson. Depts. Fisheries and Wildlife Sci., and Biological Systems Engineering, Virginia Tech., Blacksburg, VA 24061. Mule deer at the USAF Academy base, Colorado Springs, CO, had a high frequency of antler breakages and high concentrations of fluoride were noted in hard tissues (bones, teeth, and antlers) of deer at the base. Antlers are difficult to procure and bones were considered as substitutes for study. Bones (fifth ribs) from hunter-harvested deer were removed intact and had strength measurements done using an MTS Syntech 5/G in a 3-point test configuration. Fluoride concentrations were measured. The strength test was a three-point bending test to failure on a mid-point of intact fifth ribs. Data on strength measurements of ribs of 229 deer were obtained and related to bone fluoride concentrations. Data on strength measurements are presented in relation to fluoride concentration, and sex and age of deer. Fluoride concentrations were higher in females of comparable age groups and also increased with age in both sexes. Shear force was higher in males but was influenced by larger rib size. Shear force increased to age 3.5 years but declined thereafter. Sex and age had interactive effects on the 'strength' measurements as these related to fluoride concentrations.

INFLUENCE OF HYDROPEAKING ON THE ABUNDANCE AND DISTRIBUTION OF INVERTEBRATES IN THE SMITH RIVER, VIRGINIA. Katherine M. Hanna, Tammy J. Newcomb & Marcy R. Anderson. Dept. of Fish. & Wildlife Sciences, Virginia Polytechnic Inst. & State Univ., Blacksburg, VA 24061-0321. Benthic macroinvertebrates were sampled at 12 fish sampling stations in the tailwater of Philpott Dam in July 2000 and April 2001. A riffle within each site was stratified into top, middle, and bottom sections and surber samples were collected at two randomly selected locations within each section. Samples were preserved in 70% ethanol and returned to the lab for identification to family and measures of wet weight. Species richness, ANOVA, and linear regression were used to evaluate longitudinal trends with increasing distance from the dam and to determine significant differences between sites and years. Richness was low near the dam but increased at site 4.2 km and remained high downstream. Both wet weight and abundance were significantly greater in April than July and Ephemerellidae dominated the samples in April. Abundance of aquatic invertebrates in this tailwater was similar to abundances found in Appalachian streams where trout growth was limited. All sites, with the exception of sites 4, 8, and 12 in April, had lower densities than what is commonly found in trout streams in Virginia.

Geography

(No Abstracts Submitted)

Geology

STUDY OF THE LITHOLOGY AND SOURCE OF THE STONE WALL ALONG SUNKEN ROAD AT FREDERICKSBURG, VIRGINIA. Stephen P. Flora, W.C. Sherwood, Department of Geology & Environmental Science. The stone wall along Sunken Road was an important feature at the Battle of Fredericksburg, during the Civil War and is now a landmark of that site. The dominant rock type making up the stone wall is a coarse sandstone from the Aquia Formation. The Aquia sandstone consists of coarse quartz sand of various colors with a feldspar matrix. A few large quartz pebbles up to 2 inches in diameter are included. Other rocks found in the wall are composed of various igneous and metamorphic lithologies. The percentages of each rock type in the wall were determined by conducting linear traverses at set intervals along the wall. In addition to lithologic identifications, evidence of mechanical quarrying was noted on a small number of the rocks indicating that some of the rocks were mined from quarries in the area. Several abandoned quarries in the Aquia were identified within two miles of the stone wall. Wadel Roundness Values were used to rate the degree of roundness of the rocks in the wall and it was determined that the majority of the rocks in the wall are subrounded to subangular blocky in shape. In addition to shape, sizes of the rocks were also measured. The lithology, roundness, and size of the rocks in the stone wall are being statistically analyzed and the results used to determine if other rocks may have been brought in to rebuild sections of the wall at a later time.

DETERMINING THE ORIGIN OF SULFATE IN SELECTED WATERS OF SHENANDOAH VALLEY, VIRGINIA. Robert H. Greenlaw, S.J. Baedke, Department of Geology & Environmental Science. Sulfate is an ubiquitous constituent in most reservoirs of water on Earth. However, in the Shenandoah Valley waters with significant concentrations of sulfate are seemingly rare. Statistical analyses of previously published data indicate that a few springs and wells in the Valley have significantly higher concentrations of sulfate. In order to determine the origin of sulfate in these waters, samples were collected and analyzed for predominant chemical character and isotopes of sulfur. Sulfur isotopes can be used to discriminate between natural (e.g. pyrite, limestone, gypsum, organic matter) and anthropogenic sources of sulfur. The results of this study will provide previously unknown data about the origin of sulfate in waters of the Valley and how it relates to Valley geology and/or identify pollutants if present.

SOIL PEDOGENESIS OF DEBRIS FANS, GRAVES MILL, VA. Brian H. Neely, L. Scott Eaton, Department of Geology & Environmental Science. Debris-flow fan deposits in the upper Rapidan River Basin record a long and complex history of activity. During a storm on June 27, 1995, 30.5 inches of rain in 16 hours initiated approximately 1000 slope failures, and incised stream channels and debris fans. The Generals debris fan complex, located 1 km west of Graves Mill in the Blue Ridge physiographic province, was partially impacted by the deluge, and the scouring from the floodwaters exposed prehistoric fluvial and mass movement deposits. This event has allowed the study of soil pedogenesis and geomorphic activity of prehistoric debris flow fans. Cosmogenic 10Be dating and soil chronosequence studies reveal a minimum of five distinct ages of debris fans, spanning from approximately 500,000 YBP to present. The oldest debris fan exhibits two distinct debris flows. The upper unit has a 1.0 m argillic horizon, a 2.5 YR Munsell color, and a clay content of 72%. The lower unit has an argillic horizon that exceeds 0.8 m, a 10R Munsell color, and a clay content of 40%. In contrast, the youngest fan surface lacks significant soil pedogenesis, shows Munsell colors of 10YR, and a clay content of only 3%. XRD analyses of the soil profiles reveal a range of clay mineralogy that includes Kaolinite, Illite, Chlorite, and Vermiculite. Additional research of the debris fan should help further elucidate the landscape evolution of this region.

THE KARST DEVELOPMENT OF THE UNION AND HURRICANE RIDGE CAVE SYSTEMS, DICKSON SPRING DRAINAGE BASIN, MONROE COUNTY, WEST VIRGINIA. Christopher M. Printz, L. Scott Eaton, Department of Geology & Environmental Science. The Dickson Spring drainage basin of northern Monroe County, West Virginia, is a well-developed karst basin that encompasses an area of approximately 25 mi2. Until recently, little has been known about subsurface flow routes of waters within the Dickson drainage basin, other than their eventual emergence at the Dickson Spring. However, two recent cave discoveries have revealed significant portions of this complex karst drainage system. These are the Hurricane Ridge and Union Cave systems, which currently comprise more than five miles of known cave passage, and new passages are progressively being surveyed and explored. Union Cave has been positively dye-traced to the Dickson Spring, 6.5 miles to the north. Mapping of cave passages within Union cave reveals that many of the active tributaries, as well as abandoned paleo-passages, flow (or once flowed) southward, whereas the active main river passage flows almost due north to the Dickson Spring. These two cave systems lie near the southern boundary of the Dickson drainage basin, south of which waters drain southward to Indian Creek. The research assesses the possible roles of headward erosion and stream piracy of the Dickson drainage basin in capturing waters that once drained to the south. The study also examines the evolution of the Hurricane Ridge and Union Cave systems, with emphasis on their hydrology, and structural and lithological controls on passage development and passage orientation. This aspect of the study is accomplished through the ongoing surveying and mapping of cave passages and subsurface geology. The results of this study should help elucidate the subsurface hydrology of this region.

A GIS CONSTRUCTION OF A GEOLOGIC MAP OF THE GEORGE WASHINGTON NATIONAL FOREST. Mark Villa, W.C. Sherwood, Department of Geology & Environmental Science. The purpose of this project was to compile a geologic map of the George Washington National Forest in a GIS environment. Quadrangle maps of 1:100,000 scale and state geologic maps of 1:250,000 scale were digitized in Abicas, a GIS program for personal computers by Innovative Technologies of America, Inc.. Lithological formations were attributed to areas created using the digitized maps. Area files were converted to shape files for use in ArcExplorer and ArcInfo. There was extensive edge matching required at this stage to form a complete map of the National Forest from individual shape files of quadrangles. At the same time that the bedrock geology was being compiled in the GIS format, a database containing acid resistivity linked to lithology was created in spreadsheet form. This data can be imported into ArcInfo and added as an attribute to formations. The GIS environment offers the ability to attach numerous other attributes to formations and overlay multiple layers containing any mappable features.

USE OF ELECTRICAL RESISTIVITY TO DISTINGUISH EARTH MATERIALS IN THE APPALACHIANS WITH SPECIAL APPLICATION TO DEBRIS FLOW DEPOSITS, GRAVES MILL, VIRGINIA. Erin F. Sutton & C.F. Watts, Dept. of Geol., Radford Univ., Radford, VA 24142. Mapping and characterizing debris flows are important to obtain information useful in identifying locations and conditions prone to future debris flow events and identifying and characterizing actual debris flow deposit material. Two-dimensional electrical resistivity profiling was completed at 16 sites in Virginia and Northeast Tennessee to determine if this geophysical technique was useful in identifying debris flow deposits from six different geological substrata. The resistivity of debris flow material was found to be dependent upon the clay content and the void ratio; "young" debris flows with low clay contents and high void ratios have average resistivities exceeding 1,250 Wm. Residual soils developed in the period of time between flow events. Therefore in areas of accumulation, debris flows are commonly seen as layers of high resistivity above soils of low resistivity. The wenner array produced a more accurate model of the subsurface than the dipole-dipole array. This was due to the extremely high lateral and vertical variability of subsurface conditions associated with layering and boulders.

PETROLOGY, MACRO- AND MICRO-STRUCTURES, AND SCANNING ELECTRON MICROSCOPY OF THE DEVONIAN MILLBORO AND NEEDMORE SHALES, HIGHLAND COUNTY, VIRGINIA. L. L. Combs & P. S. Sethi, Department of Geology, Radford University, Radford, VA 24142-6939. The Devonian Millboro and Needmore Shales outcrop in Highland County, Virginia, along US Route 250 on Bullpasture Mountain. This project seeks to characterize the petrology, macro- and micro-structures, and SEM (Scanning Electron Microscopy) fabric of the two shale units. Methods of analysis include the determination of relative indices of bioturbation in hand sample, thin section analysis and microphotography, and SEM. Both field and lab results indicate that the Millboro Shale is significantly more laminated and pyritiferous than the bioturbated Needmore Shale. Interestingly SEM studies of the relatively more bioturbated Needmore Shale do, however, reveal presence of clearly visible, micron-scale framboids of pyrite disseminated through the rock. Additional data being collected will serve to explore cause-and-effect relationships between the mineralogy and petrology of such shales and their environmental properties/behavior, specifically – potential for acid drainage and problems related with slope instability.

A MULTIMEDIA, INTERACTIVE CD-ROM FOR TEACHING/LEARNING GEOLOGY OF THE VALLEY AND RIDGE AND THE APPALACHIAN PLATEAUS PROVINCES OF VIRGINIA. P. S. Sethi[1], R. C. Whisonant[1], K. K. Cecil[2], P. L. Newbill[1] & L. L. Combs[1], [1]Department of Geology, Radford University, Radford, VA 24142-6939 and [2]New River Community College and Radford High School, Radford, VA 24141. This paper presents an interactive, multimedia CD-ROM focusing on the geology of the Valley and Ridge and the Appalachian Plateaus physiographic provinces of Virginia. Latest models pertaining to learning psychology and educational theory were merged with modern multimedia instructional technology tools for creation of this unique teaching/learning resource. The two CD-ROM set includes coverage of the topics of physiography, geology, economic resources, environmental issues and connections between geology and human history of the two provinces. The CD-ROM contains state-of-the-art slide shows, video clips, animations, narratives and user-friendly text screens with a intuitive navigational scheme and is designed for the ninth grade Earth Science students and interested laypeople. A detailed Teacher's Guide (including worksheets for students) accompanies the CD-ROM and discusses coverage of the Earth Science SOLs. For ordering please contact the Virginia Division of Mineral Resources at (804) 951-6340.

DENUDATION FROM CATASTROPHIC FLOODING IN NORTHERN VENEZUELA, DECEMBER, 1999, L. Scott Eaton, Dept. of Geology and Environmental Science, James Madison University. Landslides and catastrophic flooding occurred in the Coast Range of northern Venezuela in December 1999. The state of Vargas received 300 mm of rainfall during the first two weeks of December, followed by an additional 900 mm during December 14 to 16. This deluge triggered thousands of landslides, including debris flows, and caused flash flooding in communities along a 40 km coastal zone north of Caracas. Estimates of fatalities range from 10,000 to as high as 50,000. In the disaster zone, the mountain range rises to 2,600 m within 6 km of the coast, producing steep slopes commonly in excess of 45°. Most of the initial landslides occurred in thin soils overlying weathered schist and gneiss bedrock and developed into debris flows as they moved downslope into secondary drainages. A survey of 10 impacted watersheds shows that the spatial distribution of flood and debris-flow deposits is highly variable. Although most watersheds exhibited evidence of both processes, some experienced either debris flows or water floods. Historically, the region experienced major flooding and landsliding events, though of lesser magnitudes in 1936 and 1951. Legends originating from indigenous inhabitants tell of the mountain range periodically "spitting out" rocks onto the fans. Stratigraphic studies of deposits that predate the 1999 event suggest that flooding events of equal or greater magnitude than the 1999 flood have occurred in the last 500 to 1000 years. Soils of some prehistoric debris-flow deposits are probably no older than Holocene as they show a near absence of pedogenesis, including minimal rubification, clay coatings of peds, and clast weathering. The debris fans on the north coast of Venezuela will almost certainly continue to be sites of catastrophic flooding and landsliding.

Materials Science

NANO-PHASE OXIDES FORMED ON HPS STEELS. R Balasubramanian[1], D C Cook[2], [1]Department of Physics, James Madison University, Harrisonburg, VA 22807, USA, [2]Department of Physics, Old Dominion University, Norfolk, VA 23529, USA. Nanophased oxides found in the corrosion coatings of atmospherically weathered steels have properties that are scientifically significant and industrially important. Samples of high strength steels of varying composition were exposed in Campeche, along the Gulf of Mexico for up to one year and the development of corrosion products as a function of steel type and exposure time were studied using Mössbauer Spectroscopy and X-ray diffraction. Both X-ray diffraction and transmission Mössbauer spectroscopic (TMS) results indicated that lepidocrocite, maghemite and goethite were the dominant oxides. TMS analysis at 77K indicated that for up to three months of exposure, lepidocrocite and maghemite accounted for nearly 80% of the relative amount, with goethite contributing only 20% to the mixture. However, as the exposure time increased to 6 months, the relative contribution of goethite increased at the expense of decreasing amounts of maghemite. Monitoring the environment during the exposure time indicated that the average time of wetness decreased. The decrease in the relative contribution of maghemite to the total oxide concentration is related to the decreasing time of wetness, with increasing exposure time. Nearly 20% of the goethite was nanophase exhibiting the superparamagnetic behavior.

A GAS FLOW PROPORTIONAL COUNTER FOR SCATTERING GEOMETRY MOSSBAUER STUDIES OF TIN. L. E. Brown, C. D. Robinson, W. C. McDermott, Department of Physics and Astronomy, Hampden-Sydney College, Hampden-Sydney, Virginia, 23943, USA. A gas flow proportional was constructed and tested for use in nondestructive testing of a banjo tone ring that is made of a tin alloy. The goal was to produce a counter out of readily available material that could be used in back scattering geometry Mossbauer experiments. The detector was constructed using a square body design which should produce a uniform gain over the detector except at the corners where the electric field is non-uniform. The construction criteria, design, and gain uniformity measures are presented and the data show that this detector operates according to the design specifications.

OPTICAL SPECTROSCOPY OF EUROPIUM DOPED GALLIUM NITRIDE PREPARED BY SOLID SOURCE MOLECULAR BEAM EPITAXY. Ei Ei Nyein[1], U. Hommerich[1], J. T. Seo[1], J. Heikenfeld[2], & A. J. Steckl[2], [1]Research Center for Optical Physics, Hampton University, VA 23668 and [2]Nanoelectronics Laboratory, University of Cincinnati, Ohio 45221-0030. Thin Film Electroluminescent (TFEL) Displays are all solid-state devices and offer several advantages over well known LCD's including increased brightness and viewing angle. We are currently investigating Eu doped GaN as a potential red phosphor for TFEL display applications. Eu doped GaN films were grown by solid source molecular beam epitaxy on Si (111) substrates. The material was optically characterized through temperature dependent emission spectroscopy using an Argon laser at 336-363 nm for above band gap excitation. A strong red emission was obtained at ~622 nm, which corresponds to a Eu^{3+} inner 4f-shell transition from the $^5D_0$ to $^7F_2$ state. A temperature dependent study of the red Eu^{3+} line showed that the integrated emission intensity decreased by a factor of 13 between 13 K and 300 K. On the contrary, the emission lifetime changed only slightly (~10-20%) for the same temperature range suggesting that non-radiative decay processes are small. Therefore, the observed thermal quenching of red Eu emission is assigned to a strongly temperature dependent pumping process.

RECENT RESULTS ON THE OPTICAL PROPERTIES OF CR DOPED II-VI MATERIALS. A. G. Bluiett[1], U. Hommerich[1], J. T. Seo[1], R. Shah[1], S.B. Trivedi[2], S.W. Kutcher[2], R.J. Chen[2], C.C. Wang[2], & H. Zong[2], [1]Research Center for Optical Physics, Department of Physics, Hampton University, Hampton, VA 23661. [2]Brimrose Corporation of America 5020 Campbell Blvd., Baltimore, MA 21236. Cr^{2+} in tetrahedrally coordinated CdTe and $Cd_{0.55}Mn_{0.45}Te$ crystals are under investigation as potential host materials for tunable, mid-infrared (MIR) laser development. The small crystal field splitting of the degenerate free ion energy levels of Cr^{2+} induces absorption (approximately 1900nm) and stokes shifted emission (approximately 2500nm) bands in the MIR. Also, the relatively large ionic mass and tetrahedral environment of Cr^{2+} in these host materials contributes to high luminescence efficiency. Preliminary data from the Cr^{2+}:CdTe free running laser will be reported.

AFM ANALYSIS OF THE BIODEGRADATION OF PHA THIN FILMS. Justinn F. Arceo, Dr. Brian Augustine, Department of Chemistry, James Madison University, MSC 7701, Harrisonburg, VA 22807. A co-polymer mixture of polyhydroxybutyrate (PHB) and polyhydroxyvalerate (PHV) were spun-cast onto glass slides to create thin films. These films are naturally biodegradable by a variety of bacterially-produced enzymes. In our study, the P(HB-HV) thin films were degraded by concentrated *Streptomyces* enzyme. Atomic force microscopy (AFM) was used to image and analyze the data. Both *ex-situ* and *in-situ* experiments were performed on the P(HB-HV) to determine the kinetics of the biodegradation. The *in-situ* experiment was performed using a liquid cell, which allowed for real-time analysis. Degradation was observed uniformly across the surface of the P(HB-HV). We have also developed a height standard using soft-lithography techniques to microfabricate P(HB-HV) structures with dimensions as small as 3 μm.

Medical Science

SERRATIA MARCESCENS: PRODIGIOSIN PRODUCTION. Shana N. Levine & M.C. Simurda. Department of Biology, Washington and Lee University, Lexington, VA 24450. *Serratia marcescens* bacterial colonies can vary in color from deep red to light red to white depending on the growth conditions and medium composition. The color is due to the presence of prodigiosin, a tripyrrylmethene molecule that is also known to be a pH indicator. In basic conditions prodigiosin is white, therefore, the purpose of our research was to determine if the white and red bacterial colonies are both producing prodigiosin. Hexane extractions of bacterial broth cultures separated the prodigiosin from the bacterial cell envelope. Thin layer chromatography of the extracts, using 1:1::acetone:*n*-hexane as the solvent, resolved that white bacteria do not but red bacteria do produce prodigiosin. TLC of extracts from two mutant strains 933 and WCF that are block in each of the two arms of the bifurcated metabolic pathway leading to prodigiosin production, show that neither mutant is capable of prodigiosin production. However, growth of these mutants in proximity to each other on an agar plate show that the intermediate metabolite produced by the WCF strain can be used by the 933 strain to produce the complete prodigiosin molecule. In this situation strain 933 bacteria are red and the TLC analysis shows identity with the red prodigiosin molecule produced by the wild type bacteria.

ISOLATION AND SEPARATION OF CELL POPULATIONS FROM THE MOUSE ANTERIOR PROSTATE. Rachel E. Hess-Yoder, Sarah M. Herr, & Roman J. Miller, Dept. of Biol., Eastern Mennonite Univ., Harrisonburg, Va. 22802. A method to isolate and separate a heterogeneous cell population from mouse anterior prostate tissue into viable, homogeneous populations is being developed to better characterize the unique function of individual cell types. Cell suspensions with an average yield of 2,266 cells/mg tissue were obtained through a two hour incubation of minced prostate tissue in an enzyme mix of collagenase, deoxyribonuclease, and protease made in Earl's Balanced Solution with Bovine Serum Albumin (BSA). A unit density gradient of 0.3-2.4% BSA in Hanks Balanced Salt Solution separated the mixed cell suspension via cell mass and density. Four aliquots that denoted semi-purified homogeneous cell types were selected: (1) Red blood and connective tissue cells predominated the first 50-ml aliquot (9,625 cells, 86.1 percent viable). (2) Stem cells, fibroblasts, and smooth muscle cells were the majority in the third 50-ml aliquot (17,175 cells, 86.2 percent viable). (3) Vacuoled secretory epithelial cells predominated the fourth 50-ml aliquot (10,100 cells, 79.2 percent viable). (4) Large epithelial cells dominated the eighth 50-ml aliquot (6,100 cells, 83.7 percent viable). Obtaining a mixed cell suspension with high percent viability and separation in four semi-purified aliquots illustrates progress toward the goal of this research. (Supported by: Daniel B. Suter Endowment, EMU).

NICOTINE STIMULUS GENERALIZATION TO BUPROPION. T. Bondareva, Richard Young & Richard A. Glennon, Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, VA 23298. Bupropion is a clinically available agent used both as an antidepressant *Welbutrin* and as a smoking cessation agent *Zyban*. Its mechanism of action is still unknown in both instances. Its chemical structure is unique and unrelated to those of nicotinic agents. Although bupropion does not bind at brain a4b2-type nicotinic acetylcholinergic (nACh) receptors, it can block nicotine-induced hypothermic and antinociceptive effects in the mouse. Bupropion is suggested to be a nicotinic antagonist. We used drug discrimination, with rats trained to discriminate 0.6 mg/kg of nicotine from vehicle, to determine whether or not bupropion is a nicotinic antagonist. If bupropion is a nicotinic antagonist, it should block the stimulus effect of nicotine. However, bupropion failed to antagonize this effect; it produced nicotine-like effects in stimulus generalization studies (ED_{50} = 5.50 mg/kg). Perhaps bupropion is effective in smoking cessation therapy because it can antagonize certain of nicotine's effects while mimicking certain other of its effects. [Supported by DA-05274.]

EFFICACY OF g- VERSUS a-TOCOPHEROL IN ATHEROSCLEROSIS PREVENTION. W. Ross Brown & Kathryn E. Loesser, Dept. of Biol., Mary Washington College, Fredericksburg, VA 22401. The g- form of tocopherol has recently been proposed to have a greater effect on the progression of atherosclerosis than that of a-tocopherol. The purpose of this experiment was to explore whether g-tocopherol would have a greater effect than a-tocopherol on prevention of atherosclerotic plaque development and on lowering cholesterol levels. Three groups of hyperlipidemic mice were fed high cholesterol feed: 1 group's food was supplemented with g-tocopherol, another group's food was supplemented with a-tocopherol and no supplements were added to the control group feed. Animals were sacrificed at the end of 14 weeks and blood along with heart and aorta samples were evaluated. Blood cholesterol and triglyceride levels were significantly higher ($p < 0.05$) in the control group than in the tocopherol-supplemented groups. The semi-quantitative evaluation of plaque development in heart and artery cross sections showed that the g-tocopherol-supplemented mice had the lowest atherosclerotic plaque development, the a-tocopherol fed group showed a slightly higher presence of plaques, and the control group showed the highest plaque occurrence. Gamma tocopherol supplementation seems to slow plaque development but more studies need to be done to confirm these findings.

THE ROLE OF CADMIUM IN THE INDUCTION AND EXACERBATION OF AUTOIMMUNITY IN TWO RODENT MODELS. Elizabeth K. Leffel & Kimber L. White, Jr. Dept. of Pharm. & Tox., Va. Commonwealth Univ., Richmond, VA, 23298. The Brown Norway rat (BN) is an induction model for SLE. A self-limiting increase in IgE levels occurs after exposure to autoimmune-inducing compounds. We utilized this characteristic to develop a "mercury challenge" model. Female BN were exposed via drinking water to 3, 30, 300 ppm cadmium chloride (Cd) for 9 weeks then challenged with 1 mg/kg mercuric chloride (Hg). There were increases in urinary Cd levels in the 30 and 300 ppm dose groups. Hg resulted in significant increases in IgE levels, compared to pre-challenge levels. No differences were observed between control and treated rats. This data does not reflect the inverse dose response expected. Therefore, cadmium in this model does not appear to induce autoimmunity, at the doses tested. The NZB/W mouse is an exacerbation model for SLE. Female NZB/W mice were exposed to 0, 0.03, 0.3, 3, and 10 ppm via drinking water for 4 or 31 weeks. Kidney cadmium levels were significantly increased in the 3 and 10 ppm groups. Proteinuria developed at 15 weeks of exposure in treated groups and 27 weeks in vehicle mice. This early onset of disease indicates that Cd may exacerbate autoimmune disease in those genetically predisposed.

TRYPTAMINES AS 5-HT$_6$ SEROTONIN RECEPTOR LIGANDS. Manik R. Pullagurla[1], Bryan L.Roth[2] & Richard A.Glennon[1], [1]Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, VA 23298 and [2]Department of Biochemistry, Case Western Reserve University. To date, 14 distinct types of mammalian 5-HT receptors have been identified. 5-HT$_6$ receptors belong to the family of seven transmembrane helix GPCRs and are positively coupled to an adenylate cyclase second messenger system. There is growing interest in this receptor because of the high affinity of several antidepressants and antipsychotics, suggesting a role in psychiatric disorders. 5-HT$_6$ receptor antagonists might be useful in the treatment of neuropsychiatric disorders, memory and cognitive dysfunction, and as anticonvulsants. We have earlier reported N$_1$-(benzenesulfonyl)tryptamines as 5-HT$_6$ receptor antagonists. In the present investigation we have further studied the structural requirements for the binding of the N$_1$-(benzenesulfonyl)tryptamines at the 5-HT$_6$ receptors and have identified structural modifications that influence affinity.

STATUS EPILEPTICUS RESULTS IN AN INCREASE IN BASAL AND MAXIMAL CALCINEURIN ACTIVITY IN RAT FOREBRAIN HOMOGENATE. JE Kurz. Dept. of Biology, VA Commonwealth Univ., Richmond, VA 23284. We studied the effects of status epilepticus (SE) on calcineurin, a neuronally enriched, calcium-dependent phosphatase. Calcineurin is an important modulator of many neuronal processes, including learning and memory, induction of apoptosis, receptor function and neuronal excitability. Therefore, a SE-induced alteration of calcineurin activity could have significant physiological implications. SE was induced by pilocarpine injection and allowed to continue for 60 minutes. Brain region homogenates were assayed for calcineurin activity by dephosphorylation of p-nitrophenol phosphate. A significant SE-dependent increase in both basal and Mn^{2+}-dependent calcineurin activity was observed in homogenates isolated from the cortex and hippocampus, but not the cerebellum. This increase was specific to calcineurin. Both maximal dephosphorylation rate and substrate affinity were increased following status epilepticus. However, increased calcineurin activity was not due to an increase in calcineurin enzyme levels. Finally, increase in calcineurin activity was found to be NMDA-receptor activation dependent. The data demonstrate that SE resulted in a significant increase in both basal and maximal calcineurin activity.

REGIONAL IMMUNIZATION WITH PEPTIDE-PULSED DENDRITIC CELLS LEADS TO COMPARTMENTALIZED IMMUNE RESPONSES AND ENHANCED ANTITUMOR EFFICACY. D.W. Mullins, S.L. Sheasley, T.N.J. Bullock, T.A. Colella, and Victor H. Engelhard, Carter Immunology Center, Univ. of VA, Charlottesville VA. To evaluate the efficacy of immunization maneuvers in a context relevant to human cancer therapy, we established a preclinical model using HLA-A*0201/H-2D^d recombinant (AAD) mice. IV immunization of AAD$^+$ mice with dendritic cells (DC) pulsed with YMDGTMSQV (human A2-restricted tyrosinase antigen) induced protection against challenge with an AAD-transfected murine B16-F1 melanoma. Tumor outgrowth was significantly decreased and survival extended by tyrosinase epitope-pulsed DC immunization as compared with unpulsed DC or untreated controls. Interestingly, SQ immunization was more efficacious at controlling tumor than IV immunization. Antigen-specific assays revealed an expanded population of tyrosinase-specific CTL in both the proximal draining nodes and spleen following SQ immunization with peptide-pulsed DC; IV immunization induced a larger total population of Ag-specific CTL, but these cells were detected only in the spleen. Thus, enhanced tumor control may be achieved with a small cohort of reactive CTL, provided these cells reside in the appropriate compartment.

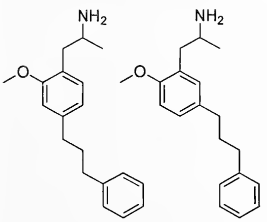
TRYPTAMINES AND *ISO*TRYPTAMINES: BINDING AT 5-HT$_2$ RECEPTORS. J. Chang-Fong[1], Milt Teitler[2] & Richard A. Glennon[1], [1]Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, VA 23298 and [2]Department of Pharmacology, Albany Medical College, Albany, NY 12208. The 5-HT$_2$ serotonin receptors are now well characterized and their distribution is established. Some 5-HT$_2$ receptor ligands are currently undergoing clinical assessment as potential treatment for a range of CNS-related diseases including schizophrenia, anxiety, sleep, feeding disorders, and migraine. We have previously shown that *iso*trytamines represent a novel class of 5-HT$_2$ agonists. Hoffman La Roche Pharmaceuticals has now developed *iso*tryptamines with selectivity for 5-HT$_{2C}$ versus 5-HT$_{2A}$ receptors. Because most of these agents display <100-fold selectivity, we conducted structure-affinity studies to determine what structural features might be responsible for selectivity. Tetrahydropyrazinoindoles, bridged derivatives of phenylpiperazine combining the structural features of mCPP (a potent 5-HT$_{2C}$ agonist) and the *iso*tryptamines, were also prepared and examined.

EXPLORATION OF POTENTIAL 5-HT$_5$ SEROTONIN RECEPTOR LIGANDS. Nantaka Khorana[1], Malgorzata Dukat[1], Carol Smith[2], Milt Teitler[2] & Richard A. Glennon[1], [1]Department of Medicinal Chemistry, Virginia Commonwealth university, Richmond, VA 23298 and [2]Department of Pharmacology, Albany Medical College, Albany, NY 12208. Diversity of the neurotransmitter serotonin receptor may be one of the reasons which causes serotonin to be involved in a variety of pathological changes. One of the newest receptor subtypes, 5-HT$_{5A}$ receptors, has been speculated to be involved in brain development and non-neuronal function. The lack of selective ligands for 5-

HT_{5A} receptors has hampered understanding the functional role of these receptors. A pyridoindole was identified as a possible lead for the development of such agents. Structural manipulation has resulted in analogs with low nanomolar affinity for 5-HT_{5A} receptors.

MITOGENIC EFFECT OF LIPOTEICHOIC ACID ON HUMAN EPITHELIAL CELLS. Robert B. Becker & Linda Ameen, Dept. of Biol., Mary Washington College, Fredericksburg, VA 22401. *Enterococcus faecalis* is a gram-positive bacterium existing as normal flora within the human colon and is implicated in many significant infections. We determined whether a component of gram positive bacterial cell walls called Lipoteichoic Acid (LTA) acted as a mitogen on human epithelial cells. Blocking studies utilizing Genistein, a tyrosine kinase inhibitor, were conducted to elucidate the pathway LTA may use to stimulate mitosis. LTA extracted from *E. faecalis* was incubated at a concentration of 10 mg per ml with ME-180 cervical, P-69 prostate, and Caco-2 colorectal carcinoma cells lines for 24, 48, and 72 hours. Additional trials treated with 250 mM genistein were also conducted. Proliferation was assessed at each time point by measuring BrdU incorporation. The results indicate that LTA may have a mitogenic effect on all of the cell lines. However, statistical differences were only found with the Caco-2 colorectal cells ($p < .05$). Results from the blocking studies show that proliferation was inhibited by genistein, suggesting that a protein tyrosine kinase signal transduction pathway may be used by the mitogen.

NOVEL AND UNEXPECTED PHENYLISOPROPYLAMINE 5-HT_2 RECEPTOR LIGANDS. Jagadeesh B. Rangisetty[1], Malgorzata Dukat[1], Milt Teitler[2], & Richard A. Glennon[1], [1]Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, VA 23298 and [2]Dept. of Pharmacology, Albany Medical College, Albany, NY. 5-HT_2 serotonin ligands are being evaluated for the treatment of schizophrenia and depression. Phenylisopropylamines such as 1-(4-bromo-2,5-dimethoxyphenyl)-2-aminopropane (DOB) are high affinity 5-HT_2 agonists. We have investigated the structure-affinity relationships (SAFIR) for binding and the structural requirements for agonist activity. A 2,5-dimethoxy pattern seems optimal for agonism, and affinity is broadly modulated by the nature of the 4-position substituent. The present study examined several related analogs of **1**, which lack a 5-methoxy group. It was additionally found that shifting the phenylalkyl substituent from the nuclear 4- to the 5-position results in retention of affinity. For example **2** unexpectedly binds at 5-HT_{2A} receptors with high affinity (Ki =13 nM) but now possesses 5-HT_{2A} antagonist character.



1 **2**

GROWTH FACTOR-LIKE ACTIVITY OF LTA ON CERVICAL AND PROSTATE. Alexandra C. Racanelli & Linda C. Ameen, Dept. of Biol., Mary Washington College, Fredericksburg, VA 22401. The relationship between prokaryotic and eukaryotic cells and the effects prokaryotes have on their hosts is not fully understood. Bacteria are classified as either gram-positive or gram-negative. These microorganisms possess several properties, known as virulence factors, which facilitate their pathogenicity, or ability to cause a disease. Lipoteichoic acid (LTA) is a virulence factor within the cell wall of gram-positive bacteria. It is composed of a glycolipid with an attached fatty acid n(glycosyl) polyglycerophosphate. This research used ME-180 and P69 cells to study LTA's possible mitogenic effects. Cell proliferation was quantified using an enzyme assay. T-test analysis indicated that LTA acted as a mitogen. Further studies are needed to confirm LTA's long-term effects on the host cell and to elucidate the pathway LTA may utilize to induce cellular proliferation.

ACCURATE IN VITRO END-JOINING OF A FREE RADICAL MEDIATED DOUBLE STRAND BREAK BEARING A 3'OVERHANG WITH COHESIVE ENDS AND 3'-PHOSPHOGLYCOLATES: EFFECT OF KU ON REPAIR FIDELITY. K.V. Inamdar, S.Chen, P.Pfeiffer,S. Lees-Miller, M.F.Hannah, Jae Wan Lee, Tong Zhou and L.F.Povirk. Dept. of Pharmacology and Toxicology, Medical College of Virginia, VCU. The subunits of DNA-dependent protein kinase(DNA-PK; Ku86, Ku70, and DNAPKcs) are critical for the mammalian nonhomologous end-joining pathway of double-strand break repair. A plasmid substrate containing a model of a staggered free radical-mediated double-strand break, with cohesive phosphoglycolate-terminated 3'overhangs and a one base gap in each strand , was constructed. Our results indicate that Ku, but not DNA-PKcs, is required for accurate end-joining of staggered free radical-mediated DNA double-strand breaks in vitro . Ku plays specific roles in protecting DNA termini from degradation, and maintaining the alignment of short partial complementarities during gap-filling and ligation. The role of DNA-PK may be primarily regulatory, initially inhibiting both end alignment and end processing by displacing Ku from the extreme end of DNA. This inhibition appears to be subsequently relieved as a result of specific, DNA- PK- catalysed phosphorylation events, the details of which remain to be defined.

AZANICOTINE ANALOGS AS nACh RECEPTOR LIGANDS. Giovanni Ferretti[1], Malgorzata Dukat[1], Billy R. Martin[2], M. Imad Damaj[2] & Richard A. Glennon[1,2], [1]Department of Medicinal Chemistry, School of Pharmacy, and [2]Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA 23298. Nicotine is an alkaloid with a wide spectrum of biological activities – some beneficial, some detrimental – perhaps due to its inability to discriminate between the different subtypes of nicotinic acetylcholinergic (nACh) receptors. Although the antinociceptive effects of nicotine have been known for over 60 years, the recent discovery of epibatidine, a potent nACh agonist, has prompted a search for analogs that might be potential analgesic agents without unacceptable toxicity. On the basis of structure-activity relationships formulated in our laboratories we synthesized a series of chain lengthened and imidazoline analogs of nicotine. Preliminary data show that azanicotine (K_i=206 nM) and homoazanicotine (K_i=7.8 nM) possess analgesic activity (ED_{50}=21 and 19 mg/mouse, respectively) and potency comparable to that of nicotine (K_i=2.1 nM; ED_{50}=12 mg/mouse). However, their antinociceptive actions, unlike those of nicotine, do not seem to involve a4b2 nACh receptors. These compounds may represent a novel mechanistic class of analgesics worthy of further investigation. [Supported by DA-05274.]

THE EFFECTS OF IL-1 AND IL-6 ON RAT SMOOTH MUSCLE CELL PROLIFERATION AND CYTOKINE PRODUCTION. Dahlia Peterson & Kathryn E. Loesser, Dept. of Biol., Mary Washington College, Fredericksburg, VA 22401. Interleukins 1 and 6 may be involved in the exacerbation of atherosclerosis. In this experiment, the effects on rat vascular smooth muscle cell

(VSMC) proliferation *in vitro* following incubation with either IL-6 or IL-1b were evaluated using ELISA BrdU Proliferation assays, cell counts, and tritium incorporation. RNA extraction, followed by RT-PCR was also performed to determine if rat VSMC produced IL-6 and/or IL-1 in a positive feedback response to treatment with these cytokines. Increase in cell number was measured using the trypan-blue exclusion method. The results indicate that IL-1b-treated cells had the lowest increase in cell number (29.5%) followed by control (49.7%), then 0.2 ng/ml IL-6 (91.1%) and highest in 0.4 ng/ml IL-6 (112.4%). Similar trends were obtained with tritium-labeled thymidine incorporation. However, very different results were obtained with ELISA BrdU; IL-1b and 0.4 ng/ml IL-6 showed a decrease in cell proliferation compared to the control and 0.2 ng/ml IL-6 increased cell proliferation. RT-PCR did not yield any consistent results. These experiments do not convincingly support a role of the cytokines Il-6 and IL-1b in atherosclerosis, although further research needs to be done to clarify these roles.

IS *PORPHYROMONAS ASACCHAROLYTICA* SUPEROXIDE DISMUTASE CAMBIALISTIC? Erin A. Clark & E.M. Gregory, Department of Biochemistry, Virginia Tech, Blacksburg, VA. Superoxide dismutase (SOD) from *Porphyromonas asaccharolytica* was a 42,000 mw dimeric protein. Inhibition of enzymatic activity by 2 mM NaN_3 and time-dependent inactivation by 100 mM H_2O_2 were consistent with iron at the active site. Denaturation of SOD from cell extracts in 5 M guanidinium chloride, 20 mM 8-hydroxyquinoline, and renaturation in 20 mM Tris (pH 7.0) but without added metal salt abolished SOD activity. Renaturation in the Tris buffer supplemented with either $MnCl_2$ or $Fe(NH_4)_2(SO_4)_2$ restored 50% and 42%, respectively, of the SOD activity initially denatured. Electropherograms of the native, Fe-reconstituted and Mn-reconstituted SODs revealed that each had a single band of SOD activity with the same relative mobility. SOD activity in native and Fe-reconstituted samples was inhibited >70% by 2 mM NaN_3 whereas Mn-reconstituted SOD was inhibited <20%. These data are consistent with the insertion of either Fe or Mn into the active site with restoration of catalytic activity. This ability to express activity with either metal defines a cambialistic enzyme.

Natural History & Biodiversity

POPULATION DECLINES OF MADTOM CATFISHES IN NORTH AMERICA AND POTENTIAL RESTORATION APPROACHES. John M. Kilpatrick, Department of Fisheries and Wildlife Sciences, Va. Polytechnic Inst. & State Univ., Blacksburg, Va 24061. Madtoms (*Notorus* spp.) are an extremely imperiled group of North American catfish, with 19 of the 25 described species protected in one or more of 19 states. 5 of these species are federally listed as threatened or endangered. My objectives were to determine reasons for madtom declines, identify common threats, and suggest possible restoration methods. Habitat loss, population fragmentation, predator introduction, poor water quality, and siltation have been the most common causes cited for declines in this genus. Biological traits such as low fecundity, narrow habitat requirements, and short life spans have contributed to madtom imperilment. Habitat preferences of madtoms usually include clean riffle-type areas of streams with availability of cavities for nesting. Recovery efforts such as habitat restoration and reintroductions have been directed at individual species, but efforts directed at preservation and restoration of entire systems would likely prove more effective. Artificial propagation and subsequent reintroduction into historic ranges will be important, but factors limiting madtom populations (e.g. adult survival, reproductive success, etc) must be identified for restoration efforts to be effective.

DISTRIBUTIONS OF FISHES IN RIVER DRAINAGES OF GREECE, WITH COMMENTS ON ASSESSING FISH BIODIVERSITY IN THE SOUTHERN BALKAN PENINSULA. W. C. Gretes[1] & E. G. Maurakis[1,2], [1]Biology Department, Univ. of Richmond, VA 23173 & [2]Science Museum of VA, 2500 W. Broad St., Richmond, VA 23220. Distributions and species diversity of freshwater fishes were determined relative to physical factors (stream order, width, depth, elevation, river km, and water temperature) in four river drainages (Aliakmon, Aoos, Axios, and Strymon-Aggitis) in Greece. Stream order was negatively correlated with elevation and gradient, and positively correlated with stream width and depth. Number of species was positively correlated with stream order, width and depth, and negatively correlation with elevation and gradient. Changes in species composition among stream orders were related more to replacement rather than addition of species. Funded in part by the Thomas F. and Kate Miller Jeffress Memorial Trust, Science Museum of Virginia, and University of Richmond.

COMPARISON OF MYOMERE COUNTS IN LARVAL *HEMITREMIA FLAMMEA* AND POPULATIONS OF FOUR SPECIES OF *SEMOTILUS*. E. G. Maurakis[1,3], R. Katula[2], & W. C. Gretes[3], [1]Science Museum of VA, 2500 W. Broad St., Richmond, VA 23220, [2]North American Native Fishes Assoc., 308 16th Ave. N., Onalaska WI 54650, & [3]Biology Department, Univ. of Richmond, VA 23173. Larval preanal, postanal, and total myomere counts of *Hemitremia flammea* were compared to those of *Semotilus corporalis*, *Semotilus lumbee*, *Semotilus thoreauianus* and GA (Altamaha drainage) and VA populations of *Semotilus atromaculatus*. Total myomere counts can be used effectively to separate species and populations of *S. atromaculatus*, *S. corporalis*, *S. lumbee*, *S. thoreauianus*, and *H. flammea*. Preanal myomere counts distinguish *S. corporalis* and VA *S. atromaculatus* but do not discriminate *H. flammea* from *S. thoreauianus*, nor *S. lumbee* from GA *S. atromaculatus*. Altamaha *S. atromaculatus* differs from VA *S. atromaculatus* in preanal, postanal, and total myomere counts, some adult meristic and morphometric characters, and has two unique undescribed *Dactylogyrus* species of gill parasites, characters that may warrant specific status for the GA Altamaha drainage population of *S. atromaculatus*. This study was funded in part by Science Museum of VA and Univ. of Richmond.

TIDE SPRING VIRGINIA-A REGIONAL TREASURE. James D. Lehman, Department of Physics, James Madison University, Harrisonburg VA. 22807. The presence of an ebb-and-flow spring in the Shenandoah Valley has intrigued observers for the past 240 years. Thomas Jefferson wrote about this phenomenon in his "Notes On The State Of Virginia". For many years the spring was an attractive objective for school field trips and social outing. In 1927, O.E. Meinzer identified and described the various flow patterns. Our five-year survey, beginning in 1982 monitored the site, logged seasonal changes, and attempted to model an underground profile. The spring is located in the outcrop belt of the Beekmantown formation. A triangular watershed to the south nourishes the spring activity. At present he area is a mixture of grazing land and mixed hardwoods – totally undeveloped. There are no immediate threats to the spring and watershed. Some local interest has been expressed in the recognition and preservation of this unusual natural phenomenon.

BENTHIC MACROINVERTEBRATE RESPONSES TO PHYSICALLY-COMPLEX HABITAT IN THE JAMES RIVER, VIRGINIA: LINEAR OR CURVILINEAR RELATIONSHIPS? Timothy W. Stewart, Tammy Shumaker, & Tom Radzio, Department of Natural Sciences, Longwood College, Farmville, VA 23909. We quantified response of a macroinvertebrate community to changes in habitat-structure abundance. Concrete slabs with stones attached were placed in the James River and retrieved after 28 days. Taxonomic richness and densities of macroinvertebrates were positively affected by stones. Total invertebrate density and densities of several taxa were linearly related to stone density. However, nonlinear relationships occurred between stone density and both macroinvertebrate taxonomic richness and chironomid (*Eukiefferiella* sp.) densities. Nonlinear relationships were characterized by increased macroinvertebrate densities and taxonomic richness across a gradient of increasing stone density when stones were rare (0-45 stones$\cdot$0.08 m^{-2}; 0-40% of slab faces covered by stones), but weak responses to additional stones at higher stone densities (83-160 stones$\cdot$0.08 m^{-2}; 89-96% coverage). We conclude that low levels of habitat structure greatly increase densities and taxonomic richness of benthic macroinvertebrates on hard substrata in the James River, and interstices provided by densely-packed substratum particles are not critical habitat for most taxa.

RELATIONSHIPS BETWEEN SUBSTRATUM PARTICLE SIZE AND MOLLUSK ABUNDANCE IN THE APPOMATTOX RIVER, VIRGINIA. Robert M. Northington, Timothy W. Stewart, & Joseph E. Garcia, Department of Natural Sciences, Longwood College, Farmville, VA 23909. Substratum particle size is an important determinant of the distribution and abundance of mollusks and other benthic invertebrates in aquatic ecosystems. For example, certain gastropods (i.e., snails) have adapted their foraging behavior to graze the surfaces of rocks for patches of freshwater algae. Rocks need to have sufficient surface area and algal coverage for these gastropods to graze effectively. In contrast, most bivalves (i.e., clams) require substrata consisting of small particles that allow for burrowing. We quantified relationships between substratum particle size and densities of two mollusks in the Appomattox River: the gastropod *Leptoxis carinata*, and the asian clam, *Corbicula fluminea*. We used a Surber sampler to collect mollusks from three broad habitat types characterized by dominant substratum particle size and flow regime. Habitat types included 1) sand/pool, 2) cobble/run, and 3) cobble/riffle (n = 3 replicates each). Results from statistical analysis showed that *Leptoxis* densties increased along a gradient of increasing particle size. Trends also suggested that *Corbicula* densities increased as a function of decreasing particle size.

PROPAGATION AND CULTURE OF ENDANGERED JUVENILE MUSSELS (UNIONIDAE) AT THE VIRGINIA TECH AQUACULTURE CENTER. Jess W. Jones, Richard J. Neves and Jeff J. Allen. Virginia Cooperative Fish and Wildlife Research Unit, Department of Fisheries and Wildlife Sciences, Virginia Tech, Blacksburg, VA 24061. Although North America contains the greatest diversity of freshwater mussels in the world, roughly 300 species, this family of mollusks is the most imperiled taxon in the United States. Biologists at the Virginia Tech Aquaculture Center have developed methods to propagate and culture endangered juvenile mussels for release into rivers of the upper Tennessee River drainage. Without immediate efforts to recover the 25 federally protected species in this drainage, the extinction of additional species is likely. The Tennessee Wildlife Resources Agency, U.S. Fish and Wildlife Service, and Virginia Tech have entered into a 5 year cooperative program to fund the production, culture, and release of numerous juvenile mussel species into rivers in eastern Tennessee. The goal of this project is to augment natural reproduction at sites with these species and to release juvenile mussels at historic sites within those rivers to expand the population ranges. In 1998-2000, a total of 243,000 juvenile mussels of 9 species were released into the Clinch, Powell and Hiwassee rivers in Tennessee. Annual releases will continue, and an evaluation of the success of the releases will begin in the year 2001.

THE ECOLOGICAL SIGNIFICANCE OF TAIL SPOTS AND PLUMAGE IN FEMALE PROTHONOTARY WARBLERS. Jonathan R. Moore, Charles R. Blem & David W. Podlesak, Department of Biology, Virginia Commonwealth Univ., Richmond, VA 23284. One-year-old (SY) female Prothonotary Warblers (*Protonotaria citrea*) have distinctive subadult plumages, including a variable amount of white on the webbing of their rectrices. The extent of white varies from elongated spots only on the webbing of r5 and r6 to large, bright-white areas on r2-r6 (similar to the male pattern). Older females tended to have more white than first-year birds and changes between years always resulted in more extensive or equal areas of white on rectrices. Retrix patterns are not rigorous indicators of the age of female Prothonotary Warblers, but may function in signaling changes in maturity and potential fitness of older females. SY females with smaller amounts of white in their rectrices produced significantly smaller clutches, but nested earlier and had nestlings with faster growth rates, and greater fledging masses than first-year females with more male-like plumages. SY females tended to be absent from the study area in their first breeding seasons and may have skipped nesting or been forced to breed in marginal habitat away from study area.

STUDIES OF MAMMALS ON THE VIRGINIA BARRIER ISLANDS: BIOGEOGRAPHY, GENETICS, AND ECOLOGY. Nancy D. Moncrief[1] & Raymond D. Dueser[2], [1]Virginia Museum of Natural History, Martinsville, VA 24112 & [2]College of Natural Resources, Utah State University, Logan, UT 84322. The Virginia barrier islands, which extend 150 km along the southern seaward margin of the Delmarva Peninsula, comprise the only undeveloped barrier system on the eastern seaboard. These islands are separated by deep inlets, tidal lagoons, and extensive salt marshes. They are subject to frequent storms; islands often are overwashed and sometimes inundated, creating a dynamic ecological and evolutionary environment. We are studying the mammals that inhabit these islands and the adjacent Delmarva Peninsula mainland. Our research is focused on four major areas: 1) historical and current biogeography; 2) ecological and genetic comparisons of island and mainland populations of rodents; 3) recent erosion events on the northern end of Myrtle Island; and 4) population dynamics and distributions of mammalian predators.

Psychology

DOES THE TEMPORAL DEVELOPMENT OF READING PROCESSING SKILLS INFLUENCE LETTER DETECTION FAILURES? Breton Harris Permesly, Washington and Lee University. This experiment traces the temporal development of reading-processing skills in attempts at gaining insight into the effects reading proficiency has on the "Unitization" and "Phonetic-Recoding" hypotheses approach to letter detection errors. The experimenter presented 30 Washington and Lee undergraduates with two sets of passages in which they were to encircle instances of target letter. One set of passages was used to assess the effects of proficiency on the "Unitization" hypothesis, and the other set examined the effects of proficiency on the "Phonetic-Recoding" hypothesis. The observed pattern of detection errors in the "Unitization" study was found to significantly depend not only on the presentation of the search passage, but also on the attained level of the subject's reading-processing skills. In the second study, although trends were observed that supported the hypothesized effects of temporal development of read-processing skills on phonetic factors in detection errors, no significant effects were observed.

THE EFFECTS OF GENDER AND STATUS ON THE ACCURACY OF DETERMINING LIES AND TRUTHS. Lisa A. Chew, Washington and Lee University, Lexington, Va. 24450. Do gender and status affect the accuracy of determining lies and truths? Participants were shown four 2-minute videos (each viewed twice) of targets with different status levels – a male professor (high), a female professor (high), a male student (low), and a female student (low). Each video included each of the following descriptions: someone the person liked, someone the person disliked, someone the person liked as if he/she disliked him/her, and someone the person disliked as if he/she liked him/her. The participants (males and females) were told that the targets lied between zero and four times and were then asked to determine whether each description was a lie or the truth and indicate their confidence in those determinations. Male participants were found to be significantly more accurate in their determinations of lies/truths for low status targets than for high status targets. Overall, the male participants were more confident in their determinations than were the female participants. A significant interaction indicated that the female participants had significantly higher confidence in the determinations for the high status target when it was a male and significantly higher confidence in the determinations for the low status target when it was a female.

ORIGINS AND MECHANISMS OF SEMANTIC MEMORY RETRIEVAL INHIBITION. Nathan R. Hoot & David G. Elmes, Department of Psychology, Washington and Lee University, Lexington, VA, 24450. Retrieval blocks can be induced in semantic memory by a variety of methods, including part-list cuing or priming with information semantically related to a target. Mechanisms underlying retrieval blocks may involve automatic spreading inhibition, but other interfering cognitive processes seem to play a role in this phenomenon as well. The first study attempted to evoke memory retrieval blocks using an indirect means of priming. Subjects studied a part list of United States with their capitols and were asked to recall the capitol of a given state after being primed with a studied or non-studied state. Results showed that studied primes inhibited retrieval more than non-studied primes, indicating that significant retrieval blocks may indeed be induced by indirect means. A new model was devised using a connectionist approach to illustrate one possible means of such retrieval inhibition. In the second study, studied and non-studied capitols were included as the possible primes. The results showed that state primes tended to inhibit retrieval more than capitol primes. To explain this finding, the concept of immediate memory was proposed to explain a possible mechanism underlying the cognitive process of memory retrieval.

HOW DOES RESPONSE MODALITY AFFECT FREE RECALL? Michael T. Morrow, Dept. of Psych., Washington and Lee Univ., Lexington, Va. 24450. Although significant studies have examined the interaction between presentation modality and encoding modality in memory performance, this research has continuously overlooked response modality as a significant variable. Therefore, this study investigates the interaction between presentation modality, encoding modality, and response modality in order to reexamine previous findings and proposals such as the translation hypothesis. Participants were given an incidental instruction before viewing a wordlist. Words were presented visually and aurally, encoded by writing and speaking, and recalled through writing or speaking. A significant interaction appeared between encoding and response modality illustrating that regardless of presentation modality, when verbal encoding and response are paired, comparatively lower recall is observed. Also, responding in a different modality than the encoding modality, generated higher recall than responding in the same modality. Contrary to previous findings and the translation hypothesis, intermodal processing only produced higher recall in the auditory presentation condition. The results observed in this study demonstrate that response modality is a variable to consider.

THE ROLE OF RATE OF PRESENTATION AND TYPE OF ESTIMATION ON ACCURACY OF TIME PERCEPTION. Shelby R. Fierke, Department of Psychology, Washington and Lee University, Lexington, VA 24450. What affect does both rate of presentation and type of estimation have on the accuracy of time perception? In the present experiment 60 participants, 28 males and 32 females, were assigned to one of four groups in this 2x2 between subjects design. The four groups were divided between the two independent variables, rate of presentation (fast or slow) and type of estimation (prospective or retrospective). After a short slide show, absolute error values and estimations of time were taken from the participants by the reproduction method. A significant main effect for type of estimation was found, in that prospective estimates of time were longer than retrospective estimates of time. A significant interaction was also found. Prospective estimates were longest in the fast presentation group and shortest in the slow presentation group, while retrospective estimates were longest in the slow presentation group and shortest in the fast presentation group. Explanations for these results are presented and discussed using various models of subjective time perception.

THE EFFECT OF COMBINATION OF INSULIN-PRODUCED HYPOGLYCEMIA AND ETHANOL ON RAT PERFORMANCE IN THE RADIAL-ARM MAZE. J. Constantine, J. Kenney, & P. Duncan, Department of Psychology, Old Dominion University, Norfolk, VA 23529-0267. Male Long-Evans rats were trained to criterion performance on a win-shift procedure in a radial-arm maze (RAM) for food reward. The effects of two units/kg of insulin, 1500 mg/kg ethanol, and the combination of these drugs were then tested with a 2 X 2 factorial experimental design with both injections given IP 15 min before testing.. Insulin was a between-subjects variable (two groups, n=10) and ethanol was a within-subjects manipulation given to half of each group on the first test day and to remaining rats on the second test day. Insulin produced a state of hypoglycemia in that blood glucose levels were reduced to approximately 65% of baseline value. Both ethanol and insulin significantly increased time required to complete the RAM trials, and ethanol caused an impairment of correct arm-choice. Insulin did not cause RAM errors. The combination of ethanol and insulin caused a non-significant tendency for potentiation of the increase in time of RAM completion. These results demonstrate that ethanol can impair working memory and ethanol or hypoglycemia produce general behavioral depression.

THE EFFECTS OF REPEATED ADMINISTRATION OF ETHANOL, AND OF INSULIN ON RAT PERFORMANCE IN THE RADIAL-ARM MAZE. B. Parris & P. Duncan, Department of Psychology, Old Dominion University, Norfolk, VA 23529-0267. In order to determine whether tolerance develops to the behavioral effects of ethanol, and of insulin-produced hypoglycemia, these drugs were administered on three days of radial-arm maze (RAM) tests. Ten rats were administered 1600 mg/kg ethanol, and ten were administered two units/kg insulin. Each drug was injected IP 15 min prior to the RAM tests and all rats had been given extensive training in the RAM. A no-drug control day preceded each drug test and drugged performance was compared to these control tests. Ethanol initially increased time to complete the RAM, but this effect was not seen on test days 2 and 3. Significantly increased RAM errors also occurred after ethanol treatment and persisted for all test days. Insulin treatment did not cause RAM errors, but did greatly increase RAM-completion time and this effect did not decrease on days 2 or 3. These results demonstrate that some effects of ethanol are subject to tolerance and do not persist after chronic treatment, but a similar effect of hypoglycemia does persist for at least three hypoglycemic episodes. The degree of hypoglycemia did not vary significantly over the three days of RAM tests.

THE EFFECTS OF TEAM PROCESS TRAINING AND PERCEIVED TASK AND LIFE WORK LOAD ON TEAM PERFORMANCE. Hope S. Hanner, Old Dominion University. The effects of task load and life load on team performance were examined in a time series design. Research participants were 55 students of an I/O Psychology course who were randomly assigned to teams. The 11 teams completed team assignments for which each individual received team grades three times per week for the entire semester. In addition to the team assignments, all individuals completed self-report questionnaires on the level of difficulty of the task (task load) and the level of current stress in their lives (life load). Midway through the course, students in a 3-hour behaviorally based team process training whose goal was to make the teams aware of the team processes, observed team processes in the context of the team assignments, and practiced with the teamwork processes. The hypotheses were that team training would bring about an increase in performance by teams, and alleviate the effect of work load and life load on team training. Results from autoregressive integrated moving average (ARIMA) analyses indicated that team training improved team performance and moderated the relationship between the task and life load variables with team performance. It appeared as though teamwork controlled the effects of both task (internal) and life (external) stressors on team performance. Implications of this study for future research were discussed.

AN INVESTIGATION OF CONTROL CONDITIONS IN ASCH-TYPE EXPERIMENTS: II. Lynn M. McGeein, Karen P. Craig & James P. O'Brien, Tidewater Community College, Virginia Beach VA 23456. The results are reported for the second year of a standardization protocol for stimuli used in Asch's (1951,1956) independence-conformity paradigm (first year: *Va. J. Sci.*, 51 (2), p. 132). Asch's control condition is replicated for participants' sex and college (4-yr. college vs. community college) and experimenters' sex and status (authority vs. peer) constituting a 2x2x2x2 design. So far, only white male college undergraduates (the same type of subjects Asch used) err as seldom as Asch's controls (regardless of whether a male experimenter is authoritative or a peer). For many other conditions, percent error-free and mean error measures are more extreme than those found by Asch. In fact, the mean error for the sample least like Asch's, 55 community college women with a same-sex peer experimenter, is 0.91; or 11.4 times greater than Asch's 0.08. Most replications in the 50 years since Asch, using college undergraduates (men & women), replicate only the group pressure experimental conditions and not controls. Therefore, the typical conclusion that women are more conforming than men in the Asch paradigm may be erroneous. As Asch demonstrated, the more ambiguous the stimuli the more conformity occurs.

THE EFFECTS OF EDUCATIONAL COURSES ON INMATE DEPRESSION. Karen E. Herrera, Tidewater Community College, Virginia Beach, Virginia 23456. In order to test the effects of educational courses on inmate depression, a modified version of the Geriatric Depression Scale (GDS) was administered to volunteer prisoners at Bexar County (Texas) Adult Detention Center who were either enrolled in educational courses (n=100) or who were not enrolled (n=100). APA ethical guidelines were implemented, and special safety precautions for working in a correctional facility were taken. Male inmates taking intense (college level or multiple) courses showed a 40% lower average depression score than male inmates taking no classes, but males taking parenting courses scored 24% higher (more depressed) than males taking no courses at all. Female inmates taking parenting courses scored 27% lower depression scores than females taking no courses.

THE EFFECTS OF SOCIAL CONTACT ON BEHAVIORAL RESPONSES FOLLOWING STRESSFUL AND ENRICHING ENVIRONMENTS. Jennifer K. West and Kelly G. Lambert, Dept. of Psychology, Randolph-Macon College, Ashland, VA 23005. Social contact and enrichment have been shown to act as buffers against some of the detrimental effects of chronic stress. This study sought to examine the role each plays on reducing emotional reactivity and increasing learning abilities in rats. Following exposure to a 10 day chronic unpredictable stress paradigm, results suggested that animals housed with a social partner were less anxious in an open field test. On the first day of testing in a dry land maze, exposure to an enriched environment enhanced learning. On the second day of testing in a dry land maze, a significant interaction between social contact and enriched environment was observed. Specifically, the rats housed in a standard environment demonstrated impaired learning ability when raised in isolation whereas the standard environment animals housed with a social partner demonstrated no learning deficits. Thus, social contact seems to be able to compensate for impoverished environmental conditions.

EFFECTS OF GENDER SCHEMAS ON OCCUPATIONAL JUDGEMENTS. Shawna J. Maio & Kelly B. Cartwright, Psychology Department, Christopher Newport University, Newport News, VA. Literature currently indicates that there are persistent wage and promotional inequities between males and females in the United States. These inequalities seem to favor men over women in occupational settings, and several studies have examined possible causes for these observed disparities. Theoretical explanations have included Human Capital Theory and Devaluation Theory. This research proposes that Gender Schema Theory may provide additional explanation for sex-based inequities. Participants were classified as schematic (masculine or feminine) or aschematic (androgynous or undifferentiated) based on the Bem Sex Role Inventory. The current study examined aschematic and schematic individuals' occupational judgements regarding fictitious resumes, predicting that gender schemas would affect judgements even when human capital and devaluation variables were controlled. No evidence of sex-based inequities or devaluation emerged. However, consistent with predictions, gender schemas appeared to affect individuals' suitability judgements of female applicants for schema-inconsistent positions, while no such effect was found for males.

EFFECTS OF REWARDS AND STANDARDS OF CRITERIA ON CREATIVITY IN THE ELEMENTARY SCHOOL CLASSROOM. Daniel M. Birdwhistell[1], David G. Elmes[1] & Paul Notaro[2]. [1]Dept. of Psychology, Washington and Lee Univ., Lexington, VA 24450 and [2]Department of Psychology, Univ. of Missouri at St. Louis, St. Louis, MO 63121. Investigates how the promise of non-synergistic extrinsic rewards (candy) and quantitative standards of production (minimum levels of production for specific tasks) affect the creative performance of 41 3rd and 42 5th grade students relative to normal creative ability. The Average Standard Score on the Torrance Test of Creative Thinking, Figural Form B was used to measure overall creative performance. Individual creative behaviors were measured by fluency, originality, elaboration, abstractness of title, and resistance to closure on the same test. The promise of reward increased creative production in both 3rd and 5th grade students irrespective of ability, with higher increases in the 5th grade students. The reward significantly improved fluency, abstractness of title, and resistance to closure. The standard of production negatively affected creative production for students with normally low creative abilities. This result was especially negative with the 5th grade students.

A SECONDARY ANALYSIS OF LEADERSHIP BEHAVIORS AND VIRTUAL TEAM EFFECTIVENESS. J. Bryant & D. Davis, Department of Psychology, Old Dominion University, Norfolk, VA 23529-0267. No research to date has attempted to explore effectiveness of virtual teams with respect to specific leadership traits or practices. This study is an initial step toward understanding the interrelationship between the domains of team effectiveness and leadership. Transcripts of interviews with 41 managers of three international companies with offices in Asia were analyzed qualitatively with "team" (regional or global) as the unit of analysis. Ratings of team effectiveness from managers and this investigator were correlated. Further, specific leader behaviors assessed by the LMX-7 and MLQ were correlated with both ratings of team effectiveness. Results were generally inconsistent with regard to specific, identifiable leadership behaviors conducive to effectiveness in virtual teams. However, one LMX item, "quality of working relationship with leader", was found to be significantly correlated with ratings of team effectiveness from both managers and this investigator. Implications of this finding, as well as limitations of the research design and analytic methods, are discussed.

ESTIMATING STRESS, PERSONAL RELATIONHSHIPS, AND VIOLENT TENDENCIES IN JAPANESE AND AMERICAN STUDENTS. S. Hinnefeld, M. Gibbons, J. Gibbons, K. Cartwright, & T. Marshall. Psychology Department, Christopher Newport University, Newport News, VA 23606. In the current study a self-report survey was administered to 419 junior high school and college students in Japan and America. Students reported their stress, violent thoughts, violent actions, and their relationships with parents, teachers, and peers. As expected, American students reported less stress, fewer violent thoughts, and better relationships with parents and peers than Japanese students. Interestingly, American junior high school students reported fewer violent behaviors than Japanese junior high school students.

THE EFFECTS OF COLOR AND EXPOSURE ON ACCURACY AND SPEED OF IDENTIFICATION. S. Wood & T. Betts, Department of Psychology, Christopher Newport University, Newport News, VA 23606. The goal of the current experiment was to determine if presentation color affects accuracy and reaction time when identifying old and new words. Specifically, participants were shown a set of 40 words to recognize later. The words were either colored or black. At test, participants made recognition ratings for 80 words (40 old and 40 new) and their accuracy and reaction times were recorded. Surprisingly, color affected recognition and reaction time for new words, but not old words. Apparently, colored words focused participants on identifying old words, which distracted them from identifying new words. Future research will replicate the current study across longer retention intervals.

AN EXAMINATION OF NEED FOR COGNITION AND DEFINING MOMENTS. R. Page, B. Sander, & J. Gibbons, Department of Psychology, Christopher Newport University, Newport News, VA 23606. The current study examined existential moments, or defining moments in the lives of the participants. Specifically, 16 participants reported ten of their most defining moments, and for each event estimated frequency of recall. They also rated memories of the events and pleasantness and importance of the events. Participants also filled out a need for cognition scale. The defining events and contrived existential moments were then incorporated into a computer-simulated program. One week later, participants were asked to correctly identify their events from the contrived events. Reaction times were recorded. Unexpectedly, participants showed faster reaction times to the contrived events. Interestingly, defining events became more pleasant over time for high need-for-cognition participants.

MALE CHARACTERS ARE MALICIOUS FOR GENDER SCHEMATIC INDIVIDUALS. B. Sander, E. Pope, & N. Traxel, Department of Psychology, Christopher Newport University, Newport News, VA 23606. The current study examined how participants' gender schemas determined the gender of the main and supporting characters in positive and negative stories. Schematic participants identified character gender best when the story was negative. Moreover, schematic participants remembered character gender best in negative stories with male lead characters. Future experiments will balance gender and analyze the data for gender effects.

Statistics

MODEL ROBUST REGRESSION BASED ON GENERALIZED ESTIMATING EQUATIONS. Seth K. Clark, Jeffrey B. Birch, & Oliver Schabenberger, Dept. of Stat., Va. Polytechnic Inst. & State Univ., Blacksburg, VA 24061. One form of model robust regression (MRR) predicts mean response as a convex combination of a parametric and a nonparametric prediction. MRR is a semiparametric method by which an incompletely or an incorrectly specified parametric model can be improved through adding an appropriate amount of a nonparametric fit. The combined predictor can have less bias than the parametric model based estimate alone and, as shown in previous work for uncorrelated data with linear mean function, can converge faster than the nonparametric predictor alone. We propose extending the MRR technique to the problem of predicting the mean response for clustered non-normal data. We review parametric and nonparametric GEE methods then combine them through a mixing parameter. As a special case where data are uncorrelated, this amounts to mixing a local likelihood estimate with predictions from a global generalized linear model. Bandwidth selection, mixing parameter estimation, and asymptotic convergence rates are discussed.

BAYESIAN MODIFICATION TO THE TWO-STAGE EXPERIMENTAL DESIGN PROCEDURE WITH NEAR-SATURATED DESIGNS AND HETEROGENEOUS VARIANCE. D'Arcy P. Mays, Department of Mathematical Sciences, Virginia Commonwealth University, Richmond, VA 23284-2014. Experimental designs are critical in the quality improvement of the manufacturing process and to the development of new processes. Experimental methods are often used to identify the most important controllable variables in the product. The relationship of the product response and the controllable variables can be determined by experimental data. Therefore, the empirical mean model should be established using multiple regression. Experimental designs often are evaluated with regard to estimating coefficients in the regression model. However, with dispersion effects many standard designs are not optimal for estimation of the mean model. A two-stage experimental design procedure developed by Mays and Myers, in which the first stage estimates the heterogeneous variance structure and the second stage augments for estimating the mean model, is beneficial in many situations. However, the first stage variance estimation is not reliable when small first stage experiment sizes are used. A modified two-stage procedure using a Bayes estimator indicates that the procedure is beneficial for the small first stage experiment sizes. This study focuses on the application of this modified procedure for several small designs. Near saturated and saturated designs such as Hybrid designs, Koshal designs, and small composite designs are chosen for the investigation. The examination of the variance estimation in the first stage for these designs will be performed in order to find the optimal number of replicates to make at each design location. The Bayesian procedure will be compared to the non-Bayesian procedure for the various variance structures as well as the different variance ratios, and is shown to be beneficial.

CAPTURING NONCONFORMITY POINTS IN REGRESSION. James E. Mays, Department of Mathematical Sciences, Virginia Commonwealth University, Richmond, VA 23284-2014. A study of the regression problem of developing confidence intervals around the estimate of mean response when there is both a small sample size and a possible misspecification of the form of the underlying model. Of particular interest is capturing the true mean response for the specific points that do not conform to the user's chosen model. For these points, classical parametric methods such as ordinary least squares (OLS) yield inaccurate fits and undesirable confidence interval coverage probabilities. Nonparametric alternatives such as local linear regression (LLR) yield large variances and undesirably wide confidence intervals due to the small sample size. The solution to this dilemma is to use semiparametric model-robust techniques that combine the parametric information available with a nonparametric adjustment to capture the structure missed by the parametric fit. Confidence intervals for two of these methods (based on linear estimates) are compared to those from OLS and LLR. An example and a simulation study establish the benefits of the model-robust confidence intervals by comparing coverage probabilities and widths. These benefits are apparent for fits based on recently developed data-driven criteria. Results are presented for the specific nonconforming points and across all points in the data sets.

AN ALTERNATIVE ESTIMATE OF LOCATION FOR CIRCULAR DATA. Bennett Sango Otieno & Christine M. Anderson-Cook, Dept. of Statistics, Va. Polytechnic Inst. & State Univ., Blacksburg, Va, 24061. Circular- or angular -data are dealt with in many fields of applied statistics. A well-known biological example is the migration of birds, or, as medical examples, the time (month) of onset of lymphatic leukemia and circadian rhythms like time of maximal blood pressure could be mentioned. A common problem of interest in circular data is estimating a "preferred direction" or "center" and its corresponding error estimate. This problem is complicated by the fact that there is no minimum or maximum on the circle-the so-called wrap-around effect. The usual statistics employed for linear data are inappropriate for directional data, as they do not account for the circular nature of directional data: the fact that 1^0 and 359^0 are only 2^0 apart. Common choices for summarizing the preferred direction are the sample circular mean (quite robust, especially if data is from Von Mises distribution), and sample circular median (very insensitive to the tails of the distribution). A circular analogue of the celebrated Hodges-Lehmann estimator for linear data is proposed, as an alternative estimate of "preferred direction" (center). The new measure of center is robust under general distributions, down weights outliers sparingly and gives some influence to the tail of the distribution.

LINEAR MIXED MODEL ROBUST REGRESSION. Megan J. Waterman, Jeffrey B. Birch & Oliver Schabenberger, Department of Statistics, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061. Mixed models are powerful tools for the analysis of clustered data and many extensions of the classical linear mixed model with normally distributed response have been established. As with all parametric models, correctness of the assumed model is critical for the validity of the ensuing inference. Model robust regression techniques predict mean response as a convex combination of a parametric and a nonparametric model fit to the data. It is a semiparametric method by which incompletely or incorrectly specified parametric models can be improved through adding an appropriate amount of a nonparametric fit. In this talk we apply this idea of model robustness in the framework of the linear mixed model. The mixed model robust regression (MMRR) predictions we propose are convex combinations of predictions obtained from a standard normal-theory linear mixed model, which serves as the parametric model component, and a locally weighted maximum likelihood fit which serves as the nonparametric component. An application of this technique with real data is provided.

VIRGINIA JUNIOR ACADEMY OF SCIENCE
2001 AWARD WINNERS

Presented at James Madison University, Harrisonburg, VA
May 23, 2001

AGRICULTURAL AND ANIMAL SCIENCE

Honorable Mention:
KATHERINE M. FOSTER
Yorktown High School

Honorable Mention:
CHARLES UPSHAW
Stonewall Jackson Middle School

Third Place:
KRISTIN J. SHAFFER
Chesterfield County Mathematics and Science High School

Second Place:
MEAGHAN O'BRIEN & MARTA TOMIC
Chesterfield County Mathematics and Science High School

First Place:
ZACHARY J. CAPPELLO
Stonewall Jackson Middle School

ANIMAL BEHAVIOR (ETHOLOGY)

Honorable Mention:
MAEVE E. GOULD
Tuckahoe Middle School

Honorable Mention:
JAMILLE Y. ROBINSON
Richmond Community High School

Honorable Mention:
SARAH H. SHAHMORADIAN
J.R. Tucker High School

Third Place:
BRETT M. WILSON
Atlee High School

Second Place:
ELIZABETH S. MAJETTE
Harry F. Byrd Middle School

First Place:
ASHLEY E. ALBERS & LAUREN N. CARPENTER
Chesterfield County Mathematics and Science High School

BOTANY 'A'

Honorable Mention:
EMILY M. ANDREE
Roanoke Valley Governor's School

Honorable Mention:
ROGER B. ARNOLD, JR.
Tuckahoe Middle School

Honorable Mention:
KEITH E. GOODMAN
I.C. Norcom High School

Third Place:
MARY B. GAYLE
Chickahominy Middle School
Second Place:
TRUE BECK
Tuckahoe Middle School
First Place:
ERIN B. FRACKLETON
Central Virginia Governor's School

BOTANY 'B'

Honorable Mention:
MAY S. HELAL
Tuckahoe Middle School
Honorable Mention:
NOHA HELAL
Tuckahoe Middle School
Honorable Mention:
JOHN R. KENNEDY
Bishop Denis J. O'Connell High School
Third Place:
TAREQ MALIKYAR
Yorktown High School
Second Place:
DEREK R. MILLER
Central Virginia Governor's School
First Place:
LESLIE A. NEELY
Blacksburg High School

BOTANY 'C'

Honorable Mention:
LAURA L. ROTHENBERG
Tuckahoe Middle School
Honorable Mention:
KATIE N. SAMAY
Yorktown High School
Honorable Mention:
KAYLA I. SCHEINER
Yorktown High School
Third Place:
ERIN B. STRANG
Stonewall Jackson Middle School
Second Place:
JEFFREY S. TUCKER, JR.
Central Virginia Governor's School
First Place:
DAVID C. SCHAFER
Tuckahoe Middle School

CHEMISTRY 'A'

Honorable Mention:
JONATHAN S. CHAN
Manchester Middle School

Honorable Mention:
MARTHA L. CLATTERBUCK
Atlee High School
Honorable Mention:
ROBERT FISHER
Governor's School for Government and International Studies
Third Place:
TREVOR E. CORBIN
Harry F. Byrd Middle School
Second Place:
MAURICE S. FISHER
Atlee High School
First Place:
LAUREN M. CONNOLLEY
Atlee High School

CHEMISTRY 'B'

Honorable Mention:
LEAH M. HARRIS
Liberty Middle School
Honorable Mention:
CATHERINE E. HILL
Bishop Denis J. O'Connell High School
Honorable Mention:
ALISA KHAN
Thomas Jefferson High School for Science and Technology
Third Place:
REBECCA R. POMPANO
Atlee High School
Second Place:
SHELLY L. JONES
Homer L. Hines Middle School
First Place:
MICHELLE MINASKANIAN
Mills E. Godwin High School

CHEMISTRY 'C'

Honorable Mention:
CAROLINE M. RASMUSSEN
Yorktown High School
Honorable Mention:
COLLEEN M. SWEENEY
Bishop Denis J. O'Connell High School
Honorable Mention:
LANE E. VAN ARSDALE
Harry F. Byrd Middle School
Third Place:
ANDREA V. SAEVOON
Bishop Denis J. O'Connell High School
Second Place:
SURAVI SIRCAR
Mills E. Godwin High School
First Place:
ZHOU YI
Thomas Jefferson High School for Science and Technology

COMPUTER SCIENCE

Honorable Mention:
RONALD O. FORBES
Chesterfield County Mathematics and Science High School
Honorable Mention:
JOSEPH E. GONZALEZ
Governor's School for Government and International Studies
Honorable Mention:
LING-LUN B. HSIA
Mills E. Godwin High School
Third Place:
MICHAEL J. DOBBS
Yorktown High School
Second Place:
SETH D. JOHNSON
Central Virginia Governor's School
First Place:
WILLIAM S. FITHIAN
New Horizons Governor's School

CONSUMER SCIENCE 'A'

Honorable Mention:
JENNIFER C. BIBBS
Ocean Lakes High School
Honorable Mention:
STEPHANIE L. BYERS
Governor's School for Government and International Studies
Honorable Mention:
JENNIFER M. DAVIS
Ocean Lakes High School
Third Place:
MATTHEW F. CANCIAN
Swanson Middle School
Second Place:
LINDSAY M. DENEAULT
James River High School
First Place:
LAURA M. BEACH
Roanoke Valley Governor's School

CONSUMER SCIENCE 'B'

Honorable Mention:
REBECCA L. EASLEY
Central Shenandoah Valley Governor's School
Honorable Mention:
CHIKEZIE I. ESEONU
Governor's School for International Studies
Honorable Mention:
ELIZABETH K. JETTON
Cave Spring High School
Third Place:
JANE M. GURNICK
Chesterfield County Mathematics and Science High School

Second Place:
NORA E. GRAY
Yorktown High School
First Place:
ELIZABETH J. HAMED
Cave Spring High School

CONSUMER SCIENCE 'C'

Honorable Mention:
ANN K. MAJOR
Atlee High School
Honorable Mention:
LAURA R. MOONEY
Governor's School for Government and International Studies
Honorable Mention:
ROSEMARY O. OVERBEY
George H. Moody Middle School
Third Place:
JAMES W. NORWOOD
Atlee High School
Second Place:
JEREMY C. MULLINS
Manchester Middle School
First Place:
JOHN S. O'GRADY
Atlee High School

CONSUMER SCIENCE 'D'

Honorable Mention:
MEAGEN C. SMITH
Atlee High School
Honorable Mention:
KARA N. TOWLES
Prince George High School
Honorable Mention:
BRITTNEY A. TOWNSEND
J.R. Tucker High School
Third Place:
MATTHEW R. ZEDLER
Chesterfield County Mathematics and Science High School
Second Place:
STEPHEN P. SOJKA
Mills E. Godwin High School
First Place:
JOHN B. WILKINS
Central Shenandoah Valley Governor's School

EARTH AND SPACE SCIENCE

Honorable Mention:
JEFFREY S. DAVIDHIZAR
Booker T. Washington Middle School
Honorable Mention:
SHELLY T. DEVEREAUX
Southwest Virginia Governor's School

Honorable Mention:
YOLANDA L. ROBERTS
Chesterfield County Mathematics and Science High School
Third Place:
ANEESH K. VENKAT
New Horizons Governor's School
Second Place:
RAVI A. DEEPAK
Poquoson Middle School
First Place:
JENNIFER E. SLOANE
Atlee High School

ENGINEERING 'A'

Honorable Mention:
SARA K. BURCH & ROBBIE C. JONES
Roanoke Valley Governor's School
Honorable Mention:
KIMBERLY A. EVERETT
Williamsburg Middle School
Honorable Mention:
WILLIAM A. FUCHS
Swanson Middle School
Third Place:
MICHAEL J. FRASER
Swanson Middle School
Second Place:
JOSEPH R. DAVIS
Central Shenandoah Valley Governor's School
First Place:
IAN K. BARNES
Gildersleeve Middle School

ENGINEERING 'B'

Honorable Mention
MICHAEL T. LOTT
Harry F. Byrd Middle School
Honorable Mention:
TRACY P. NG
Blacksburg High School
Honorable Mention:
JONATHAN D. RAMALEY
Gildersleeve Middle School
Third Place:
XIAOMING YIN
Yorktown High School
Second Place:
BART M. JOHNSON & ROBERT D. PEACOCK
Yorktown High School
First Place
DEREK E. SLAUGHTER
Roanoke Valley Governor's School

ENVIRONMENTAL SCIENCE 'A'

Honorable Mention:
MEGHAN L. BERNIER
Chesterfield County Mathematics and Science High School
Honorable Mention:
MICHELLE R. BRIGHT
Cave Spring High School
Honorable Mention:
REBECCA L. DROKE
Yorktown High School
Third Place:
JONATHAN D. CHARLESWORTH
Governor's School for Government and International Studies
Second Place:
JULIANNA M. CONNELLY
Thomas Jefferson High School for Science and Technology
First Place
CURTIS C. COPELAND
Gloucester High School

ENVIRONMENTAL SCIENCE 'B'

Honorable Mention:
STAVROULA K. HATZIOS
Blacksburg High School
Honorable Mention:
LINDSEY A. HERSHNER
Manchester Middle School
Honorable Mention:
ERIN C. LADD
Mills E. Godwin High School
Third Place:
STEPHANIE N. HASSELL
Mills E. Godwin High School
Second Place:
PETER L. GRIMALDI & PATRICK W. RAINEY
Yorktown High School
First Place:
BRENDA N. GOGUEN
Thomas Jefferson High School for Science and Technology

ENVIRONMENTAL SCIENCE 'C'

Honorable Mention:
DEBORAH S. LYU
Central Virginia Governor's School
Honorable Mention:
CLARE D. MCLEAN
Yorktown High School
Honorable Mention:
ANNA R. MOORE
Southwest Virginia Governor's School
Third Place:
KRISTIN A. PEDERSON
Central Shenandoah Valley Governor's School

Second Place:
KIMBERLY A. MEUER
Roanoke Valley Governor's School
First Place:
KATIE MCCOWN
Roanoke Valley Governor's School

ENVIRONMENTAL SCIENCE 'D'

Honorable Mention:
COURTNEY M. SNODGRASS
Massaponax High School
Honorable Mention:
ANDREW R. SPOTTS
Mills E. Godwin High School
Honorable Mention:
MORGAN R. WOOD
Warwick High School
Third Place:
ANNA P. TSUCHITANI
Yorktown High School
Second Place:
BETHANY B. SKELTON
Washington-Lee High School
First Place:
NGOC B. VU
Gloucester High School

GENETICS AND CELLULAR BIOLOGY

Honorable Mention:
BRANCH W. TRENT
Mills E. Godwin High School
Honorable Mention:
JESSICA L. WARNKE & NICOLE M. KESSINGER
Chesterfield County Mathematics and Science High School
Third Place:
DANIEL B. FISHER
Stonewall Jackson Middle School
Second Place:
JINA KIM
Thomas Jefferson High School for Science and Technology
First Place:
MARISSA R. KESSLER
Mills E. Godwin High School

MATHEMATICS AND STATISTICS

Honorable Mention:
MICHAEL B. DEATON
Central Shenandoah Valley Governor's School
Honorable Mention:
CHRISTOPHER U. EMBA
Mills E. Godwin High School
Honorable Mention:
DAVID A. MASSAND & GREG C. NIGHTINGALE
Yorktown High School

Third Place:
BERNARD E. FARLEY
Mills E. Godwin High School
Second Place:
FINALE P. DOSHI
Governor's School for Government and International Studies
First Place:
MONIKA H. SCHLEIER-SMITH
Thomas Jefferson High School for Science and Technology

MEDICINE AND HEALTH 'A'

Honorable Mention:
KATI K. CROUCH
Stonewall Jackson Middle School
Honorable Mention:
HILARY M. HOLMAN
Liberty Middle School
Honorable Mention:
CAITLIN ANDERSON
Moody Middle School
Third Place:
MATTHEW B. ALLEN
The Collegiate School
Second Place:
NOAH B. EISENBERG
Chesterfield County Mathematics and Science High School
First Place:
BENJAMIN D. EASTER
Governor's School for Government and International Studies

MEDICINE AND HEALTH 'B'

Honorable Mention:
ANNY K. KIM
J.R. Tucker High School
Honorable Mention:
SUSAN M. LAMB
Menchville High School
Hnorable Mention:
AMY R. LONG
Cave Spring High School
Third Place:
EDWARD J. MITCHELL
Mills E. Godwin High School
Second Place:
DIANE LEE
Yorktown High School
First Place:
SARAH K. LONGEST
Governor's School for Government and International Studies

MEDICINE AND HEALTH 'C'

Honorable Mention:
LAURA S. SAUVAIN
Manchester Middle School

Honorable Mention:
CHRISTOPHER J. SIONS
Chickahominy Middle School
Hnorable Mention:
MICHAEL D. STRATMOEN
Bishop Denis J. O'Connell High School
Third Place:
DAVID G. WEISZ
Chesterfield County Mathematics and Science High School
Second Place:
MARY K. SPRINKEL
Atlee High School
First Place:
GAURI R. RAVAL
Mills E. Godwin High School

MICROBIOLOGY 'A'

Honorable Mention:
KRISTIN P. BIBEE
Central Virginia Governor's School
Honorable Mention:
COLIN P. FREEMAN
Gildersleeve Middle School
Honorable Mention:
CAITLIN M. GOLD
Yorktown High School
Third Place:
PETER D. ELBAOR
Swanson Middle School
Second Place:
ANNA M. LIGHT
Roanoke Valley Governor's School
First Place:
CHARLES HAWKINS
Wakefield High School

MICROBIOLOGY 'B'

Honorable Mention:
KELLY M. O'BRIANT
Swanson Middle SchoolmicroBHM-1 sch
Honorable Mention:
DONIKA K. PATEL
Southwest Virginia Governor's School
Honorable Mention:
SAMUEL R. PROTICH, AARON K. CECIL & JAMIE R. SMITH-GEORGE
Homer L. Hines Middle School
Third Place:
DONNA N. SIMMONS
Hayfield High School
Second Place:
SARAH M. MYERS
Yorktown High School

First Place:
PRAVEEN K. RAO & GENE M. BERDICHEVSKY
Mills E. Godwin High School

PHYSICS 'A'

Honorable Mention:
GARRETT L. BRADLEY
Lee-Davis High School
Honorable Mention:
DESIREE R. BROOKE
Lee-Davis High School
Honorable Mention:
ALLISON J. BRUCE & MICHAEL A. SWIGERT
Washington-Lee High School
Third Place:
LUKE M. DAVIS
Patrick Henry High School
Second Place:
BENJAMIN P. CRIDER
Lee-Davis High School
First Place:
ANNA K.M. BEST
Patrick Henry High School

PHYSICS 'B'

Honorable Mention:
SYDNI GOULD
Chickahominy Middle School
Honorable Mention:
DANIEL P. HARLAN
Yorktown High School
Honorable Mention:
PETER L. HARLAN
Yorktown High School
Third Place:
ANDREW M.R. HOPKINS
Atlee High School
Second Place:
AFTON P. GROSSMAN
Governor's School for Government and International Studies
First Place:
MEREDITH A. ELLIOTT
Manchester Middle School

PHYSICS 'C'

Honorable Mention:
DANIEL E. JONES
Central Virginia Governor's School
Honorable Mention:
ROBERT B. KOLICK
Yorktown High School
Honorable Mention:
MANA K. OTA
Yorktown High School

Third Place:
JOEL A. MORGAN
Central Virginia Governor's School
Second Place:
ROBERT D. HAGAN
Harry F. Byrd Middle School
First Place:
MATTHEW L. MALLORY
Central Virginia Governor's School

PHYSICS 'D'

Honorable Mention:
NICHOLAS R. SKAPERDAS
Manchester Middle School
Honorable Mention:
MATTHEW E. WALKER
Mills E. Godwin High School
Honorable Mention:
MICHAEL E. WALLACE
Central Virginia Governor's School
Third Place:
JESSICA M. YOUNG
Hayfield High School
Second Place:
THOMAS H. RUSCHER
Roanoke Valley Governor's School
First Place:
JONATHAN H. YOKE
Manchester Middle School

PSYCHOLOGY - GENERAL

Honorable Mention:
SARGON M. DE JESUS
Washington-Lee High School
Honorable Mention:
MARISSA S. HARRIS
Cave Spring High School
Honorable Mention:
JAMES E. VAUGHAN
Tuckahoe Middle School
Third Place:
JUSTIN A. GAYLE
Atlee High School
Second Place:
NIKKI G. SANDERS
Blacksburg High School
First Place:
JERZY A. WIECZOREK
Central Virginia Governor's School

PSYCHOLOGY - LEARNING & PERCEPTION 'A'

Honorable Mention:
THOMAS L. BATEMAN & JONATHAN L. GREEN
Patrick Henry High School

Honorable Mention:
ANDREW L. BECKER
Central Virginia Governor's School
Honorable Mention:
ANNE M. BOWMAN
Governor's School for Government and International Studies
Third Place:
ERIN R. EAHEART & DEANNA M. ARBLE
Mills E. Godwin High School
Second Place:
PAUL N. BUI & J. ALEXANDER MEDITZ
Yorktown High School
First Place:
JOSHUA A. DUNFORD
Central Virginia Governor's School

PSYCHOLOGY - LEARNING & PERCEPTION 'B'

Honorable Mention:
JENNIFER F. LOGAN
Central Virginia Governor's School
Honorable Mention:
MEGHAN E. SPIGLE
Central Shenandoah Valley Governor's School
Honorable Mention:
EAMING WU & VALERIE M. HOANG
Mills E. Godwin High School
Third Place:
CLAIRE E. PAISLEY-JONES
Washington-Lee High School
Second Place:
KRISTIN M. PRZELOMIEC
Central Virginia Governor's School
First Place:
COLLIN D. RILEY
Gloucester High School

PSYCHOLOGY - SOCIAL

Honorable Mention:
ANITA A. BACHLANI
Governor's School for Government and International Studies
Honorable Mention:
EMILY C. OSWALD
James River High School
Honorable Mention:
MARA S. ROSENKRANTZ
Harry F. Byrd Middle School
Third Place:
ZOE K. LUKIC
Ocean Lakes High School
Second Place:
BETHANY B. SKELTON
Washington-Lee High School
First Place:
CARLA S. POOL
Governor's School for Government and International Studies

ZOOLOGY 'A'

Honorable Mention:
TOMMY W. DIERKES
Yorktown High School
Honorable Mention:
ASHLEY L. FOREHAND
Crittenden Middle School
Honorable Mention:
JOHN P. KENNEDY
Governor's School for Government and International Studies
Third Place:
MICHAEL C. DUBAY
Dozier Middle School
Second Place:
JARED A. FISHER
Southwest Virginia Governor's School
First Place:
LINDSEY A. CONKLIN
Yorktown High School

ZOOLOGY 'B'

Honorable Mention:
SARAH E. MERRYMAN
Gloucester High School
Honorable Mention:
LAUREN K. TROTT
Atlee High School
Honorable Mention:
BRYAN C. WITT
Heritage High School
Third Place:
CHIDI J. ONYESO
Mills E. Godwin High School
Second Place:
JACOB P. NEAL
Chesterfield County Mathematics and Science High School
First Place:
NISHEETH PANDEY
Warwick High School

SPECIAL AWARDS

Botany Section Award, given by the Botany Section of the VAS, to the best paper on a botanical subject.($150.00)
LESLIE A. NEELY
Blacksburg High School

VJAS Neuroscience Awards supported by the Virginia Neurological Society and the Auxiliary of the Virginia Neurological Society are given to three outstanding papers in the field of neuroscience $100.00 each).
GAURI RAVAL
Mills E. Godwin High School

Speleological Society Award given to the best paper addressing karst or topics related to speleology given by the Richmond Area Speleological Society. ($200.00)

PETER L. GRIMALDI & PATRICK RAINEY

Yorktown High School

Mathematics and Statistics Award for the paper that evidences the most significant contribution in the field of Mathematics. ($200.00)

MONIKA H. SCHLEIER-SMITH

Thomas Jefferson High School for Science and Technology

Smith Shadomy Infectious Disease Award in honor and memory of Dr. Smith Shadomy given by the Virginia Chapter of the National Foundation of Infectious Diseases. ($50.00)

GAURI RAVAL

Mills E. Godwin High School

Roscoe Hughes Award for the best paper in the field of Genetics. ($150.00)

MARISSA R. KESSLER

Mills E. Godwin High School

Rodney C. Berry Chemistry Award for the paper that evidences the most significant contribution in the field of chemistry.($200.00)

LAUREN CONNOLLEY

Atlee High School

The Dr. and Mrs. Preston H. Leake Award in Applied Chemistry ($200.00) will be given to the author of a research paper which best exemplifies how chemicals, chemical principles, or chemistry have been used, are used, or might be used to enhance or even to save life. ($200.00)

ROBERT FISHER

Governor's School for Government and International Studies

Russell J. Rowlett Award for the Best Research Paper of the Year.($300.00)

CURTIS COPELAND

Gloucester High School

The Virginia Psychological Foundation Meritorious Research Awards recognize outstanding presentations of research in the various fields of psychology. $100.00

JERZY A. WIECZOREK

Central Virginia Governor's School

JOSHUA A. DUNFORD

Central Virginia Governor's School

COLLIN D. RILEY

Gloucester High School

CARLA S. POOL

Governor's School for Government and International Studies

Virginia Sea Grant College Program Award is given by the Virginia Sea Grant College Program for outstanding marine or coastal research. ($100.00)

CURTIS COPELAND

Gloucester High School

American Cancer Society Award - This award is to recognize outstanding science papers related to cancer research. A certificate to each and to 1st place - $500, 2nd place $300, 3rd place $125, and honorable mention $75. There will be a ribbon with a pin for each winner. These awards are provided by the American Cancer Society (Virginia Council).

Honorable Mention - ($75)
PETER L. GRIMALDI & PATRICK RAINEY
Yorktown High School
Second Place - ($300)
KELLY M. O'BRIANT
Swanson Middle School
First Place - ($500)
GAURI RAVAL
Mills E. Godwin High School

The Gamma Sigma Delta Award (Agriculture). Presented by the VPI & SU Chapter of the Honor Society of Agriculture. This award is presented in recognition of excellence in research dealing with application of new technologies and/or concepts in agriculture forestry, or veterinary medicine. ($100.00)

ZACHARY J. CAPPELLO
Stonewall Jackson Middle School

Dominion - W.W. Berry Award. This award is given by Dominion Virginia Power in honor of Mr. W. W. Berry who was a past Chairman of the Board of VA Power. This award of a $500.00 Savings Bond will be presented to the best engineering paper.

DEREK E. SLAUGHTER
Roanoke Valley Governor's School

The Joyce K. Peterson Award is presented for the outstanding paper by a middle school student. It is presented in honor of Mrs. Joyce K. Peterson who has been an outstanding teacher in the Arlington County Schools. ($50.00)

KELLY O'BRIANT
Swanson Middle School

The Ann M. Hancock Award - ($500 - 1999, 2000, 2001) This award is given to the best paper in genetics and is given in memory of Anne Hancock who retired from Patrick Henry High School in Hanover County and who gave many years of service to the Jr. Academy not only by teaching but also serving on the Jr. Academy Committee.

MARISSA KESSLER
Mills Godwin High School

The Dorothy Knowlton Award - ($50)

KIRSTEN STUBER
Patrick Henry High School

VABE Award in Zoology - On behalf of the Virginia Association for Biological Education, we would like to present this award to you as a Zoology Section winner. VJAS students such as you represent the future of Biology in Virginia.

LINDSAY A. CONKLIN
Yorktown High School

The Office of Naval Research Naval Science Award - Congratulations on winning the U.S. Navy and Marine Corps Distinguished Achievement Award at VJAS this year. The Office of Naval Research (ONR) is the organization that sponsors scientific research for the Navy and Marine Corps. ONR is proud to recognize your achievement and encourage your continuing interest in the sciences and engineering.

FINALE P. DOSHI
Governor's School for Government and International Studies
ALEX GRIFFITH & SCOTT C. HENRY
Mills E. Godwin High School
BRENNAN P. LOWERY
Gildersleeve Middle School
ERIC V. MAI
Gildersleeve Middle School

Trip to AJAS - AAAS Meeting for two students and two alternates for presenting outstanding papers. The 1999 meeting will be held in Boston.

Winner:
CURTIS C. COPELAND
Gloucester High School
Winner:
MARISSA R. KESSLER
Mills E. Godwin High School
Alternate:
ANNA K.M. BEST
Patrick Henry High School
Alternate:
ACHARY J. CAPPELLO
Stonewall Jackson Middle School

Honorary Membership - AAAS given to two students.

ALEX GRIFFITH
Mills Godwin High School
JESSICA HAWTHORNE
Hermitage High School

Honorary Membership - VAS given to a student.

KASHAUNA GILL
Atlee High School

Bethel High School Scholarship - This $1,000 Scholarship Award comes from the interest earned rom a $10,000 endowment contributed by the students of Bethel High School, Hampton, VA, over two year period. This award is based on both the students presentation and paper.

TRICIA L. LOBO
Berkeley Middle School

Frances and Sydney Lewis Environmental Scholarship: A $14,000 scholarship ($3,500 per year for our years) for the best effort by a student in grades 9 to 12 in the field of environmental science. This cholarship is in the name of Frances and Sydney Lewis and is given by the Virginia Environmental ndowment.

NGOC VU
Gloucester High School

E.C.L. Miller Science Teacher of the Year Award is given to an outstanding science teacher. An ll-expense-paid trip to next year's AAAS which will be in Boston

PAMELA GENTRY
Patrick Henry High School

VJAS Distinguished Service Award, most prestigious award given by the VJAS, is presented to a person for exceptionally outstanding service to the VJAS.

Dr. D. Rae Carpenter, Jr.
VMI, Retired

Actions of the VAS Research Committee for 2000-2001

J. Shelton Horsley Research Award

The Horsley Award was not given in 2000-2001 because no papers were submitted for consideration.

Horsley Cancer Research Fund Grants

A total of $4,000.00 in funding was recommended.
In December, 2000, the Research Committee of the Virginia Academy of Science recommended the funding of the following new proposal by the Horsley Cancer Research Fund provided by the trust of Mary Louise Old Andrews.

Lizabeth A. Allison, Ph. D., Department of Biology, College of William and Mary. $4000.00. "Ontogeny of an oncogene: Oncogenic conversion of the thyroid hormone receptor"

Small Project Research Funds

A total of nine proposals were funded out of a total of 12 proposals submitted, for a total of $9,500.00. $ 2,500.00 was retained to be awarded in the new undergraduate research grant program. In May, 2001, the Research Committee of the Virginia Academy of Science recommended the funding of the following proposals by the Virginia Academy of Science.

E. F. Benfield, Dept. of Biology, Virginia Polytechnic Institute and State University was awarded $1250.00 for his proposal titled " Quantifying nitrogen spiraling in anthropogenically enriched stream ecosystems" (Proposal Number 01-1794-03).

Jennifer A. Clevinger, Dept. of Biology, James Madison University was awarded $1244.00 for her proposal titled " Phylogenetic analysis of the *Silphium asteriscus* complex (Asteraceae) using intersimple sequence repeat markers (ISSR)".

Daniel Cristol, Dept. of Biology, College of William and Mary, was awarded $1156.00 for his proposal titled " Are Virginia's created saltmarshes valuable replacement habitat for wetland birds?"

Can M. Eristi and Charles L. Rutherford, Dept. of Biology, Virginia Polytechnic Institute and State University were awarded $1250.00 for their proposal titled " Analysis of transcriptional regulation of the 5'nucleotidase gene in *Dictyostelium*".

Mundy Hackett and John F. Pagels, Dept. of Biology, Virginia Commonwealth University, were awarded $603.70 for their proposal titled "The northern flying squirrel (*Glaucomys sabrinus*), an endangered Pleistocene relict in southwestern Virginia" (VCU proposal number 01-1045-00).

Kristi Niehaus and Khidir Hilu, Dept. of Biology, Virginia Polytechnic Institute and State University, were awarded $1250.00 for their proposal titled " A study of speciation in *Nymphaea odorata* based on molecular, morphological, and ecological information".

Erik T. Nilson, Dept. of Biology, Virginia Polytechnic Institute and State University was awarded $1178.00 for his proposal titled " Analysis of interference between invasive *Ailanthus altissima* (Miller) Swingle and native *Robinia pseudoacacia* L. ".

Timothy W. Stewart, Dept. of Natural Sciences, Longwood College, was awarded $568.30 for his proposal titled " A demographic study of the snail, *Leptoxis carinata*, in a tributary of the Appomattox River: effects of physicochemical features on densities, biomass, and body size".

Roy L. Williams, Dept. of Chemistry and Biochemistry, Old Dominion University , was awarded $1000.00 for his proposal titled " Black goo: A grapevine trunk disease that is infecting the Virginia vineyards".

AUTHOR INDEX

Notes

Notes

VIRGINIA ACADEMY OF SCIENCE

APPLICATION FOR MEMBERSHIP

Name (Please Print) ______________________

Phone (   ) ____________ FAX(   ) ____________

E-mail ______________________

Address ______________________

City ____________ State ________ Zip ________

Institution or Business ______________________

Position -- Title ______________________

Interest -- Section No.(s) ________ First no. indicates major interest

Contacted by: ______________________

Make check payable to Virginia Academy of Science and send to: Virginia Academy of Science, 2500 W. Broad St., Richmond, VA 23220-2054.

Membership in the Academy is organized into sections representing various scientific disciplines as follows:

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4. Biology
5. Chemistry
6. Materials Sciences
7. Biomedical & General Engineering
8. Geology
9. Medical Sciences
10. Psychology
11. Education
12. Statistics
13. Aeronautical & Aerospace Sciences
14. Botany
15. Environmental Science
16. Archaeology
17. Computer Science
18. Geography
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Mammals of Fort A. P. Hill, Caroline County, Virginia and Vicinity

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ABSTRACT

Fort A.P. Hill (APH) is a 30,329 ha military training installation (U.S. Army) located in the upper Coastal Plain of Caroline County, Virginia. It was formed in 1941 and named in honor of Civil War Confederate Lt. General Ambrose Powell Hill. The current landscape includes a mosaic of habitats that range from old fields to hardwood forests. Forty species of mammals are known to exist on or near the installation. These include one marsupial, five insectivores, 9 chiropterans, one lagomorph, 12 rodents, 10 carnivores, and one cervid. We have studied many of the species on APH since 1997. In this paper we describe the physical environment of the area and 7 important habitats used by mammals. We also summarize the ecology and natural history of each species and provide statistical summaries of original measurements from mammals caught on the installation. The results of several recent studies on APH allow us to describe habitat affiliations and relative abundance of most of the mammals native to the mid-Atlantic region. Old fields and clearcuts support a total of 20 species, including several found predominately in this habitat. Pine stands and pine plantations support the fewest number of mammal species (17) of any habitat on the installation. Mixed pine and hardwood forests, hardwood forests, and riparian forests support the largest number of species (29-36). With the possible exception of pine plantations, the habitat mosaic found on APH provides abundant resources for mammal communities. We also include an evaluation of age and health attributes of the deer population and describe the hunting program on the base. Number of deer harvested annually 1985-2000 varied from 460 to 1765. Management activities since 1996 when the deer population exceeded carrying capacity have improved herd health. Because much of Caroline County and eastern Virginia is in extensive agriculture and the remaining hardwood forests are

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being clearcut, APH is becoming a valuable habitat island for the mammalian fauna of the upper Coastal Plain of Virginia and the mid-Atlantic region.

Key Words: Biotic Communities, Coastal Plain, Ecology, Hunting, Life History, Mammals, Management, Military, Natural History, Virginia.

INTRODUCTION

Mammals in the mid-Atlantic region of North America play key functional roles in terrestrial and freshwater ecosystems. They act as predators, prey, and consumers of vegetation and thus regulate many aspects of these ecosystems, including affecting the structure of forest and stream habitats and population regulation of some small vertebrates. Some are conspicuous and well known to most people, and others are secretive and only detected with specialized inventory techniques. Declines of populations of some mammal species can have serious effects on many other animals. Some mammals are part of the human food chain and are important economically. Active management is mandatory for some of these species, or else they become so overpopulated that they alter habitats and interfere with human endeavors. The many ecological and economic roles played by mammals create the need to know which species occur in an area of interest and how they may be managed effectively.

Many species of mammals interact with humans directly or indirectly. Some, like white-tailed deer and gray squirrels, are designated as game animals and their harvests are regulated. Others, like raccoon, mink, muskrat, and beaver, are harvested for their fur. Woodchucks and beavers indirectly influence humans because of their habitat-altering behaviors. For example, beaver activity can create and maintain of certain types of wetlands that support a wide diversity of plants and animals (Naiman et al., 1988; 1994). Beaver ponds are used extensively by amphibians and reptiles (Mitchell, 1994; 2000) and by waterfowl, such as wood ducks (*Aix sponsa*), black ducks (*Anas rubripes*), and mallards (*Anas platyrhynchos*) (Merendino et al., 1995). Some mammal predators help to control insect pests and mammal pests, such as some rodents (Godin, 1982; Samuel and Nelson, 1982; Toweill and Tabor, 1982). Diseases such as rabies can be carried by any mammal and are potentially fatal to humans. Lyme disease transmitted by ticks carried on white-tailed deer and white-footed mice is a growing human health concern. The large number of people engaging in outdoor recreation has increased the value of many mammals as watchable wildlife. Thus, the aesthetic, economic, and ecological values of mammalian fauna the region cannot be underestimated.

Removal of old-growth stands during the 19th century and the beginning of the 20th century, and human activities since then, have transformed the landscape of the mid-Atlantic region into a mosaic of habitat types (Handley, 1992; Pagels et al., 1992; Sharitz et al., 1992). Because short-term and long-term disturbances played roles in the evolution of temperate mammals, the mosaic is advantageous for many species (Kirkland, 1988, 1990). In addition to anthropogenic activities, short-term factors, such as fire, wind storms, and floods have long reshaped the landscape creating ever-changing mosaics of successional habitats (Sharitz et al., 1992). Pleistocene glaciations brought on long-term shifts in climate and vegetation assemblages (Kirkland, 1990) and the concomitant introduction of many northern species to what is now Virginia

(Handley, 1992). The present statewide mammal community represents a mix of boreal and indigenous austral species (Handley, 1992).

Following Linzey's (1998) summary, 78 mammal species, exclusive of marine and domesticated species, occur in Virginia. They represent 8 orders, 18 families and 50 genera. For comparison, 40 species are known or suspected to occur on APH representing 7 orders, 14 families, and 31 genera. No federal or state threatened or endangered mammal species are known to occur on the base or in its vicinity.

The United States Department of Defense is the fifth largest landowner of the federal landholding agencies and currently manages over 25 million acres of land (>10.1 million ha) (Boice, 1997). Most of the land is contained in military installations that have large areas protected from activities that adversely affect local flora and fauna. As a result, military installations such as APH serve as islands of biodiversity (Mitchell and Roble, 1998) for native species and provide opportunities to study their ecologies and distributions. Such opportunities are becoming increasingly important as land-use practices on surrounding properties become more complex.

In this paper we summarize our observations on the ecology and natural history of the mammals of APH. We provide new data on morphometrics for many of the species and an assessment of their habitat affinities and relative abundance. We also summarize the literature pertinent to the mammals of the mid-Atlantic Coastal Plain.

FORT A.P. HILL AND ITS ENVIRONMENT

Fort A.P. Hill Military Reservation (APH), Caroline County, Virginia is located in the upper Coastal Plain Physiographic Province (Fig. 1). Northern and southern boundaries of APH are located at 38^0 12' 46" and 38^0 01' 5.6" N latitude, and eastern and western boundaries are at 77^0 08' 13" and 77^0 23' 11" W longitude, respectively. APH includes 30,329 ha, mostly in Caroline County, with fewer than 41 ha in Essex County. U.S. Route 301 divides the base into north and south sections (Fig. 1).

APH was established for military training in June 1941 and named in honor of Civil War Confederate Lieutenant General Ambrose Powell Hill. Lands available for direct access training and where our surveys were conducted are divided into 30 Training Areas; most are north of U.S. Rt. 301. An additional 28 Controlled Access Areas occur south of U.S. Rt. 301 around the periphery of the Impact Area. The Impact Area receives live ordnance from small arms, light artillery, and helicopter firing exercises. Controlled Access areas may be opened to hunters and other persons on a limited basis but few people enter the active Impact Area due to potential hazards of unexploded ordnance. Thus, much land south of U.S. Rt. 301 is unmanaged with restricted access by humans. Most of APH is now used for infantry-related training activities. The Controlled Access Areas and the Impact Area were off-limits to our field research. APH has hosted the Boy Scouts of America National Jamboree every four years since 1981.

Drainages

Two drainages divide APH into two watersheds. The Mattaponi River watershed includes about a third of the base in the western and southwestern portions of the installation. The rolling topography in the watershed includes the highest point on APH (76 m [249 ft]). Tributaries of the Mattaponi River on APH drain to the south and southwest. The remainder of APH is drained by the Rappahannock River. Tributaries

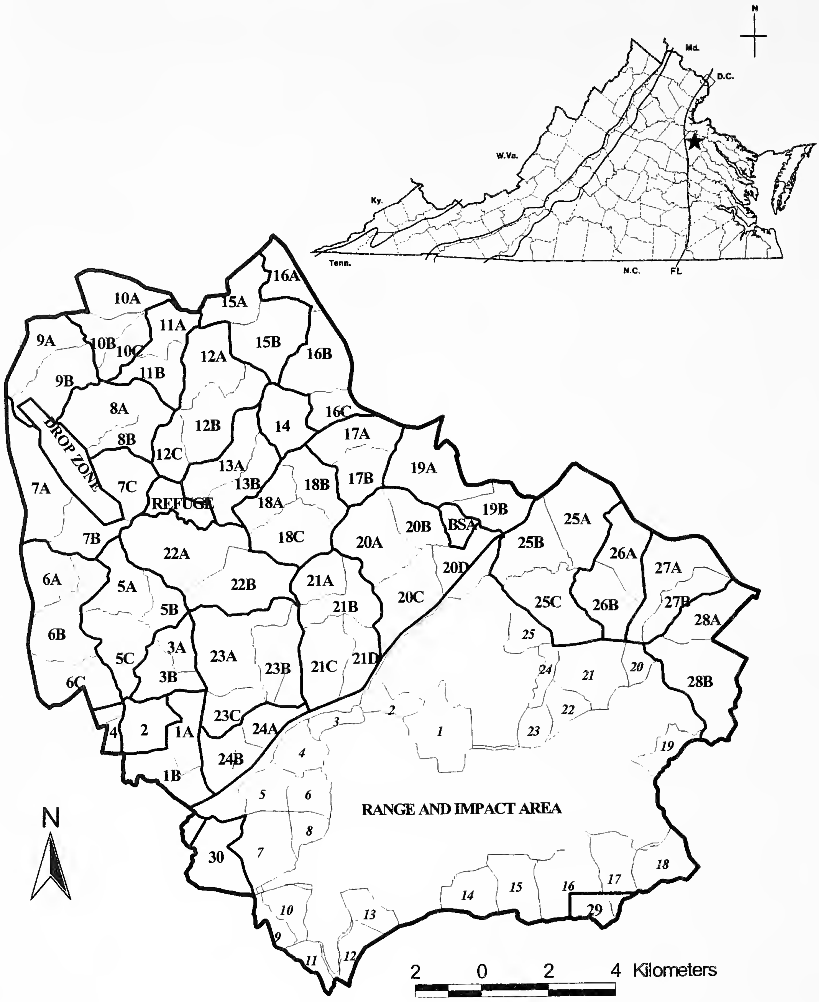

FIGURE 1. Map of Fort A. P. Hill, Caroline County, Virginia, illustrating major roads, training areas (large numerals), and controlled access areas (small italicized numerals). Boundaries of Virginia's physiographic regions are indicated on the insert map (FL = fall line).

of the Rappahannock drain to the northeast. Some of the topographic relief in this area is steep, especially around Devil's Bottom where highest and lowest elevations are separated by 46 m (151 ft).

Climate

Climate of APH and vicinity is humid subtropical and similar to the rest of Virginia east of the Blue Ridge Mountains (Woodward and Hoffman, 1991). Summers are warm to hot (maximum temperature reaches 38.3 C) and winters are cool to cold (minimum temperature reaches –18.3 C) (1997-1999, NOAA) (Fig. 2). Temperature extremes are ameliorated by the proximity of APH to the Chesapeake Bay.

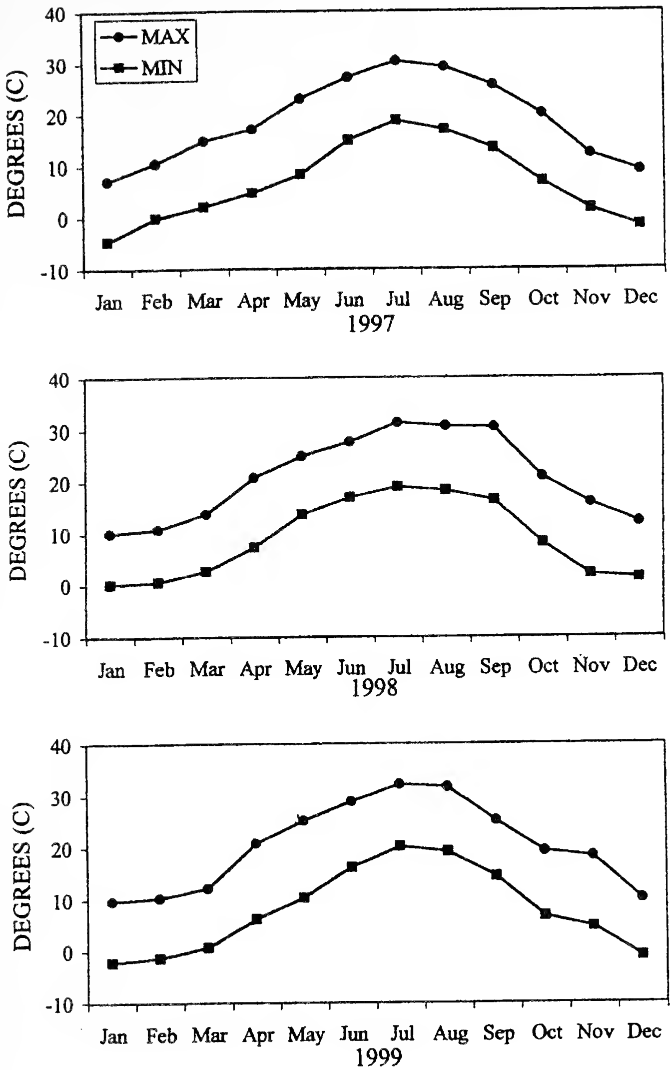

FIGURE 2. Average minimum and maximum monthly temperatures (°C) for 1997 through 1999 for Corbin, Virginia (U.S. Route 17, adjacent to training area 9a) (from NOAA Climatological Data).

Precipitation is usually in the form of rain but some snow accumulates during winter months. Average annual precipitation is 108.2 cm (42.6 inches) (based on 30-yr average, 1961-1990, NOAA). Monthly precipitation is generally higher in winter and spring than in summer and fall (Fig. 3). Droughts occasionally affect surface moisture. A drought in 1999 peaked in May with only 1.3 cm (0.5 inches) of rainfall, and continued through August. Precipitation in September 1999 from hurricanes Dennis

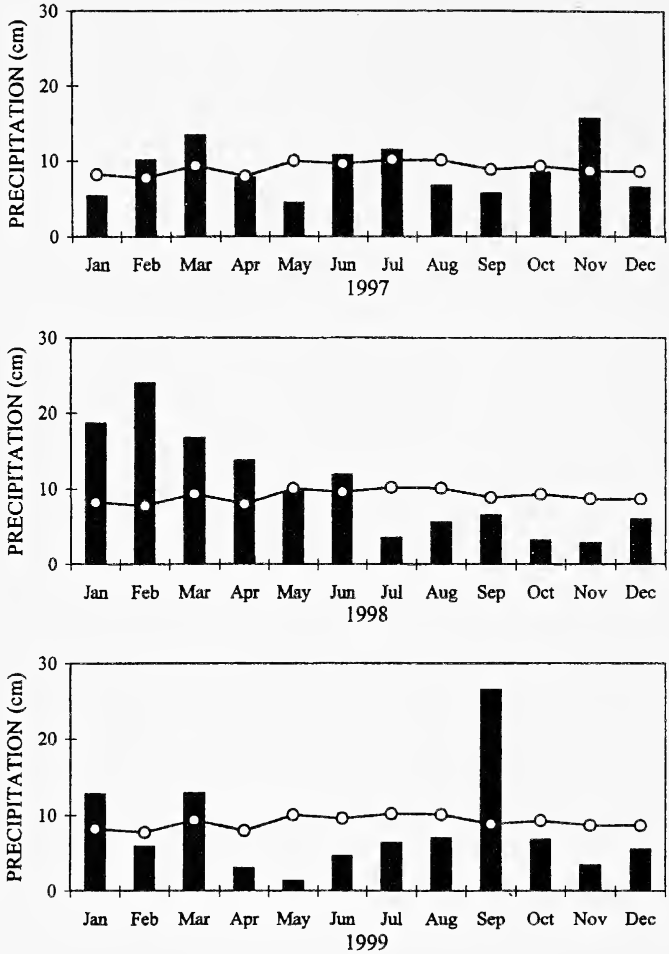

FIGURE 3. Average monthly precipitation (cm) for 1997 through 1999 for Corbin, Virginia (U.S. Route 17, adjacent to training area 9a). Thirty-yr averages (1961-1990) are indicated by the line. (taken from NOAA Climatological Data).

and Floyd replenished many ponds and vernal pools that had become dry. Total rainfall that month was 26.5 cm (10.4 inches). Such events modify the average monthly precipitation pattern illustrated in Fig. 3.

Habitats

Prior to 1941 the lands of APH were used primarily for farming and timber harvesting. APH is currently about 80% forested from natural re-growth and forestry management. The present landscape of APH is comprised of grasslands, pine forests, mixed pine and hardwood forests, and hardwood forests, all in various stages of succession. Management has converted some of the pine and hardwood forests into loblolly pine (*Pinus taeda*) plantations. Riparian forests usually support more diverse flora and are more corridor-like than adjacent upland forests. Some grasslands are kept open by mowing operations and prescribed burns, usually for military training purposes, and, for example, wildlife food plots. Aquatic habitats include large to small impoundments, beaver ponds, vernal pools, streams, and seepages. For general description of plant communities and associated mammal communities, the habitats of APH can be grouped into 7 general habitat types.

Old fields

Perennial grasses, notably broomsedge (*Andropogon virginicus*) and redtop (*Agrostis alba*), characterize old-field habitats (Fig. 4). Other common herbs in old-fields are bushclover (*Lespedeza cuneata*), yarrow (*Achillea millefolium*), spotted knapweed (*Centaurea maculosa*), goldenrod (*Solidago* spp.), thistle (*Cirsium* spp.), and partridge pea (*Chamaecrista fasciculata*). Common vines include Japanese honeysuckle (*Lonicera japonica*), common greenbrier (*Smilax rotundifolia*), bullbrier (*Smilax bona-nox*), Virginia creeper (*Parthenocissus quinquefolia*), and less often, poison ivy (*Rhus radicans*). Bramble (*Rubus* spp.) was abundant in more mature old fields, often forming thick mats that provide important cover for small mammals. An exotic, Russian olive (*Elaeagnus augustifolia*), was the most abundant shrub found in old-field habitats on APH. Young *Pinus taeda* was the most common tree species found in this habitat, but winged sumac (*Rhus copallina*), persimmon (*Diospyros virginiana*), and saplings (e.g., oak [*Quercus* spp.], sweetgum [*Liquidambar styraciflua*], black cherry [*Prunus serotina*]) were also frequent and abundant inhabitants. Early successional stages (grasslands) were abundant under maintained (mowed regularly) and unmaintained (unmowed) management programs. Some of the latter are managed extensively by burning and disking to provide wildlife food plots.

Pine Forests

Virginia (scrub) pine (*Pinus virginiana*), was the primary tree in naturally occurring pine forests, however, in many locations *P. taeda* was also abundant (Fig. 5). Understory trees were generally a mix of saplings of overstory species (e. g., pines [*Quercus* spp.], *Liquidambar styraciflua*, *Fagus grandifolia*) and understory species like American holly (*Ilex opaca*) and less frequently flowering dogwood (*Cornus florida*) and sassafras (*Sassafras albidum*). Common shrubs in these habitats were blueberry (*Vaccinium* spp.) and huckleberry (*Galussacia* spp.). *Lonicera japonica* and *Smilax rotundifolia* were the most common vines in the pine forests. Except for partridgeberry (*Mitchella repens*), ground-level herbs were sparse in pine forests.

Pine Plantations

Pine plantations were almost all comprised of *Pinus taeda*. Recent plantings are usually thick and impenetrable (Fig. 6) but older stands that have been thinned mechanically or naturally have an open understory and a carpet of pine needles (Fig. 7). Trees associated with these plantations included sapling *Quercus* spp., hickories (*Carya* spp.), tulip-tree (*Liriodendron tulipifera*), *Liquidambar styraciflua*, and understory species such as *Ilex opaca*. Vines sometimes common in pine plantations included *Lonicera japonica* and *Smilax rotundifolia*. Ground-level herbs were scarce.

Mixed Pine and Hardwood Forests

Mixed pine and hardwood communities dominated the forest types on APH (Fig. 8). Pines (*Pinus taeda* and *P. virginiana*), southern and northern red oaks (*Quercus falcata* and *Q. rubra*), *Liquidambar styraciflua*, red maple (*Acer rubrum*), *Liriodendron tulipifera*, and princess tree (*Ailanthus altissima*) generally comprised the canopy. Saplings of overstory tree species, especially hardwood saplings, were abundant in older stands, but *Ilex opaca* was usually the dominant understory tree species in this habitat, with *Cornus florida* less common. Vine and shrub communities were similar to those found in naturally occurring pine stands. Common herbs included *Mitchella repens*, spotted wintergreen (*Chimaphila maculata*), and hog-peanut (*Amphicarpa bracteata*).

Hardwood Forests

Two tree species, white oak (*Quercus alba*) and *Liriodendron tulipifera* (Fig. 9), dominated hardwood forest communities on APH. Less frequently the dominant tree species was *Acer rubrum*, *Liquidambar styraciflua*, or American beech (*Fagus grandifolia*). More mature upland stands tended to be dominated by *Q. alba* or *F. grandifolia* and younger upland stands by *L. tulipifera* or *L. styraciflua*. Mesic stands were often dominated by *A. rubrum*. Other important overstory tree species are pignut hickory (*Carya glabra*), mockernut hickory (*C. tomentosa*), *Q. rubra*, *Q. falcata*, and black gum (*Nyssa sylvatica*). Understory tree species in hardwood forest communities were often comprised of saplings of overstory species. Other understory tree species included *Ilex opaca*, *Cornus florida*, and less frequently ironwood (*Carpinus caroliniana*). Shrub communities were primarily comprised of *Vaccinium* spp. and *Gaylussacia* spp., which in general were less abundant in older stands. Vines were less frequent in this habitat than other habitats, but common species included *Lonicera japonica*, *Smilax rotundifolia*, *S. bona-nox*, *Parthenocissus quinquefolia*, *Rhus radicans*, and less frequently groundnut (*Apios americana*). Common ground-level herbs included *Mitchella repens*, *Chimophia maculata*, *Amphicarpa bracteata*, tree clubmoss (*Lycopodium obscurum*), ebony spleenwort (*Asplenium platyneuron*), and Christmas fern (*Polystichum acrostichoides*).

Riparian Corridors

Overstory trees that occurred in these corridors were primarily hardwood species (Fig. 10). Several overstory tree species commonly observed in the riparian corridors (e.g., *Acer rubrum*, river birch [*Betula nigra*], sycamore [*Platanus occidentalis*], and *Nyssa sylvatica*) were classified as facultative wetland species in this region by Reed (1988). Other common overstory trees in riparian corridors (e.g., *Quercus alba*, chestnut oak [*Q. prinus*], and *Fagus grandifolia*) were classified as facultative upland species in this region. *Ilex opaca*, *Cornus florida*, *Carpinus caroliniana*, and sweetbay (*Magnolia virginiana*), and saplings of overstory trees comprised the understory tree community. Common shrubs in APH wetland habitats were *Vaccinium* spp., *Gaylussacia* spp., coast pepperbush (*Clethra alnifolia*), and spicebush (*Lindera benzoin*). Common forbs included obligatory wetland species (e. g., royal fern [*Osmunda regalis*], Lizard's tail [*Saururus cernuus*], skunk cabbage [*Symplocarpus foetidus*], golden club [*Orontium aquaticum*], and broad-leaved arrowhead [*Sagittaria latifolia*]). Facultative wetland forb species common in riparian corridors were cinnamon fern

(*Osmunda cinnamomea*), netted chain fern (*Woodwardia areolata*), sensitive fern (*Onoclea sensibilis*), and false nettle (*Boehmeria cylindrica*). *Mitchella repens* was the only facultative upland forb we observed in riparian corridors at APH.

Wetlands

Tributaries of the Mattaponi River in APH tended to be broad and shallow with a slow flow rate, whereas those of the Rappahannock River tended to be narrow, deeper, and faster. Exceptions were the marshes and ponds created by beaver activity (Fig. 11). Aquatic vegetation was well established in the marshes, ponds, and floodplains associated with slow streams. Yellow pond lily (*Nuphar variegatum*), golden club, swollen bladderwort (*Utricularia inflata*), fragrant water lily (*Nymphaea odorata*), pickerelweed (*Pontederia cordata*), arrow arum (*Peltandra virginica*), and arrowhead (*Sagittaria latifolia*) were common emergent vegetation in most ponds. Cattails (genus *Typha*) were less commonly observed. Impoundments usually lacked emergent vegetation except along shallow margins or in the upstream portion of the drainage where *N. variegatum* was common. There are 19 named impoundments on APH and most are stocked with game fish. Other often temporary wetlands on APH include naturally acidic vernal pools in hardwood forests, water-filled depressions in old fields, and road ruts and depressions.

Structures

There are relatively few buildings on APH and most occur in clusters. They vary from old structures of World War II vintage to modern brick buildings that serve as offices and barracks. Occupied buildings have not been surveyed for mammals but undoubtedly some species use them for shelter.

TAXONOMIC CHECKLIST OF THE MAMMALS OF APH, VIRGINIA

Class Mammalia
- Order Didelphimorphia (Marsupialia)
  - Family Didelphidae
    - *Didelphis virginiana* (Kerr)—Virginia opossum
- Order Insectivora
  - Family Soricidae
    - *Blarina brevicauda* (Say)—northern short-tailed shrew
    - *Cryptotis parva* (Say)—least shrew
    - *Sorex hoyi* (Baird)—pygmy shrew
    - *Sorex longirostris* (Bachman)—southeastern shrew
  - Family Talpidae
    - *Condylura cristata* (Linnaeus)—star-nosed mole
    - *Scalopus aquaticus* (Linnaeus)—eastern mole
- Order Chiroptera
  - Family Vespertilionidae
    - *Eptesicus fuscus* (Palisot de Beauvois)—big brown bat
    - *Lasionycteris noctivagans* (LeConte)—silver-haired bat
    - *Lasiurus borealis* (Müller)—eastern red bat
    - *Lasiurus cinereus* (Beauvois)—hoary bat
    - *Myotis austroriparius* (Rhoads)—southeastern myotis

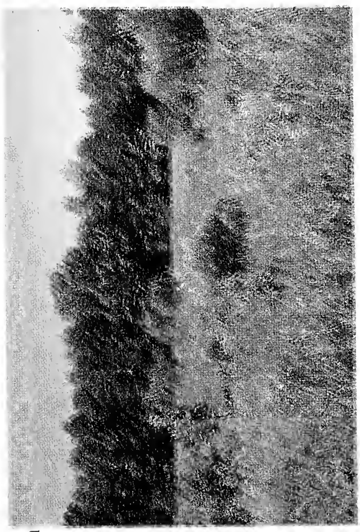

FIGURE 4. Example of an old-field habitat on Fort on Fort A. P. Hill. (Photo by A. S.Bellows)

FIGURE 5. Example of a pine forest habitat on Fort A. P. Hill. (Photo by J. C. Mitchell)

FIGURE 6. Example of a young pine plantation on Fort A. P. Hill. (Photo by A.S. Bellows)

FIGURE 7. Example of an older pine plantation on Fort A. P. Hill. (Photo by A. S. Bellows)

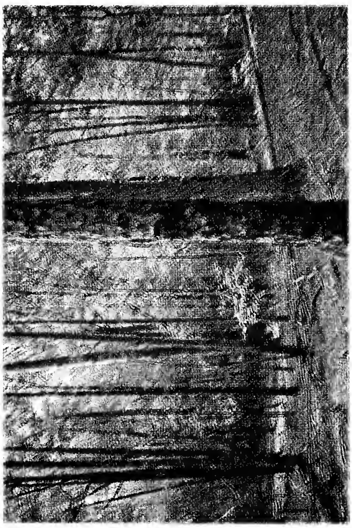

FIGURE 8. Example of a mixed hardwood-pine forest on Fort A. P. Hill. (Photo by A. S. Bellows)

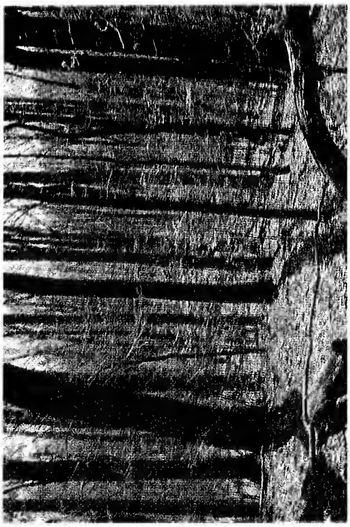

FIGURE 9. Example of a hardwood forest on Fort A. P. Hill. (Photo by J. C. Mitchell)

FIGURE 10. Example of a riparian corridor on Fort A. P. Hill. (Photo by A. S. Bellows)

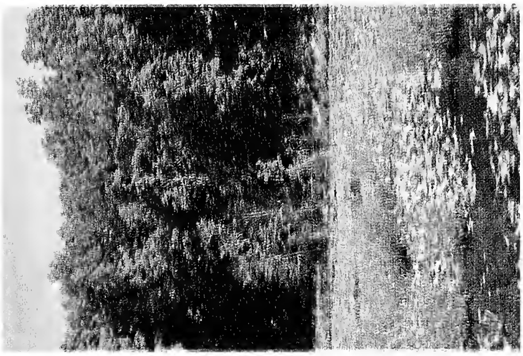

FIGURE 11. Example of a wetland (beaver pond) on Fort A. P. Hill (Photo by A. S. Bellows)

Myotis lucifugus (LeConte)—little brown myotis
Myotis septentrionalis (Trouessart)—northern myotis
Nycticeius humeralis (Rafinesque)—evening bat
Pipistrellus subflavus (F. Cuvier)—eastern pipistrelle

Order Lagomorpha
Family Leporidae
Sylvilagus floridanus (Allen)—eastern cottontail

Order Rodentia
Family Sciuridae
Glaucomys volans (Linnaeus)—southern flying squirrel
Marmota monax (Linnaeus)—woodchuck
Sciurus carolinensis (Gmelin)—gray squirrel
Tamias striatus (Linnaeus)—eastern chipmunk

Family Castoridae
Castor canadensis (Kuhl)—beaver

Family Muridae
Microtus pennsylvanicus (Ord)—meadow vole
Microtus pinetorum (LeConte)—woodland vole
Mus musculus (Linnaeus)—house mouse (introduced)
Ondatra zibethicus (Linnaeus)—muskrat
Oryzomys palustris (Harlan)—marsh rice rat
Peromyscus leucopus (Rafinesque)—white-footed mouse
Rattus norvegicus (Linnaeus)—Norway rat (introduced)
Rattus rattus (Linnaeus)—black rat (introduced)
Reithrodontomys humulis (Audubon and Bachman)
eastern harvest mouse

Family Zapodidae
Zapus hudsonius (Zimmermann)—meadow jumping mouse

Order Carnivora
Family Canidae
Canis latrans (Say)—coyote
Urocyon cinereoargenteus (Baird)—gray fox
Vulpes vulpes (Linnaeus)—red fox

Family Procyonidae
Procyon lotor (Linnaeus)—raccoon

Family Mustelidae
Lontra canadensis (Schreber)—river otter
Mephitis mephitis (Schreber)—striped skunk
Mustela frenata (Lichtenstein)—long-tailed weasel
Mustela nivalis (Linnaeus)—least weasel
Mustela vison (Schreber)—mink

Family Felidae
Lynx rufus (Schreber)—bobcat

Order Artiodactyla
Family Cervidae
Odocoileus virginianus (Rafinesque)—white-tailed deer

SPECIES ACCOUNTS

INTRODUCTION

The following provides a brief account for all 40 mammal species that occur on APH. Although some species were not captured in our study (e.g., long-tailed weasel, *Mustela frenata*), published records (e.g., Handley and Patton, 1947, and others) indicate that each species should be present on APH. These accounts provide basic descriptions and diagnostic characteristics that aid field identification. Morphometric measurements are presented in a consistent format. Dental formulae should be interpreted as follows: (i.) incisors, (c.) canines, (p.) premolars, and (m.) molars; upper jaw dentition is presented over lower jaw dentition (i.e., 3/3). Whenever possible, measurements provided are from animals collected on APH. For these data, we provide minimum and maximum values with means (avg.) ±1 standard deviation in parentheses. When local data were not available, measurements were included from populations as near to APH as possible. In some instances where sample size from APH was small, we provided measurements from other regional populations for comparison. We note the geographic distribution of each species, as well as the subspecies present at APH. Order of species accounts follows the taxonomic checklist.

Order Didelphimorphia (Marsupialia)

Didelphis virginana Kerr (Virginia Opossum)

The Virginia opossum is North America's only marsupial. It is a relatively recent arrival, not appearing in North America until the late Pleistocene (Whitaker and Hamilton, 1998). Thick underfur, white basally and black at the end, and long white guard hairs give the pelage a grizzled appearance. The tail is prehensile and is used for grasping and carrying various items including nesting materials (Pagels, 1996). Weights of 9 adults captured on APH ranged from 1.6 to 3.2 kg (avg.=2.0 ±0.4). Means and ranges of measurements for 10 adults from Indiana, New York, North Carolina, and Pennsylvania were: total length 686-836 mm (avg.=767); tail length 290-348 mm (avg.=321); length of hind foot 60-74 mm (avg.=68.5) (Whitaker and Hamilton, 1998). Dentition: i. 5/4, c. 1/1, p. 3/3, m. 4/4 =50 (Gardner, 1982).

The range of the Virginia opossum extends northward into southern Ontario and British Columbia, west to the central Great Plains, and southward to Costa Rica, except central and northern Mexico (Hall, 1981). All populations within our region are the subspecies *D. v. virginiana* (Cothran et al., 1991).

Virginia opossums are not particularly selective about habitat, but streams associated with deciduous forests appear to be preferred. This habitat generalist has been observed in marshlands and a variety of forest, grassland, suburban, and agricultural habitats (Gardner, 1982; Hallett et al., 1991). We captured Virginia opossums in both riparian and upland habitats; upland habitats include pine-hardwood and hardwood forests. All 30 individuals taken by trappers on APH during the 1998/1999 Virginia trapping season were captured in riparian habitats. The omnivorous diet consists of carrion, human refuse, fungi, insects, worms, snakes, and a diversity of plant matter (Lay, 1942; Gardner, 1982). Few predators eat Virginia opossums, but raptors may contribute to the mortality of young of the year (Fitch and Shirer, 1970).

Food and sport values of Virginia opossums are limited. Management concerns often stem from this species being mislabeled as a nuisance by farmers and sportsmen (Gardner, 1982).

Order Insectivora

Blarina brevicauda Say (Northern Short-tailed Shrew)

The northern short-tailed shrew is the largest shrew in the United States. The snout is somewhat elongate, eyes minute, ears inconspicuous, and its short, noticeably hairy tail is less than half the total body length. Pelage is soft, mole-like in winter and color is slate to nearly black; it is lighter in summer. Dorsal pelage is generally darker than ventral pelage. External body measurements for 50 adults captured on APH were: total length 91-113 mm (avg.=101.3 ±4.9), tail length 15-25 mm (avg.=20.8 ±1.8), length of hind foot 13-15 mm (avg.=13.9 ±0.5). Dentition: i. 4/2, c. 1/0, p. 2/1, m. 3/3 =32 (Hall, 1981).

Northern short-tailed shrews occur from southern Canada throughout much of the eastern United States except that in the southeastern portion of its range it occurs only at higher elevations (George et al., 1986). They range throughout Virginia, except south-central and southeastern areas and the eastern portion of the Northern Neck Peninsula and Gwynn's Island, where the smaller southern short-tailed shrew (*B. carolinensis*) occurs (Tate et al., 1980; Pagels and French, 1987). Of 11 recognized subspecies, three (*B. b. churchi*, *B. b. telmalestes*, and *B. b. kirtlandi*) occur in Virginia; only *B. b. kirtlandi* is known from the northern Coastal Plain, including APH (George et al., 1986).

Northern short-tailed shrews occur in deciduous and coniferous forests and exhibit little affinity for any vegetation type (Jameson, 1949; Wrigley et al., 1979). This species occurs in somewhat higher numbers in forests than in clearcuts in Virginia's Piedmont (Pagels et al., 1992), and has been described as a habitat generalist (George et al., 1986). On APH, they were abundant in old fields and in riparian and upland forests. They were found in relatively low abundance in shelterwood treated forests. Habitat selection was positively associated with downed woody debris (Bellows et al., in press). Diet consists primarily of earthworms and myriapods, but also includes insects, snails, plant material, and occasionally small mammals, such as meadow voles (Hamilton, 1941; Eadie, 1952; Linzey and Linzey, 1973). Predators of the northern short-tailed shrew that occur on APH include Great Horned Owls (*Bubo virginianus*), Eastern Screech-Owls (*Otis asio*), Barred Owls (*Strix varia*), Barn Owls (*Tyto alba*), American Kestrels (*Falco sparverius*), Northern Harriers (*Circus cyaneus*), Red-tailed hawks (*Buteo jamaicensis*), black rat snakes (*Elaphe obsolete*), mole kingsnakes (*Lampropeltis calligaster*), copperheads (*Agkistrodon contortrix*), foxes, mustelids, and domestic free-ranging and feral cats (*Felis catus*) (Jackson et al., 1976; Mumford and Whitaker, 1982; Mitchell and Beck, 1992; Mitchell, 1994; Johnston, 2000).

Cryptotis parva Say (Least Shrew)

The least shrew is a short-tailed shrew similar in appearance to the northern short-tailed shrew, but smaller and lighter in color. Dense, almost velvety, pelage is brownish dorsally and paler ventrally. Measurements of 14 adults captured on APH were: total length 61-84 mm (avg.=74.7 ±5.6), tail length 12-17 mm (avg.=15.5 ±1.7), length of hind foot 9-11 mm (avg.=9.9 ±0.5). Dentition: i. 3/1, c. 1/1, p. 2/1, m. 3/3 =30 (Cothran et al., 1991).

The distribution of the least shrew is centered in southeastern to south-central United States and extends southward through Mexico. It occurs statewide in Virginia (Handley and Patton, 1947), except rarely at high elevations (elevation record, 1,524 m) (Pagels, 1991). Of 9 recognized subspecies, only *C. p. parva* is found in Virginia (Hall, 1981).

Least shrews are most commonly found in open grassy or brushy habitats with dense herbaceous vegetation (Whitaker, 1974). They are also found in edge habitats such as power lines and along roadsides (Golley et al., 1965). Pagels et al. (1992) described this shrew as an old-field/edge form. We captured them on APH most frequently in old fields, but some were taken in forested habitats, including very low numbers in shelterwood-treated forests. Diet consists of insects, spiders, earthworms, and myriapods, and they have been known to eat more than their body weight daily (Whitaker, 1974; Whitaker and Mumford, 1972). Owls, including Barn Owls, Eastern Screech Owls, Short-eared Owls (*Asio flammeus*), and Great Horned Owls, are primary predators of the least shrew, however, hawks (e.g., Northern Harriers, Red-tailed Hawks), copperheads, predatory mammals, and domestic free-ranging cats, will eat it opportunistically (Whitaker, 1974; Linzey and Linzey, 1971; Jackson et al., 1976; Deibler, 1988; Mitchell and Beck, 1992; Mitchell, 1994; Whitaker and Hamilton, 1998; Johnston, 2000).

Sorex hoyi Baird (Pygmy Shrew)

The pygmy shrew is North America's smallest mammal. Pelage is reddish or grayish brown dorsally and paler ventrally, and the tail is bicolored. Measurements for 44 adults captured on APH were: total length 62-82 mm (avg.=75.0 ±3.8), tail length 20-30 mm (avg.=24.6 ±1.9), length of hind foot 8- 9 mm (avg.=8.1 ±0.3). Dentition: i. 3/1, c. 1/1, p. 3/1, m. 3/3 =32 (Long, 1974).

The pygmy shrew is a boreal species found throughout most of Canada and the northern United States southward into the Appalachians (Hall, 1981). Of 7 recognized subspecies, only *S. h. winnemana* occurs in Virginia (Hall, 1981). The pygmy shrew was once considered to be extremely rare in Virginia. As recently as 1980 this shrew was known from only 7 locations and a total of 8 specimens (Handley et al., 1980). Results from pitfall trapping techniques have shown that it is widely distributed and common in Virginia (Pagels, 1987; Pagels et al., 1992; Erdle and Pagels, 1995). Our trapping efforts at APH yielded 107 captures during the period of 1997-2000.

Wrigley et al. (1979) observed pygmy shrews in marsh, bog, shrub thicket, deciduous and coniferous forest habitats. They have been captured in a range of habitats, including riparian situations, upland sites, and old fields (Long, 1974; Pagels, 1987; Mitchell et al., 1993). This species was described by Pagels et al. (1992) as a habitat generalist. We captured them on APH more often in forested habitats than in old fields. Pygmy shrew populations on APH are positively associated with downed woody debris and dense forest canopies (Bellows et al., in press). Diet consists of insects, earthworms, spiders, carrion, and other small mammals (Long, 1972; Ryan, 1982; Webster et al., 1985). Predation on this little shrew is rarely reported, but Long (1974) reported that raptors and domestic cats may take this species opportunistically.

Sorex longirostris Bachman (Southeastern Shrew)

The southeastern shrew is a long-tailed shrew with reduced ears, an elongated rostrum, and brown-tipped teeth. Pelage is brown dorsally and cinnamon to tawny ventrally. Measurements for 50 individuals captured on APH were: total length 73-88 mm (avg.=80.1 ±2.9), tail length 23-31 mm (avg.=27.0 ±1.8), length of hind foot 9-10 mm (avg.=10.0 ±0.1). Dentition: i. 3/1, c. 1/1, p. 3/1, m. 3/3 =32 (French, 1980a).

The geographic range of the southeastern shrew centers in the southeastern United States and extends westward into Missouri, Arkansas, and Louisiana, and northward into Iowa and Illinois (Hall, 1981). They are found throughout Virginia except in higher elevations (Pagels and Handley, 1989). Two of the three recognized subspecies (*S. l. fisheri* and *S. l. longirostris*) occur in Virginia. The larger *Sorex. l. fisheri* (the Dismal Swamp southeastern shrew) is restricted to the Great Dismal Swamp and surrounding suitable habitat in the Coastal Plain of southeastern Virginia and adjacent North Carolina (Handley, 1991; Jones et al., 1991); only *S. l. longirostris* is known from APH.

Southeastern shrews occur in a diverse range of habitats but most frequently in moist areas (French, 1980a; 1980b). Pagels et al. (1992) captured them in Cumberland County, Virginia, more frequently in three- and six-year-old clearcuts than in 40-year-old hardwood forests. Pagels and Handley (1989) captured them most frequently in edge habitats. At APH, we captured southeastern shrews in all habitats, including old fields and riparian zones. It was the most frequently captured insectivore in forests treated with the shelterwood silvicultural technique—32% of all small mammal captures in that habitat. Presence of southeastern shrews was positively associated with ground-level shrub density (Bellows et al., in press). They feed on insects, myriapods, snails, spiders, and some herbaceous vegetation (Whitaker and Mumford, 1972). Known predators include Barn Owls and Barred Owls, snakes, Virginia opossums, and feral cats (French, 1980a; French, 1980b; Whitaker and Hamilton, 1998).

Condylura cristata Linnaeus (Star-nosed Mole)

The star-nosed mole is characterized by its elongate snout that bears 22 fleshy appendages (11/nostril) that contain sensitive tactile organs called Eimer organs (Petersen and Yates, 1980). The tail is scaled, hairy, and roughly equal to its body length. Forelimbs are short and powerful and its forefeet are greatly enlarged, an adaptation for a fossorial or burrowing lifestyle. Pelage is brown to black and velvety. We captured 7 individuals on APH but external measurements were not obtainable on three of them. Measurements of four remaining specimens were: total length 149-165 mm (avg.=156.8 ±7.1), tail length 48-53 mm (avg.=51.5 ±2.4), length of hind foot 25-27 mm (avg.=25.8 ±1.0). For comparison, adult measurements from 9 individuals from the southern Appalachians and southern coastal areas were: total length 158-170 mm (avg.=165), tail length 57-63 mm (avg.=60), length of hind foot 24-26 mm (avg.=25) (Whitaker and Hamilton, 1998). Dentition: i. 3/3, c. 1/1, p. 4/4, m. 3/3 =44 (Petersen and Yates, 1980).

Star-nosed moles occur throughout most of eastern Canada and northeastern United States (Petersen and Yates, 1980). Two of the three recognized subspecies (*C. c. cristata* and *C. c. parva*) occur in Virginia, and marginal records from Hall (1981) suggest that both subspecies may occur at APH. Measurements of the four specimens captured on APH fall within the range Handley and Pagels (1991) reported for *C. c. parva.*

Star-nosed moles are most commonly found in or near marshy habitats or streams, and rarely in dry situations (Hamilton, 1931; Handley, 1991). At APH, one was captured in a 5-10- year-old clearcut, three were captured in upland mixed or hardwood forests, one was in an upland pine forest, and two were captured in upland forests modified by shelterwood treatment. As with eastern moles, star-nosed moles may be more common on APH than our capture data indicate. Pitfalls and snap trap captures often misrepresent the abundance of fossorial species. Over 80% of their diet consists of insects and annelid worms (Handley, 1991). They are also known to eat minnows, mollusks, and crustaceans (Hamilton, 1931). Raptors, including the Red-Tailed Hawks, Great Horned Owls, and Barn Owls, are major predators on the star-nosed mole (Hamilton, 1931). Snakes and skunks will also consume this species (Mitchell, 1994; Linzey, 1998). Mitchell (1994) found a star-nosed mole in a copperhead from Giles County, Virginia.

Scalopus aquaticus Linnaeus (Eastern Mole)

The eastern mole can be easily distinguished from the star-nosed mole by its plain nose and relatively short, scantily haired tail. As in other moles, the eyes are tiny and concealed in the pelage and ear pinnae are absent. Pelage is thick and velvety, with color ranging from silver to black dorsally and paler ventrally. Males are generally larger than females (Whitaker and Hamilton, 1998). Two individuals were captured on APH, however, one specimen was partially eaten and measurements could not be taken. External measurements for the one individual were: total length 134 mm, tail length 20 mm, length of hind foot 16 mm. For comparison, body measurements for 15 adult males and 8 adult females, respectively, from Washington D.C. were: total length 154 to 175 mm (avg.=163.4), 146 to 168 mm (avg.=152.6); tail length 22 to 29 mm (avg.=26.5), 21 to 28 mm (avg.=26.0); length of hind foot 18 to 21 mm (avg.=19.8), 18 to 20 mm (avg.=19.0) (Jackson, 1915). Dentition: i. 3/2, c. 1/0, p. 3/3, m. 3/3 =36 (Hall, 1981).

The eastern mole has the broadest distribution of any North American mole, occurring throughout much the eastern and central United States (Yates and Schmidly, 1978). It is more abundant in southern than in northern portions of its range (Whitaker and Hamilton, 1998). Two of the 16 recognized subspecies (*S. a. aquaticus* and *S. a. howelli*) occur in Virginia (Hall, 1981), however, only *S. a. aquaticus* occurs on APH.

Eastern moles occur in most environments where the soil is well drained and relatively free of clay and gravel, especially open fields and pastures (Whitaker and Hamilton, 1998). One of two individuals captured on APH inhabited an oak-pine upland forest; the other was collected in a shelterwood-treated forest. Diet consists primarily of earthworms, insects, and other invertebrates, although some plant matter is eaten (Whitaker and Hamilton, 1998). Home ranges are relatively large compared to other fossorial mammal species, probably due to a demanding diet. Male home ranges (average 1.1 ha) are generally larger than those of females (0.3 ha) (Harvey, 1976). Their fossorial habits prevent predation from many species; however, Barn Owls and domestic free-ranging cats will take it opportunistically (Jackson et al., 1976; Mitchell and Beck, 1992; Whitaker and Hamilton, 1998). Mole kingsnakes and black racers (*Coluber constrictor*) probably enter its tunnels (Mitchell, 1994).

Order Chiroptera

Eptesicus fuscus Beauvois (Big Brown Bat)

This bat can be distinguished from all other bats of the region by its large size and coloration. The pelage is long, lax (>10 mm mid-dorsally), uniformly dark brown on the dorsal surface and paler on the ventral surface. Ears are rounded and short and just reach the nostrils when laid forward. The tragus is blunt and bends forward slightly at the tip. The calcar is keeled. Weights of big brown bats captured on APH ranged from 13.0 to 18.0 g for 11 males (avg.=15.7 ±1.5 g) and 18.0 to 24.0 g for 18 females (avg.=20.5 ±1.8 g). Measurements for the same individuals were: total length 107.0-120.0 mm (avg.= ±3.9), 108.0-124.0 mm (avg.=117.2 ±4.2); tail length 39.0-51.0 mm (avg.=45.1 ±3.2), 39.0-52.0 mm (avg.=46.3 ±3.1); wingspan 309.0-340.0 mm (avg.=324.1 ±9.5), 321.0-355.0 mm (avg.=334.5 ±8.8); forearm length 43.0-49.0 mm (avg.= ±1.6), 42.0-50.5 mm (avg.=47.0 ±2.2); length of hind foot 10-12 mm (avg.=11.0 ±0.6), 9.5-13.0 mm (avg.=11.0 ±0.9); ear length 11.0-17.0 mm (avg.=14.4 ±1.8), 11.0-18.0 mm (avg.=14.0 ±1.7); tragus length 3.5-8.0 mm (avg.=6.4 ±1.5), 3.0-8.0 mm (avg.=5.6 ±1.7). Dentition, i. 2/3, c. 1/1, p. 1/2, m. 3/3 =32 (Kurta and Baker, 1990).

The North American distribution of the big brown bats extends across southern Canada and throughout the contiguous United States (Hall, 1981). They occur state-wide in Virginia (Handley and Patton 1947). Of the 11 recognized subspecies, only *E. fuscus fuscus* occurs in the mid-Atlantic region (Hall, 1981).

The big brown bat is frequently one of the last species to enter hibernation, which lasts from November until it emerges in March (Whitaker and Hamilton, 1998). Hibernacula are usually in old buildings and caves (Handley and Patton, 1947). They usually roost singly and often share hibernacula with *Myotis* and *Pipistrellus* (Whitaker and Hamilton, 1998). Big brown bats are partially migratory, but do not undertake long seasonal migrations between hibernating and summer sites (Paradiso, 1969). Summer roosts are usually located in old buildings, such as barns, churches, hollow trees, and houses (Barbour and Davis, 1969). They can also be found roosting in hollow trees (Padgett and Rose, 1991). This was one of the most common bat species on APH. We collected big brown bats in old fields and in upland and riparian forests.

Big brown bats forage throughout the night with most activity beginning about an hour after sunset (Kunz, 1973). Diet consists primarily of small beetles (Freeman, 1981). Known opportunistic predators include Common Grackles (*Quiscalus quiscula*), American Kestrels, long-tailed weasels, house cats, and bullfrogs (*Rana catesbeiana*) (Rysgaard, 1942; Mumford, 1969; Long, 1971; Black, 1974; Kirkpatrick, 1982).

Lasionycteris noctivagans LeConte (Silver-haired Bat)

This medium-sized bat can be distinguished from other bats of the region by its coloration. Pelage is generally blackish brown with white-tipped dorsal hairs that give it a slight silver-haired appearance. The frosting is more pronounced in young and older individuals may appear yellowish (Barbour and Davis, 1969). Wings, ears, and interfemoral membrane are black; and half of the dorsal surface of the interfemoral membrane is furred. The ears are short and rounded and the tragus is broad and blunt. The calcar is not keeled. Weight of three individuals captured on APH during April 2000 was 11.0 for a single male and 12.0 and 13.0 g for the two females, respectively.

Measurements for the same three individuals were: total length 103.0 mm (male), 97.0 and 97.0 mm (females); tail length 30.5 mm, 39.0 and 41.0 mm; wingspan 292.0 mm, 273.0 and 290.0 mm; forearm length 42.0 mm, 41.0 and 43.0 mm length of hind foot 8.0 mm, 8.0 and 10.0 mm; ear length 11.0 mm, 10.0 and 10.0 mm; tragus length 3.0 mm in all three individuals. Dentition, i. 2/2, c. 1/1, p. 2/3, m. 3/3 =36 (Kunz, 1982).

The geographic range of the silver-haired bat includes all of Virginia and extends over most of North America from southeastern Alaska to southern Canada and all of the contiguous United States except extreme southern regions (Kunz, 1982). There are no recognized subspecies.

Silver-haired bats are migrants, spending summers in the northern tier of states and in Canada (Handley and Patton, 1947). Based on Linzey's (1998) summary, there are no records of this bat in Virginia during the summer months. They occur in Illinois, Indiana, Ohio, Pennsylvania, Connecticut, and Rhode Island in spring and fall only during migration (Whitaker and Hamilton, 1998). Winters are spent hibernating in southern regions as far south as coastal Georgia. Padgett and Rose (1991) commonly observed them during winter in the Great Dismal Swamp. Winter roosts include caves, mines, hollow trees, and houses (Padgett and Rose, 1991). Silver-haired bats are usually solitary and there are no reports of large winter or summer colonies (Barbour and Davis, 1969; Whitaker and Hamilton, 1998). On APH, we captured them during migration in or near pine-hardwood and hardwood riparian forests during April 2000.

Kunz (1973) considered silver-haired bats to be relatively late flyers, emerging in the evening after other species have begun foraging. In contrast, Whitaker and Hamilton (1998) refer to them as early flyers. The individuals we captured on APH were netted well after sunset. Diet is variable. Kunz's (1982) summary of various studies includes records of more than a dozen insect orders, as well as some arachnids. Records of predation on silver-haired bats in the Commonwealth are few, however, Johnston (2000) provides a single record of a Great Horned Owl taking this bat in Virginia.

Lasiurus borealis Müller (Red Bat)

The red bat is a medium-sized bat that can be easily distinguished from other bats of the region by its coloration. Pelage is brick red to rusty red. Hairs on the back and chest are white-tipped resulting in a frosted appearance. The underparts are paler. The anterior portion of the shoulder has a whitish patch. This is one of the few mammals where coloration varies between the sexes; males are generally brighter red and less frosted than females. The dorsal surface of the interfemoral membrane is thickly furred. Ears are low, broad and rounded, and the tragus is triangular. The calcar is keeled. Weight of adults captured on APH ranged from 9.0 to 15.0 g for 34 males (avg.=10.9 ±1.5 g) and 10.0 to 20.0 g for 48 females (avg.=14.4 ±2.1 g). Measurements for the same individuals, male and females respectively, were: total length 95-115 mm (104 ±4.7), 99-117 mm (108±4.4); tail length 41.0-52.0 mm (48.0 ±2.5), 42.0-57.0 mm (49.5 ±3.2); wingspan 276-330 mm (300 ±10.6), 288-341 mm (314 ±11.0); forearm length 36.0-42.5 mm (40.2 ±1.5), 37.0-45.5 mm (42.1 ±1.9); length of hind foot 7.0-11.0 mm (8.6 ±0.8), 8.0-11.0 mm (9.1 ±0.7); ear length 8.0-12.0 mm (9.4 ±1.1), 8.0-13.0 mm (10.3 ±1.4); tragus length 3.0-6.0 mm (4.5 ±1.0), 3.5-6.5 mm (4.9 ±0.9). Dentition, i. 1/3, c. 1/1, p. 2/2, m. 3/3 =32 (Cothran et al., 1991).

Red bats occur throughout the midwestern and eastern United States, including all of Virginia, and throughout the south, along the Pacific Coast, and south through

Mexico and much of Central and South America (Shump and Shump, 1982a). Of the five recognized subspecies, only *L. b. borealis* is found in eastern North America.

Red bats are considered to be highly migratory and, although they are generally solitary, they may migrate in groups (Shump and Shump, 1982a). They generally travel south in winter but little is known about movement patterns or hibernacula selection. However, Padgett and Rose (1991) recorded the year-round presence of males in the Great Dismal Swamp. Whitaker et al. (1997) reported that male red bats were actively feeding, mostly on moths, during moderate winter evenings in the Great Dismal Swamp. Summer roosts are usually on trees or shrubs (Cothran et al., 1991).

Although red bats are generally solitary, Barbour and Davis (1969) reported that they are known to forage with big brown bats, hoary bats, silver-haired bats, evening bats, eastern pipistrelles, and little brown myotis. It was by far the most abundant of the 9 bat species that we collected on APH. We captured red bats in old fields and all forested, riparian and upland, habitats.

Kunz (1973) reported that red bats begin foraging 1 to 2 h after sunset. At APH, we have frequently captured them<1 h after sunset. Padgett and Rose (1991) found that daily emergence times changed with time of year, earliest in winter months, latest during summer, and around sunset in spring. Secondary foraging occurs throughout the night but with less intensity than during the initial period (Kunz, 1973). Diet includes moths, crickets, flies, bugs, beetles, and cicadas (Shump and Shump, 1982a). Predators include Virginia opossums, domestic cats, raptors, and most importantly (to young) Blue Jays (*Cyanocitta cristata*) (Elwell, 1962; Shump and Shump, 1982a).

Lasiurus cinereus Beauvois (Hoary Bat)

The hoary bat is the largest bat in the region. Pelage is dark brownish with hairs tipped in white, producing a frosted or hoary effect. Shoulder and wrist patches are whitish. The ears are edged in black and are short and rounded; the tragus is short and broad. The interfemoral membrane is heavily furred on the dorsal surface. The calcar is keeled. Adults usually weigh from 18 to 38 g (Whitaker and Hamilton, 1998). Weight of a single adult female captured on APH was 33.3 g. Weight of a single adult male captured on APH was 24.0 g. Measurements for the same two individuals, male and female respectively, were: total length 137.0 mm, 134.0 mm; tail length 61.0 mm, 60.0 mm; wingspan 415.0 mm, 408.0 mm; forearm length 55.0 mm, 54.0 mm; length of hind foot 15.0 mm, 12.0 mm; ear length 13.0 mm, 15.0 mm; tragus length 5.0 mm, 8.0 mm. For comparison, mean external measurements of 41 adult females from Indiana were: total length 135 mm, tail length 57 mm, wingspan 400 mm, forearm length 55 mm, tragus 8.0 mm (Whitaker and Hamilton, 1998). Dentition, i. 1/3, c. 1/1, p. 2/2, m. 3/3 =32 (Whitaker and Hamilton, 1998).

The hoary bat has the broadest distribution of all American bats. It occurs to the northern limits of trees in Canada and southward to Guatemala (Shump and Shump, 1982b). In South America, it ranges from Brazil south to Argentina and Chile; it is the only bat known from Hawaii. The hoary bat probably occurs throughout Virginia, at least seasonally, but is not common (Handley and Patton, 1947, and unpublished records). Of the three recognized subspecies, only *L. c. cinereus* occurs in North America (Shump and Shump, 1982b).

Although there is much evidence supporting the migratory habits of hoary bats, little is known about migration routes and wintering sites. They have been reported passing through southern states (e. g., Florida) from late October to late November heading south to wintering sites and again from February to early May bound for northern summer roosting sites (Zinn and Baker, 1979). They are all but absent in the south the remainder of the year (Zinn and Baker, 1979). Findley and Jones (1964) suggested that they overwinter south of the United States. There have been a few observations of hoary bats overwintering in more northern areas of its range. The solitary summer roost is frequently located in foliage, especially in coniferous trees, 3 to 5 m above the ground (Whitaker and Hamilton, 1998). All three individuals captured on APH, one adult female, one subadult female and one adult male were netted in close proximity to or within riparian habitats.

Similar to red bats, hoary bats do not seem to associate with other bat species except when foraging. Shump and Shump (1982b) summarized reports that they forage with the red bats, big brown bats, Indiana bats (*Myotis sodalis*), eastern pipistrelles, silver-haired bats, and evening bats. They are often the last bat species to begin foraging, frequently not emerging until well after sunset. Hoary bats appear to prefer moths, but will also eat bugs, beetles, and flies, and are known to attack pipistrelles (Whitaker and Hamilton, 1998). Wiseman (1963) reported predation on this bat by a Texas rat snake (*Elaphe obsoleta linderheimi*). Hawks and owls probably take them opportunistically (Jackson, 1961; Barbour and Davis, 1969; Lowery, 1974).

Myotis austroriparius Rhoads (Southeastern Myotis)

The southeastern myotis is similar in appearance to the little brown bat but can be distinguished from this species by its shorter woolly pelage and pink face. Pelage is gray or drab brown dorsally and white or tan ventrally. The calcar is not prominently keeled. Weight of a single adult female captured on APH was 9.0 g. Measurements for the same individual were: total length 91.0 mm, tail length 41.0 mm, wingspan 269 mm, forearm length 39.0 mm, length of hind foot 10.0 mm, 14.0 mm, tragus length 7.0 mm. Dentition, i. 2/3, c. 1/1, p. 3/3, m. 3/3 =38 (Jones and Manning, 1989).

The range of this species is disjunct and centered in the southeastern United States (Jones and Manning, 1989). No subspecies are recognized.

Summer roosts and hibernacula are commonly located in caves, abandoned buildings, sewers, and hollow trees; and summer roosts are always near permanent bodies of water (Webster et al., 1985). The female collected on APH was captured over a gravel access road adjacent to a beaver pond. Southeastern myotis are occasionally found associated with other bats such as other *Myotis* spp., eastern pipistrelles, and Rafinesque's big-eared bats (*Corynorhinus rafinesquii*) (Jones and Manning, 1989).

Southeastern myotis emerge from day roosts at late in the evening. Common prey include beetles, moths, mosquitos, and crane flies (Whitaker and Hamilton, 1998). Known predators include Virginia opossums, black rat snakes, corn snakes, and owls (Jones and Manning, 1989).

Myotis lucifugus LeConte (Little Brown Myotis)

The little brown myotis is a medium-sized bat for this region. Pelage varies in color from dark sooty brown to paler golden brown with a glossy appearance. The face, ears, and membranes are dark brown. Young individuals are darker in color than adults. The interfemoral membrane is not furred, the calcar is not keeled, and the tragus is slender and pointed. Weight of 8 individuals captured on APH ranged from 7.0 to 9.0 g for three males (avg.=8.3 ±1.2 g) and 8.5 to 10.5 g for five females (avg.=9.7 ±0.8 g). Measurements (ranges, means in mm) for the same individuals, males and females respectively, were: total length 85.0-90.0 mm (avg.=88.3 ±2.9), 85.0-93.0 mm (avg.=88.8 ±2.9); tail length 36.0-41.0 mm (avg.=38.7 ±2.5), 38.0-40.0 mm (avg.=39.6 ±0.9); wingspan 250.0-255.0 mm (avg.=253.3 ±2.9), 245.0-271.0 mm (avg.=259.6 ±11.4); forearm length 36.5-37.5 mm (avg.=37.0 ±0.5), 37.0-40.0 mm (avg.=38.7 ±1.2); length of hind foot 8.0-10.0 mm (avg.=9.2 ±1.0), 9.0-10.0 mm (avg.=9.8 ±0.4); ear length 10.0-14.0 mm (avg.=12.3 ±2.19), 6.0-7.5 mm (avg.=6.9 ±0.5); tragus length 3.5-7.0 mm (avg.=5.7 ±1.9), 6.0-7.5 mm (avg.=6.9 ±0.5). Dentition, i. 2/3, c. 1/1, p. 3/3, m. 3/3 =38 (Paradiso, 1969).

The species ranges from Alaska to eastern Canada, and it occurs in most of the east as far south as southern Alabama and Georgia (Fenton and Barclay, 1980). It is one of the most common bats in the Commonwealth and occurs throughout the state (Handley and Patton, 1947). Of six recognized subspecies, only *M. l. lucifugus* occurs in Virginia.

Roost preference is dependent on season and setting. Hibernacula (winter roosts) are usually located in caves and abandoned mines, and less frequently in buildings (Paradiso, 1969). Migration distances between hibernacula and summer roosts can be several hundred kilometers (Fenton and Barclay, 1980). Little brown myotis are gregarious, especially in winter when they have been found in colonies numbering 15,000 (containing several species) (Kurta and Teramino, 1994). Colonies generally have many fewer individuals. In the southern part of its range, little brown myotis enters hibernacula as late as November and emerges as early as mid-March (Fenton and Barclay, 1980). Day roosts and night roosts (spring, summer, and fall) are located in buildings, hollow trees, under the bark of trees, under bridges, and in caves (mostly males) (Whitaker and Hamilton, 1998). On APH, little brown bats have been captured most frequently in upland forests and less frequently in old-field and riparian habitats.

These bats emerge from a day roost at dusk to feed for an hour or two and continue to feed intermittently throughout the night, each time returning to night roosts. Diet, like all bats of the region, consists almost entirely of insects (Webster et al., 1985). Common prey are beetles, moths, and midges (Griffith and Gates, 1985). Size of prey generally falls between 3 and 10 mm in body length and 0.2 and 15.0 mg in weight (Gould, 1955; Anthony and Kunz, 1977). Small carnivores, birds, mice, and snakes opportunistically consume little brown bats (Gillette and Kimbrough, 1970).

Myotis septentrionalis Trouessart (Northern Myotis)

This bat was formally known as *Myotis keenii septentrionalis* (Keen's myotis), but based on morphological characteristics it is now considered to be a separate species (Jones et al., 1992). The species is similar in size and color to little brown myotis but lacks the glossy appearance and has longer ears, long for *Myotis*, that extend well beyond the muzzle when laid forward. Pelage is brown and darker dorsally than ventrally. The calcar is not keeled, and the tragus is long, pointed, and slightly curved. Weights of three individuals captured on APH were 7.0 g for a single male and 9.0 and 10.0 g for two females, respectively. Measurements of the same three individuals, male and females respectively, were: total length 95.0 mm, 87.0 and 92.0 mm, tail length 42.0 mm, 40.0 and 41.0 mm; wingspan 244.0 mm, 252.0 and 253.0 mm; forearm length 37.0 mm, 38.5 and 42.0 mm; length of hind foot 9.0 mm, 10.0 and 10.5 mm; ear length 17.5 mm, 17.0 and 18.0 mm; tragus length 9.5 mm, 9.5 and 9.5 mm. For comparison, weights of 20 adults from various parts of the range were from 5 to 10 g (Whitaker and Hamilton, 1998). External body measurements for the same 20 individuals were: total length 79.2-87.8 mm (avg.=84.1), tail length 36.4-43.0 mm (avg.=41.7), forearm length 34.6-38.8 mm (avg.=36.7), length of hind foot 7.2-9.4 mm (avg.=8.4), ear length 17-19 mm, wingspan 228-258 mm. Dentition, i. 2/3, c. 1/1, p. 3/3, m. 3/3 =38 (Barclay, 2000).

Northern myotis range broadly in northeastern North America from portions of Canada to north Florida, although they are absent in the coastal areas of the South (Caceres and Barclay, 2000). They occur statewide in Virginia but vary from uncommon to locally abundant, making up 35 to 50% of late summer populations of *Myotis* inventoried at the mouths of caves (Handley, 1979). Northern myotis may be the least abundant species of those that hibernate in Virginia, but may be more abundant than winter cave inventories suggest (Dalton, 1987).

Northern myotis hibernate October or November through March or April. Winter hibernacula are usually in caves or mines (Caceres and Barclay, 2000). They often winter with other species, such as big brown bats, eastern pipistrelles, little brown myotis, and Indiana myotis (Whitaker and Hamilton, 1998). Favored summer roost sites are in trees; they will occasionally roost in man-made structures (Caceres and Barclay, 2000). We captured only three northern myotis during the 2000 APH bat survey. We captured them in riparian and upland habitats.

Northern myotis begin to forage shortly after dusk and probably use night roosts. They forage again before dawn and then retire to day roosts (Barbour and Davis, 1969). Diet consists primarily of moths, but beetles, flies, caddisflies and spiders are also taken (Whitaker and Hamilton, 1998). Typical of North American bats, northern myotis have few natural enemies, although many predators will take them opportunistically (Whitaker and Hamilton, 1998).

Nycticeius humeralis Rafinesque (Evening Bat)

The evening bat looks much like the larger big brown bat (*Eptesicus fuscus*), but can be distinguished from the latter by its smaller size. This bat also resembles bats of the genus *Myotis*, but can be distinguished from this group by its short, sparse pelage, which is dull brown dorsally with plumbeous bases, and paler on the ventral surface. Also in contrast with *Myotis*, the ears of the evening bat are short and the tragus is blunt. Interfemoral membrane is furred only at the base, and the calcar is not keeled. Ear and tail and wing membranes are leathery. The weight of a single adult male captured at APH was 9.5 g. Measurements for the same individual were: total length 86.0 mm, tail length 36.0 mm, 246.0 mm, forearm length 34.5 mm, length of hind foot 9.0 mm, ear length 11.0 mm, tragus length 5.0 mm. For comparison, weights of 19 males and 50 females from South Carolina were 6.1-12.0 g (avg.=8.2) for males and 6.6-13.0 g (avg.=8.8) for females (Cothran et al., 1991). Measurements for these males and females, respectively, were: total length 79-94 mm (avg.=88), 76-99 mm (avg.=92); tail length 31-39 mm (avg.=36), 33-43 mm (avg.=38); forearm length 33-38 mm (avg.=36), 31-40 mm (avg.=36); length of hind foot 6.0-9.0 mm (avg.=7.1), 5.5-8.0 mm (avg.=6.9) (Cothran et al., 1991). Dentition, i. 1/3, c. 1/1, p. 1/2, m. 3/3 =30 (Watkins, 1972).

Evening bats occur throughout the East as far north as Pennsylvania, west to Nebraska, and south into northeastern Mexico, and within these boundaries in all regions except the Appalachians (Whitaker and Hamilton, 1998). Linzey (1998) summarized that they are found at lower elevations in Virginia and have not been reported from the mountains. Of the three recognized subspecies, only *N. h. humeralis* occurs in the mid-Atlantic region (Watkins, 1972).

Little is known about migration patterns of this primarily southern species, but it likely reaches the northern limits of its distribution (e.g., Pennsylvania, Michigan, Illinois) during summer (Whitaker and Hamilton, 1998). Summer colonies in Indiana usually disband by late September to October and reactivate by the beginning of May (Humphrey and Cope, 1968; Clem 1992; Clem, 1993). They migrate distances exceeding 500 km (Watkins, 1969), but where they actually hibernate is unknown. Summer roosts occur most frequently in buildings or hollow trees and uncommonly in caves (Watkins, 1972). We have captured this bat on APH in upland and riparian hardwood forests.

Evening bats leave daytime roosts to forage as darkness falls (Whitaker and Hamilton, 1998). Diet consists mainly of beetles, moths, and leafhoppers (Whitaker and Clem, 1992). Predation on evening bats is not well known. Domestic and feral cats are the most important predators in rural settings and raccoons and black rat snakes inhabiting the same buildings that serve as summer roosts would no doubt take them, especially young (Watkins, 1972).

Pipistrellus subflavus F. Cuvier (Eastern Pipistrelle)

The small size and light coloration of this bat distinguishes it from all other eastern bats. Pelage consists of tricolored hairs that are dark at the base, lighter and yellowish brown in the middle, and dark tipped. The anterior third of the interfemoral membrane is sparsely furred on the dorsal surface. The tragus is blunt, ears longer than they are wide, and reach the end of the nose when laid forward. The calcar is not distinctly keeled. Weights of bats captured on APH were 5.5 and 6.0 g for two males and 5.5 to 9.5 g for 10 females (avg.=7.3 ±1.3 g). Measurements for the same males and females, respectively, were: total length 78.0 and 85.0 mm, 77.0-92.0 mm (avg.=84.6 ±4.1); tail length 38.0 and 40.0 mm, 31.0-45.0 mm (avg.=39.2 ±3.7); forearm length 34.0 and 35.0 mm, 32.5-35.5 mm (avg.=34.3 ±0.9); length of hind foot 9.0 and 9.0 mm, 7.5-11.0 mm (avg.=9.0 ±1.1); ear length 10.5-11.0, 9.5 to 12.0 mm (avg.=11.3 ±0.8); tragus length 5.5 and 6.5 mm, 4.5-7.0 mm (avg.=5.9 ±0.8). Dentition, i. 2/3, c. 1/1, p. 2/2, m. 3/3 =34 (Fujita and Kunz, 1984).

Eastern pipistrelles occur throughout most of the eastern half of the United States and south into Central America (Hall, 1981). They occur throughout Virginia (Dalton, 1987). Of four recognized subspecies, only *P. s. subflavus* occurs in Virginia (Hall, 1981).

Eastern pipistrelles hibernate from late October until April and early May (Whitaker and Hamilton, 1998). Winter hibernacula include caves, mine shafts, rock crevices, and various other man-made structures (Handley and Patton, 1947; Jones and Pagels, 1968). They generally hibernate singly or in relatively small numbers. Eastern pipistrelles frequently share hibernacula with other species, such as little brown myotis, northern myotis, Indiana myotis, and big brown bats, but usually in side passages where other bats are absent (Whitaker and Hamilton, 1998). This is the least specialized cave bat in Virginia with regard to microhabitat requirements of the hibernaculum (e.g., temperature, humidity, and cave configuration) (Dalton, 1987). Winter and summer roosts are in similar habitats and are geographically close (Cothran et al., 1991). On APH, we captured eastern pipistrelles in riparian and upland forests and in old-field habitats.

Eastern pipistrelles begin to forage relatively early in the evening. The erratic, slow flight often results in this species being mistaken for a large moth (Handley and Patton, 1947). Diet includes moths, tiny flies, beetles, and hymenopteran insects (Cothran et al., 1991; Whitaker and Hamilton, 1998). Little is known about predation on this bat. Hoary bats and northern leopard frogs (*Rana pipiens*) have been reported to prey on this species (Bishop, 1947; Creel, 1963).

Order Lagomorpha

Sylvilagus floridanus Allen (Eastern Cottontail)

The eastern cottontail is the only lagomorph on APH. Pelage is long and dense, brown to gray dorsally, and white ventrally, including the tail. Chapman and Morgan (1973) reported that average measurements for 35 males and 42 females, respectively, from western Maryland and West Virginia adult were: total length 427.0 mm, 433.2 mm; tail length 44.9 mm, 44.8 mm; length of hind foot 95.4 mm, 95.4 mm; length of ear 61.5 mm, 61.1 mm. Dentition, i. 2/1, c. 0/0, p. 3/2, m. 3/3 =28 (Chapman et al., 1980).

The distribution of the eastern cottontail includes portions of southern Canada, all of the central and eastern United States, and northwestern South America (Chapman et al., 1980). Of the 23 recognized subspecies of *S. floridanus*, only *S. f. mallurus* occurs throughout the Coastal Plain of the mid-Atlantic region (Handley, 1991).

Eastern cottontails occur in a variety of disturbed, early successional, and transitional habitats, but seem to prefer those with weedy forbs and bunch-type perennial grasses that provide thickly vegetated escape routes. On APH, eastern cottontails have been observed in old fields, clearcuts, grassy roadside firebreaks, forests with open canopy, and edges of pine-hardwood and hardwood forests. Diet consists mainly of herbaceous vegetation such as grasses and clover, fruits when available, and woody vegetation during winter (Chapman et al., 1982).

Eastern cottontails are prey of many predators within its range. Mammalian predators include raccoons, weasels (*Mustela* spp.), red and gray foxes, coyote, bobcats, and feral cats (Martin et al., 1961; Mitchell and Beck, 1992). Known snake predators of eastern cottontails that occur on the post include mole kingsnakes and black rat snakes (Mitchell, 1994). Other predators include hawks (e.g., Rough-legged Hawks [*Buteo lagopus*]), and owls (e. g., Barn Owls, Great Horned Owls) (Jackson et al., 1976; Johnston, 2000).

Eastern cottontails are the most widely hunted game species in the United States (Chapman et al., 1982). They sometimes causes damage to orchards and crops and can be a garden pest in suburban areas.

Order Rodentia

Glaucomys volans Linnaeus (Southern Flying Squirrel)

Southern and the northern flying squirrels are the only nocturnal North American squirrels (Dolan and Carter, 1977). Flying squirrels are easily identified by their furred gliding membrane, or patagium, which extends from the wrists to the ankles. Pelage of the southern flying squirrel is dense and soft, slate gray dorsally and white below. Stapp (1992) found no differences in body weight between the sexes. Weights of three males captured on APH ranged from 59 to 68 g (avg.=63.0 ±4.6 g). Measurements for flying squirrels captured on APH, five males and three females, respectively, were: total length 217-239 mm (avg.=225.0 ±8.3), 210-240 mm (avg.=229.0 ±16.5); tail length 80-94 mm (avg.=87.6 ±6.2), 90-94 mm (avg.=91.7 ±2.1); length of hind foot 30-31 mm (avg.=30.8 ±0.4), 31 mm (avg.=31.0 ±0.0); ear length 18-19 mm (avg.=18.6 ±0.5), 18-20 mm (avg.=18.7 ±1.2). Dentition, i. 1/1, c. 0/0, p. 2/1, m. 3/3 (Hall, 1981).

Southern flying squirrels occur throughout the eastern United States, southeastern Canada, and in southern Mexico south into Honduras. Of the recognized subspecies, only *G. v. volans* is known from this region (Hall, 1981).

Southern flying squirrels occur most often in deciduous forests, but also inhabit mixed pine-hardwood and coniferous forests (Cothran et al., 1991). On APH, we found them in pine, mixed pine-hardwood, and hardwood forests, and in riparian and upland habitats. They are social, often forming winter aggregations of five to six individuals (Stapp et al., 1991). Such aggregations result in a form of social thermoregulation (Merritt et al., 2001). Diet consists primarily of nuts, seeds, and berries, but they will eat insects, carrion, birds, and eggs opportunistically (Dolan and Carter, 1977). Owls, domestic free-ranging cats, and black rat snakes are known predators of southern flying squirrels (Pearson, 1954; Mumford and Handley, 1956; Hall and Blewett, 1964; Mitchell and Beck, 1992). Other potential predators include mustelids, bobcats, raccoons, and hawks (Dolan and Carter, 1977).

Marmota monax Linnaeus (Woodchuck)

The woodchuck (groundhog) is the largest member of the squirrel family in the region. Pelage is gray to reddish brown dorsally and paler ventrally, feet and tail brown to black. Long white guard hairs over entire body give the woodchuck a grizzled appearance. Adult body measurements are: total length 418-665 mm, tail length 100-155 mm, length of hind foot 66-88 mm (location not provided, Hall, 1981). Dentition: i. 1/1, c. 0/0, p. 2/1, m. 3/3 =22 (Lee and Funderburg, 1998).

Woodchucks have the greatest distribution of any North American marmot (genus *Marmota*). They range from eastern Alaska, across southern Canada, and southward through the central and eastern United States, except many regions of the southeastern United States (Kwiecinski, 1998). Of the 9 recognized subspecies of *M. monax*, only *M. m. monax* is known from the mid-Atlantic region (Hall, 1981).

Woodchucks most frequently occur in woodland-field ecotones, rocky slopes, or clearings, preferring to hibernate in woodlands and to breed and forage in nearby fields (Lee and Funderburg, 1982; Kwiecinski, 1998). We observed them throughout APH, but they were especially abundant in all of the early successional habitats. We captured a subadult in an open canopy forest.

They are active from early spring until late fall, at which time they enter deep torpor until late February or early March (Webster et al., 1985). Periods of hibernation are generally longer in the northern regions of its range. Woodchucks are solitary and aggressively defend burrows, associating with conspecifics only to reproduce (Lee and Funderburg, 1982). Woodchuck burrows provide refuge for cottontails, skunks, Virginia opossums, raccoons, mice, chipmunks, weasels, and many bird and snake species (Grizzell, 1955). Woodchucks feed on a variety of plant materials, especially alfalfa, clover, chickweed, and grasses, but will also eat invertebrates opportunistically (Grizzell, 1955; Lee and Funderburg, 1982; Kwiecinski, 1998). Woodchucks sometimes cause extensive damage to crops, but often the damage is only localized due to their solitary habits (Kwiecinski, 1998). Foxes are primary predators on young woodchucks, but owls, hawks and snakes sometimes take young (Grizzell, 1955).

Sciurus carolinensis Gmelin (Gray Squirrel)

The gray squirrel is a medium-sized tree squirrel. Pelage is gray in appearance due to alternating bands of brown, white, and black on the hairs. The mid-dorsal section and cheeks are slightly darker, and the chin, throat, and belly are white. Measurements for 179 adults from Indiana were: total length 404-530 mm (avg.=469), tail length 177-285 mm (avg.=210), length of hind foot 50-76 mm (avg.=65) (Whitaker and Hamilton, 1998). Dentition: i. 1/1, c. 0/0, p. 2/1, m. 3/3 =22 (Koprowski, 1994).

Gray squirrels range throughout the eastern United States and extend west to the limits of the deciduous forest and north to southeastern and south-central Canada (Hall, 1981; Koprowski, 1994). Of the five recognized subspecies, two (*S. c. carolinensis* and *S. c. pennsylvanicus*) are known from Virginia (Koprowski, 1994). Records from Handley and Patton (1947) indicate that that the range of *S. c. carolinensis* is to the south and east of APH.

Habitat of gray squirrels is primarily mature hardwood forests, but they also occur in mixed pine-hardwood forests and urban areas with abundant trees (Flyger and Gates, 1982; Cothran et al., 1991). On APH, we have observed them in numerous habitats, including pine, mixed pine-hardwood, and hardwood forests, riparian hardwood forests, and manicured areas around post facilities. Diet consists mainly of mast crops, such as acorns, hickory nuts, and walnuts (Brown and Batzli, 1984). They also eat fungi, herbaceous plants, tree bark, crops, and insects (Flyger and Gates, 1982; Koprowski, 1994). Predators are numerous, and include snakes (e.g., black rat snakes), owls (Barred Owls), hawks (e.g., Cooper's Hawks [*Accipiter cooperii*], Red-tailed Hawks), weasels, red and gray foxes, bobcats, coyotes, domestic and free-ranging cats and dogs (Koprowski, 1994; Mitchell and Beck, 1992; Mitchell, 1994; Johnston, 2000).

The gray squirrel is an important game species, especially in the southern part of its range. Adjustment of hunting regulations is the primary form of management. Gray squirrels can cause damage to gardens and ornamentals, particularly in urban areas, and are sometimes considered a pest (Flyger and Gates, 1982).

Tamias striatus Linnaeus (Eastern Chipmunk)

The eastern chipmunk is a small, stout-bodied, terrestrial squirrel. It is readily identified by five dark brown, longitudinal stripes. The two lateral pairs of dark stripes are separated by a yellowish stripe. Background pelage is reddish-brown and yellowish on the sides. Measurements for chipmunks we captured at APH, six males and 7 females, respectively, were: total length 180-204 mm (avg.=193 ±10.2), 178-235 mm (avg.=203 ±23.8); tail length 53-74 mm (avg.=66 ±7.2), 62-89 mm (avg.=62 ±9.2); length of hind foot 32-34 mm (avg.=33 ±0.8), 31-34 mm (avg.=32 ±1.2); ear length 12-18 mm (avg.=14 ±2.4). Dentition, i. 1/1, c. 0/0, p. 1/1, m. 3/3 =20 (Snyder, 1982).

Eastern chipmunks occur from southeastern Canada throughout much of the eastern United States, except most of Florida and lower areas of the Carolinas, Georgia, and Florida (Hall, 1981). Two of 11 recognized subspecies of *T. striatus* (*T. s. striatus* and

T. s. fisheri) occur in Virginia (Hall, 1981). *Tamias s. striatus* is found only in extreme southwestern Virginia and *T. s. fisheri* is uncommon in many coastal counties (Handley, 1991).

Eastern chipmunks most frequently occur in deciduous forests, especially areas that provide crevices for protection and elevated surveillance stations (Snyder, 1982). On APH, we observed them in upland forests. Chipmunks enter burrows and are largely inactive from late fall until spring. Hibernation is longer in the northern regions and sporadic in southern regions of its range (Webster et al., 1985). Important cool season foods are seeds, nuts, and acorns (Snyder, 1982). During the growing season, fungi, invertebrates, and mast leftover from winter caches are consumed (Wrazen and Svendsen, 1978; Snyder, 1982). Eastern chipmunks are prey for many predators, including snakes (e.g., black rat snakes, copperheads, black racers), hawks (e. g., Cooper's Hawks), weasels, foxes, bobcats, and domestic and free-ranging cats (Mitchell and Beck, 1992; Mitchell, 1994; Whitaker and Hamilton, 1998; Johnston, 2000).

Castor canadensis Kuhl (Beaver)

The beaver is the largest native North American rodent (Hall, 1981). Pelage is rich glossy brown. Coarse guard hairs are underlain with very dense fine underfur. Males tend to be slightly larger than females (Cothran et al., 1991). Weight of 58 individuals captured on APH during the 1998/1999 furbearer trapping season ranged from 5.0 to 25.0 kg for 36 males (avg.=11.3 ±4.2 kg) and 4.4 to 20.1 kg for 22 females (avg.=12.5 ±5.2 kg). External body measurements for the same males and females, respectively, were: total length 653-1,151 mm (avg.=903 ±124), 620-1,101 mm (avg.=924 ±155); tail length 155-320 mm (avg.=238 ±37), 178-295 mm (avg.=241 ±37); length of hind foot 130-207 mm (avg.=159 ±16), 117-187 mm (avg.=160 ±21); ear length 25-36 mm (avg.=31 ±3), 24-36 mm (avg.=31 ±3). Dentition: i. 1/1, c. 0/0, p. 1/1, m. 3/3 =20 (Jenkins and Busher, 1979).

The native beaver was extirpated from Virginia by 1911 due to unrestricted trapping (Echternach and Rose, 1987). Reestablishment began in 1932 and beavers have since become common in Virginia. The present range, inclusive of all 24 recognized subspecies, includes most of North America south to the most northern regions of Mexico and Florida. *Castor c. canadensis*, is the subspecies found in Virginia's Coastal Plain. *Castor c. carolinensis* occurs in mid- to southwestern Virginia and is the only other subspecies known from the mid-Atlantic region (Hall, 1981).

Good beaver habitat includes impoundments, rivers and streams, and lakes with relatively constant water levels, and small tributaries with enough flow for damming (Hill, 1982). On APH, beavers are present in a variety of wetland types, including impoundments, riparian swamps, and ponds created from seepages and creeks. The large number of beavers captured during the 1998/1999 furbearer trapping season on APH (n=157), in conjunction with the high captures per unit effort (13.0/100 trap nights), reflects the large population size on APH. Beavers feed on a diversity of seasonally abundant plant material. In winter, they rely primarily on the bark and twigs of trees, and in summer herbaceous vegetation becomes a major dietary component (Jenkins and Busher, 1979). Beavers have few predators due to its large size; however, Linzey (1998) summarized observations that dogs (*Canis familiaris*), foxes, bobcats, and otters occasionally eat young beavers.

Woodcutting and dam building by beavers modify hydrology and drainage network morphology by retaining organic matter and sediments (Naiman et al., 1994). These activities have a strong influence on plant and animal community composition and, thus, are responsible for the creation and maintenance of a wide diversity of wetland types and associated floras and faunas. Jenkins and Busher (1979) summarize effects of beaver activity on flora and other fauna.

Beavers are economically important for both positive and negative impacts. They are commercially valued for pelt quality. Activities such as woodcutting and damming often benefit native wildlife, however, these activities may result in the destruction of valuable timber and flooding of roads and pastures (Hall, 1981). Due to the relatively remote nature of much of APH, beaver activity has only a small economic impact (e.g., small-scale timber destruction and road damage).

Microtus pennsylvanicus Ord (Meadow Vole)

The meadow vole is a relatively large member of the genus *Microtus*. Pelage is chestnut brown dorsally and gray ventrally. Underhair is three-banded, gray at the base, orange to yellow brown in the middle, and black-tips. Guard hairs have gray bases with darker tips. Measurements for 11 males and 8 females, respectively, captured on APH were: total length 129-160 mm (avg.=146 ±8.8), 121-183 mm (avg.=159 ±21.0); tail length 31-41 mm (avg.=36 ±2.9), 19-40 mm (avg.=32 ±7.7); length of hind foot 20-22 mm (avg.=21 ±0.8), 20-44 mm (avg.=27 ±10.4); ear length 9-12 mm (avg.=10 ±1.0), 9-11 mm (avg.=10 ±0.8). Dentition: i. 1/1, c. 0/0, p. 0/0, m. 3/3 =16 (Johnson and Johnson, 1982).

The meadow vole has the broadest distribution of any American *Microtus*. This species ranges through Canada, most of Alaska, and southward through northern and eastern regions of the United States to South Carolina and northern Georgia (Reich, 1981). Of 26 recognized subspecies, two (*M. p. pennsylvanicus* and *M. p. nigrans*) are known from Virginia (Hall, 1981). Only *M. p. pennsylvanicus* occurs on APH.

Meadow voles are most commonly associated with grass-dominated habitats, in particular those with abundant perennial grasses and forbs that provide protective cover (Adler, 1985; Dooley and Bowers, 1996). The greatest numbers of this species observed on APH were in old fields; few individuals were taken in forested habitats. The presence of meadow voles in forested habitats is consistent with the observation of Pagels et al. (1992) that this species travels through "atypical environments" in the absence of old-field corridors and that the ephemeral nature of grassy habitats in the eastern United States drives this dispersal behavior (Kirkland, 1988). Diet consists primarily of grasses, sedges, and seeds, but fungi and insects are also eaten (Reich, 1981; Zimmerman, 1965). Although typically confined to ground-level runways, they have been captured above ground-level in traps attached to Japanese honeysuckle vines (Wright and Pagels, 1977). Meadow voles are important prey for many carnivorous forms (Whitaker and Hamilton, 1998). Known snake predators of meadow voles that are known to occur or may occur on APH include mole kingsnakes, black rat snakes, black racers, corn snakes (*Elaphe guttata*), eastern kingsnakes (*Lampropeltis getula*), eastern milk snakes (*L. triangulum*), and copperheads (Mitchell, 1994). Avian predators of meadow voles on APH include Barn Owls, Eastern Screech Owls, Great Horned Owls, Barred Owls, Long-eared Owls (*Asio otus*), Northern Harriers, Cooper's Hawks, Red-tailed Hawks, American Kestrels (Rageot, 1957; Jackson et al., 1976; Johnston, 2000). Mammalian predators found on APH include domestic free-ranging cats, raccoons, foxes, bobcats, weasels and mink (Mitchell and Beck, 1992; Linzey, 1998).

Microtus pinetorum LeConte (Woodland Vole)

The semi-fossorial lifestyle of the woodland vole (or pine vole) is reflected in its smooth silky mole-like pelage, reduced eyes and ears and short tail. Dorsal coloration of this small robust mouse is russet to chestnut brown, with a lighter ventral surface. Measurements of 11 males and 7 females, respectively, captured on APH were: total length 104-125 mm (avg.=112 ±7.8), 109-126 (avg.=116 ±6.2); tail length 17-23 mm (avg.=19 ±2.0), 14-24 mm (avg.=19 ±4.4); length of hind foot 15-17 mm (avg.=16 ±0.8), 15-21 mm (avg.=17 ±2.1); ear length 7-8 mm (avg.=7 ±0.5), 7-8 mm (n=4, avg.=8 ±0.5). Dentition: i. 1/1, c. 0/0, p. 0/0, m. 3/3 =16 (Smolen, 1981).

Woodland voles occur throughout most of the eastern United States, except most of Maine and Florida and coastal regions of other Gulf Coast states. Of 7 recognized subspecies, two (*M. p. pinetorum* and *M. p. scalopsoides*) occur in Virginia (Hall, 1981). Only the latter occurs on APH.

Woodland voles have been collected in most terrestrial habitats, including edge habitat (Rose et al., 1990). Whitaker and Hamilton (1998) remarked that few small mammals use such a variety of habitats. On APH, this species was captured primarily in forested habitats, but also in old fields. This vole was captured in relatively low numbers in all habitats we studied. Its semi-fossorial habits reduce its susceptibility to traps. However, its abundance is positively correlated with downed woody debris (Bellows et al., in press). Diet consists of bulbs, tubers, roots, seeds, fruit, bark, leaves, the fungus *Endogone*, and to a lesser degree, animal matter (Linzey and Linzey, 1973; Smolen, 1981). In Virginia, forbs and grasses comprise the major portion of the diet (Cengel et al., 1978). The largely fossorial habits of the woodland vole may provide some measure of protection from predation. Owls (e.g., Barn Owl, Barred Owls, Eastern Screech Owls), and hawks (e.g., Red-tailed Hawks, Northern Harriers) are primary predators (Jackson et al., 1976; Smolen, 1981). Other predators include snakes (e. g., black rat snakes, eastern milk snakes, copperheads), foxes, domestic and free-ranging cats, and Virginia opossums (Smolen, 1981; Mitchell and Beck, 1992; Mitchell, 1994). They are sometimes considered an agricultural pest due to damage to orchard trees, commercial bulbs, potatoes, and peanuts (Smolen, 1981).

Ondatra zibethicus Linnaeus (Muskrat)

The muskrat is a large semiaquatic rodent with relatively small eyes and a laterally flattened, nearly hairless tail. Pelage is brown to nearly black, thick and soft, with long guard hairs and waterproof underfur. Weight of 9 individuals captured on APH ranged from 1.1 to 1.5 kg for five males (avg.=1.3 ±0.2 kg) and 1.3 to 1.6 kg for four females (avg.=1.5 ±0.1 kg). Measurements for the same individuals, males and females respectively, were: total length 552-611 mm (avg.=576 ±26), 528-638 mm (avg.=579 ±45); tail length 238-257 mm (avg.=246 ±9), 227-253 mm (avg.=244 ±11); length of hind foot 81-86 mm (avg.=82 ±3), 75-85 mm (avg.=82 ±4); ear length 8-17 mm (avg.=13 ±4), 13-21 mm (avg.=18 ±3). Dentition: i. 1/1, c. 0/0, p. 0/0, m. 3/3 =16 (Willner et al., 1980).

The range of the muskrat includes most of North America and northern Mexico, although they are absent in Florida (Hall, 1981). There are 16 subspecies of *O. zibethicus* in North America; *O. z. zibethicus is* the only subspecies found on APH.

In the Chesapeake Bay drainage, muskrats are most abundant in low salinity and freshwater marshes along the Rappahannock, Piankatank, Mattaponi, Pamunkey, and James Rivers (Wass, 1972). Marshes provide optimum habitat for muskrats, although most permanent bodies of water are suitable (Cothran et al., 1991). All individuals captured on APH were in riparian habitats. The muskrat is herbivorous and in the southeastern United States feeds primarily on emergent aquatic vegetation, marsh grasses, and sedges (Wass, 1972; Perry, 1982). Aquatic invertebrates become important foods in winter when vegetation is scarce or in habitats with little aquatic vegetation (Neves and Odom, 1989). Major predators of muskrat are mink, raccoons, red foxes, Barn Owls, Barred Owls, Marsh Hawks, and largemouth bass (*Micropterus salmoides*) (Wass, 1972; Perry, 1982).

Muskrat exceed all other furbearers in numbers caught and marketed and, as a result, is the most valuable fur animal in North America. Muskrats are easily managed when compared to other furbearers, due mostly to prolific breeding and readily-met habitat requirements (Perry, 1982).

Oryzomys palustris Harlan (Marsh Rice Rat)

The marsh rice rat is a medium-sized generalized rat with a long, scantily haired tail and large hind feet. Pelage is gray to grizzled brown mixed with black dorsally and buff to white below. Underfur is dense, soft, and water repellent. Two adult males were collected on APH, but measurements for only one individual are available: total length 214 mm; tail length 102 mm; length of hind foot 29 mm; ear length 16 mm. For comparison, measurements for 7 adults from Chincoteague, Virginia were: total length 252 mm; tail length 121 mm; length of hind foot 30 mm (Whitaker and Hamilton, 1998). Dentition: i. 1/1, c. 0/0, p. 0/0, m. 3/3 =16 (Cothran et al., 1991).

The range of the marsh rice rat includes most of the south-central and southeastern United States and extends northward to southeastern Pennsylvania and southern New Jersey.

In Virginia, the marsh rice rat occurs as far west as the Piedmont in Cumberland County (Pagels et al., 1992) and throughout the Coastal Plain, including barrier islands (Moncrief and Dueser, 1994). Of 23 recognized subspecies, only *O. p. palustris* is known from the mid-Atlantic region (Wolfe, 1982).

Marsh rice rats are found in greatest numbers in wetland habitats, including swamps and freshwater marshes but most frequently in Gulf coastal marshes and tidal creek areas (Wolfe, 1982; Cranford and Maly, 1990). One adult male from APH was captured in a minnow trap set in shallow water in a thickly vegetated beaver pond; another was captured in a mixed pine-hardwood upland forest. We also captured a subadult male in an upland hardwood habitat in a pitfall trap. Because the marsh rice rat prefers riparian habitats, this individual could have been a transient, a scenario supported by Wolfe (1982). Marsh rice rats are excellent swimmers and divers (Esher et al., 1978). They feed on a wide range of plants and animals, but most predominately on seeds, succulent plant parts, mollusks, and arthropods such as insects, crayfish, and crabs (Sharp, 1967; Whitaker and Hamilton, 1998). Owls are the most commonly documented predators, especially Barn Owls (Blem and Pagels, 1973; Jackson et al., 1976).

Other predators include snakes (corn snakes), hawks, raccoons, red foxes, mink, weasels, and striped skunks (Svihla, 1931; Harris, 1953; Lowery, 1974; Brown, 1979; Whitaker and Hamilton, 1998).

Peromyscus leucopus Rafinesque (White-footed Mouse)

The white-footed mouse is a relatively small member of the genus *Peromyscus*. The tail is usually less than half the total body length. Pelage is brown to yellowish-brown dorsally, whitish ventral hairs are dark-based, and the feet are white dorsally. Juveniles are gray dorsally. Weight of mice captured on APH ranged from 12.7 to 24.4 g for 43 males (avg.=17.7 ±2.9) and 12.0 to 24.8 g for 30 females (avg.=17.2 ±3.3). Measurements for the same individuals, males and females respectively, were: total length 130-179 mm (avg.=150.9 ±8.9), 132-182 mm (avg.=148.2 ±12.1); tail length 56-80 mm (avg.=66.0 ±5.4), 55-81 mm (avg.=64.1 ±6.0); length of hind foot 12-20 mm (avg.=18.4 ±1.3), 17-20 mm (avg.=18.3 ±0.7); ear length 13-19 mm (avg.=16.2 ±1.2), 15-19 mm (avg.=17.1 ±1.1). Dentition: i. 1/1, c. 0/0, p. 0/0, m. 3/3 =16 (Cothran et al., 1991).

The white-footed mouse occurs in most of the eastern two-thirds of the United States and southward through eastern Mexico into the Yucatan Peninsula (Hall, 1981). Of the 17 recognized subspecies, two (*P. l. easti* and *P. l. noveboracensis*) occur in Virginia. Records from Hall (1981) indicate that only *P. l. noveboracensis* occurs on APH.

The white-footed mouse is the most common mammal in Virginia (Handley and Patton, 1947). It is found in a wide range of habitat types and considered a habitat generalist (Pagels et al., 1992). At APH, this mouse was abundant in all habitats, including old fields, clearcuts, pine plantations, shelterwood treatments, and pine, mixed pine-hardwood, hardwood, and riparian forests. Insects constitute the majority of its diet throughout the year but mast crops, seeds, fruits, and greens are seasonally important foods (Hamilton, 1941). Major predators are numerous and include owls (e.g., Eastern Screech Owls, Long-eared Owl), hawks (e.g., Northern Harriers), weasels, red foxes, domestic and free-ranging cats and snakes (Mitchell and Beck, 1992; Mitchell, 1994; Whitaker and Hamilton, 1998; Johnston, 2000). Known snake predators that occur or may occur on APH include black racers, black rat snakes, corn snakes, eastern kingsnakes, eastern milk snakes, mole kingsnakes, and copperheads (Mitchell, 1994).

Reithrodontomys humulis Audubon and Bachman (Eastern Harvest Mouse)

The eastern harvest mouse resembles the house mouse but is easily distinguished from it by the grooved upper incisors. Pelage is rich dark brown dorsally with a darker mid-dorsal stripe. Sides are lighter than the dorsum, and underparts are ashy with a cinnamon to pinkish suffusion. Weight of harvest mice captured in southeastern Virginia averaged 7.0 ± 0.1 g for 42 males and 8.2 ± 0.3 g for 35 females (Cawthhorne and Rose, 1989). Measurements for harvest mice captured on APH, 9 males and two females respectively, were: total length 111-134 mm (avg.=123 ±8.0), 117 and 118 mm; tail length 50-61 mm (avg.=56 ±3.7), 60 and 56 mm; length of hind foot 14-17 mm (avg.=15 ±1.0), 11 and 16 mm; ear length 7-10 mm (avg.=8 ±1.0), 9 and 11 mm. External measurements for 12 individuals from Louisiana were similar: males and females combined - total length 115-132 mm (avg.=120), tail length 52-65 mm (avg.=56), length of hind foot 11-18 mm (avg.=16) (Lowery, 1974). Dentition: i. 1/1, c. 0/0, p. 0/0, m. 3/3 =16 (Stalling, 1997).

Eastern harvest mice are found throughout much of southeastern United States and portions of the south-central United States, except southern regions of Florida and Louisiana. Of the three recognized subspecies of *R. humulis*, only *R. h. virginianus* is known from the northern Coastal Plain of Virginia (Stalling, 1997).

Old fields are the preferred habitat of eastern harvest mice, especially the late herbaceous and early perennial grass stages of old-field succession (Stalling, 1997), including clearcuts (Pagels et al., 1992). We collected them on APH most frequently in old fields, however, some were collected 5 to10 year old clearcuts, upland hardwood forests, and in forests managed with the shelterwood treatment. Diet consists of seeds (particularly seeds of grasses), green shoots, and insects such as grasshoppers and crickets (Dunaway, 1968; Stalling, 1997; Whitaker and Hamilton, 1998). Reports of predation on eastern harvest mice are rare, but harvest mouse remains are regularly found in Barn Owl pellets (Handley, 1949; Wolfe and Rogers, 1969; Jackson et al., 1976). Johnston (2000) reported a harvest mouse in the stomach of a Great Horned Owl from Wythe County, Virginia.

Zapus hudsonius Zimmermann (Meadow Jumping Mouse)

Several characters of the meadow jumping mouse reflect their saltatory locomotion—hind limbs, legs, and feet are much longer and larger than forelimbs, arms, and feet, and the tail is very long. Pelage is coarse with a broad, longitudinal dorsal band of yellow-brown hairs darkened with brownish-black hairs. Sides are paler than the dorsum and underparts are buff to light yellow. Measurements for mice captured on APH, 26 males and 19 females respectively, were: total length 172-309 mm (avg.=193 ±8.5), 166-215 mm (avg.=190 ±11.7); tail length 99-126 mm (avg.=113 ±7.4), 102-125 mm (avg.=113 ±5.8); length of hind foot 25-30 mm (avg.=28 ±1.3), 23-29 mm (avg.=27 ±1.5); ear length 9-15 mm (avg.=13 ±1.9), 9-15 mm (avg.=11 ±2.0). Dentition, i. 1/1, c. 0/0, p. 0/0, 3/3 =16 (Whitaker, 1972).

The range of the meadow jumping mouse extends from southern Alaska to central and southern Canada and south through north-central and eastern United States, except the extreme southeastern regions (Hall, 1981). This mouse may be locally abundant throughout its range (Hamilton, 1935). Of the 11 recognized subspecies, only *Z. h. americanus* occurs in the mid-Atlantic region and APH.

The hibernation period of meadow jumping mice is longer than for most other hibernating mammals and in some parts of its range the duration may be as much as six months (Whitaker, 1972). No other species of small mammal found on APH hibernates. Meadow jumping mice most frequently occur in open meadows and grasslands, but also in forests with abundant herbaceous cover (Hamilton, 1935; Whitaker, 1972; Adler, 1985; Pagels et al., 1992). They are often abundant in thick brush bordering ponds, streams, and marshes (Whitaker, 1972). Most meadow jumping mice collected on APH were captured in old-field habitats, however, we captured 22 individuals in open canopy forests (e.g., shelterwood treatments), several in forest habitats, and one on the edge of a swamp. Diet consists primarily of seeds (especially of grasses), insects, and the fungus *Endogone* (Hamilton, 1935; Whitaker, 1963). Predators of meadow jumping mice are many, and include owls, (Barn Owls, Long-eared Owls, raptors (Red-Tailed Hawks), foxes, skunks, weasels, and snakes (e.g., copperheads) (Whitaker, 1963; Eaton and Grzybowski, 1969; Mitchell, 1994; Whitaker and Hamilton, 1998).

Order Carnivora

Canis latrans Say (Coyote)

The word "coyote" is taken from the Aztec word "Coyotyl," meaning "barking dog" (Bekoff, 1982). The coyote is often mistaken for other canids, such as gray wolf (*Canis lupus*), red wolf (*C. rufus*), and domestic dog. They hybridize successfully (but rarely) with those species and produce fertile offspring (Bekoff, 1982). Pelage is reddish, brown, and sometimes almost pure gray above, dorsal hairs are black-tipped, the head is grizzled gray, and the throat and belly are pale. An adult female from APH weighed 12 kg. Measurements were: total length 1295 mm, tail length not available, length of hind foot 184 mm, ear length 96 mm. Males are slightly heavier than females; males range from 8 to 20 kg, females 7 to 18 kg (location not provided, Bekoff, 1977). Measurements for adults were: total length 1,075-1,200 mm, tail length 300-390 mm, length of hind foot 175-220 mm (location not provided, Whitaker and Hamilton, 1998). Dentition, i. 3/3, c. 1/1, p. 4/4, m. 2/3 =42 (Cothran et al. 1991).

Coyotes range throughout the contiguous United States and most of Canada (Hall, 1981). They are becoming more common in the eastern United States as a result of natural range expansion due to land use changes and transplantations (e.g., Florida and Georgia) (Bekoff, 1982). There are 19 recognized subspecies of *C. latrans*, but only *C. l. latrans* is found in the mid-Atlantic region (Whitaker and Hamilton, 1998).

Coyotes inhabit a variety of grassland, desert, and montane habitats (Bekoff, 1982). Agricultural and rural land-use patterns of the mid-Atlantic region provide suitable habitat (Webster et al., 1985). They have been observed on APH in pine and hardwood upland forests. The adult mentioned above was killed by a hunter in an upland forest. Another killed by a car in the fall of 2000 near Smoot's Pond was a juvenile male, suggesting that a breeding population is present on or near APH. It is an opportunistic predator that feeds on a variety of prey; diet varies both seasonally and locally (Bekoff, 1977). Coyotes are often accused of killing livestock and have been considered a nuisance by ranchers and farmers (Cothran et al., 1991). There is little doubt that coyotes are responsible for some livestock mortality, but their diet consists primarily of wildlife (Whitaker and Hamilton, 1998). These authors summarized the results of the examination of 1,500 coyote scats collected in the Adirondacks and wild mammals were the most frequent component (78%), followed by fruits (21%), insects (10%), and birds (3%). The remaining components were reptiles, amphibians, and grasses. Carrion from large mammals such as deer is an important winter food source (Whitaker and Hamilton, 1998). Predation on young coyotes by dogs has been reported by Berg and Chesness (1978).

Movement patterns are generally within undefended territories, or home ranges, that vary geographically and seasonally (Bekoff, 1977). In western Tennessee, Babb and Kennedy (1988) reported that homes ranges averaged 31 km^2 for males and 60 km^2 for females.

Urocyon cinereoargenteus Schreber (Gray Fox)

The gray fox, a medium-sized canid, is slightly smaller than the red fox. Pelage has a "salt and pepper" appearance on the upperparts, a cinnamon neck and underparts, a dorsal median black stripe on a black-tipped tail, and black-tipped ears. Measurements of four adults from Georgia were: total length 921-955mm (avg.=935), tail length 280-351 mm (avg.=322), length of hind foot 120-150 mm (avg.=132) (Cothran et al., 1991). Dentition, i. 3/3, c. 1/1, p. 4/4, m. 2/3 =42 (Fritzell and Haroldson, 1982).

The range of the gray fox extends into southern Canada, westward to the Pacific Coast, and southward to northern Venezuela and Columbia (Fritzell and Haroldson, 1982). There are 16 recognized subspecies of *U. cinereoargenteus*, but only *U. c. cinereoargenteus* is native to the mid-Atlantic region (Samuel and Nelson, 1982).

Gray foxes inhabit areas with a diversity of fields and woods, but are most common in areas that are mostly forested (Samuel and Nelson, 1982). All 18 individuals captured on APH were trapped in riparian forests; however, we have observed them in old fields and upland pine, mixed, and hardwood forests. Average home range size varies according to sex and geographic location. In West Virginia, male (n=3) home ranges averaged 97 ha and the home range of a single female was 75 ha (Yearsley and Samuel, 1980). Home ranges are stable throughout the year and do not shift as seasonal food becomes available (Greenburg and Pelton, 1994). Home ranges often include a heterogeneous group of habitat types that provides a wide range of foods in all seasons (Greenberg and Pelton, 1994). Mammals, primarily cottontails and rodents, comprise the bulk of the winter diet (Samuel and Nelson, 1982). Mammals remain important year-round as prey, but during summer insects and fruit predominate (Wood, 1958). Natural predators of gray foxes are few, but include raptors and bobcats (Mollhagen et al., 1972; Whitaker and Hamilton, 1998).

Like red foxes, gray foxes may sometimes kill game species and livestock (Samuel and Nelson, 1982). Gray foxes are economically important furbearers. Management plans for both gray and red foxes have shifted from "bounties" designed to reduce their numbers to regulation and maintenance of their populations for continued hunting and trapping.

Vulpes vulpes Linnaeus (Red Fox)

The soft dense pelage of the red fox is reddish-yellow on the head, back, and tail, darkest on the shoulders, and white on the tip of the tail, belly, and throat and the ears are often tipped in black. An adult male captured on APH weighed 4.9 kg. Measurements were: total length 970 mm, tail length 360 mm, length of hind foot 149 mm, ear length 79 mm. The average weight of three adults from Georgia was 3.0 kg (Cothran et al., 1991). Ranges of measurements for the same three adults were: total length 639-940 mm (avg.=776), tail length 245-335 mm (avg.=282), length of hind foot 117-150 mm (avg.=133) (Cothran et al., 1991). Dentition, i. 3/3, c. 1/1, p. 4/4, m. 2/3 =42 (Samuel and Nelson, 1982).

Red foxes have the broadest distribution of any carnivore in the world (Samuel and Nelson, 1982). They occur throughout Europe, all but the most northern regions of Asia, and they have been introduced to Australia. In North America, its range includes all of Alaska and Canada and extends south throughout most of the United States, except the extreme southwestern regions, southern Florida, and parts of the Great Plains (Hall, 1981). Of numerous subspecies (Larivière and Pasitschniak-Arts, 1996), only *Vulpes v. fulva* occurs in the eastern United States and APH (Samuel and Nelson, 1982).

Red foxes frequent open habitats, such as old fields, edges, and croplands, and unlike gray foxes they are seldom seen in heavily wooded areas (Samuel and Nelson, 1982). Five individuals captured on APH were trapped in riparian habitats, an impoundment, and forested streamside habitats. They have also been observed on APH in upland forests and old fields. Diet consists primarily of small mammals, but fish, reptiles, insects, garbage, carrion, and plant matter may be seasonally or locally important (Cothran et al., 1991; Mitchell, 1994). Various carnivores including raptors and coyotes occasionally eat young foxes; coyotes are known to kill adult red foxes (Whitaker and Hamilton, 1998).

Most current management of foxes involves hunting and trapping regulations, however, habitat management has been suggested (Samuel and Nelson, 1982). The red fox probably benefits from early successional habitats.

Procyon lotor Linnaeus (Raccoon)

The raccoon is one of the most recognizable mammals of the region due to its conspicuous facial mask and ringed tail. Weights of adult females generally average 10 to 15% less than males (Kaufmann, 1982). Weight of 16 individuals captured on APH ranged from 3.4 to 6.0 kg for 10 males (avg.=4.8 ±0.8 kg) and 3.4 to 6.0 kg for six females (avg.=4.5 ±1.0 kg). Measurements for the same males and females, respectively, were: total length 754-890 mm (avg.=819 ±38), 580-938 mm (avg.=758 ±144); tail length 199-284 mm (avg.=232 ±29), 200-290 mm (avg.=241 ±35); length of hind foot 104-114 mm (avg.=110 ±4), 98-116 mm (avg.=108 ±7); ear length 29-64 mm (avg.=50 ±11), 35-58 mm (avg.=48 ±9). Dentition: i. 3/3, c. 1/1, p. 4/4, m. 2/2 =40 (Lotze and Anderson, 1979).

The raccoon ranges from southern Canada through Central America (Kaufmann, 1982). Of the 25 recognized subspecies, only *P. l. maritimus* is known from the region (Hall, 1981).

Raccoons are generally associated with riparian habitats such as swamps, coastal salt marshes, and along the banks of streams and lakes, but it will readily frequent moist uplands, suburban neighborhoods, and agricultural areas (Wass, 1972; Webster et al., 1985). We captured raccoons on APH in upland and riparian habitats. We have also been observed in clearcuts, old fields, and roadside fire-breaks. Raccoons are opportunistic omnivores, however, preferred foods are arthropods (especially crayfish and crabs), small rodents, fish, berries, acorns, fruits, eggs and adults of freshwater turtles, and in agricultural areas, grains such as corn, wheat, and millet (Whitney, 1931; Lotze and Anderson, 1979; Mitchell, 1994). The raccoon is a major predator of young muskrats (Wass, 1972). Predation on adult raccoons is likely infrequent, however, bobcats, coyotes, dogs, foxes, and Great Horned Owls will occasionally consume them (Kaufmann, 1982).

Raccoons are often considered a vector for disease and in the southeastern United States are known to carry at least 13 pathogens that are transmittable to humans and their pets (Bigler et al., 1975). Among the most notable are leptospirosis, rabies, tularemia, and canine distemper (Lotze and Anderson, 1979). Raccoons harbor a wide variety of internal and external parasites. In southwestern Georgia, one of the most common external parasites is the tick *Dermacentor variabilis*, which is known to transmit tularemia (Lotze and Anderson, 1979).

Although the demand for pelts fluctuates, raccoons remain an important commercial fur species. Males tend to be more trappable than females, two to one in most studies or censuses, probably because males tend to move more and have larger home ranges (Kaufmann, 1982). Results from the 1998/1999 trapping season on APH were consistent with this result, males (n=45) outnumbered females (n=19).

Lontra canadensis Schreber (River Otter)

Pelage of this large, semiaquatic mammal is brown on upperparts, paler below, with dense gray-tipped underfur. The lower jaw and throat are whitish. Females are slightly smaller than males. Weight of six individuals captured on APH ranged from 5.8 to 7.7 kg for three males (avg.=6.8 ±1.0 kg) and 4.6 to 9.5 kg for three females (avg.=6.9 ±2.5 kg). Measurements for these males and females, respectively, were: total length 999-1,120 mm (avg.=1,080 ±70), 963-1,170 mm (avg.=1,071 ±104); tail length 335-423 mm (avg.=397 ±54), 339-425 mm (avg.=376 ±44); length of hind foot 111-126 mm (avg.=120 ±7), 113-121 mm (avg.=116 ±4); ear length 12-25 mm (avg.=20 ±7), 14-24 mm (avg.=19 ±5). Dentition: i. 3/3, c. 1/1, p. 4/3, m. 1/2 =36 (Toweill and Tabor, 1982).

The historical distribution of the river otter encompassed most of North America. They were present in all waterways of the United States and Canada until at least the 18^{th} century, however, overtrapping extirpated them from many states and portions of others (Toweill and Tabor, 1982). There are 7 recognized subspecies of *L. canadensis*, but only *L. c. lataxina* is known from the region (Hall, 1981).

River otters occur in a wide variety of aquatic habitats, especially those maintained by beavers (Newman and Griffin, 1994). In the mid-Atlantic region, they are present in coastal estuaries and the lower reaches of most river systems (Webster et al., 1985). Fish and crayfish are the most important dietary components, but they will consume mammals, especially muskrats, turtles, and amphibians (Gilbert and Nancekivell, 1982). No predator has been shown to seriously affect river otter populations (Toweill and Tabor, 1982).

Due to the thick and durable pelage, river otters have been commercially important furbearers since the European settlement of North America. In the past they has been erroneously accused of depleting game fish populations, in fact, otters are often a benefit to game fish through predation of non-game fish species that can potentially displace game fish (Toweill and Tabor, 1982).

Mephitis mephitis Schreber (Striped Skunk)

The striped skunk is readily identified by its odor and its pelage, which ranges from black with a large white patch that extend from the back of the head to the rump to all black. Females are roughly 15% smaller than males (Hall, 1981). Body measurements of 26 individuals from Indiana were: total length 447-635 mm (avg.=586), tail length 159-290 mm (avg.=221), length of hind foot 48-71 mm (avg.=63) (Whitaker and Hamilton, 1998). Dentition: i. 3/3, c. 1/1, p. 3/3, m. 1/2 =34 (Wade-Smith and Verts, 1982). This species was well represented on APH (n=11) during the 1998/1999 furbearer trapping season but weights and measurement data were not made available.

The distribution of the striped skunk includes southern Canada, northern Mexico, and all regions of the contiguous United States except some arid areas of the extreme southwest (Wade-Smith and Verts, 1982). There are 13 recognized subspecies of *M. mephitis* and both *M. m. nigra* and *M. m elongata* are known from Virginia. *Mephitis m. elongata* is most prevalent in the Piedmont and mountains (Hall, 1981) and *M. m. nigra* occurs on the Coastal Plain (Whitaker and Hamilton, 1998).

Striped skunks inhabit many habitat types, including old fields, forests, and croplands (Verts, 1967). On APH, they were frequently captured in beaver-maintained habitats and observed in upland forests. Striped skunks are omnivores that feed primarily on insects, but also eat fruits and crops in season (Verts, 1967). In spring and winter, when preferred insects are scarce, small mammals, snakes, bird eggs and nestlings, and freshwater turtles and their eggs become important dietary components (Wade-Smith and Verts, 1982; Mitchell, 1994). Great Horned Owls, Barred Owls, bobcats, foxes, coyotes, and dogs are known to prey on striped skunks (Godin, 1982).

Insect control, especially those insects that negatively affect crop production, is the primary economic value of striped skunks. They are commercially important fur-bearers, particularly in northern states where they are often protected within management guidelines (Godin, 1982).

Mustela frenata Lichtenstein (Long-tailed Weasel)

The long-tailed weasel has the typical tubular mustelid body with short legs. Its tail reaches 44-70% of body length (Sheffield and Thomas, 1997). Pelage is brown dorsally with underparts yellow or buff continuous from the chin to the inguinal area. Southern populations lack white winter pelage (Sheffield and Thomas, 1997). Females are approximately two-thirds the size of males (Cothran et al., 1991). Measurements for 20 adult males and 13 adult females from a population in New York, respectively, were: total length 374-447 mm (avg.=405), 306-362 mm (avg.=325); tail length 124-157 mm (avg.=135), 95-117 mm (avg.=108); length of hind foot 42-50 mm (avg.=45), 35-41 mm (avg.=37) (Whitaker and Hamilton, 1998). Dentition: i. 3/3, c. 1/1, p. 3/3, m. 1/2 =34 (Cothran et al., 1991).

Long-tailed weasels have the broadest distribution of any mustelid in the Western Hemisphere and occur in all life zones except desert (Sheffield and Thomas, 1997). They occur in North America in southern Canada, throughout the United States except the extreme Southwest, and throughout Mexico and Central America. Of 35 recognized North American subspecies, only *M. f. novaboracensis* occurs in Virginia (Hall, 1981). Long-tailed weasels have not yet been collected on APH, although they should be present (Handley and Patton, 1947; Whitaker and Hamilton, 1998).

As with least weasels, habitat use is primarily based on prey abundance, but ecotones, habitats with dense brush or vegetation, and those close to standing water seem to be preferred (Gamble, 1981). Diet consists mainly of small rodents (particularly *Microtus* and *Peromyscus* spp.), shrews, and young cottontails (Hamilton, 1933). Occasionally moles, bats, birds, bird eggs, and rarely snakes, lizards, and insects are eaten (Polderboer et al., 1941; Teer, 1964; Mumford, 1969). Predators of long-tailed weasels include snakes, raptors, and foxes (Hamilton, 1933).

Mustela nivalis Linnaeus (Least Weasel)

The least weasel is the smallest member of the order Carnivora. Summer pelage is brown above and white below; winter pelage is white in northern regions and brown above and below in southeastern regions (Sheffield and King, 1994). Males are larger than females. A single female collected on APH weighed 34.5 g; measurements were: total length 180 mm, tail length 32 mm, length of hind foot 21 mm, length of ear 10 mm (Bellows et al., 1999b). Weights of five males and two females from West Virginia and from the Piedmont and montane areas of Virginia ranged from 34 to 64 g (avg.=44

g) for males and 25 g for females (Handley, 1991). Measurements of these males and females, respectively, were: total length 184-217 mm (avg.=196); 174-175 mm; tail length 28-42 mm (avg.=34), 26-34 mm; length of hind foot 22-25 mm (avg.=23), 18-20 mm. Dentition: i. 3/3, c. 1/1, p. 3/3, m. 1/2 =34 (Sheffield and King, 1994).

The species has a circumboreal distribution, which in North America includes all of Alaska, Canada except extreme western areas, north-central United States, and southward down the Appalachian Mountains (Sheffield and King, 1994). Of the four recognized subspecies of *M. nivalis*, only *M. n. allegheniensis* occurs in the Eastern United States (Hall, 1981). The female captured at APH represented the first least weasel reported from Virginia's Coastal Plain (Bellows et al., 1999b). It is not clear whether this capture represents a recent range expansion or is the result of trapping this animal in an area previously unstudied. Other Virginia records for this species are from the mountains and Piedmont (Handley, 1991).

Least weasels are known to occupy many habitats, and home ranges are primarily determined by prey availability (Sheffield and King, 1994). The female collected on APH was captured in a mixed pine-hardwood forest (Bellows et al., 1999b). Small mammals comprise 50 to 80% of the diet, mostly rodents (over 90% in autumn and winter), birds, lizards, and insects are also eaten (Hamilton, 1933). Predators of the least weasel are similar to (and include) the long-tailed weasel (Hamilton, 1933; Handley, 1949; Jackson, 1961).

Mustela vison Schreber (Mink)

This medium-sized member of the weasel family is well known for its soft, lustrous pelage that in wild forms is primarily chestnut brown. Underparts are somewhat lighter than the dorsal surface. Underfur is dense, overlain with oily guard hairs that serve as waterproofing (Lowery, 1974). Females average 10% smaller in external body measurements and about 50% less in weight than males (Hall, 1981). Weight of a single adult male captured on APH was 1.8 kg. Measurements were: total length 641 mm, tail length 225 mm, length of hind foot 68 mm, ear length 19 mm. Weights from throughout its range for males range from 0.9 to 1.6 kg and females from 0.7 to 1.1 kg (Jackson, 1961). Adult measurements for these individuals, males and females, respectively, were: total length 580-700 mm, 460-575 mm; tail length 190-230 mm, 150-190 mm; length of hind foot 68-80 mm, 60-70 mm (Jackson, 1961). Dentition: i. 3/3, c. 1/1, p. 3.3, m. 1/2 =34 (Linscombe et al., 1982).

The range of the mink includes all of Canada south of the tree line and all of the lower 48 United States except portions of southwestern states. Of 16 recognized subspecies, two (*M. v. vison* and *M. v. mink*) occur in Virginia. *Mustela v. vison* is restricted to southwestern montane regions of Virginia and *M. v. mink* is found in the Piedmont and Coastal Plain (Hall, 1981)

Mink occur in all types of aquatic habitats, including human constructed habitats (e.g., drainage ditches and canals) (Clark et al., 1985; Linscombe et al., 1982). During the 1998/1999 trapping season on APH, mink were trapped in streamside riparian forests and forests surrounding impoundments. They are opportunistic carnivores that feed on most small aquatic and terrestrial animals; readily eating whatever prey is abundant (Gilbert and Nancekivell, 1982; Linscombe et al., 1982). Wilson (1954), who examined the stomach contents of 335 mink in northeastern North Carolina, recorded the following percentages: fish 61%, mammals 34%, arthropods 30%, birds 18%, amphibians 13%, and reptiles 5%. Mink are occasionally eaten by red and gray foxes, bobcats, and Great Horned Owls (Linscombe et al., 1982).

Mink have long been considered one of the most desired furs on the commercial market, mostly due to the perceived prestige of owning a mink garment. Mink ranching practices that produce large quantities of perfectly matched pelts have reduced trapping pressure on wild populations (Linscombe et al., 1982).

Lynx rufus Schreber (Bobcat)

The bobcat is roughly twice the size of the domestic cat, but is readily distinguished by the very short tail, and otherwise taller and muscular build. Males are larger than females. Pelage is yellowish to reddish brown and is streaked or spotted with black to dark brown. Guard hairs are black-tipped. Underparts are black with white spots. One bobcat was collected on APH during the 1998/1999 trapping season, however, measurements for this individual were not made available. Adult measurements for 37 males and 51 females, respectively, from North Carolina were: total length 610-940 mm (avg.=823), 610-864 mm (avg.=728); tail length 113-178 mm (avg.=138), 90-152 mm (avg.=121); length of hind foot 137-178 mm (avg.=159), 127-165 mm (avg.=145) (Whitaker and Hamilton, 1998). Dentition: i. 3/3, c. 1/1, p. 2/2, m. 1/1 =28 (McCord and Cardoza, 1982).

Bobcats are distributed from southern Canada to most of Mexico. They were extirpated from much of the Ohio Valley, upper Mississippi Valley, and southern Great Lakes region. Two of 12 recognized subspecies (*L. r. floridanus* and *L. r. rufus*) are found in the mid-Atlantic region (Hall, 1981). Only *L. r. rufus* is present on APH.

Bobcats frequent a diverse array of habitats ranging from dry (xeric) to moist (mesic), however, the principal factor that defines habitat quality is prey abundance (Larivière and Walton, 1997; Koehler and Hornocker, 1989). A strict carnivore, bobcats are known to eat sciurids (squirrels) and microtines (voles and mice) opportunistically, but feed primarily on lagomorphs (rabbits and hares) (Whitaker and Hamilton, 1998). They will occasionally attack poultry and young livestock (Cothran et al., 1991). Adult bobcats are rarely victims of predation, however, kittens may be killed by foxes, coyotes, and owls (McCord and Cardoza, 1982).

Bobcats are secretive animals that avoid interaction with humans and are rarely seen even in areas where tracks are commonly observed. Bobcat pelts have remained commercially valuable since the European settlement of North America (McCord and Cardoza, 1982).

Order Artiodactyla

Odocoileus virginianus Rafinesque (White-tailed Deer)

The white-tailed deer is a large even-toed ungulate and the largest herbivore occurring in Virginia. Males have antlers that are grown annually and shed in winter following the breeding season. Summer pelage is thin and wiry, red-brown to bright tan dorsally, but underparts, including tail, inside of legs, belly, and chin are white. Pelage is longer and thicker in winter, with upperparts blue-gray to gray-grown. Weight and external adult measurements vary greatly depending on subspecies and location with high elevation and northern populations reaching generally larger sizes. Average dressed weights for aged adult deer (1.5 yrs and older) harvested on APH during three hunting seasons on APH were: bucks (1997/1998, n=502, 28.6 kg; 1997/1998, n=390, 37.2 kg, 1999/2000, n=286, 31.3 kg) (Table 1), and does (1997/1998, n=629, 28.7 kg; 1997/1998, n=480, 29.6 kg, 1999/2000, n=278, 29.8 kg) (Table 2). Average antler beam diameter for the same group of bucks was: 1997/1998, 24 mm; 1998/1999, 26 mm; 1999/2000, 23 mm (Table 1). Dentition: i. 0/3, c. 0/1, p. 3/3, m. 3/3 =32 (Hesselton and Hesselton, 1982).

In North America, the white-tailed deer is found from southern Canada throughout the contiguous United States (except some southwestern regions), and throughout Mexico and Central America (Hall, 1981). Of the 30 recognized, only *O. v. virginianus* occurs in Virginia (Smith, 1991). White-tailed deer are the only remaining native member of the deer family (Cervidae) occurring in the mid-Atlantic region (Webster et al., 1985). Another cervid, the elk or wapiti (*Cervus canadensis*) disappeared from Virginia in 1855 (Handley and Patton, 1947).

During the Colonial period, white-tailed deer were abundant throughout the state, but agricultural expansion and uncontrolled hunting severely reduced Virginia's deer herd, reaching lowest numbers by 1925 (Handley and Patton, 1947; Barick, 1951). Between 1930 and 1950 over 1,800 white-tailed deer were introduced into Virginia from nearby states (Engle, 1951). In conjunction with restocking programs, strict hunting laws, shorter hunting seasons, and abundant suitable habitat, the white-tailed deer has become abundant statewide. White-tailed deer distribution and abundance appears to positively correlate with the distribution of forest openings, riparian zones, and farmlands. As a result of abundant habitat, white-tailed deer are common throughout APH. Their diet tracks the seasonal availability of plant material. Succulent green plants and mushrooms are preferred in summer. In autumn, acorns are dominant, but Japanese honeysuckle, grapes (*Vitis* spp.), sumac, blueberries, and green brier are also important foods. Winter foods are primarily acorns, grasses, and honeysuckle (Hesselton and Hesselton, 1982). Most agricultural crops are readily eaten when available. Major predators of the white-tailed deer are feral dogs and the coyote.

Historically, counts of yearly buck and doe harvests were the only data available on the deer herd on APH. Wildlife biologists on APH were more interested in determining if deer have exceeded the carrying capacity of the installation's habitat than in an absolute estimate of the size of the herd. A primary indicator of the herd's relationship with carrying capacity is overall herd health. Therefore, in recent years, extensive data have been collected to determine the health of the deer herd on APH. The data collected on harvested deer include body weight/age class, evidence of hemorrhagic disease (splitting or sloughing hooves), antler beam diameter (ABD) and number of antler points (bucks), and evidence of lactation (does) (Tables 1-3). In more extensive health checks, complete necropsies were performed and bone marrow consistency, fat covering of body organs, and the number of fetal fawns per doe were also documented (Table 2). In 1996, the deer herd was determined to have exceeded the carrying capacity and the deer were in poor health.

To improve deer herd health, APH wildlife biologists increased the harvest, in particular the doe harvest, by increasing the number of days that does could be harvested and by adding a special muzzleloader season. After four years of intensive harvesting (1996-2000), improved health was realized in deer 2.5 yrs-old and younger. For a more detailed description of the current deer management plan refer to the "Hunting and Trapping Programs" section.

TABLE 1. Summary of age and three health attributes for a sample of white-tailed deer bucks harvested during three consectutive hunting seasons (1997/1998, 1998/1999, 1999/2000) on Fort A. P. Hill, Virginia. Hemmorhagic disease = HD. Antler beam diameter = ABD.

Mean	Aged (*n*)			Mean dressed weight (kg)			Individuals with HD			Mean ABD (mm)		
Age (yrs)	97/98	98/99	99/00	97/98	98/99	99/00	97/98	98/99	99/00	97/98	98/99	99/00
0.5	150	118	83	15.0	16.8	17.7	2	2	4			
1.5	211	119	104	29.5	29.9	31.3	3	4	8	14	15	15
2.5	87	101	65	39.5	39.5	40.4	4	4	5	22	22	23
3.5	43	45	28	43.1	42.2	44.5	0	1	1	26	24	26
4.5	9	3	6	47.6	49.9	50.8	0	0	0	31	32	30
5.5	2	3		49.0	49.9		0	0		28	36	
6.5		1			52.2			0			32	
Mean (all classes)				37.3	40.1	36.9						
Mean ($\geq$ 1.5 yrs)				41.7	42.3	41.8				24	26	23
Total aged		502	390	286			9	11	18			
Total unaged		264	317	309								
Total harvest		766	707	595								

TABLE 2. Summary of age and four health attributes for a sample of white-tailed deer does harvested during three consectutive hunting seasons (1997/1998, 1998/1999, 1999/2000) on Fort A. P. Hill, Virginia. Hemmorhagic disease = HD. Data for mean number of fetuses/doe were not available for the 1997/1998 and 1998/1999 hunting seasons.

Mean	Aged (*n*)			Mean dressed weight (kg)			Individuals with HD			Lactating does (*n*)			Fetus/doe
Age (yrs)	97/98	98/99	99/00	97/98	98/99	99/00	97/98	98/99	99/00	97/98	98/99	99/00	98/99
0.5	177	117	66	14.5	15.9	16.3	4	6	3				
1.5	131	106	62	25.4	26.3	28.1	2	5	4	7	25	3	1.4
2.5	113	113	71	29.5	30.4	31.3	2	3	7	31	62	29	0.7
3.5	98	63	46	30.8	31.8	31.8	1	4	4	27	31	4	1.2
4.5	44	29	14	31.3	32.7	31.8	1	1	1	16	17	1	2.0
5.5	27	21	6	30.8	32.2	34.0	0	0	0	5	15		1.0
6.5	14	13	6	30.4	31.3	32.2	0	0	0	2	6		1.3
7.5	5	4	3	33.1	33.6	31.8	1	0	0	0	2		1.2
8.5	17	9	3	29.5	32.2	33.6	0	0	0	3	3		
9.5	1	2	1	29.5	33.1	27.2	0	0	0	1	2		
10.5	2	2		30.8	28.1		0	0		0	0		
12.5		1			27.2			0			0		
Mean (all classes)				28.7	29.6	29.8							
Mean (< 1.5 yrs)				30.1	30.8	31.3							1.3
Total aged	629	480	278				11	19	19	92	163	37	
Total unaged	293	382	295										
Total harvest	922	862	573										

TABLE 3. Description of health parameters for white-tailed deer.

Weight:	An indicator of the health and nutritional status. Low mean weights, or declines in weight, as evidenced by long-term data, is an indication that a population has exceeded the carrying capacity of the environment.
Antler Beam Diameter (ABD):	An indicator of the amount of food consumed and a deer's nutrition; ABD is positively correlated with nutrition.
Number of antler points:	For the most part, the number of antler points is only of interest to the hunter, the exception is that well nourished bucks may go from a button buck to a forked (four point) buck in a year. A high percentage of yearling (1.5 yrs-old) spike bucks (50% or more) and low body weights indicate a nutritional deficit that often results from a population too large for the amount of available forage (Coggin, 1987).
Hemorrhagic Disease (HD):	This disease is caused by two different strains of virus. Deer infected with more virulent strains die within 72 hours. Those that survive typically show lesions and/or sloughing and/or splitting of the hooves (Davidson and Nettles, 1997). If many deer with HD are harvested, the size of the deer harvest for the next year(s) may need to be reduced or modified.
Productivity:	Although possible, doe fawns (6-7 months) do not typically breed (Smith 1991). Yearling does usually produce one offspring and older does usually twin until they are about 7-8 years old, when they cease reproduction. However, reproductive status can be confounded by health. Triplets may be born to does receiving adequate nutrition, and fawns in excellent health have are known to produce a single offspring (Halls, 1974). Conversely, older does that are in poor health produce fewer offspring.

TABLE 4. Summary of the occurrence of 40 terrestrial mammal species in generalized habitat types on Fort A. P. Hill, Virginia. See text for descriptions of the habitat types. An "X" indicates an original capture or an observation for the species in these habitats.

	Habitat type				
Species	Old Field & Clearcut	Pine*	Pine-hardwood	Hardwood	**Riparian & Wetland
Didelphis virginiana			X	X	X
Blarina brevicauda	X	X	X	X	X
Cryptotis parva	X	X	X	X	
Sorex hoyi	X	X	X	X	X
Sorex longirostris	X	X	X	X	X
Condylura cristata	X	X	X	X	
Scalopus aquaticus			X		
Eptesicus fuscus	X		X	X	X
Lasionycteris noctivagans			X	X	X
Lasiurus borealis	X	X	X	X	X
Lasiurus cinereus				X	X
Myotis austroriparius			X	X	X
Myotis lucifugus	X		X	X	X
Myotis septentrionalis			X	X	X
Nycticeius humeralis				X	X
Pipistrellus subflavus	X		X	X	X
Sylvilagus floridanus	X		X	X	
Glaucomys volans		X	X	X	X
Marmota monax	X			X	X
Sciurus carolinensis		X	X	X	X
Tamias striatus		X	X	X	
Castor canadensis			X	X	X
Microtus pennsylvanicus	X		X	X	
Microtus pinetorum	X	X	X	X	X
Ondatra zibethicus			X	X	X
Oryzomys palustris			X	X	X
Peromyscus leucopus	X	X	X	X	X
Reithrodontomys humulis	X			X	
Zapus hudsonius	X	X		X	X
Canis latrans		X	X		X
Urocyon cinereoargenteus	X	X	X	X	X
Vulpes vulpes	X			X	X
Procyon lotor	X	X	X	X	X
Lontra canadensis				X	X
Mephitis mephitis		X	X	X	X
Mustela frenata					
Mustela nivalis			X		
Mustela vison				X	X
Lynx rufus				X	X
Odocoileus virginianus	X	X	X	X	X

* Includes pine plantations

** May be a subset of successional habitats that include beaver ponds, swamps, impoundments, and streamside floodplain

Introduced Species

In addition to domestic pets and livestock, several species of mammals closely associated with humans have been introduced into North America from the Old World. Two species, Norway rat and house mouse, likely occur from time to time in parts of the APH complex. Both species are most common in or near human-made structures but are sometimes found in more natural settings. The typical laboratory rat and laboratory mouse are albinistic forms of the Norway rat and house mouse, respectively.

Another introduced rat, the black rat or roof rat, *Rattus rattus* (Linnaeus), has been collected at widely scattered localities in the Commonwealth, but its occurrence at APH is unlikely. It can be distinguished from the Norway rat (*Rattus norvegicus*) by its somewhat darker coloration and from both *R. norvegicus* and native rats by its long slender and tapered tail, which is greater than half the total length. Jackson (1982) provides an overview of these introduced species.

Rattus norvegicus Linnaeus (Norway Rat)

Also sometimes called sewer or barn rat, Norway rats are well known for the damage they do to structures, and especially their consumption and contamination of stored dry goods, fruit, and grains. Although closely associated with human-made structures (e.g., garbage dumps and general trashy areas) they are sometimes found nearby in overgrown fields and stream banks. The Norway rat can be easily distinguished from other rats or rat-like forms in the study area by its prototypic rat form, which includes scruffy fur, small ears, and a heavy, long and nearly hairless scaly tail. It has a much thicker tail than the marsh rice rat or the hispid cotton rat, (see below), and adults are larger than adults of these native species. Young Norway rats that approximate the adult size of the rice rat and cotton rat will have the conspicuously large "puppy feet" that are characteristic of young mammals.

Mus musculus Linnaeus (House Mouse)

The house mouse is very much at home in human dwellings, granaries, barns, and other such dwellings, and yards and cultivated fields, but it is also found sometimes in more natural settings including meadows and marshes. This mouse has not been captured on APH but it is likely present in or near some of the buildings. The house mouse is easily distinguished from the somewhat similar eastern harvest mouse by the absence of the longitudinally grooved upper incisors. Adult white-footed mice are larger, have larger eyes and ears, body and tail are much more distinctly bicolored, and the species has a much sharper line of demarcation between dorsal and ventral coloration than is found in the house mouse. When viewed laterally, the incisors of *Mus musculus* reveal a distinct notch on the back near the tip. The notch is not present in other rodents of the area.

Species Recorded From The Vicinity of APH

Several species of mammals are known to occur in the mid-Atlantic region that encompasses APH. At least two of them may be found on the base in future research efforts.

Sigmodon hispidus Say and Ord (Hispid Cotton Rat)

Hispid cotton rats are small, native rodents found primarily in old field-habitats. In much of their range in the Commonwealth they are especially abundant in late-stage old fields that include patches of honeysuckle and blackberries and oftentimes saplings of pioneer trees. As the name hispid suggests, elongate guard hairs in its dorsal pelage have a spiny appearance and are tipped with black; its hair is otherwise relatively long and coarse. It has relatively uniform gray pelage ventrally and its feet are dark brown to blackish. Adults are much smaller than adult Norway rats and their tails are much thinner. Hispid cotton rats superficially resemble marsh rice rats but tails on rice rats are much longer and the belly and feet of marsh rice rats are whitish.

Hispid cotton rats have expanded their range northward in much of North America. They were not collected in Virginia until the early 1940s (Patton, 1941). Subsequently, Pagels and Adleman (1971) reported this species from Chesterfield County, and later it was found in northern Powhatan County to the west and north of the James River in eastern Henrico County (Pagels, 1977). More recently, cotton rats were found farther north in the Piedmont in Cumberland County (Pagels et al., 1992), and they has been collected at relatively high elevations in the Blue Ridge in Nelson County (J. Cranford and J. Pagels, personal observations). Humans have played critical roles in the range expansion of the hispid cotton rat in their creation of an abundance of early successional habitat and the introduction of Japanese honeysuckle, an obvious component of old-field habitats at most Virginia sites (Wright and Pagels, 1977). If range expansion continues, it seems likely that the hispid cotton rat will be found at APH.

Ochrotomys nuttalli Harlan (Golden Mouse)

The golden mouse is known for its aboveground nesting and feeding. It occurs in many habitats but is most common in forest edge or disturbed areas in forests that include abundant vine growth. It has a typical mouse-like form and has a soft, thick pelage that ranges from a deep, goldish-yellow on the back, to yellowish on the sides and creamy white ventrally. Golden mice somewhat resemble white-footed mice but the above characters, as well as smaller eyes and ears and no distinct line of demarcation between dorsal and ventral coloration, easily separate the two species.

The golden mouse occurs across much of the southern half of Virginia but not as far north as APH (Handley and Patton, 1947; Webster et al., 1985). Although there is no evidence of range expansion as in the hispid cotton rat, the golden mouse may sometime in the future occur on APH.

HUNTING and TRAPPING PROGRAMS

A hunting program has been in place on APH since 1945. As a federal military installation, U.S. Army regulations require that recreational activities such as hunting, trapping, and fishing be allowed to the fullest extent possible where there are no conflicts with the military mission. Daily assessments by the Directorate of Plans, Training, Mobilization, and Security during the hunting and trapping seasons determine which areas will be open to recreational use. Areas with any type of military training activity are closed to hunting.

Thirty Training Areas are open to general hunting and 28 Controlled Access Areas are open to hunting with certain restrictions. The Wildlife Refuge and the Impact Area are closed to hunting (Fig. 1). After successfully completing the Virginia Hunter Safety Course (regulation #APH CIR 200-00-2), licensed hunters can hunt most of the training areas with an APH hunting permit. Controlled Access Areas are considered the buffer for the Impact Area. People hunting Controlled Access interiors must be familiar with the areas through current or past employment or training and must pass yearly tests on local navigation and Installation, State, and Federal hunting regulations (regulation #APH CIR 200-00-3). Hunters can hunt locations along the perimeter of Controlled Access Areas after attending a briefing. There is currently a pilot program for disabled hunters (regulation #APH CIR 200-00-4, Disabled hunter pilot program regulations) with areas that have accessible stands designated for handicapped hunters.

Hunters are permitted to harvest deer with bow and arrow, muzzleloading rifles during special muzzleloader season, and shotguns. The use of dogs to hunt deer is prohibited, however, dogs may be used to hunt small game, quail, and waterfowl. In Controlled Access Areas, the use of dogs is permitted for waterfowl hunting only.

The installation sets its own hunting seasons. Most conform and run concurrent with state and federal seasons; differences are in accordance with base management needs. Although many game animals are pursued (e.g., gray squirrels, eastern cottontails, quail [*Colinus virginianus*], and wild turkey [*Meleagris gallopavo*]), the white-tailed deer is the primary focus of installation game managers. Harvest numbers for the 1999/2000 hunting season were typical for most years: 312 gray squirrels, 105 eastern cottontails, one red fox, one coyote, and 1,168 white-tailed deer.

In the hunting seasons 1985/1986 through 1999/2000, the deer harvest on APH ranged from 460 individuals (282 bucks and 178 does) in 1988 to 1,765 individuals (918 bucks and 847 does) in 1995 (Fig. 12). For the same time period, hunter effort (days in the field) ranged from 7,848 d in 1989 to 14,501 d in 1996 (Fig. 13). At the Savannah River Site, South Carolina, a comparably large federal facility, where dogs may be used, Cothran et al. (1991) reported 29% hunter success for hunting deer with dogs and 11% for still-hunting (without dogs). Still-hunter success was approximately 9% at APH, and is comparable to the success for still-hunters at the Savannah River Site.

In 1996 it was determined from data on deer health (Tables 1-3) that the deer population was exceeding the carrying capacity for APH. Does were then targeted in order to reduce the deer herd. To increase the overall harvest, while still focusing on increasing doe harvest, a special muzzleloader season was added and the number of doe days was increased. Bonus tag use for antlerless deer only was permitted three years before the state adopted a similar policy. Controlled Access Areas presented a special problem because they surround the Impact Area, a large area (10,925 ha) that cannot be hunted. To resolve this, the number of deer/day a hunter can harvest in Controlled Access Areas was increased from one to two—in Training Areas, the limit remained one deer/day. Deer of either sex may be harvested from Controlled Access Areas during the entire deer hunting season. In addition, APH enrolled the 28 Controlled Access Areas in the Commonwealth's Deer Management Assistance Program (DMAP). Through DMAP, the Virginia Department of Game and Inland Fisheries (VDGIF) provides antlerless deer tags in exchange for data collected (e.g., weight, indication of disease, reproductive status [evidence of lactation], age [using tooth wear], antler beam diameter) on harvested deer. These actions have collectively improved the health of the deer herd on APH.

The trapping program on APH is relatively small compared to the hunting program. It runs concurrently with the Virginia State trapping season, and most state and federal regulations are observed. Currently, 8 trappers participate in the program; all are employees or former employees of the post. Trapping is permitted in Training Areas

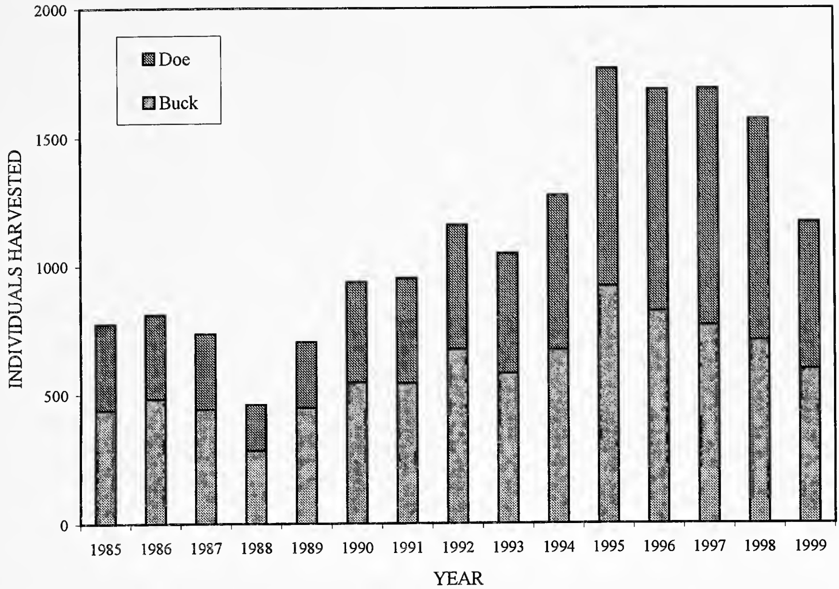

FIGURE 12. Summary of deer harvest for the 1985/1986 through 1999/2000 Virginia deer hunting seasons on Fort A. P. Hill, Virginia. Bars represent total harvest of bucks (light) and does (dark).

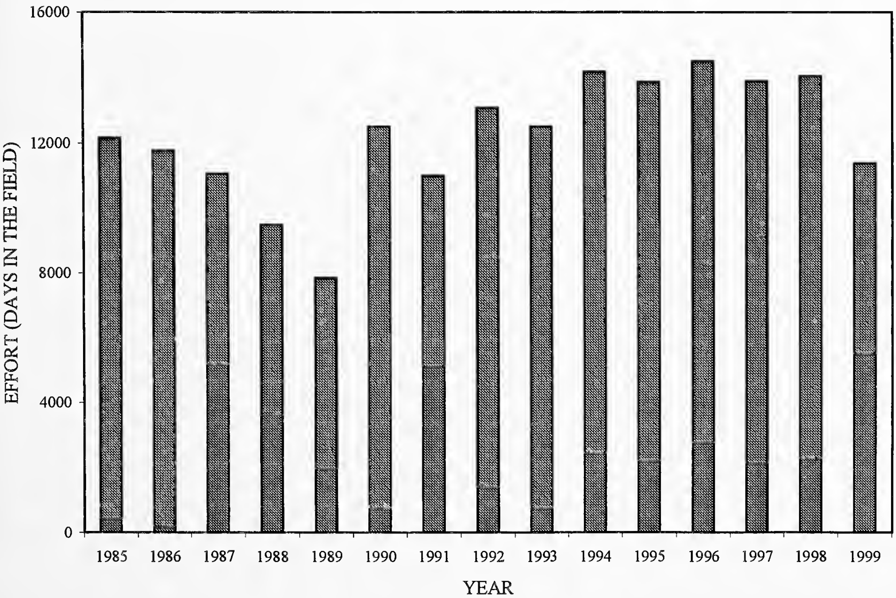

FIGURE 13. Summary of hunter effort (one day in the field = one day hunting for one hunter) for the 1985/1986 through 1999/2000 Virginia deer hunting seasons on Fort A. P. Hill, Virginia.

and Controlled Access Areas, and prohibited in the Impact Area. Most furbearer species are trapped but the beaver is targeted because it has a relatively high pelt value and because of the potential damage it can impose on the road system on the post.

DISCUSSION

Mammals occupy a wide variety of habitats in the mid-Atlantic region. Our research on APH has focused largely on habitat affiliations, community structure, and body size variation of many of the mammals on this military installation. We have evaluated small mammal habitat use of successional habitats, riparian, and upland habitats (Bellows and Mitchell, 2000; Bellows et al., in press ; Bellows et al., 2001). We also evaluated furbearer capture records and habitats targeted by local trappers, and we have obtained measurements on many of the trapped furbearer species. We are continuing our evaluations of small mammal communities in four forest types, as well our survey of the bat community. Collectively, the results we have accumulated to allow us to examine the habitat affiliations of most of mammal species of APH.

Table 4 summarizes the species documented for each of five generalized habitats on the installation. Old fields support a total of 20 species, including several species found predominately in this habitat. Pine stands and pine plantations support the fewest number of mammal species (15) of any habitat on APH. Mixed pine and hardwood forests, hardwood forests, and riparian forests support the largest number of species (27-34). Based on species richness, hardwood forests are the most important habitats for mammals on APH. With the possible exception of pine plantations, the habitat mosaic found on this installation provides abundant resources for mammal communities. This area should be representative of the upper Coastal Plain. However, much of Caroline County and eastern Virginia is in extensive agriculture and the remaining hardwood forests are being clearcut (A.S. Bellows and J.C. Mitchell, personal observations). Thus, APH is fast becoming a habitat island for the mammalian fauna of the upper Coastal Plain of Virginia and the mid-Atlantic region.

Our initial survey of small mammals on APH involved the study of small mammal communities in habitat types representing seral stages ranging from grasslands to mature hardwood forests (Bellows et al., 1999a; Bellows et al., in press; Bellows et al., 2001). The primary objective was to describe small mammal communities among 11 Society of American Foresters (SAF) habitat types that are common on the post. This survey also served to test the effectiveness of multiple trapping techniques (pitfall traps with drift fences and snap traps) and the importance of the duration of sampling periods. Bellows et al. (1999a) provided a detailed description of the small mammal communities in these habitats and the criteria used for site selection. We collected a total of 1,164 individuals representing 15 small mammal species during this study. Captures were dominated by two species, white-footed mouse (n=786) and northern short-tailed shrew (159), which together represented nearly 80% of all individuals collected. These two species, as well as southeastern shrews and pygmy shrews, were present in all 11 habitat types. Pine voles were captured in all but one habitat type—60-90-yr-old mixed pine forest. Least shrews, eastern harvest mice, meadow voles, and meadow jumping mice were captured primarily in grasslands. We found variation in small mammal community composition to be minimal among forested habitat types and attributed these results to the abundance of habitat generalist species, such as the white-footed mouse and the northern short-tailed shrew, that were present in all habitats.

We also studied small mammal communities in riparian and nearby upland habitats (Bellows and Mitchell, 2000). The primary objective was to compare small mammal communities between stream corridor and adjacent uplands in a forested ecosystem in the upper Coastal Plain of Virginia. Small mammal species richness and relative abundance were assumed to be higher in stream corridors because they frequently serve as dispersal corridors for many species. To test this possibility, we sampled 14 sites, 7 riparian sites and 7 upland sites, during 12 trapping sessions conducted from 9 April 1998 to 24 January 1999. Riparian sites were located in floodplains of 7 different tributaries, two in the Mattaponi River drainage and five in the Rappahannock River drainage. Upland sites were located between 150-250 m from their associated riparian site. Detailed descriptions of these habitats are in Bellows and Mitchell (2000). We collected a total of 162 small mammals representing four species of insectivores and three of rodents. Captures of insectivores were dominated by northern short-tailed shrews (n=23) and southeastern shrews (15). Captures of rodents were dominated by a single species, the white-footed mouse (115), representing all but three rodent individuals captured and 71% of total captures. The number of small mammals captured/100 trapnights in riparian habitats was greater than in upland habitats but the difference was not significant, nor were any small mammal species captured in significantly higher numbers in either habitat type. Although there was no significant difference in small mammal species richness between riparian (5 species) and upland habitats (6), Bray-Curtis ordination showed that there was more variation in small mammal community composition among riparian habitats than among adjacent uplands. This elevated variation in community composition was probably due to the isolated nature of the riparian sites compared to the contiguity of upland sites on the post and to the distribution of riparian sites between the two different river drainages. However, lack of a significant difference in average small mammal species richness and captures between riparian and upland trapping sites showed that both habitats are important to the long-term survival of the small mammal fauna in the upper Coastal Plain of Virginia.

Silvicultural treatment of forest stands to manage both timber and wildlife is a common approach used by foresters and wildlife biologists. Several of the pine-dominated stands on APH have recently been timbered using the shelterwood treatment with the goal of creating early successional habitat for bobwhite quail without having to resort to clearcutting. In this type of forest management, a portion of the trees on the site is removed and a varying proportion is left. Measurement of the remaining trees generates an estimate of the amount of area left covered by the trees, expressed as basal area. Low basal areas reflect few trees standing, whereas higher numbers indicate a larger number of trees or larger diameter trees left standing. Changes in the microenvironments in such stands are related to the amount of remaining canopy and directly affect small vertebrates living on the forest floor.

We designed a study using pitfall traps with drift fences to evaluate the small mammal communities in four forest types: (1) pine stands, (2) pine stands that were partially cleared with the shelterwood treatment, (3) stands with a mix of pine and hardwood, and (4) hardwood stands. The objective was to elucidate differences in community composition, species richness, and relative abundances of these animals, and to determine if the shelterwood treatment was adversely or positively affecting populations of these forest floor vertebrates. During March-September 2000 we captured a total of 427 small mammals representing 14 species. Three species, Virginia opossum, eastern cottontail, woodchuck, were represented by one juvenile each. The remaining species were insectivores (4 species), rodents (6), and a single southern flying squirrel. More individuals (172) were captured in shelterwood sites, all other forest types had similar but lower numbers. The number of species in the four forest types varied from 7 to 9 (hardwood, 7 species; mixed, 8; pine, 9; shelterwood, 8). The shelterwood site had more downed woody debris due to recent logging operations and more herbaceous ground cover than the other forest types. This microhabitat provides more resources for seed and grass-eating rodents, especially the white-footed mouse, a habitat generalist species, and the meadow jumping mouse and eastern harvest mouse, two species well adapted to grassland habitats. The other forest types do not provide as much of the forest floor microhabitat favored by these mammals. Reducing forest canopy cover favors these rodents much more other insectivores, except the least shrew that commonly frequents open habitats.

The primary objective of our furbearer survey was to provide a first-level assessment of furbearer mammals on APH through analysis of trapping success and measurements of captured individuals. We worked with local licensed trappers and the Environmental and Natural Resources Division of APH. We collected data from animals trapped on APH between 5 December 1998 and 9 February 1999. Trappers provided daily capture success reports during this period. We recorded data from 345 individuals representing 10 furbearer mammal species. The external measurements and age for these individuals are provided in the associated species accounts. All of the 10 species of mid-sized mammals represented in this survey are known to occur in this area (Webster et al., 1985). Four other mid-size mammals that occur on APH, eastern cottontail, woodchuck, gray squirrel, and long-tail weasel, were not captured and were not addressed in this survey. The trappers reported no captures of wild domestic dogs or feral cats, although we have commonly observed them on APH.

The large geographic area encompassed by APH (30,329 ha) provides an excellent opportunity for examining population trends and habitat use of furbearer mammals. Population densities of many of these species fluctuate greatly from year-to-year, especially those species dependent on small mammal populations for food.

Our bat inventory on APH was designed to assess composition and variation in bat communities in different habitats. Eight of the 9 species of bats captured thus far in this survey (April 2000-June 2001) are known to occur in the region—no additional species are expected. All species are insectivorous and are members of the family Vespertilionidae, the most widely distributed family of bats (van Zyll de Jong, 1985). Two of the 8 species, silver-haired bat and hoary bat, should be present on the post in spring and fall only as they pass through the region on migratory routes. Another migratory species, the red bat, spends the summer here but generally migrates south where it overwinters (Shump and Shump, 1982b). Male red bats have been reported to overwinter and forage in the Great Dismal Swamp (Padgett and Rose, 1991). Little is known about migratory patterns of evening bats, but published records suggest that southern winter movements are frequently quite distant (Humphrey and Cope, 1968). Four species, little brown myotis, northern myotis, eastern pipistrelle, and big brown bat, are likely year-round residents in Virginia, although short seasonal migrations to and from hibernacula within the region are commonplace (Fenton and Barclay, 1980).

The southeastern myotis, a bat we recently discovered on APH (May 2001), reaches the northernmost limits of its range in Virginia (D. Webster, personal communication).

Bats were captured using mist nets set in likely corridors of bat movement at sites ranging from ground or water level to a height of 10 m. Our most successful sets were positioned over logging roads and road rut puddles, in openings in riparian/upland interfaces, and over woodland creeks with little or no emergent vegetation. Other productive sets included those under streetlights, over and under bridges, and across small power line openings. The red bat was the species most frequently captured. The big brown bat and eastern pipistrelle were also commonly captured. The remaining 7 species were captured in low numbers; two of these, silver-haired bat and hoary bat, are seasonal migrants.

Taken together, our research on APH allows for a robust evaluation and description of the mammalian fauna representative of the upper Coastal Plain of Virginia and the mid-Atlantic region. The value of such research in a military installation is obvious when one realizes that relatively intact landscapes and habitats are fast disappearing from eastern Virginia. Such public lands should be evaluated for all taxa, although we should not ignore other lands being deforested for agriculture and urban and suburban growth. Comparisons of mammalian faunas and those of other taxa between habitat islands, like APH, and altered landscapes would help explain the differences created by such anthropogenic changes.

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ABSTRACT

Sporadic, high mortality in test populations of wedge clams (*Rangia cuneata)* has limited the potential for using this otherwise desirable test organism in biomonitoring studies. To determine whether high mortality was due to ontogenic or experimental variables, a two-phased study was conducted. In phase I, mortality of collected and re-deployed wedge clams, subjected to varying transport conditions, was determined at 7, 14, 21 and 60 days re-deployment. The use of three transport times (1, 2, 3 hr.), two vehicle conditions (open, closed) and three transport treatments (open, closed, iced containers) yielded 18 test groups. Individual test group mortalities were below 10% through the 21 day re-deployment period and peaked at 13% at the 60 day re-deployment point. The low rates of mortality observed in phase I of this study indicate that reasonable collection and transport of wedge clams does not significantly increase natural mortality and suggests other parameters are more strongly correlated to test population mortality. In phase II of this study, percent survival of collected and "acutely" redeployed and "acclimated" redeployed wedge clams was determined. Acclimated re-deployment is the transfer of *R. cuneata* from saline to freshwater in decrements of 3-4 ppt/day in accord with recommendations in Bedford and Anderson (1972). Acute re-deployment is the placement of *R. cuneata* in lower salinity waters or freshwater without acclimation. Although percent survival of clams acutely deployed to the freshwater test site was significantly ($p<0.05$) less than the percent survival at other test sites, mortality was only 3.3 %. No significant differences ($p<0.05$) were recorded in the percent survival of acclimated redeployed wedge clams.

Keywords: *Rangia cuneata*, wedge clam, mortality, bioconcentratable pollutants

INTRODUCTION

Interest in developing biological monitoring systems to monitor pollutant bioaccumulation has increased over the past ten years due in part to the continued emphasis on bioconcentratable pollutants by the U.S. Environmental Protection Agency (EPA)(USEPA 2000, VDEQ 1994, USEPA 1991). The potential for *Rangia cuneata* (wedge clam) to be used as a biomonitoring organism was described approximately 25 years ago (Croonenberghs 1974), and was based on the animal's occurrence in many oligohaline systems in U.S. and portions of Mexico, it's ease of collection, and it's large tissue yields for analysis (Pfitzenmeyer and Drobeck 1964, Gooch 1971, Pinkney et al. 1995).

Effectiveness of a wedge clam system to monitor pollutant bioaccumulation was recently demonstrated in field studies (Grimes 1992; VDEQ 1994; Pinkney et. al. 1998, 1997, 1995). Notably, it's use in the characterization of bioaccumulation potentials associated with PCB and pesticide discharges from an old leaking landfill provided regulatory agencies the basis for instituting a fish consumption advisory for protection of human health (U.S. Marine Corps Base-Quantico 1996).

However, other wedge clam biomonitoring studies have had varying success rates because of wide ranging mortality in control and test populations in the absence of acutely toxic conditions (Grimes 1996). Causal factors associated with these sporadic high mortalities are unknown, in part because there have been no studies to identify natural mortality rates of *R. cuneata*.

Determination of natural mortality rates, and mortality rates associated with the collection (e.g. dredge), transport [1-3 hr. with varying handling methods (i.e., clams with or without ice in various containers held in or outside vehicle)], and re-deployment of *R. cuneata* was needed to understand these characteristics of the wedge Clam biomonitoring system. Accordingly, objectives of this study were to (1) define natural mortality, (2) determine effects of collection and transport treatment on natural mortality of *R. cuneata*, and (3) test the efficacy of deploying clams acclimated and not acclimated to aquatic environments where water quality parameters differ from those of the collecting site.

MATERIALS AND METHODS

Phase I Collection and transport studies:

A total of 570 *R. cuneata* (size range 33-75 mm) were collected by hand from oligohaline reaches of the James River, VA on 28 October 1999. This collection time was two months later than the latest occurrence of mature gonads as reported by Pfitzenmeyer and Drobeck (1964), and was selected to minimize effects (e. g. low post-spawning biomass) of spawning on mortality. Collected wedge clams were held underwater in plastic mesh baskets while randomly sorted into control and test groups. Each replicate (15 clams) of control and test clams was placed in a 23 x 46 cm, 19.4 mm stretch mesh bag which was tagged for travel time and transport treatment parameters. Control clams were returned to the collection area after zero travel time and no transport treatment. Test clams were exposed to the following conditions (Table 1): 1) transport travel speed=80 km/h; 2) open or closed vehicle conditions (i.e., on top of or inside vehicle, respectively); 3) 1-hr, 2-hr, and 3-hr transport times; and 4) "open" test treatment (bushel baskets), "closed" test treatment (covered Styrofoam coolers), and "iced" test treatment (covered Styrofoam coolers with 1-liter of ice placed on top of clams). These ranges of exposure conditions are based on combinations of conditions that clams experienced in previous studies. Because of the wide distribution of *R. cuneata*, maximum travel times to redeployment test sites have not exceeded 3 hr. in previous studies (pers. obs). Each test treatment was equipped with minimum-maximum thermometers. At the end of each travel time, mortality and minimum and maximum temperatures (C) were recorded from each test treatment.

TABLE 1. Minimum, maximum and ΔT temperatures (C) of containers (open, closed, and closed with ice) of *Rangia cuneata* held inside (initial temperature=13.4 C) and outside (initial temperature=14 C) of vehicle at 1, 2, and 3-hour travel times on 28 October 1999.

Vehicle Placement	Test Condition	Travel Time (hr)	Temp (C)	ΔT
Inside	open	1	16.1	2.7
Inside	open	2	18.6	5.2
Inside	open	3	20.5	7.1
Inside	closed	1	13.5	0.1
Inside	closed	2	16.0	2.6
Inside	closed	3	20.0	6.6
Inside	closed with ice	1	13.9	0.5
Inside	closed with ice	2	10.8	-2.6
Inside	closed with ice	3	13.3	-0.1
Outside	open	1	14.4	0.4
Outside	open	2	15.6	1.6
Outside	open	3	17.8	3.8
Outside	closed	1	13.9	3.9
Outside	closed	2	15.3	1.3
Outside	closed	3	15.6	1.6
Outside	closed with ice	1	11.1	-2.9
Outside	closed with ice	2	15.3[a]	1.3
Outside	closed with ice	3	12.8[b]	-1.2

a-cooler with large crack
b-cooler with small crack

TABLE 2. Air and water quality parameters at study control site in James River, Virginia from October 28 –December 23, 1999.

Date	Temp (C) Air	Temp (C) Water	pH (mg/L)	Alkalinity (mg/L)	Hardness (mg/L)	DO	Salinity (ppt)
10-28	13.9	24.5	7.2	42	280	8.8	3
11-04	11.1	18.0	7.5	90	600	10.5	3
11-11	9.4	17.5	7.4	60	510	10.0	10
11-18	9.4	10.5	7.3	60	560	10.2	3
12-23	3.3	7.5	7.2	80	520	11.8	4

Replicates from each test treatment were placed into 0.2 x 0.3 m square mesh bags and then into one meter square mesh bags, which were tagged for travel time. Travel time mesh bags containing individual "test treatment" mesh bags were then returned to the collection area alongside control clams. Clam mortality, water temperature, pH, dissolved oxygen, salinity, alkalinity and hardness and air temperature were measured at the 0, 7, 14, 21 and 60 day re-deployment intervals (detection levels of: pH ±0.5; dissolved oxygen, ±0.2 mg/L; salinity, ±1 ppt; alkalinity, ±4 mg/L; and hardness, ±4 mg/L (Table 2). Dead clams were measured for maximum shell length (mm) and removed from bags.

Spearman correlation analysis (SAS, 1996) was used to identify variables significantly ($p \leq 0.05$) correlated with mortality. A one-way analysis of variance followed by Duncan's Multiple Range Test (SAS 1996) was used to determine significant ($p < 0.05$) differences between variables significantly correlated with mortality.

Phase II Acute and Acclimated Redeployment Studies:

A total of 180 *R. cuneata* (size range=35-63 mm), collected by the same methods and in the same locations as used in the phase I studies, were divided randomly and placed in twelve mesh bags (15 clams/mesh bag). Two of these were placed back into the water at the collection site as control replicates. The 10 remaining bags of clams were deployed at three locations in the James River after 0.5, 1, and 2 hour transport times, respectively: James River at mouth of Chickahominy River (two replicates for acute re-deployment and two replicates for acclimation re-deployment; 21 km upstream of control site); Rt. 155 bridge at Jordan Point (two replicates each for acute re-deployment and acclimation re-deployment; 58.5 km upstream of control site); and Heugenot Bridge (two replicates for acute re-deployment; 94.5 km upstream of control site). Clams acutely deployed were left *in situ* for the duration of the 21-day study to compare percent survival between acute and acclimated re-deployment. The two acclimation replicates at the Chickahominy and Jordan Point sites remained at their respective sites for three days based on recommendations in Bedford and Anderson (1972), that transferring *R. cuneata* from saline to freshwater may be less stressful to the animals when made in decrements of 3-4 ppt/day. Acclimation clams at Jordan Point were transferred to the Heugenot Bridge on 27 October 2000. Acclimation clams at Chickahominy River were transported to Jordan Point on 27 October, and then from Jordan Point to Heugenot Bridge on 30 October 2000. Percent survival of clams at each site was determined on October 24, 27, and 30, and November 2, 7 and 14. Water quality parameters measured in acute and acclimation re-deployment studies were the same as those taken in phase I studies (Table 3). A one-way analysis of variance followed by Duncan's Multiple Range Test (SAS 1996) was used to determine significant ($p \leq 0.05$) differences in percent survival between control and test sites.

RESULTS

Phase I Collection and transport studies:

After 60 days re-deployment, total control mortality of *R. cuneata* was 0% while individual test group mortalities ranged from 0-13% ($p \leq 0.05$). Total test group mortality was not significantly different between: vehicle conditions ($F=0.57$; $p>F=0.56$; df=2); travel treatments ($F=1.35$; $p>F=0.2553$; df=3); or travel treatments by vehicle conditions ($F=1.61$; $p>F=0.1542$; df=6). Total mortality of *R. cuneata* in the 1-hr travel time-closed transport treatment test group was significantly greater than other time-transport treatment test groups ($F=1.04$; $p>F=0.4129$; df=2)(Table 4). Although there were significant differences in mean clam sizes between some test groups, clam size (mean=48.26 mm, min=33, max=75) was not significantly correlated with mortality ($R=-0.00891$; $p>R=0.67$). Mortality was significantly correlated with pH ($R=-0.1102$; $p>R=0.0001$), hardness ($R=-0.06378$; $p>R=0.0024$), dissolved oxygen ($R=0.1505$; $p>R=0.0001$), and water temperature ($R=0.10676$; $p>R=0.0001$).

Phase II Acute and Acclimated Redeployment studies:

Acute re-deployment survival (96.7%) of *R. cuneata* at the Heugenot Bridge test site was significantly lower ($F=3.67$; $p>F=0.019$; df=3) than survival at other re-deployment test locations (Table 5).

TABLE 3. Air and water quality parameters for acute and acclimation re-deployment studies of *Rangia cuneata* at control station and test stations in James River (mouth of Chickahominy River; Jordan Point; and Heugenot Bridge in Richmond, VA) from 24 October – 14 November 2000.

Station	Date		
	10-24	11-7	11-14
Air temp (C)	12.7	10.5	4.4
Control			
Water temperature (C)	21.0	15.0	18.0
pH	7.7	8.0	7.7
Salinity (ppt)	6.0	10.0	16.0
Alkalinity (ppm)	40.0	68.0	82.0
Hardness (ppm)	530.0	520.0	560.0
D.O. (ppm)	9.2	9.2	8.6
Chickahominy			
Water temperature (C)	18.0	15.0	14.0
pH	7.0	7.5	7.3
Salinity (ppt)	3.0	3.5	4.0
Alkalinity (ppm)	40.0	78.0	70.0
Hardness (ppm)	240.0	440.0	400.0
D.O. (ppm)	8.0	10.2	10.0
Jordan Point			
Water temperature (C)	19.5	14.5	14.5
pH	7.7	7.3	7.3
Salinity (ppt)	1.0	1.5	2.0
Alkalinity (ppm)	44.0	80.0	78.0
Hardness (ppm)	100.0	96.0	130.0
DO (ppm)	11.6	9.0	9.0
Heugenot Bridge			
Water temperature (C)	18.0	14.0	13.0
pH	7.7	7.3	7.4
Salinity (ppm)	0	0	0
Alkalinity (ppm)	10.0	100.0	110.0
Hardness (ppm)	18.0	100.0	80.0
D.O. (ppm)	9.6	10.2	9.0

The acclimation re-deployment test groups showed no significant differences in percent survival of *R. cuneata* ($F=5.18$; $p>F=0.60$; $df=2$).

DISCUSSION

Studies of bioconcentratable pollutants typically are conducted over a 28-day period (USEPA and USACE, 1998). Our studies indicate the efficacy of using *R. cuneata* as a biomonitoring tool in studies of up to 60 days. Using protocols outlined in this study, *R. cuneata* can be collected, transported and redeployed from oligohaline (i.e., water temperature range=15-21 C; salinity range=6-16 ppt; pH range=7.7-8.0; alkalinity range=40-82 ppm; hardness range=520-560 ppm; and D.O. range=8.6-9.2

TABLE 4. Control, test group mortality and % mortality of *Rangia cuneata* at 0, 7, 14, 21 and 60 days re-deployment in James River, Virginia from October 28-December 23, 1999.

	Re-deployment Interval (Days)						
Test Group	0	7	14	21	60	Total	Mortality
Control	0	0	0	0	0	0/30	(0%)
Inside Vehicle:							
1 hr. in-open	0	0	1/30	1/29	1/28	3/30	(10%)
1 hr. in-closed	0	0	0	0	4/30	4/30	(13%)
1 hr. in-ice	0	0	0	0	0	0/30	(0%)
2 hr. in-open	0	0	0	1/30	1/29	2/30	(7%)
2 hr. in-closed	0	0	0	0	2/30	2/30	(7%)
2 hr. in-ice	0	0	0	0	0	0/30	(0%)
3 hr. in-open	0	0	0	0	0	0/30	(0%)
3 hr. in-closed	0	0	0	0	0	0/30	(0%)
3 hr. in-ice	0	0	0	0	0	0/30	(0%)
all inside groups	0	0	1/270	2/269	8/267	11/270	(4%)
Outside Vehicle:							
1 hr. out-open	0	0	0	0	1/29	1/29	(3%)
1 hr. out closed	0	0	0	0	4/30	4/30	(13%)
1 hr. out-ice	0	0	0	0	3/30	3/30	(10%)
2 hr. out-open	0	0	0	0	0	0/30	(0%)
2 hr. out closed	0	0	0	0	1/30	1/30	(3%)
2 hr. out-ice	0	0	0	0	1/30	1/30	(3%)
3 hr. out-open	0	0	0	0	0	0/30	(0%)
3 hr. out closed	0	0	0	0	0	0/30	(0%)
3 hr. out-ice	0	0	0	0	0	0/30	(0%)
all outside groups	0	0	0	0	10/269	10/269	(4%)

ppm)(Table 2) to freshwater conditions (i.e., water temperature range=13-18 C; salinity=0 ppt; pH range=7.3-7.7; alkalinity range=10-110 ppm; hardness range=18-100 ppm; and D.O. range=9.6-10.2 ppm)(Table 3) without significant mortality even when acutely deployed to freshwater conditions. Although *R. cuneata* does not inhabit freshwater environments because it requires a minimum salinity of 6 ppt to initiate gamete release (Chanley, 1965, Gainey and Greenberg, 1977), its ability to significantly osmoregulate allows *R. cuneata* to survive in both hypo-osmotic and hyper-osmotic environments (Saintsing, 1979; Saintsing and Towle, 1978a, 1978b). When transferred from 10 ppt to 0 ppt salinity, conditions requiring significant osmoregulation, *R. cuneata* reach equilibrium within 12 hours (Saintsing, 1979; Saintsing and Towle, 1978a, 1978b).

In our collection and transport studies, there were no significant short-term (21 days) effects of collection, travel time or transport conditions, as mortalities were below 0.1 %. Although the 10 % mortality threshold was exceeded in some test treatments 60 days after re-deployment, we suspect this late increase in mortality may have been the result of their inability to escape freezing air temperatures when exposed during periods of unexpectedly low tides. The spatial arrangement of these test treatments in the travel time re-deployment bags during the later stage of the study placed them on

TABLE 5. Control, test group, and percent survival of *Rangia cuneata* at 0, 3, 6, 9, 14, and 21 days deployment and re-deployment in James River, Chickahominy River, Jordan Point, and Heugenot Bridge from October 24 – November 14, 2000.

	Re-deployment Interval (days)						Survival	
Group	0	3	6	9	14	21	Total	%
Control	30/30	30/30	30/30	30/30	30/30	30/30	30/30	100
Deployment								
Chickahominy	30/30	30/30	30/30	30/30	30/30	30/30	30/30	100
Jordan Point	30/30	30/30	30/30	30/30	30/30	30/30	30/30	100
Heugenot	30/30	30/30	30/30	29/30	29/30	29/30	27/30	90
Re-Deployment								
Control	30/30							
Chickahominy	30/30	30/30						
Jordan Point			30/30					
Heugenot				30/30	30/30	30/30	30/30	100
Control	30/30							
Jordan	30/30	29/30						
Heugenot			29/30	29/30	29/30	29/30	29/30	96.7

top of other test treatments and thereby precluded significant burrowing into the substrate.

This study addresses some priority issues and research needs for the field of bioaccumulation monitoring as outlined by EPA (2000) (e.g. identification and development of additional species for water quality and sediment bioaccumulation test methods; guidance on how reference sites can be selected; and how this species should be collected and transported for bioaccumulation testing). Based on results of our investigations into the collection, maintenance, transport, and re-deployment of *R. cuneata*, we recommend the following protocol for using *R. cuneata* in bioconcentratable pollutant studies.

1. Collect specimens and conduct bioconcentratable pollutant studies in autumn to minimize effects (e. g. low post-spawning biomass) of spawning on mortality (see Pfitzenmeyer and Drobeck 1964).
2. Maintain specimens in collection site water until ready for transport.
3. Transport specimens in closed containers with ice outside the vehicle; or in open containers inside an air-conditioned vehicle to re-deployment sites within 3 hr. of collection;
4. Maintain specimens in containers specified above while distributing specimens into mesh-bags prior to re-deployment (nylon mesh allows burrowing whereas metal fabrics inhibit burrowing).
5. Deploy 15 clams/2600 cm^3 replicate mesh-bag at sampling sites to safeguard against density limitations to burrowing.

Our future studies include determining rates of bioconcentration in hypo-osmotic and hyper-osmotic environments and ascertaining whether *R. cuneata* continues to feed after collection, transport, and re-deployment to various sites. Such data are requisite for making comparisons between control and test groups redeployed at sites with different water quality parameters.

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An Examination of the First Sediment Cores from Mountain Lake, Giles County, Virginia, for Diatoms and Pollen

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ABSTRACT

Mountain Lake, Virginia is a small, unique, oligotrophic, subalpine ecosystem in the southern Appalachians. Its geology, origin, climate, and history have influenced its morphometry, and therefore its sedimentology and algal flora. Radiocarbon dates establish specific Mountain Lake sediment ages at 1800, 4100, and 6100 years BP. Sediment core analysis suggests at least 6 prolonged periods when Mountain Lake probably was nearly dry or very small in size. These individual low-water periods (at approximately 100, 400, 900, 1200, 1800 and 4100 years BP) are evidenced by changes in diatom and pollen content, sedimentary erosion features, and the presence of wood fragments, plant fibers, and abundant *Sphagnum* and fern spores. The ratio of planktonic-to-benthic diatom taxa was used to estimate approximate past water depths from sediment. One or more of these low-water intervals may correspond to a drier climate coincident with solar activity minima. Resolution of prolonged low water intervals probably has been enhanced by the continuous loss of water through the crevice or fault at the lake bottom. The sediment core record suggests also that some eutrophication has occurred during the 20th century, in parallel with anthropogenic impacts (i.e. increased sedimentation, induced eutrophication, and diatom diversity changes). These findings provide the first published diatom, pollen and sedimentology-based paleolimnology for this lake.

Key words: Mountain Lake, paleolimnology, diatoms, sedimentology, water fluctuation, solar minima.

INTRODUCTION

Mountain Lake is located at an elevation of 1177 m (3862 feet) near the summit of Salt Pond Mountain, Giles County, Virginia, in the Ridge and Valley Province of the southern Appalachians (37°27’56"N, 80°31’39"W). This lake is the only natural lake of significance in the unglaciated highlands of the southern Appalachians (Figure 1). Mountain Lake has a maximum depth of 33 m at its north end; this deep portion consists only of a narrow crack or crevice feature in the bottom of the lake revealed by sonar depth mapping and SCUBA reconnaissance (Cawley *et al.*, 2001). Most of the deeper

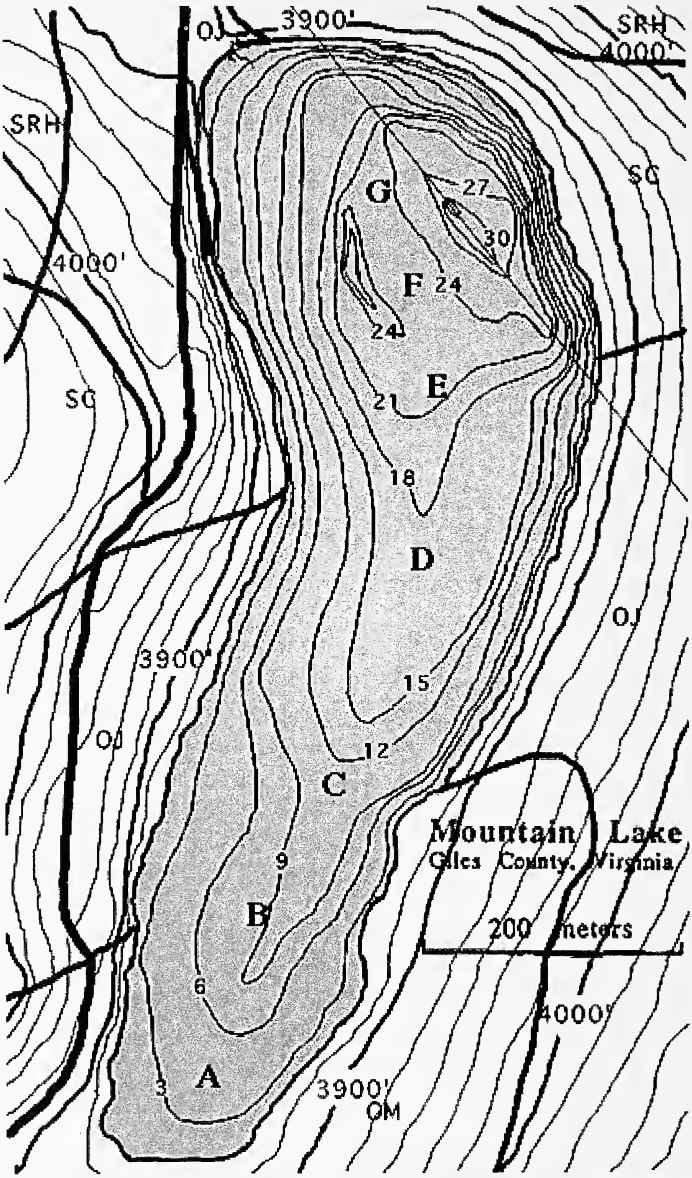

FIGURE 1. Sonar bathymetric map of Mountain Lake (37°27'56"N, 80°31'39"W) showing depth contours (m), topographic map contours of the watershed (ft.), and locations of sediment cores A through G.

north end of the lake is approximately 24 m deep, becoming shallower to the south. The lake is underlain by three geological formations previously described in detail (Parker *et al.*, 1975; Beaty and Parker, 1994): The Ordovician Martinsburg shale (OM) at the southern end, the Ordovician Juniata sandstone (OJ) near the middle, and the Silurian Clinch sandstone (SC) at the northern end. The Silurian Rose Hill sandstone (SRH) is north of the lake rim (Figure 1). All formations lack carbonates, so that the lake water maintains a circumneutral pH (6.0-7.0), low alkalinity and hardness. Today, Mountain Lake is oligotrophic and severely phosphate-limited (Cawley *et al.*, 1999).

Since the 1930s, numerous workers have addressed various aspects of the origin, geology, phytoplankton or macrophyte communities, and physicochemical limnology of Mountain Lake. (e.g., Hutchinson and Pickford, 1932; Roth and Neff, 1964; Marland, 1967; Obeng-Asamoa and Parker, 1972; Parker *et al.*, 1975; Dubay and Simmons, 1981; Parson and Parker, 1989a,b; Beaty and Parker, 1994, 1996a, 1996b; Cawley *et al.*, 1999, 2001). Deevey et. al. (1957, unpublished) produced the first bathymetric map of the lake, using lake soundings and War Department aerial photographs (Parson and Parker, 1989a). This lake bathymetry was updated using sonar depth mapping in 1997-1998 (Cawley *et al.*, 1999), and the lake origin with its periodic draining and filling was shown to relate to the 33 m depth feature (a fault) located at the north end of the lake (Cawley *et al.*, 2001). Historical records have documented that the size of the lake has varied periodically over the last 2.5 centuries (Parker *et al.*, 1975). In fact, the lake has not been full and appears to have been shrinking since 1997.

In 1997-1998 we undertook a re-evaluation of the water quality (Cawley *et al.*, 1999), lake history and origins (Cawley *et al.*, 2001), and the extant and recent historical diatom and pollen sediment components (Cawley *et al.*, In Press) of Mountain Lake. The abundance and species diversity of diatoms seen in Ekman dredge samples of the surficial sediments dating back an estimated 100 years suggested that sediment cores from Mountain Lake would be useful for interpretation and analysis of the lake's paleolimnology (Smol and Glew, 1992; Anderson, 2000). Also, the undisturbed nature of the sediments, the lack of permanent stream inputs, and the sedimentation rates estimated at <1 mm/yr (Parker *et al.*, 1975; Cawley *et al.*, 2002) further supported our viewpoint that this lake would be suitable for sediment core analysis. Accordingly, in 1998 we undertook the first study of Mountain Lake's paleohistory using cores to study diatoms, a limited spectrum of pollen, and sedimentary erosion features.

METHODS

Except for Marland's (1967) unpublished work on cladocerans, no sediment cores have been collected previously from Mountain Lake. Marland's attempt at using gravity coring in Mountain Lake was only partially successful, so we designed and built a new, diver-assisted percussion coring device for retrieving the relatively soft sediments in Mountain Lake (Cawley and Parker, In Press). Our recovery included 7 complete cores (100% of attempts) from the lake.

Cores were taken from a transect near the north-south centerline of the lake (Figure 1). All cores were made to hard resistant substrate; the final cores measured 50-60 cm in length. We estimate the average compression at about 20%, but our measurements were not corrected for this compression. Intact 2-inch (5.04 cm) diameter cores in PVC pipes were frozen, then cut longitudinally and their sedimentary characteristics described and correlated. Traverse (1994) and the correlation strategies of Anderson (1986, 1990a, 1990b) were used where applicable.

Subsamples for diatom and pollen extraction consisted of 10 g of wet lake sediment (ca. 2.5 g dry wt), selected at intervals of 3-5 cm. These subsamples comprised individual sedimentary layers from the core longitudinal section as much as possible. The spacing of the subsamples depended on the physical features of the material. Ten slides were made after processing from which random slides were counted.

For diatoms, sediment samples were oxidized with hydrogen peroxide (3% H_2O_2) followed by hypochlorite bleach (5.25% NaClO) (Barss and Williams, 1973; Brasier, 1980; Baron, 1987). Multiple strews of each sample were mounted in Histoclad mounting medium (1.54 refractive index) [Clay Adams, Parsipany, NJ 07054]. Residues were preserved in distilled water and 70% ethyl alcohol within glass vials, as suggested by Pakorny (1963). Diatom identifications were made using primarily Hustedt (1930) and Patrick and Reimer (1966, 1975), but also referring to Cox (1996), Hufford (1987), and Trumbull and Hufford (1989). Diatom counts were carried out via optical and video microscope. Typically slide mounts were viewed with oil immersion lens in an Olympus BH2 Microscope equipped with an Hitachi Digital Signal Processor Color Video Camera (VK-C370) linked to a Power Mac computer and monitor. Magnifications of several thousand facilitated identifications. Counts were then carried out at lower magnifications. Identifications were confirmed by all three co-authors. Counts of relative abundance were made; initially, counts of 300 were made along microscope slide transects until the standard deviation for the means of the most abundant taxa were no more than 20% of the means. Thereafter, counts of 100 were made with random recounts for comparison. Blind recounts of 100 rarely varied by >10% of each other.

Notations were made of diatom taxa typically associated with benthic substrates (many pennates, biraphid, monoraphid forms) and taxa more typical of a planktonic existence (many centrics, a few nonraphid pennates). These data were used to calculate planktonic to benthic ratios for different lake locations. Wolin and Duthie (1999) noted that life-form or habitat group changes based on depth distributions (i.e., planktonic vs benthic) produce the most reliable evidence of water level change.

Pollen was also examined. The maceration methodology for pollen was modified from Iverson and Faegri (1975) and Traverse (1988). This included (1) removal of carbonates with concentrated HCl (10-12N), (2) removal of silicates with concentrated HF (25N), (3) very brief oxidation with hypochlorite bleach (5.25% NaClO), and (4) acetolation with acetic anhydride/sulfuric acid [$(CH_3CO)_2$ O: H_2SO_4 = 9:1]. Pollen identifications were made using Iverson and Faegri (1975), Lewis *et al.* (1993), and Jones (1995). Relative numbers were assessed at different core depths similarly to the diatom counts but with less emphasis on actual counts.

Two samples for radiocarbon dating were taken from organic-rich layers in core G at 27 and 45 cm. An additional sample was taken from an organic-rich layer near the bottom of core D at 45 cm. Samples were submitted to Beta Analytic, Inc. Miami, FL. Sample D 45 cm contained sufficient carbon to be processed by standard radiometric analysis with extended counts. The other two samples (G27 and G45 cm) were analyzed by accelerator mass spectrometry (AMS).

RESULTS AND DISCUSSION

Comparison of Cores and ^{14}C Dates:

Of the seven cores made to hard resistant substrate, older diatoms and pollen within the shallower lake depth cores A, B, and C were in poor condition, abraded or not showing distinct layering and apparently oxidized. These materials would have been exposed repeatedly during periods of low lake levels. Thus, data from these three cores is not included. Core E taken from a sloping portion of the lake bottom, showed

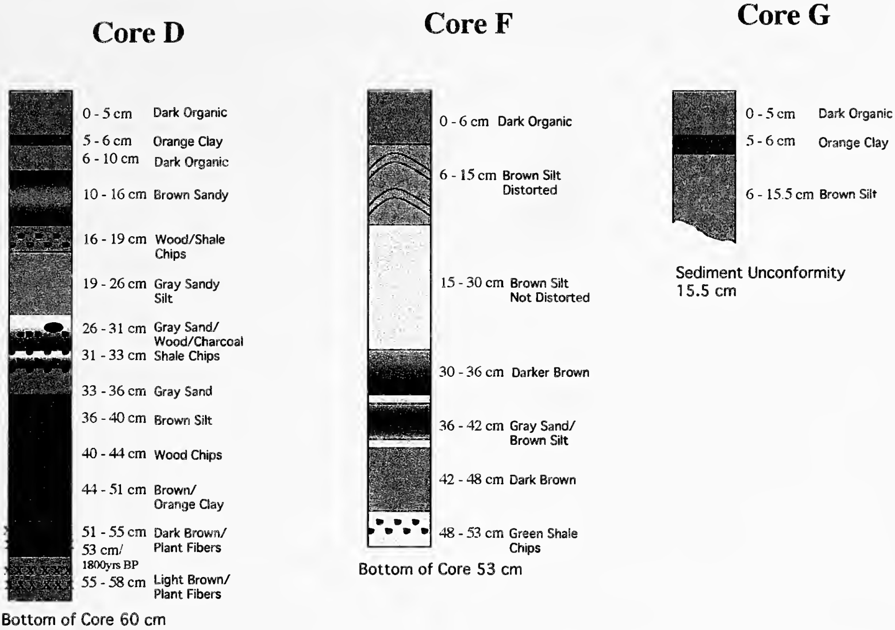

FIGURE 2. Stratigraphic column representation of Mountain Lake cores D, F, and top portion of G above sediment unconformity.

evidences of turbidity flow; that is, the layering in this core was obviously disturbed. Thus, data from this core also is not included.

In contrast, good preservation of both diatoms and pollen occurred in deeper cores D, F, and G. Individual layers of broken and abraded materials were present in these deep cores; however, these layers appear to coincide with past very low-water levels. Overall, the resolution of sediment structures was excellent in cores D, F, and G.

Core F correlated directly to core D (Figure 2). Core D was collected from a shallower region of the lake than core F, and showed consistently shallower sedimentary and diatom assemblage structure. Assemblage is used here instead of community, because it represents a mixture of diatoms from the planktonic, epipelic, epiphytic, and near terrestrial communities, which will differ in proportion depending on lake depth and other variables.

^{14}C dates established an age for sediment at 53 cm in core D at 1800 ± 100 years BP (D45). The top 15cm of core G also correlated with D and F. Below 15 cm in core G however, an interval of sediment and therefore time is absent, producing a sediment unconformity. Older material, however, was preserved in core G below the unconformity (Figure 3). ^{14}C dating at 27 cm in core G gave a date of 4100 ± 50 years BP (G27), while the core near the bottom dated at 6100 ± 70 years BP (G45). Even allowing for compression, the sedimentation rate between 27 and 50 cm in core G is considerably less (<0.2 mm/yr) than in the upper portions of the cores (≤ 1.0mm/yr) (Parker *et al.*, 1975).

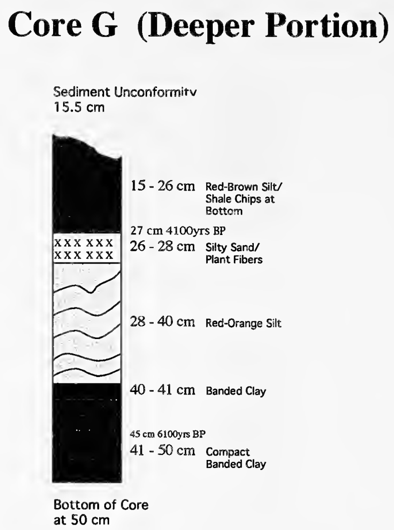

FIGURE 3. Stratigraphic column representation of Mountain Lake core G below sediment unconformity.

Table 1 summarizes diatom and pollen information for cores D, F, and top portion of G (Figure 2), including planktonic/benthic diatom ratios. The percentages with depth for the more abundant diatom genera in cores D and F are shown in Figure 4. Table 2 summarizes diatom and pollen information for the deeper portion of core G (Figure 3). The percentages with depth for the more abundant diatom genera in core G are shown in Figure 5. Many more diatom taxa were detected at lower frequency than the major taxa shown in Tables 1 and 2 and Figures 4 and 5. For example, Table 3 shows the contrast in diversity for all diatom species in core F at 45 cm (high diversity) compared to 53 cm (low diversity). Table 4 lists the diatom species identified in core G at 45 cm versus 50 cm. Additional findings, with our suggested interpretations will be discussed below.

Diatoms, pollen, and erosional features in these cores (Tables 1 and 2) suggest at least 6 extended periods when Mountain Lake probably was small or nearly dry, as has been documented historically for the last 2.5 centuries (Parker *et al.*, 1975). These periods occurred at approximately 100, 400, 900, 1200, 1800 and 4100 years BP. The sediment core diatom contents suggested progressive (albeit slow) eutrophication through time, as well as anthropogenic impacts during the 20th Century.

Zero to 1800 Years BP:

Prior to the major sediment coring in Mountain Lake, we collected Ekman dredge samples to sediment depths of >10 cm estimated to date back 100+ years. In this preliminary study, we identified 66 diatom taxa (25 genera) and showed that the plankton-to-benthic diatom ratios increased significantly with lake depth (Cawley *et al.*, In Press).

At 5-7 cm depth in the samples, we observed a well-defined red-orange inorganic silty layer also present in cores D and G (Figures 2, 3). This layer had a more viscous consistency than sediments above and below and contained few diatoms. We sug-

TABLE 1. Major diatoms and pollen for Mountain Lake cores D, F, and top 15 cm of G. Diatoms listed in order of abundance. Pollen abbreviations: A=Asteraceae, H=hardwoods, P=pine, Po=Poaceae, S=*Sphagnum*, not ranked by abundance.

Depth [cm]	Approx. Years BP	No. Diatom Species	Dominant Diatoms	Plankton/ Benthic Ratio	Dominant Pollen
5	50	66	*S. alpinus,* *C. stelligera* *Fragilaria* spp.	3.5	A, H, P, Po
10	100	10	*C. stelligera* *T. flocculosa* *M. distans*	3.5, 6.4	A, H, P, Po, S
15			*T. fenestrata* *T. flocculosa* *C. stelligeria*	3.1, 5.0	A, P, Po
17	400	23	*M. distans* *T. fenestrata*	1.6	A, P, Po
20				1.3	A, H. P, Po
28			*C. stelligeria* *M. distans*	1.5, 1.9	A, H, P
31	900	40	*C. stelligera* *M. distans* (abraded)	1.2, 2.3	A, H, P, Po, S
34		?		1.0	A, Po, S
42	1200	?	(abraded)	0.7, 1.8	S
45		28	*M. distans* *Fragilaria* spp	0.9, 1.2	None
53	1800	5	*C. stelligera* *F. pinnata*	1.8	A, H, P, S
55			*C. stelligera* *F. pinnata* (abraded)	3.4, 6.6	A, H, P, Po, S

gested that this inorganic-rich layer may represent erosion of upland areas and deposition into the lake in the early 1930's when the Mountain Lake Hotel and nearby roads skirting two sides of the lake were under construction. In addition, the once dominant American chestnut was no longer present to stabilize soil erosion from the steep hillsides around the lake. The American chestnut blight had swept through the southern Appalachians in the 1930's, bringing with it the ultimate extermination of the chestnut forests. Braun (1950) noted that "the prevailing slope forest" of Salt Pond Mountain near Mountain Lake "was dominantly chestnut before the chestnut was killed." Given

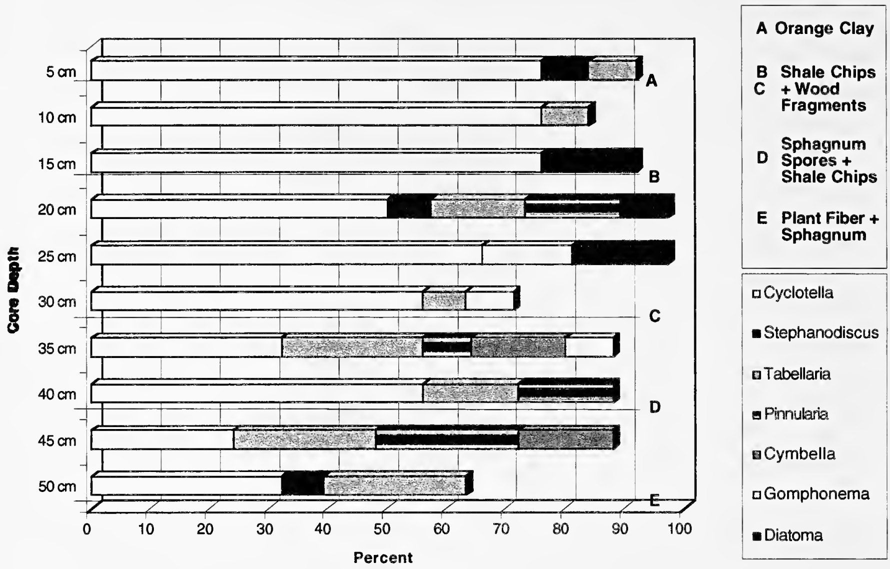

FIGURE 4. Percentages of more abundant diatom genera at increments of approximately 5 cm in cores D and F with other core content information.

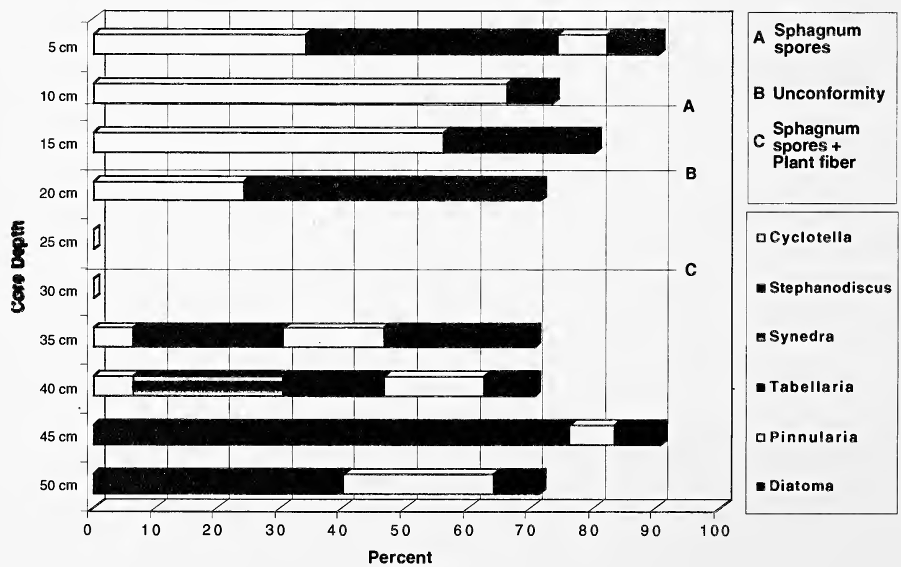

FIGURE 5. Percentages of more abundant diatom genera at increments of approximately 5 cm in core G with other core content information.

estimates of ≤1.0 mm/year of sedimentation for these shallow sediments (Parker *et al.*, 1975), an age of ca. 70 years for this red-orange layer is reasonable (Cawley *et al.*, In Press). This estimate also is supported by the qualitative observation that an order of magnitude decrease of *Castanea dentata* (American chestnut) pollen occurred above this layer in the cores.

Diatoms at this 5-7 cm level closely resembled the modern flora (Cawley *et al.*, 1999, In Press) although their diversity appears generally less than at present. The planktonic-to-benthic ratio throughout the cores (Tables 1 and 2) also is less than at present; at no time in the cores did we see a ratio >10 found in the modern surface sediments (Cawley, *et al.*, In Press). Tappan (1980) suggested that, if water depth is held constant, an increase in the ratio of centric to pennate diatoms may indicate increased eutrophication. Therefore, one interpretation of the higher ratio in Mountain Lake today is that at least some eutrophication has occurred recently. This interpretation is supported by the findings of Beaty and Parker (1994), who showed that phosphate and ammonium levels had increased in Mountain Lake during the late 1980s and early 1990s compared to the early 1980s. Also, *Stephanodiscus alpinus* has become more abundant recently in the lake flora (Tables 1, 2; Figures 4, 5; Cawley *et al.*, In Press), possibly a response to eutrophication (also see Hall and Smol, 1999).

At 10 cm (ca. 100 years BP) in core F, we noted a decrease in diatom numbers and diversity (i.e., 10 species, compared to 66 in the sample above). In addition, *Sphagnum* spores occurred at 10 cm in cores D, F and G (Tables 1, 2). These were identical to extant *Sphagnum* spores from Spruce Bog, a relict high altitude peat bog only a few km from Mountain Lake's local drainage (but not in the same basin). This change in the diatom community at 10 cm, as well as the presence of *Sphagnum* suggests that the lake was very low or nearly dry during that period of sedimentation. This interpretation agrees with anecdotal information showing that Mountain Lake has been low frequently in the past and that about 50% of the water entering Mountain Lake leaves through the fracture feature or fault in the lake's bottom (Parker *et al.*, 1975; Cawley *et al.*, 2001). Also, historic reports from the late 1800's confirm that the lake was a small pond ("Salt Pond") with surrounding meadow used for seasonal grazing of cattle, which then filled after a local earthquake toward the end of the 1890's (Parker *et al.*, 1975; Roberts, 1998 pers. comm.).

At 17 cm (ca. 400 years BP), wood (with tracheids) and shale fragments were more abundant than elsewhere in core D (Figures 2, 4); these probably were associated with a period of lake-bottom erosion. The 20 cm portion contained virtually no diatoms. These data suggest an extended period of low water and subareal exposure, during which the area around the lake/pond was at least partially wooded. This interpretation is supported by a well-preserved yellow pine (*Pinus pungens*) tree stump recovered from growth position at a depth of 10 meters in the full lake (Parker *et al.*, 1975). ^{14}C dating of this wood produced an age of 1655 ±80 AD. Tree ring analysis revealed that the tree had grown for 30 years along the shore of a much smaller analogue of Mountain Lake. Of interest is the fact that this extended low-water period at Mountain Lake coincided with a cold dry period in Europe associated with the Maunder Minimum of solar activity (Eddy, 1977; Parker *et al.*, 1982; Dreschhoff *et al.*, 1993).

A more pronounced layer of wood and shale fragments occurred especially in core D at 31 cm (Figures 2, 4), or about 900 years BP. Marland (1967) using ratios of

TABLE 2. Major diatoms and pollen for Mountain Lake core G. Diatoms listed in order of abundance. Pollen abbreviations: A=Asteraceae, H=hardwoods, P=pine, Po=Poaceae, S=*Sphagnum*, not ranked by abundance.

Depth [cm]	Approx. Years BP	No. Diatom Species	Dominant Diatoms	Plankton/ Benthic Ratio	Dominant Pollen
5	50	66	*S. alpius* C. stelligera *Fragilaria* spp.	*3.6*	
10	100	20	*T. fenestrata* *T. flocculosa* *C. stelligera*	6.4	A, H, P, Po, S
15	Unconform	10	*M. distans* *Fragilaria* spp. *T. fenestrata*	3.1	H, P
20		5	*Tabellaria* *Fragilaria* *C. stelligera* *Gomphonema* (abraded)	2.4	
25			*Cymatopleura* or *Surirella*		P, Po, S
27	4100	5	*Tabellaria* *Cymatopleura* or *Surirella*		H, P, Po, S
30			*Cymatopleura* or *Surirella*		A, H, P, Po, S
37		10	*Fragilaria* *C. kutzingiana* *Pinnularia*	0.8	H, P, Po
40		20	*T. fenestrata* *T. quadriseptata* *T. flocculosa*	1.4	
45	6100	21	*T. fenestrata* *T. quadriseptata* *C. meneghiniana*	5.4	A, H, P, Po
50		34	*T. fenestrata* *T. quadriseptata*	1.3	P, Po

TABLE 3. Diatom taxa present in core F at 45cm and 53cm (^{14}C date1800 ± 100 years BP) in Mountain Lake, Virginia: ^{p} planktonic and ^{b} benthic taxa primarily. Nomenclature follows Patrick and Reimer (1966, 1975), Hufford (1987), and Trumbull and Hufford (1989).

45cm, core F:

^{b}*Amphora ovalis* var. *affinis* (Kütz.) V. H. ex DeT.
^{b}*Anomoeoneis serians* var. *brachysira* (Bréb. ex Kütz.) Hust.
^{p}*Cyclotella comensis* Grun.
^{p}*Cyclotella stelligera* (Cl. & Grun.) V. H.
^{b}*Cymatopleura solea* (Bréb.) W. Sm.
^{b}*Cymbella lunata* W. Sm var. *lunata*
^{b}*Epithemia argus* (Ehr.) Kütz. var. *argus*
^{b}*Eunotia valida* Hust. var. *valida*
^{p}*Fragilaria constrictica* Ehr. var. *constricta*
^{p}*Fragilaria contruens* (Ehr.) Grun. var. *construens*
^{p}*Fragilaria contruens* var. *venter* (Ehr.) Grun.
^{p}*Fragilaria pinnata* Ehr. var. *pinnata*
^{p}*Fragilaria virescens*. var. *capitata* Østr.
^{b}*Frustulia vulgaris* (Thwaites) DeT. var. *vulgaris*
^{b}*Gomphonema affine* Kütz. var. *affine*
^{b}*Gomphonema angustatum* (Kütz.) Rabh. var. *angustatum*
^{b}*Gomphonema gracile* Ehr. emend V.H. var. *gracile*
^{b}*Gomphonema truncatum* var. *capitatum* (Ehr.) Patr.
^{p}*Melosira arenaria* Moore
^{p}*Melosira distans* (Ehr.) Kütz.
^{b}*Pinnularia legumen* (Ehr.) Ehr. var. *lugumen*
^{b}*Pinnularia nodosa* (Ehr.) W. Sm. var. *nodosa*
^{b}*Pinnularia parvula* (Ralfs) Cl.-Eul. var. *parvula*
^{b}*Pinnularia substomatophora* Hust. var. *substomatophora*
^{b}*Stauroneis phoenicenteron* (Nitz.) Ehr. var. *phoenicenteron*
^{p}*Synedra ulna* (Nitz.) Ehr. var. *ulna*
^{p}*Tabellaria fenestrata* (Lyng.) Kütz. var. *fenestrata*
^{p}*Tabellaria flocculosa* (Roth) Kütz. var. *flocculosa*

53 cm, core F:

^{b}*Amphora ovalis* var. *affinis* (Kütz) V.H. ex DeT.
^{p}*Cyclotella bodanica* Eulenst.
^{p}*Cyclotella stelligera* (Cl. & Grun.) V. H.
^{p}*Fragilaria pinnata* Ehr. var. *pinnata*
^{b}*Pinnularia nodosa* (Ehr.) W. Sm. var. *nodosa*

planktonic-to-littoral cladocerans reported a prolonged low-water level in a sediment core layer carbon dated at 910± 110 years BP (Parker *et al.*, 1975). The planktonic-to-benthic diatom ratio for core D (Table 1, Figure 4) at this depth was 1.2, suggesting shallow water conditions, as well. At that level diatoms were diverse with 40 species present, implying relatively high lake productivity during this shallow interval. We also found *Sphagnum* spores in these layers, supporting also the presence of a fairly shallow or nearly dry lake that may have been associated with a peat bog. This period at about 900 years BP or 1100 AD, coincides with the Wolf Minimum of solar activity, a time when historic records document that agriculture failed in both Europe and the

TABLE 4. Diatom taxa present in core G at 45cm (^{14}C date 6100 ± 70 years BP) and 50 cm in Mountain Lake, Virginia: [p] planktonic and [b] benthic taxa primarily. Nomenclature follows Patrick and Reimer (1966, 1975), Hufford (1987), and Trumbull and Hufford (1989).

45cm, core G:

[b]*Amphora ovalis* var. *affinis* (Kütz.) V. H. ex DeT.
[b]*Anomoeoneis serians* (Breb. ex Kütz.) Cl. var. *serians*
[b]*Caloneis ventricosa* (Ehr.) Meist. var. *ventricosa*
[p]*Cyclotella meneghiniana* Kütz.
[b]*Cymbella lunata* W. Sm var. *lunata*
[p]*Fragilaria contruens* var. *venter* (Ehr.) Grun.
[b]*Gomphonema gracile* Ehr. emend V. H. var. *gracile*
[b]*Gomphonema parvulum* (Kütz.) var. *parvulum*
[b]*Gomphonema truncatum* var. *turgidum* (Ehr.) Patr.
[b]*Navicula exigua* Greg. ex Grun. var. *exigua*
[b]*Nitzschia linearis* (Ag.) W. Sm.
[b]*Nitzschia tryblionella* var. *victoriae* Grun.
[b]*Pinnularia bogotensis* (Grun.) Cl. var. *bogotensis*
[b]*Pinnularia intermedia* (Lagerst.) Cl. var. *intermedia*
[b]*Pinnularia maior* var. *transversa* (A.S.) Cl.
[b]*Pinnularia parvula* (Ralfs) Cl.-Eul. var. *parvula*
[b]*Pinnularia substomatophora* Hust. var. *substomatophora*
[p]*Synedra ulna* (Nitz.) Ehr. var. *ulna*
[p]*Tabellaria fenestrata* (Lyng.) Kütz. var. *fenestrata*
[p]*Tabellaria flocculosa* (Roth) Kütz. var. *flocculosa*
[p]*Tabellaria quadriseptata* Knuds. var. *quadriseptata*

50cm, core G:

[b]*Amphora ovalis* var. *affinis* (Kütz.) V. H. ex DeT.
[b]*Caloneis ventricosa* (Ehr.) Meist. var. *ventricosa*
[p]*Cyclotella meneghiniana* Kütz.
[b]*Cymatopleura elliptica* (Bréb.) W. Sm.
[b]*Cymbella lunata* W. Sm var. *lunata*
[b]*Eunotia flexuosa* Bréb. ex Kütz. var. *flexuosa*
[b]*Eunotia pectinalis* var. *minor* (Kütz.) Rabh.
[p]*Fragilaria brevistriata* var. *inflata* (Pant.) Hust.
[p]*Fragilaria contruens* var. *pumila* Grun.
[p]*Fragilaria pinnata* Ehr. var. *pinnata*
[p]*Fragilaria pinnata* var. *lancettula* (Schum.) Hust.
[b]*Gomphonema angustatum* (Kütz.) Rabh. var. *angustatum*
[b]*Gomphonema parvulum* (Kütz.) var. *parvulum*
[b]*Gomphonema truncatum* var. *capitatum* (Ehr.) Patr.
[b]*Gomphonema truncatum* var. *turgidum* (Ehr.) Patr.
[b]*Navicula integra* (W. Sm.) Ralfs. var. *integra*
[b]*Navicula mutica* Kütz. var. *mutica*
[b]*Navicula pupula* var. *capitata* Skr. & Meyer
[b]*Navicula scutiformis* Grun. ex A.S. var. *scutiformis*
[b]*Nitzschia linearis* (Ag.) W. Sm.
[b]*Pinnularia abaujensis* (Pant.) Ross var. *abaujensis*
[b]*Pinnularia acuminata* var. *instabilis* (A.S.) Patr.
[b]*Pinnularia appendiculata* (Ag.) Cl. var. *appendiculata*
[b]*Pinnularia formica* (Ehr.) Patr. var. *formica*
[b]*Pinnularia maior* (Kutz.) Rabh. var. *maior*

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TABLE 4. continued

[b]*Pinnularia mesogongyla* Ehr. var. *mesogongyla*
[b]*Pinnularia nodosa* (Ehr.) W. Sm. var. *nodosa*
[b]*Pinnularia parvula* (Ralfs) Cl.-Eul. var. *parvula*
[b]*Pinnularia subcapitata* var. *paucistriata* (Grun.) Cl.
[b]*Pinnularia substomatophora* Hust. var. *substomatophora*
[b]*Pinnularia viridis* (Nitz.) Ehr. var. *viridis*
[p]*Synedra rumpens* Kütz. var. *rumpens*
[p]*Tabellaria fenestrata* (Lyng.) Kütz. var. *fenestrata*
[p]*Tabellaria quadriseptata* Knuds. var. quadriseptata

American southwest due to cold and dry conditions (Parker *et al.*, 1982; Dreschhoff *et al.*, 1993; Blinn and Helvey, 1994). At approximately 28 cm in core D we also found a large (ca. 1 cm) fragment of charcoal, indicating fires during this time period.

Sphagnum remains present in the cores from the 31 cm (ca. 900 years BP) to the 42 cm estimated at 1200 years BP (Figure 4). This suggests that a well-developed peat-forming community had developed in the lake basin during this interval. At the 1200 years BP level, we noted an erosional surface in the core marked by the presence of shale chips that suggest aerial exposure. The planktonic-to-benthic diatom ratio of 0.7 in this layer of core D supports this interpretation.

A layer at 53 cm in core D consisted of a mat of plant fiber associated with abundant *Sphagnum* spores (Figures 2, 4). This layer produced a ^{14}C date of 1800 ±100 years BP. Also, at this depth in core F (Table 3, Figure 4) diatoms had a low diversity or fewer taxa, and about 20% of the *Tabellaria* and *Pinnularia* were aberrant morphologic forms. These aberrant forms may have been produced under the acidified conditions of a bog, when the prolonged low-water may have accompanied higher humic acid, fulvic acid, and other hydrochemical changes (Kirschner *et al.*, 1999; Anderson, 2000). Marland (1967) also claimed a prolonged low-water interval based on cladoceran ratios for sediment layers dating to 1760 ±120 years BP (Parker *et al.*, 1975). This period of time coincided with part of the Medieval Minimum of solar activity (Parker *et al.*, 1982; Dreschhoff *et al.*, 1993).

Both cores D and F bottomed where the hard resistant substrate prevented further penetration. This may have been an erosional surface; the lowest portion of core F consisted of a layer of greenish shale chips and sand (Figure 2). The ratio of planktonic-to-benthic diatoms, however, was between 3.4 and 6.6, indicating that a period of deeper water had likely preceded the low-water interval and bog formation.

Eighteen Hundred to 6100 Years BP (core G):

As noted earlier, a sedimentary unconformity occurred in core G at approximately 15cm depth (Figures 2, 3). The underlying 35cm of core G, however, predated both cores D and F. Less organic material was noted in this older interval, and greatly reduced rates of sedimentation. We noted only one probable prolonged low-water event specifically within this section, but our detection capability most likely was reduced by the lower sedimentation rates during this earlier period.

At 27 cm in core G, a plant fiber layer provided a ^{14}C date of 4100 ±50 years BP (Figure 3). Diatom abundance and diversity at this level was reduced; five taxa were present, consisting of fragmentary remains of a few large individuals of either *Cymatopleura* or *Surirella* (Figure 5). These forms may have been mud- or soil-dwellers, representing a mud-surface assemblage. *Sphagnum* spores were present but wood fragments were lacking, suggesting a less-wooded situation around the lake. At 25 cm, other trilete (fern and/or moss) spores were abundant, indicating an open meadow surrounding the lake. Relative pollen abundance included abundant grass pollen, as well as occasional hemlock. Interestingly, this time period of 4100 years BP corresponds with climatic fluctuation leading to widespread aridity and failure of floods in Egypt (Bell, 1975).

At 37 cm in core G, approximately 10 diatom species occurred. The planktonic-to-benthic ratio was 1.8, implying relatively shallow waters, and some diatoms were abraded. The assemblage at this level was sparse, with low population density, including *Fragilaria* species, a few *Cyclotella kützingiana*, and occasional *Pinnularia* spp. (Table 2).

Core G below 37 cm consisted primarily of finely-banded silty clays. These individual bands may represent fine-scale records of lake level changes. Individual layers were thin and numerous, and a challenge to resolve. When the two radiocarbon dates at G27 and G45 were applied, the sedimentation rates during this period were considerably less (0.09 mm/yr) in the lake than during more recent times.

The 45 cm organic-rich layer of the core yielded a ^{14}C date of years 6100 ±70 years BP. The interval below 40 cm contained an assemblage dominated by *Tabellaria*, including ridged forms of *T. fenestrata*, and *T. quadrisepta* that were not seen elsewhere. At 45 cm (Table 4), the assemblage contained *Cyclotella meneghiniana*, the only samples in our study where this species was found. Moreover, the diatom diversity was high with 21 species in this layer. Such an assemblage does not typify a pioneering community of a lake early in its history. Rather, it more aptly resembles a community within a well-established lake. Therefore, the 6100 years BP maximum age near the bottom of sediment in core G probably represents only the end of our paleolimnological record for that core. Mountain Lake may have been well-established as an oligotrophic system well before 6100 BP. Marland (1967) reported a ^{14}C date of ca. 9500 years BP near the bottom of his longest core but made no further comment or interpretation. Furthermore, Clinch colluvium located at the north end of Mountain Lake is estimated to be Wisconsinian in age (12,000 years) (Mills, 1988; Cawley *et al.*, 2001).

The planktonic-to-benthic diatom ratio at the 6100 year BP 45 cm core layer suggests deep and open waters. Pine, hemlock, hardwood and grass pollen in the core suggests that the local terrestrial flora was not significantly different from that of the present. The deepest samples of core G contained more spruce pollen along with hemlock. Thus, hemlock was present in the local flora by 6100 years ago accompanied by a greater abundance of red spruce.

CONCLUSIONS

1) The clay/inorganic silt layer at 5 to 7 cm in the lake core probably represents erosion from construction of the present hotel and the two roads and/or deforestation due to the mass mortality of the dominant American chestnut in the early 1930's. A 10-fold drop in chestnut pollen above this layer supports this.

2) The core record suggests Mountain Lake is undergoing eutrophication. Early sediments show few organics and lower rates of sedimentation. Recent sediments show that organic materials have accumulated more rapidly and have accompanied more diverse diatom assemblages. Eutrophication apparently has accelerated in the past 100 years (Parson and Parker, 1989a; Beaty and Parker, 1994).

3) A drop in diatom diversity at 10 cm corresponds to the late 1800's when the lake was temporarily low ("Salt Pond") during which the surrounding unforested meadow was used as pasture. The historic record (Parker *et al.*,1975) supports this low-water period.

4) Mountain Lake probably was shallow or nearly dry for at least 30 years in the mid 1600s. The lake was also low or nearly dry for more extended periods at about 900, 1200, and 1800 years BP; two of these low-water periods also agree with Marland's (1967) findings. The increase in *Sphagnum* spores also in these layers suggests that at least the lowest portion of the lake bed was occupied by a peat bog during those periods. Some of the low-water periods appear to correspond to climatic, dry periods, which may be associated with solar minima.

5) Low-water and a peat bog and fern meadow were present in the lake basin at 4100 years BP. Diatoms in the cores at this time likely represent mud- or soil-colonizing forms.

6) Layered clays and silts in the oldest portions of the core may represent additional lake water level changes.

7) At 6100 years BP, deep, open waters occurred in Mountain Lake, and diatoms were already diverse, productive, and well-established. The lake at that time was surrounded by terrestrial flora not greatly different from today, although more red spruce coexisted with hemlock. The origin of Mountain Lake probably predates 6100 years BP, but our oldest core ended just below this period.

We have presented here the first paleolimnological account of Mountain Lake using diatoms, pollen, and stratigraphic analyses of sediment cores. That this study is but a beginning and far from the final word or a complete paleohistory should be obvious. The bottom sediments in the oldest layer of core G suggest that Mountain Lake may already have been well-established 6100 years BP. The stage has now been set for a more comprehensive study of paleolimnology at Mountain Lake. More sediment cores, more ^{14}C dates, and more analyses of sediment diatom and pollen assemblages may lead to a better understanding of the history of this unique lake and paleoclimate for the region. Two features associated with Mountain Lake make it unique for studies of paleoclimate (especially precipitation). In contrast to regions holding numerous lakes, Mountain Lake is the only natural lake in the unglaciated southern Appalachians offering opportunities to track long-term changes in climate using indicators in lake sediments (Smol and Cumming, 2000; Anderson, 2000). Second, the narrow open crevice (fault) at the deep north end of the lake (Cawley *et al.*, 2001) which accounts for 50% of the water loss enhances the magnitude of low water levels during prolonged dry periods, a feature which stands out in the sediment core record.

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Production of a Novel Copper-Binding Ligand by Marine *Synechococcus* (Cyanobacteria) in Response to Toxic Concentrations of Copper

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ABSTRACT

Marine *Synechococcus* spp. are extremely sensitive to copper toxicity. Some strains have been shown to produce high-affinity, extracellular ligands of unknown structure which form complexes with free cupric ion. They are also known to produce metallothioneins (MT) in response to cadmium and zinc stress. In the present study, marine *Synechococcus* PCC 73109 (*Agmenellum quadruplicatum* BG-1) (Van Baalen) was exposed to three concentrations of $CuSO_4$ for various times. Size exclusion chromatography, atomic absorption spectrophotometry, and reverse phase HPLC were used to isolate an intracellular copper binding ligand of low molecular weight (< 6,500 Da). The ligand was detected after exposure to $\geq$ 8 μM $CuSO_4$ for 2 hr in BG-11 medium. The intracellular ligand was characterized by electrospray mass spectrometry, amino acid analysis and a universal assay for siderophores. The ligand was not MT, phytochelatin or a siderophore. It is not a peptide but it contains lysine and an unidentified UV 254-absorbing constituent. This compound is a novel copper-binding ligand previously not reported in *Synechococcus* spp.

Key Index Words: copper toxicity, intracellular copper binding ligand, lysine-contained ligand, marine *Synechococcus*, low molecular weight non-peptide ligand.

Abbreviations: FPLC, Fast Protein Liquid Chromatography; MT, Metallothionein; OD_{450}, Optical density at 450 nm; *smt*, *Synechococcus* metallothionein encoded gene.

INTRODUCTION

Trace metals found in oceanic surface waters can control phytoplankton production and species composition (Bruland *et al.*, 1991). Some trace metals, such as copper, are essential but are toxic at high levels. Excessive copper concentrations can inhibit growth and reproductive rates of phytoplankton in the ocean (Brand *et al.*, 1986; Walsh *et al.*, 1994; Gledhill *et al.*, 1997). A number of marine bacteria, fungi and protozoa have evolved methods to control cellular metal concentrations. Intracellular metal

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detoxifying chelators known as phytochelatins, found in algae and higher plants, can effectively reduce toxic levels of free copper to nontoxic levels (Grill *et al.* 1985). Phytochelatins are produced by marine phytoplankton (Ahner *et al.,* 1995; 1997) and their concentration has been observed to vary systematically with free copper concentration in coastal waters (Ahner *et al.*, 1997). Microbial oxidation reactions also reduce the toxicity of some metals (Emerson *et al.*, 1979; Tebo, 1995). Extracellular copper complexing ligands may also function in copper detoxification (Harwood-Sears and Gordon, 1990; Harwood and Gordon, 1994; Moffett and Zika, 1983; Bruland *et al.*, 1991). *Synechococcus spp.* (WH7803 and PCC 73109) have been shown to produce a strong extracellular copper chelator of unknown structure in response to copper stress (Moffett and Brand, 1996; Gordon *et al.* 2000). Furthermore, McKnight and Morel (1979) detected a strong chelator in freshwater *Synechococcus* cultures. The principal ligand in their studies was shown to be a siderophore (McKnight and Morel, 1980). Various metal-binding compounds including H_2S, siderophores, and metallothionein, have been detected in *Synechococcus* cultures and possibly protect *Synechococcus* from copper toxicity. There is no evidence that these compounds are the strong extracellular ligands detected in cultures (Moffett and Brand, 1996). MT has been reported to be induced by cadmium and zinc, but not copper, in marine *Synechococcus* (Olafson *et al.*, 1980). In contrast, those three metals induced MT in a freshwater strain, but a mutant strain lacking the MT gene (*smt*) was sensitive to high concentrations of cadmium and zinc but not copper (Robinson *et al.*, 1990; Turner *et al.*, 1993). This observation suggests that MT is not important in determining copper tolerance in these microorganisms.

The purpose of the present study was to test the hypothesis that MT is produced in response to elevated concentrations of copper by marine *Synechococcus.* Metallothionein, phytochelatin, or siderophores were not produced by Cu-stressed cells of the strain we examined. However, a low molecular weight (<6,500 Da) molecule that bound the majority of copper contained in cellular extracts of marine *Synechococcus* exposed to copper was isolated. This component, which is not a peptide but which contains lysine, was produced in significantly higher amounts when the organisms were stressed by copper.

MATERIALS & METHODS

Source of marine *cyanobacteria.*

The strain of cyanobacteria used in this study was *Synechococcus* PCC 73109 (American Type Culture Collection ATCC 29404; *Agmenellum quadruplicatum* BG-1), that was originally collected from seawater (Rippka *et al.*, 1979).

Cultivation of marine *cyanobacteria.*

Axenic cultures of PCC 73109 were grown in BG-11 medium (Rippka, 1988), under fluorescent light (30-60 μmol quanta (par)'cm^{-2}'s^{-1}, dark/light cycle [12/12 hr]), at 28°C. Cell number was determined from optical density (450 nm) and cultures were routinely checked for bacterial contamination by streak-plating on Tryptic Soy agar (Difco) plates. Green pigmentation of cultures was used as a visual indication of cell viability. All glassware was rinsed several times with deionized water after soaking overnight in 10% nitric acid. Medium was prepared using Milli-Q water. Solutions and buffers were prepared in Milli-Q water and run through a chelex -100 (Bio-Rad Laboratory) column to remove trace metal contamination.

Induction of copper-complexing ligands in marine *Synechococcus.*

Three concentrations (2.2, 8 and 50 μM) of $CuSO_4$ were used in this study to induce the production of copper-complexing metabolites. These concentrations were selected based upon toxic response of the organism to each concentration in BG-11 medium. 2.2 μM was the concentration at which *Synechococcus* could be maintained readily after stepwise adaptation to $CuSO_4$ (described below). Eight μM was selected based upon the results of toxicity assays as an inhibitory but non-lethal concentration and 50 μM was used as an excessive, lethal dose. Control samples consisted of the organism grown without the addition of $CuSO_4$.

Stepwise adaptation. Using a stepwise adaptation method described by Gupta *et al.* (1992), marine *Synechococcus* PCC 73109 was subcultured in liquid BG-11 medium containing an initial concentration of 0.5 μM $CuSO_4$. Cells that grew in the initial concentration of copper were further subcultured to fresh medium containing higher concentrations of $CuSO_4$ (1, 1.5, 2.2 μM). After adaptation, cultures were maintained in 2.2 μM $CuSO_4$ for subsequent studies.

Inhibitory concentrations of CuSO4.

Different concentrations of $CuSO_4$ (0-20 μM) were added to tubes containing fresh subcultures (OD_{450} = 0.06-0.07) of PCC 73109. Optical density was determined daily (450 nm) until constant. A non-lethal concentration of $CuSO_4$ that caused significant growth inhibition was selected as the inhibitory concentration. The inhibitory concentration (8μM) was added to log phase cultures in subsequent induction experiments. For these experiments, cultures (80 mL) of strain PCC 73109 were grown to log phase (OD_{450} = 0.4-0.5 nm). Cultures were pooled and transferred (80 mL each) to two flasks. Eight μM $CuSO_4$ was added to one of these flasks. The growth of both cultures was monitored by reading the absorbance at 450 nm every 15 min for 2 hr, every 2 hr for 24 hr after the first 2 hr, and every day after the first 24 hr.

Lethal concentration of CuSO4.

Fifty μM copper addition was lethal as indicated by a clear and colorless culture after ten days. This concentration was also utilized in short-term induction experiments for comparison with the inhibitory concentration.

Isolation of genomic DNA and Southern blot hybridization.

Genomic DNA was isolated from *Synechococcus* PCC 73109 that had been maintained in medium containing 2.2 μM $CuSO_4$. Genomic DNA was isolated using a modification of the method described by Porter (1988). After centrifugation of the cell culture at 5,000 x g for 10 min, the cell pellet was resuspended in a solution containing 25% sucrose, 50 mM Tris, 100 mM EDTA, pH 8.0. The cells were then lysed by repeated freeze (-80°C)/ thaw (37°C) cycles. Lysozyme (10 mg• mL^{-1}) and RNase (5 mg•mL^{-1}) were added and the lysate was incubated at 37°C for 30 min. Sodium dodecyl sulfate (1%) was added. The lysate was incubated at 50°C after adding proteinase K (100 μg/mL). After phenol/chloroform extraction, the DNA was dialysed in 1X TE (10 mM Tris and 1 mM EDTA) buffer. Equal concentrations of genomic DNA isolated from marine *Synechococcus* PCC 73109 grown in the presence or absence of $CuSO_4$ were digested with restriction enzymes (Hind III, Sal I, EcoR I, BamH I) followed by Southern blot DNA transfer using a rapid downward alkaline capillary transfer technique described by Chomczynski (1992). The blot was hybridized with a non-radioisotope probe of a *smt* gene fragment. The *smt* gene probe was prepared by labeling a 1.8 Kb Hind III-Sal I DNA fragment isolated from a freshwater *Synechococcus* strain (PCC 6301). The plasmid containing the *smt* fragment was provided by Dr.Nigel Robinson, University of Durham, UK. The probe was labeled using a Rad-Free System kit for the labeling and detection of nucleic acids (Schleicher & Schuell). The hybridization and washing conditions were performed according to the instructions from the manufacturer.

Isolation of intracellular components from PCC 73109 after exposure to $CuSO_4$.

Three different concentrations (2.2, 8, 50 μM) of $CuSO_4$ were added to cultures of marine *Synechococcus* PCC 73109 during the log phase of growth. Cells were harvested at early stationary phase (cell densities of 10^7-10^8 cells•mL^{-1} as determined from acridine orange direct counts) for intracellular protein isolation when 2.2 μM $CuSO_4$ was added. The exposure period to added $CuSO_4$ was 7 to 10 d. When 8μM $CuSO_4$ was added, cells were sampled at 0 min, 30 min, 2 hr, 24 hr, 72 hr (3 d), and when the cultures reached the stationary phase of growth (OD_{450} >1 and cell number ~10^8-10^9 cells•mL^{-1}). When 50 μM $CuSO_4$ was added, cells were harvested for intracellular protein isolation after 2 hr, 24 hr, and 72 hr (3 d) of exposure.

At the time of culture harvest, the absorbance (450 nm) of each culture was read and the cultures were placed on ice for 30 min. Cells were then centrifuged at 3,000xg, at 4°C for 15 min. Cell pellets were washed in ice-cold sterile Milli-Q water three times before being suspended in 10 mL of ice-cold 0.5 M Tris Cl, pH 8.6. The cells were ruptured by using an ice cold "Bead-Beater" (Biospec Products) with 27.5 g of acid washed glass beads (0.1 mm diameter) for 3 min and the lysate was centrifuged at 3,000xg, 4°C for 15 min. The supernatant obtained was a crude cellular extract. The concentration of intracellular protein contained in crude extracts was determined by using a BCA Protein Assay (Pierce).

Separation of intracellular components produced by copper-stressed marine *Synechococcus*.

Crude extracts were separated by a size exclusion Superose 12 HR 10/30 column connected to an FPLC (Fast Protein Liquid Chromatography) system (Pharmacia). Column effluent was monitored by absorbance at 254 nm. The elution buffer was 30 mM Tris Cl with 5 mM mercaptoethanol, pH 8.6, at a flow rate of 1 mL•min^{-1}. Peak area was measured with an electronic graphic calculator (Numonics Corp). Molecular weight standards used for column calibration were bovine serum albumin (BSA, MW 66,000), egg albumin (MW 45,000), carbonic anhydrase (MW 29,000), cytochrome C (MW 12,000), and vitamin B_{12} (MW 1,355). The concentration of total copper in crude extracts and in eluate fractions from the Superose column was determined with Polarized Zeeman graphite furnace atomic absorption spectroscopy (Hitachi, Z-8100). Copper concentrations were normalized to total intracellular protein in the extracts.

Absorbance and emission characteristics of copper-binding components were determined with a spectrophotometer and a spectrofluorimeter. Eluate fractions containing the copper-complexing material collected from a Superose column were scanned (200-900 nm) using a UV-visible spectrophotometer (Varian, Cary 3 Bio). The same fractions were also scanned for absorption (200-400 nm) and emission (200-700 nm) using a spectrofluorimeter (Shimadzu, RF 5000 U).

Three-mL fractions containing the copper-complexing components of interest were collected from a Superose column, pooled and concentrated by lyophilization. The dry material was resuspended in 150 μL of Milli-Q water. The suspension (100 μL) was injected into a reversed-phase HPLC column (Macrosphere 300 RP C8 7U, Alltech) and eluted (1 mL•min^{-1}) using the gradient profile described by Klauser *et al.* (1983). The equilibration buffer (buffer A) was 0.1% trifluoroacetic acid (TFA, Sigma) and the elution buffer (buffer B) was 0.1% TFA containing 60% acetonitrile (Fisher, HPLC grade). The gradient profile was buffer B, 0-30% over 10 min, and 30-45% over 60 min. The column was washed in 100% buffer B for 5 min, and then was equilibrated in buffer A for 10 min prior to a second injection. In some experiments, 0.01 M tetrabutylammonium bromide (TBA) was added to the mobile phase as an ion-pairing agent. Fractions containing 1 mL of eluate were collected from the column (Fraction-100, Pharmacia). The eluate fractions were used for determination of -SH concentrations, Chrome azurol S (CAS) assay for siderophores, electrospray mass spectrometry, and amino acid analysis.

Determination of the concentrations of sulfhydryl groups.

-SH concentrations were determined in RP-HPLC eluate fractions by the method of Ellman (1959). The reaction volume was scaled down by 1/3.

CAS (Chrome azurol S) assay.

Intracellular crude extract and eluate fractions collected from RP-HPLC were used to determined the presence of siderophores using a universal chemical assay developed by Schwyn & Neilands (1987). Intracellular crude extract (0.5 mL) or eluate fractions collected from RP-HPLC were mixed with 0.5 mL of CAS assay solution. The reference control for intracellular crude extract was 0.5 M Tris HCl, pH 8.6. The reference control for the eluate fractions was 0.1% TFA. EDTA (0.5 M) was used as the positive control.

Mass spectrometry analysis and amino acid analysis.

A purified component in fractions collected from RP-HPLC was submitted for electrospray mass spectrometry and amino acid analysis at the W.M. Keck Biomolecular Research Facility, University of Virginia. The facility utilizes a Finnigan-MAT TSQ7000 system with an electrospray ion source interfaced to a reverse phase capillary column. Amino acid analysis was performed by HPLC analysis of PTC derivatives after hydrolysis overnight at 100°C under vacuum.

RESULTS

Determination of inhibitory concentrations of $CuSO_4$.

Three distinct types of growth were seen when cultures of *Synechococcus* PCC 73109 were treated with varying copper levels. In the presence of 1.5-4.5 μM $CuSO_4$, cell densities were lower than the cell density of the control at every time point. However, the growth rate (≈ 0.06/d) of these cultures was similar to that of the control.

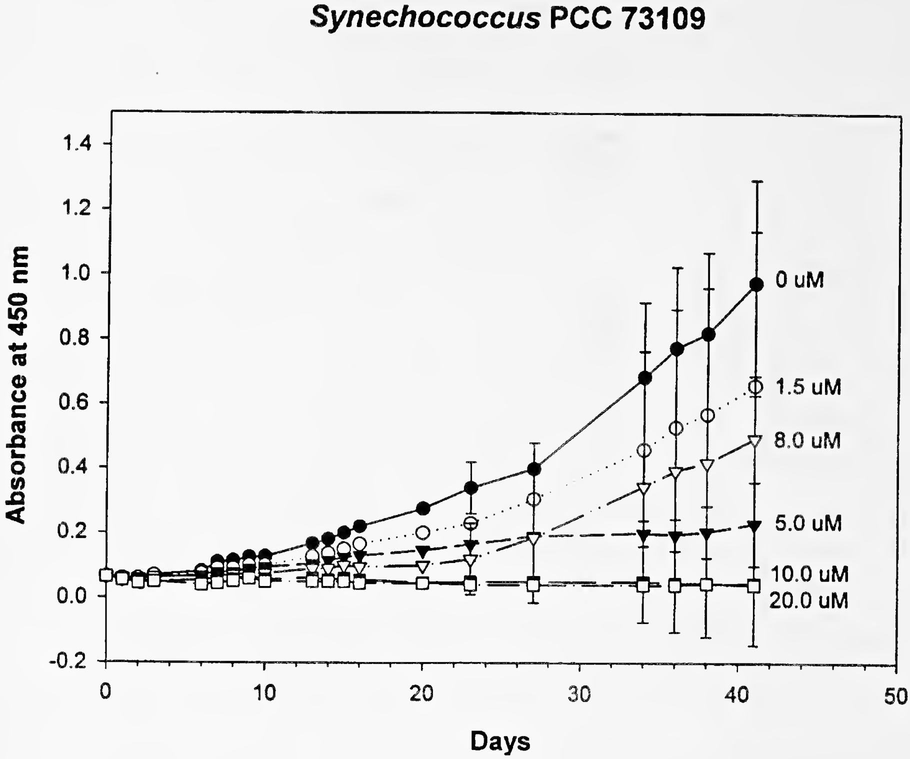

FIGURE 1. Growth of *Synechococcus* PCC 73109 in cultures containing varying concentrations of copper. $CuSO_4$ was added to cultures on day 0. 8 μM copper was selected as an inhibitory but non-lethal concentration for further studies of induction copper-complexing ligands. Error bars are standard deviations. For clarity not all copper concentrations tested are shown in the figure.

In the presence of 5.0-8.0 μM $CuSO_4$, cell densities were lower than the first group with the lowest cell densities in the presence of 8.0 μM $CuSO_4$ although the cultures remained green. After day 20, a shift to rapid growth was observed in 5.5, 6.0 and 8.0 μM $CuSO_4$ containing cultures (Figure 1). At higher concentrations of $CuSO_4$ (10 and 20 μM), the absorbance at later time points was lower than the absorbance at day 0 and the cultures were colorless. In later stages of the growth curve (after about 25 days), significant variation was observed in cell density between replicate cultures (Figure 1). The addition of 8.0 μM $CuSO_4$ to the culture resulted in significant growth inhibition during the first 20 days of incubation but cells were still alive (green color and recovery to rapid growth after 20 days). Thus growth was inhibited but cells were viable. 8.0 μM of $CuSO_4$ was chosen as the inhibitory copper concentration for further studies of the induction of copper-complexing compounds.

Southern blot of *smt* gene.

No detectable homologous (no hybridizing) band was found in *Synechococcus* PCC 73109 which hybridized to the freshwater *smt* gene probe from PCC 6301. However, hybridizing bands were readily observed in genomic DNA extracted from the freshwater strain from which the *smt* probe was derived. The intensity of a hybridizing band

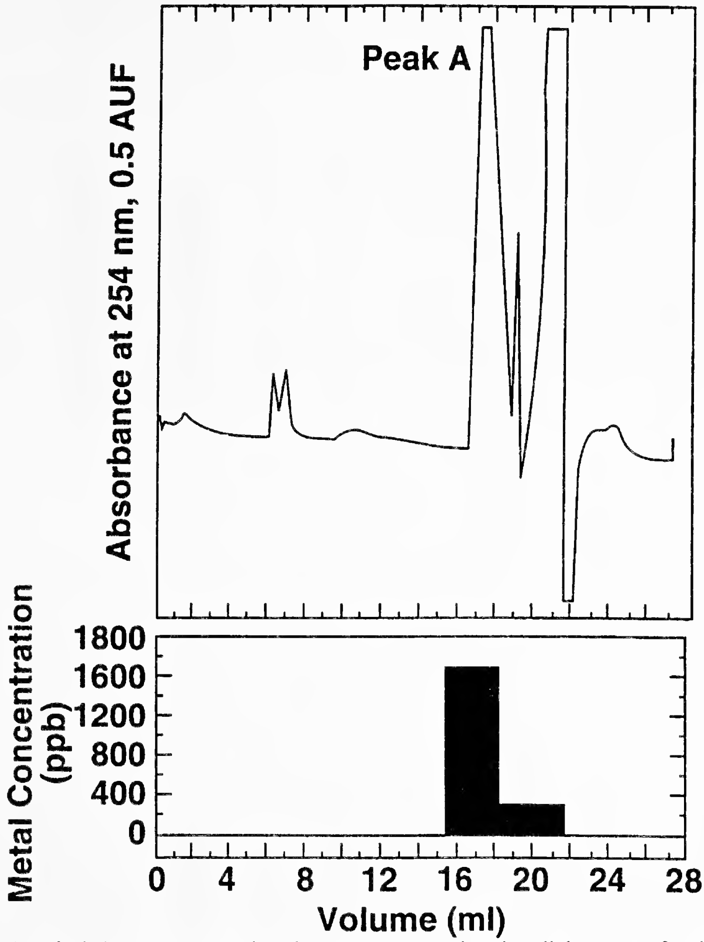

FIGURE 2. A typical chromatogram and total copper concentrations in cellular extract fractions. Extracts (200 μL) from a culture stressed with 50 μM copper were injected into a Superose size exclusion column connected to an FPLC system. The eluant buffer was 30 μM Tris Cl, pH 8.6 and 5 mM mercaptoethanol with a flow rate of 1 mL/min. Fractions of 3 mL were collected. Total copper concentration was determined

was higher when the freshwater culture was exposed to 2.2 μM $CdCl_2$ (as a positive control) possibly due to the amplification of *smt* gene induced by $CdCl_2$ (Gupta *et al.*, 1992).

Analyses of a copper-complexing component produced under copper stress.

Intracellular components isolated from cells subjected to three concentrations of copper (2.2 μM, 8μM and 50μM) were injected into a size exclusion Superose-FPLC column. Chromatograms of intracellular proteins isolated from every culture showed a distinct peak of MW <10,000 (retention volume 16-17 mL). This peak is hereafter referred to as peak A. The concentration of total copper was highest in the fraction containing this peak (Figure 2).

When cells were grown in 2.2 μM $CuSO_4$, the area of peak A was the same as peak A area from the control sample. However, when cells were exposed to 8 μM $CuSO_4$,

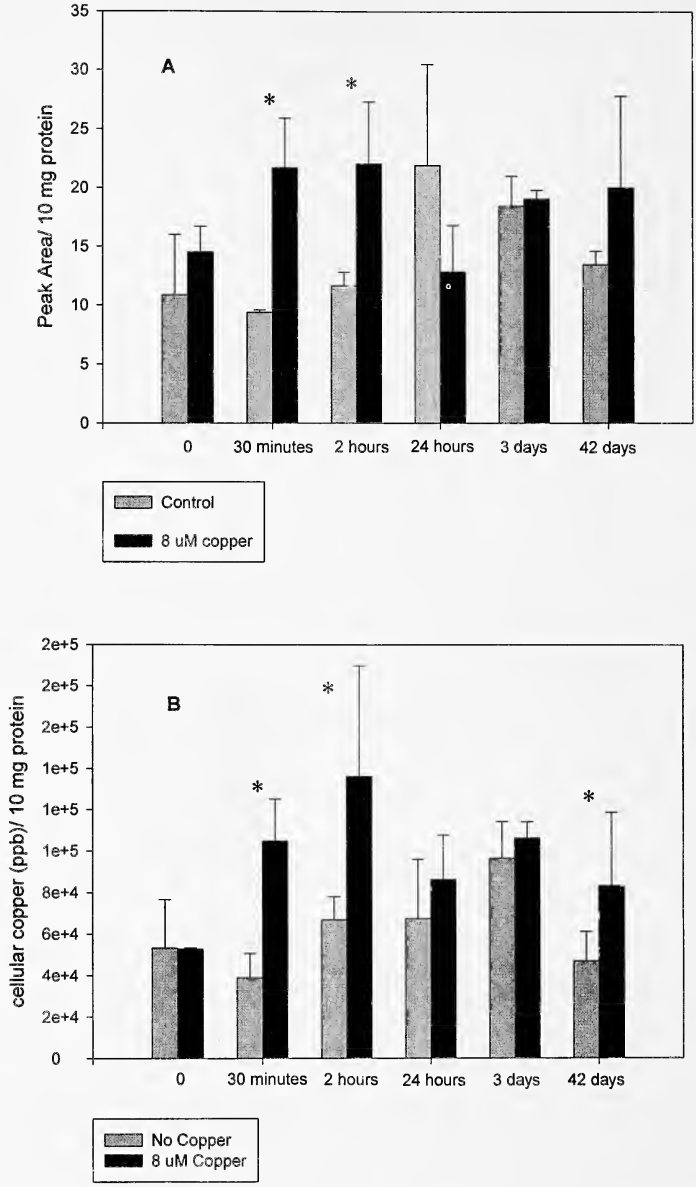

FIGURE 3a and b. Peak A area and copper concentration in cellular extracts from copper challenged (8 μM) and control cultures (a). Peak A area normalized to total protein in the cell extract. (b) Total cellular copper concentrations normalized to total protein. Error bars are standard deviations. * indicates significant ($p < 0.05$) difference from control (t-test).

peak A area from the copper amended cultures was generally larger than the control (Figure 3a) and was significantly larger at exposure times of 30 minutes and 2 hours. Total copper concentrations per mg protein in extracts of copper-treated cells were initially higher than the controls (30 min and 2 hours) but after 24 hours, intracellular copper concentrations were similar (Figure 3b).

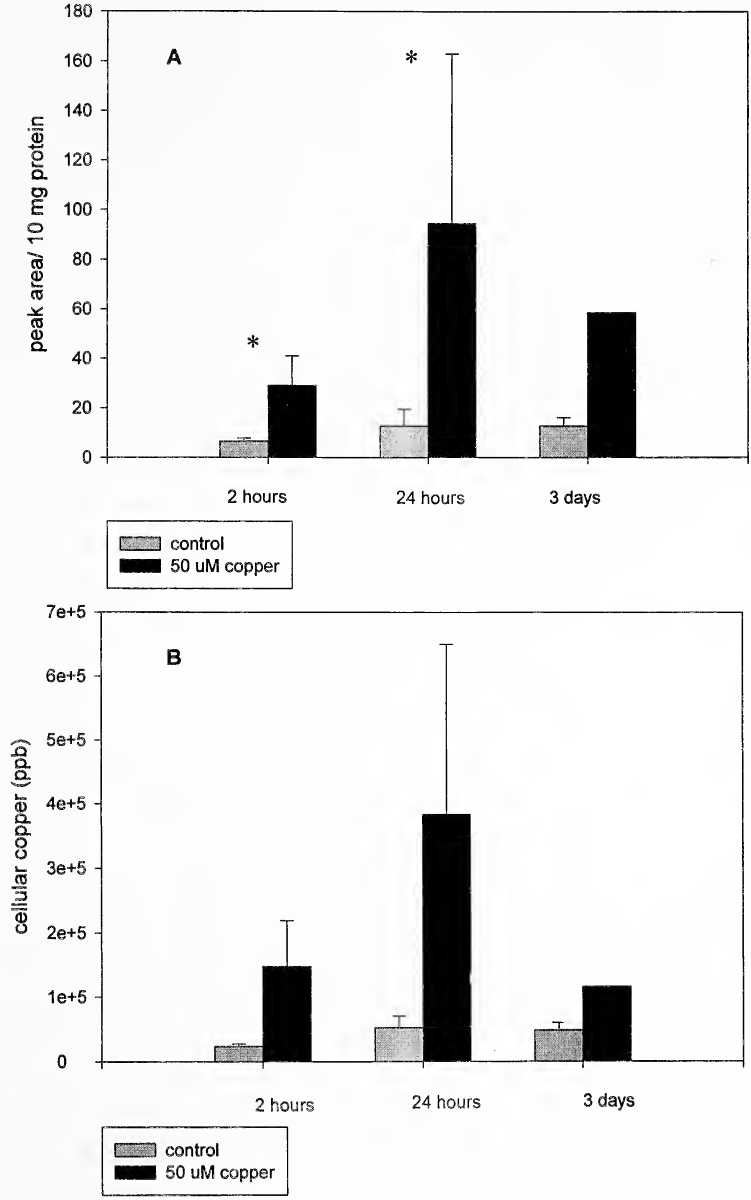

FIGURE 4a and b. Peak A area and copper concentration in cellular extracts from copper challenged (50 μM) and control cultures. (a) Peak A area normalized to total protein in the cell extract. (b) Total cellular copper concentrations normalized to total protein. Error bars are standard deviations. * indicates significant ($p < 0.05$) difference from control (t-test).

When 50 μM $CuSO_4$ was added, the culture changed color from green to yellowish green after 18 hr of exposure and then became clear and colorless after 10 d of exposure. The comparison of peak A area between the $CuSO_4$ treated sample (at 2 hr, 24 hr, and 3 d) and the control sample is shown in Figure 4a. At this higher copper concentration the area of peak A and intracellular copper concentration in copper challenged cultures

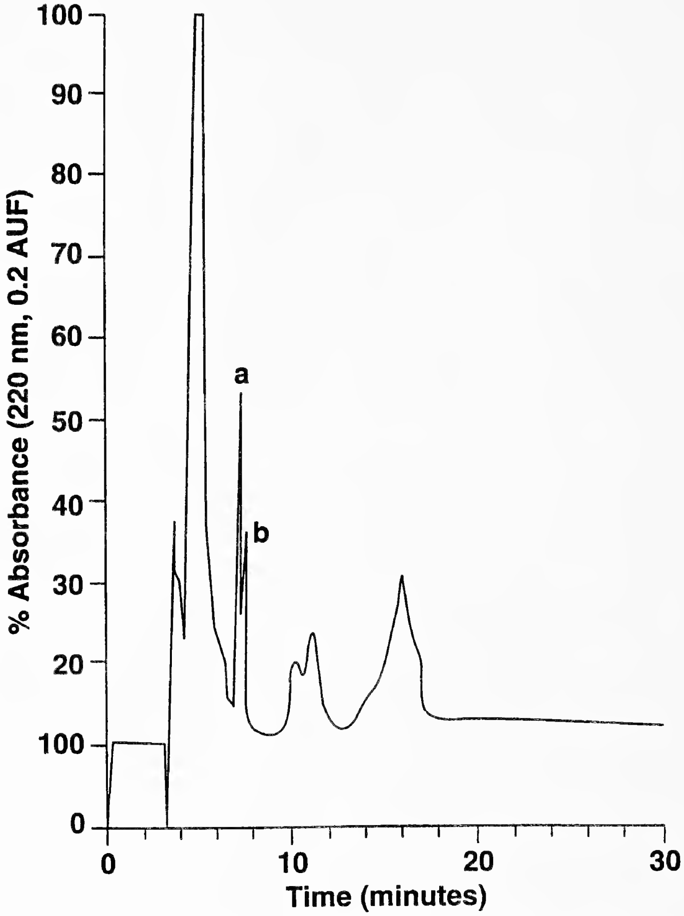

FIGURE 5. Typical reverse-phase HPLC chromatogram of peak A material collected from the Superose-FPLC separation of *Synechococcus* PCC 73109 cellular extract. Three fractions (3 mL) containing peak A eluted from a Superose-FPLC column were pooled, lyophilized, and resuspended in 150 µL of Milli-Q water. 100 µL (~78g protein) was injected into C_8-RPHPLC column. Peak a and b produced a single band (<6,500 MW) in SDS-PAGE gels.

exceeded that of the controls at each sampling time (Figure 4b). The difference was significant ($p < 0.05$) at 2 and 24 h time points. Large variation was observed in peak A area and cellular copper concentration in replicate copper-challenged cultures at the 24 hour sampling time.

Characterization of the intracellular components induced by the addition of $CuSO_4$.

The eluate fraction containing peak A was scanned using a UV-visible spectrophotometer (wavelength 200-900 nm) and a spectrofluorimeter. Maximum absorption was observed at 230 nm in all fractions containing peak A and the maximum emission was at 450 nm at excitation of 250 nm and 350 nm.

The RP-HPLC chromatogram of components contained within peak A is shown in Figure 5. The components that did not bind to the column (void volume) were eluted at approximately 4 min. The peaks at retention time 6-7 min were absent in buffer blanks and their areas were consistently larger in copper-challenged cultures when normalized to total protein in the FPLC fraction. The material comprising peaks a&b eluted about 2 min. later from the RP-HPLC column when tetrabutylammonium bromide (TBA) was added to the mobile phase buffer as an ion pair reagent.

The -SH concentration in the fraction containing peak a and b from the RP-HPLC column was similar in copper-treated samples and in the control samples, i.e. 14.2 μm per mg total protein vs. 19.9μm per mg total protein, respectively.

The CAS assay for siderophores was negative in every fraction collected from the FPLC and in the crude extract. The sensitivity of the assay (less than 7.5 nmol of chelator with high affinity for iron) is sufficient to detect siderophores had they been present in these cellular extracts; thus, siderophores were not induced in the cultures.

Electrospray mass spectrometry indicated that the compound(s) in the fraction eluting at 6-7 min from RP-HPLC was not a peptide. A charging pattern characteristic of peptide mass spectra was not observed and no peptide ion was seen in the matrix assisted laser desorption time-of-flight mass spectrum (Michael Kinter, Ph.D., Director, W.M. Keck Biomedical Mass spectrometry Laboratory, personal communication). Amino acid analysis of this compound(s) demonstrated that lysine was present along with a large peak of unknown identity which absorbed at 254 nm. No cysteine was detected.

DISCUSSION

Freshwater *Synechococcus* sp. can produce MT in response to stress from elevated concentrations of copper, cadmium or zinc (Robinson *et al.*, 1990). The gene coding for MT was reportedly amplified when cells were repeatedly exposed to high concentrations of cadmium (Gupta *et al.*, 1992). To our knowledge, no marine strain of *Synechococcus* has been reported to produce MT in response to copper stress, but some strains produce it in response to cadmium and zinc (Olafson *et al.*, 1980). The role of Cu-MT in freshwater *Synechococcus* sp. is unclear because mutant strains lacking MT retain copper resistance but become sensitive to cadmium and zinc (Turner *et al.*, 1993).

Being the same genus as the freshwater strain that produces MT, marine *Synechococcus* PCC 73109 was hypothesized to also produce MT in response to copper stress. Since the unique structure of Cu-MT has been reported to result in a labile molecule in eukaryotes (Bremner *et al.*, 1978; Bremner *et al.*, 1986; Sato & Bremner, 1984), and since a previous study which tested for MT in a marine strain used a long incubation (Olafson *et al.*, 1980), we assayed cultures at a series of time intervals. Negative results from the Southern blot hybridization technique using a *smt* probe from a freshwater strain indicate that Cu-MT in the marine strain differs from that in the freshwater strain or that Cu-MT is absent in the marine strain. Absence of cysteine in ligands isolated from cultures exposed to copper indicates that MT is not produced by PCC 73109 in response to copper stress.

The ligand detected within thirty minutes of exposure to $CuSO_4$ in log phase *Synechococcus* PCC 73109 was neither phytochelatin, siderophore nor peptide, but contained lysine and an unidentified component that absorbed at 254 nm and fluoresced at 450 nm. These spectral characteristics suggest a compound with cyclic or aromatic structure. Toxic concentrations of copper induced increased production of this ligand above the basal level. The observed copper-complexing capacity of the component, as indicated by its coelution with copper in FPLC, suggests a possible role in copper detoxification. Presence of the ligand in control cultures without an addition of $CuSO_4$ indicates a basal production at the low copper concentration present in the culture medium (0.32 μM is added to BG-11 medium). The production of the ligand rapidly increased when the cultures were exposed to high concentrations of copper, suggesting that the copper-complexing compound may be a metabolic defense at elevated copper concentrations.

Further study of the structure of the ligand isolated in this study is clearly needed. However structural characterization will require significantly more material than was obtained in the course of the present study. The influence of an ion-pairing agent on retention by RP-HPLC suggests the ligand is ionizable. Our data shows that the copper-complexing component is of low molecular weight (< 6,500), contains lysine, is probably a cyclic or aromatic component with ionizable functional groups. Lysine contains α-carboxylate, α-amino, and ε-amino group that can chelate free Cu^{2+} (Martin, 1979). This copper-complexing compound apparently functions to alleviate copper toxicity but is unlike copper binding ligands previously reported in *Synechococcus* spp.

ACKNOWLEDGEMENTS

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Spawning Behavior in *Hemitremia flammea* (Actinopterygii: Cyprinidae)

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ABSTRACT

Spawning behavior in *Hemitremia flammea* (Flame chub) is described from observations made in the field and laboratory. Spawning in the field occurred over clean gravel (size range=18-25 mm) at water temperatures from 12.8-14.4 C. Spawning in the laboratory occurred over clean gravel (11.3 mm) at water temperatures from 18.3-20 C. Males often pursued females and nudged their vents with their snouts. When the female settled to the substrate, a male moved forward and aligned himself alongside her body. Then the female moved slightly forward accompanied by the male and the pair vibrated their caudal peduncles and tails. The spawn ended as the male quickly flexed his caudal peduncle and caudal fin laterally toward the quivering caudal peduncle and caudal fin of the female. The male's flexed caudal area did not cross over but contacted the side of the female's quivering caudal peduncle, which sometimes became arched slightly upwards. Aspects of spawning behavior (males pursuing and nudging vents of females, females selecting sites for spawning, pair alignment, males vibrating caudal fin and peduncle) in *H. flammea* is similar to that described for *Couesius plumbeus* but differs significantly from that of *Semotilus atromaculatus*, a species in the hypothesized sister group of *H. flammea*.

INTRODUCTION

Hemitremia flammea (Flame chub) is restricted to spring habitats primarily in the Tennessee, Duck, and Cumberland river drainages in Tennessee and Alabama, and has been recorded in the upper Coosa River drainage (Mobile Basin) in Alabama (Etnier and Starnes, 1993). Spawning has been reported to occur from late January through May, peaking in March, with tuberculate males available from October through May (Etnier and Starnes, 1993). Etnier and Starnes (1993) observed what they thought to be a spawning aggregation in a shallow seepage area of a pasture in late February, but stated that reproductive behavior and egg deposition sites are unknown. This paper describes spawning behavior in *H. flammea* from field and laboratory observations, and compares it to that found in other lithophilous spawning cyprinids in North America, particularly in *Couesius plumbeus* and *Semotilus atromaculatus*. Recent morphological and molecular studies (Coburn and Cavender, 1992; Simons and Mayden, 1997) support a sister group relationship between *Hemitremia* and species of *Semotilus*, including *S. atromaculatus*.

MATERIALS AND METHODS

Field Studies:

Observations and videorecordings of *H. flammea* were made in a man-made riffle between two spring-fed ponds of Mountain Fork (tributary of Flint River, Tennessee River Drainage) off Winchester Road, about 5 km NE of New Market, Madison Co., Alabama, from 1000-1500 hrs CDT on 10 May 1998 and 2 May 1999; and in laboratory aquaria from January-May, 2000. Underwater videorecordings were made with a Sony VX 1000 digital camera/recorder, equipped with a built-in 100 watt light source, and mounted in a Sting Ray waterproof housing. The camera, manually manipulated or set in a fixed position on the substrate, was positioned about 0.6 m from the substrate where the fish were congregating. Stream width was 10 m, water depth where recordings were made was 45 cm, and maximum visibility was 4.5 m.

Laboratory Studies:

Adult *H. flammea*, collected from Cypress Creek, off Arlic Holt Road, Wayne Co., Tennessee in 1995, were transported to the laboratory. In January, 2000, the fish were transferred to a 40-gal aquarium kept at 7.2 C. Photoperiod was maintained at 6-8 hrs/day in January, and gradually increased with fluorescent lighting to 16 hrs/day from mid-January to late May. A submersible aquarium heater was used to increase water temperature from 7.2-18.3 C from 15 January to 8 March 2000. Groups of 6-8 male and 8-12 female adult *H. flammea*, acclimated to these conditions, were then transferred to a 151 liter aquarium for observation and videotaping. This observational aquarium was fitted with fine grain gravel (mm) and one large (8 x 12 x 15 cm) rock for cover. A single plastic tray (30.5 x 40.6 x 7.6 cm) fitted with gravel (11.3 mm size class) was placed over one part of the substrate to facilitate harvesting of eggs. Bunches of plastic grass were placed at the ends of the aquarium at various times. Routinely, females were removed from the observational aquarium after spawning. Adults were fed dry foods (Tetramin flakes in the morning) and live or frozen foods (glassworms, whiteworms, and blood worms) in the afternoon.

Behaviors recorded on 10 hrs. of videotape (2 hrs in the field and 8 hrs in the laboratory) were reviewed at normal speed, in slow motion, and frame by frame to identify specific behaviors of female and male *H. flammea* following methods in Maurakis and Woolcott (1995). Reproductive activities of male and female *H. flammea* were resolved into six chronological categories that reflected the sequence of male-female interactions characteristic of a successful spawn, following Sabaj (1992; 2000) and Maurakis and Woolcott (1993): *interim* (behavior of male between spawns), *approach* (behavior of female directed towards interim male), *alignment* (behavior affecting orientation of a spawning pair over substrate), *run* (initiated by a female, synchronized movement of aligned pair over substrate), *clasp* (spawning act, i.e., momentary flexure of male's body about that of female at end of her *run*), and *dissociation* (behaviors of male and female affecting their separation immediately following the clasp). Behaviors other than those associated with the spawning sequence were considered disruptive of a successful spawn.Accounts of spawning behavior in *H. flammea* are summarized from observations and review of videotapes made in the field and laboratory.

RESULTS

Field Studies:

Spawning occurred over clean gravel (diameter 18-25 mm) in moderate current at the head of a riffle joining two spring-fed ponds at temperatures between 12.8-14.4 C. Male *Campostoma oligolepis* (largescale stoneroller) occasionally moved over the substrate where male *H. flammea* were posturing, but were not observed spawning, digging, or foraging in this area.

Laboratory Studies:

Spawning occurred over substrates composed of primarily of 11.3 mm size gravel between 18.3 C (8 March 2000) and 20 C (9 June 2000).

Spawning Behaviors:

Interim: In the field, about 200 male *H. flammea*, swimming against the current, hovered over approximately 1 m^2 of substrate. Within this 1 m^2 area, males frequently formed two to three well-defined groups, each group maintaining position over a discrete area of substrate where they jockeyed for position. No one male consistently dominated other males. Within these sub-areas, males also foraged as they dislodged gravel with their snouts. They also dipped their heads or snouts into substrate and then expelled sand from their mouths. Aggressive behaviors observed between males were lateral head butts, body swings, chases, and short parallel swims. In the laboratory, male behavior during interim was like that observed in the field, *albeit* numbers of males in the aquarium were lower.

Approach: From a downstream position, a female swam forward against the current to the substrate regardless of the position of the majority of males. The female slowed her forward movement as she lowered her body into a downstream slope of a depression formed by natural arrangements of gravel in the substrate and rested on the substrate. Usually a female was followed by a single male, although at times a couple of other males were in close proximity.

Alignment: One to four males chased a female as she moved from one area of substrate to another, frequently nudging her genital area with their snouts. Males maneuvered themselves about the female and attempted to direct her to the substrate regardless of where other males congregated. Successful alignment occurred when a male moved forward from a downstream position and aligned himself head to tail with the female. At times, two males aligned with a female, one on each side of her body. Other times, as many as six males maneuvered themselves alongside and on top of the female and each other. The maneuvering of several males about a female often resulted in the female swimming away.

Run: After a male aligned himself aside a female, the female moved slightly forward with three to four quick tail beats and was accompanied by the male who also moved forward with quick tail beats. At the end of the short run, the pair began to vibrate their caudal peduncles and caudal fins as the female followed the contour of the substrate. At times, a couple of males, one on each side of the female, accompanied the female during her run.

Clasp: The male ended his vibrations with a quick flexing of his caudal peduncle and tail laterally toward the quivering caudal peduncle and caudal fin of the female.

The male's flexed caudal area did not cross over but contacted the side of the female's quivering caudal peduncle, which sometimes became arched slightly upwards.

Dissociation: After the clasp, the female moved off of the substrate. She either returned to the substrate to spawn again, or moved away from the spawning area. At times the female was appressed to the substrate by several males just after being clasped. She flexed her caudal fin against the substrate, springboarding herself upwards. As the female rose vertically into the water column, she often was accompanied by a male. Near the water's surface, but without breaking it, the female regained horizontal and swam away. After clasping, a male either followed the female to the same (or a different) spawning area where they spawned again, or joined other males congregating over the substrate and engaged them in aggressive behaviors.

During and after the spawning act, as many as 30 male *H. flammea* converged upon the spawning pair, most of which writhed and burrowed head first (caudal fins oriented upward) into the substrate where spawning occurred, presumably to eat eggs. Burrowing episodes, involving as many as 30 males and lasting up to 30 seconds each, also occurred when a single male butted gravel with his snout, presumably in search of food.

DISCUSSION

Spawning behaviors in *H. flammea* are somewhat similar to those described for *Couesius plumbeus* (Lake chub) by Brown (1969) and Brown et al. (1970). In both species, males and females congregate in shallow areas of streams to spawn (lake chubs also spawn along rock shores and shoals of lakes). *Hemitremia flammea* has been observed to spawn in 0.3 m of water over road gravel that had washed in from a nearby gravel drive on 5 May 1993 at a water temperature of 15 C (R. Mayden and B. Kuhajda, pers. comm.). Mayden and Kuhajda (pers. comm.) also noted when a female approached the spawning area, she was followed by 2-5 males, but spawning occurred with 1-3 males. After spawning, several individuals crowded in to eat eggs. Whereas we only observed flame chubs spawning on gravel, Brown et al. (1970) observed lake chubs spawning over a variety of substrates (i.e., on gravel, among or beneath rocks, beneath large boulders and on silt and leaves). Nest-building has not been observed and there is no evidence of parental care in either species.

In both species, males pursue females and use their snouts to nudge the female's vent region. Brown et al. (1970) noticed in aquaria that male lake chubs actively pursued, nudged and swam aside ripe females but were less interested in spent females. Hunter and Hasler (1965) demonstrated that milt and ovarian fluid of *Lepomis cyanellus* (green sunfish) attracted *Lythrurus umbratilis* (Redfin shiner) to sunfish nests and stimulated the shiners to spawn. Brown (1969) used this information to speculate that the female's ovarian fluid may elicit the male's nudging behaviors in lake chubs.

Brown et al. (1970) noted for *C. plumbeus* held in aquaria that the spawning act lasted about a second and described it accordingly: "A male would...force himself against [the female] and, as he vibrated vigorously and she appeared to struggle, the nonadhesive eggs would freely disperse." As we described in flame chubs, the female initiated a short run with a few quick tail beats after which she vibrated her caudal peduncle and tail over the substrate. The closely aligned male similarly vibrated his body as he accompanied her run and completed the act by laterally flexing his caudal peduncle and tail toward the quivering female. Although Brown et al. (1970) did not specify a run and quivering in female *C. plumbeus*, their description suggests that the spawning act in both species are quite similar. Furthermore, in both species the site of gamete deposition appears to be ultimately determined by the female as she moves toward the substrate with one or a few male swimming alongside.

Spawning behavior in *H. flammea* is significantly different from that reported for *Semotilus atromaculatus*, a species in the proposed sister-group of *H. flammea* (Coburn and Cavender, 1992; Simmons and Mayden, 1997). None of the spawning behaviors exhibited by male and female *H. flammea* during interim, approach, alignment, run and clasp, or dissociation were consistent with those described by Sabaj (1992) and Maurakis et al. (1993) for *S. atromaculatus* and *S. thoreauianus*. During interim behavior in *S. atromaculatus*, a single male uses his mouth to construct a pit-ridge nest and remains stationed in the pit unless engaged in spawning on the pit/ridge interface or aggressive behaviors with other males away from the nest (Sabaj, 1992), behaviors not observed in *H. flammea*. During the approach, a female *S. atromaculatus* always moves toward a male in his nest. The male *Semotilus* effectively determines the spawning site by constructing the nest. In contrast, female *H. flammea* may move to substrates away from congregating males for spawning. Female *H. flammea* do not retroflex as do female *S. atromaculatus* reported by Sabaj (1992) and Maurakis et al. (1993). Male *H. flammea* do not perform a spawning clasp like that described for *S. atromaculatus* by Sabaj (1992) where a male completely encircles an uplifted female.

Differences in spawning behaviors between *H. flammea* and *Semotilus* species do not conflict with the *Semotilus-Hemitremia* sister-group of Coburn and Cavender (1992) and Simmons and Mayden (1997). Pit-ridge nest building probably represents an autapomorphy that evolved within the *Semotilus* ancestral lineage after separation from the ancestral stock common to *Hemitremia* and *Semotilus*. For example, Johnston and Page (1992) indicated that the primitive *Phoxinus* and *Margariscus* and derived *Couesius, Dionda, Hybognathus* are broadcast spawners (i.e., species that scatter eggs and sperm with no previous preparation of substrate). By this definition, *H. flammea* can be considered a broadcast spawning species. However, delineation of chronological categories of spawning behavior of Sabaj et al. (2000) for closely related species (*Phoxinus*, *Margariscus*, *Couesius, Dionda,* and *Hybognathus*) will allow the use of behavioral characters to refine resolution among species relationships proposed by Coburn and Cavender (1992) and Simmons and Mayden (1997).

ACKNOWLEDGMENTS

We sincerely thank D. Etnier for sparking the collaboration among the authors, and his consultation in spawning of *H. flammea*; B.R. Kuhajda and M. H. Sabaj for their critical reviews of the manuscript, which improved its quality; and R. Mayden and B. R. Kuhajda who made us privy to their field notes on *H. flammea*. E. G. Maurakis analyzed videotaped behaviors and prepared the manuscript. R. Katula and W. Roston videotaped behaviors and assisted in manuscript preparation. Funded in part by Science Museum of Virginia and University of Richmond.

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JEFFRESS RESEARCH GRANT AWARDS

The Allocations Committee of the Thomas F. and Kate Miller Jeffress Memorial Trust has announced the award of Jeffress Research Grants to the institutions listed below to support the research of the investigator whose name is given. The Jeffress Trust, established in 1981 under the will of Robert M. Jeffress, a business executive and philanthropist of Richmond, supports research in chemical, medical and other natural sciences through grants to non-profit research and educational institutions in the Commonwealth of Virginia. The Jeffress Research Grants being announced here have been awarded in 2000.

The Jeffress Memorial Trust is administered by Bank of America. Additional information about the program of the Trust may be obtained by writing to: Advisor, Thomas F. and Kate Miller Jeffress Memorial Trust, Bank of America, Private Bank, P. O. Box 26688, Richmond, VA 23261-6688.

Lizabeth A. Allison, The College of William and Mary. Nucleocytoplasmic Transport of the Thyroid Hormone Receptor: Alternate Pathways and Determinants of Nuclear Retention. $14,950. (one year renewal).

Bao Ling Adam, Eastern Virginia Medical School. Identification and Characterization of Prostate Cancer Associated Proteins. $15,000. (one year renewal).

Todd D. Averett, The College of William and Mary. Investigations of the Structure of the Nucleon by Parity Violating Electron Scattering. $15,000. (one year renewal).

Carey K. Bagdassarian, The College of William and Mary. Enzymatic Activity and Protein-Matrix Flexibility: The Energetics and Dynamics of Catalysis by Cytidine Deaminase. $14,000 (one year renewal).

Kari E. Benson, Lynchburg College. The Adaptive Value of Female Receptivity Signals. $11,807. (one year)

David R. Bevan, Virginia Polytechnic Institute and State University. Molecular Modeling of Ligand Binding to Retinoic Acid Receptors. $18,000. (one year).

John W. Bigbee, Virginia Commonwealth University/Medical College of Virginia. Studies on a Cell-substratum Adhesive Role for Neuronal Acetylcholinesterase. $14,955. (one year renewal).

Peter Blackmore and Roy Williams, Eastern Virginia Medical School. Regulation of Platelet Aggregation by Phytoestrogens. $15,000. (one year renewal).

Milton L. Brown, University of Virginia. Design, Synthesis and Evaluation of Novel Sodium Channel Blockers. $30,000. (one year).

Amy H. Bouton and Joanna B. Goldberg. University of Virginia School of Medicine. *Helicobacter Pylori* and Its Effect on Host Cell Proliferation and Survival. $15,000. (one year renewal).

Mafia Bykhovskaia and John Hackett, University of Virginia School of Medicine. Mechanisms Underlying Time-Course of Transmitter Release During Facilitated Neurosecretion. $15,000. (one year renewal).

Judith B. Cain, Virginia Military Institute. The Determination of the Block Copolymer Exchange Rates by Nuclear Magnetic Resonance. $19,500. (one year).

Christopher Carone, The College of William and Mary. Topics in Intermediate and High Energy Particle Theory. $15,000. (one year renewal).

Vittorio Celli, University of Virginia. Opto-Electronic Processes in a Frozen Rydberg Gas. $15,000. (one year renewal).

Corey L. Cleland, James Madison University. Infleuence of Neonatal Injury on Adult Pain Sensitivity. $30,000. (one year).

Raymond J. Colello, Virginia Commonwealth University/Medical College of Virginia. Gene Delivery to the CNS: A Novel Approach Using Retroviral Vectors. $12,665 (one year renewal).

Keith P. Corodimas, Lynchburg College. Adenosine Regulators of Emotional (fear) Learning: Role of A1 Receptors in the Amygdala, Hippocampus nad Nucleus Accumbens. $26,645. (one year).

Richard M. Costanzo, Virginia Commonwealth University. Transplantation of Olfactory Neurons from Genetically Altered Mice. $30,000. (one year).

Harry Dankowicz, Virginia Polytechnic Institute and State University. Passive Gait in Anthropomorphic Bipedal Mechanisms Including Muscular Skelatal Modeling. $15,000. (one year renewal).

Robin Lee Davies, Sweet Briar College. Investigations of the Cytotoxicity of Novel Diimene Complexes of Platinum (IV) and Palladium (IV). $15,000. (one year renewal).

Cynthia J. Denbow, Virginia Polytechnic Institute and State University. Expression of Human Cystic Fibrosis Transporter Protein in Plants. $24,000. (one year).

Michael Deschenes, The College of William and Mary. Recovery of Neuromuscular Junctions from Space Flight. $14,930. (one year renewal).

Raymond Dessy, Virginia Polytechnic Institute and State University. Micro-Channel and Micro-Chip Separation and Analysis Systems Applicable to Glycoprotein Engineering. $18,000. (one year).

David A. DeWitt and John Savory, Liberty University. Aluminum Induced Mitochondrial Injury, Oxidation Stress, and Apoptosis *in vitro.* $15,000. (one year).

William B. Eggleston, Virginia Commonwealth University. Characterization of Epiallellism at the Maize rl Locus. $28,090. (one year).

Alan R. Esker, Virginia Polytechnic Institute and State University. Polymer Dynamics in Confined Geometries and Biomimetic Systems. $15,000. (one year renewal).

Erik J. Fernandez, University of Virginia. Mass Spectrometry-Detected Hydrogen Exchange: A New Method for Analysing Protein Aggregation at the Residue Level. $15,000. (one year renewal).

Mark H. Forsyth, The College of William and Mary. A Comparative Genomic Approach to Genetic Diversity Among Virulant and Avirulent *Heliobacter pylori.* $21,675. (one year).

Gregory D. Foster, George Mason University. The Use of Molecular Markers to Delineate Sources of Organic Carbon and Hydrogeochemical Processes in the Potomac River Watershed. $18,965. (one year).

Babette Fuss, Virginia Commonwealth University/Medical College of Virginia. Novel Mechanisms in CNS Mylelination: Role of PD-la/ATX. $30,000. (one year).

Shobha Ghosh, Virginia Commonwealth University/Medical College of Virginia. Cholesterol Ester Metabolism in Macrophage Foam Cells. $15,000. (one year renewal).

William F. Glass, Eastern Virginia Medical School. Regulation of Mesangial Cell Hypertrophy by c-src and Rho. $29,000. (one year).

Glenda Gillaspy, Virginia Polytechnic Institute and State University. Signal Termination in *Arabidopsis.* $15,000. (one year renewal).

Aaron S. Goldstein, Virginia Polytechnic Institute and State University. A Radial Flow Chamber to Examine the Effect of Orientation and Cell-Cell Interactions on the Strength of Adhesion of the Epithelial Cells to Chemically Patterned Model Biomaterials. $30,000. (one year).

Robert M. Granger, II, Sweet Briar College. What to do with the CO~? Studies of Novel CO2 Reduction Catalysis. $30,000. (one year).

Robert W. Granger and Robert J. Talmadge, Virginia Polytechnic Institute and State University. Role of the ATP-Dependent Ubiquitin-Proteasome Pathway in Cell Cycle Regulation of Normal and Dystrophic Muscle. $30,000. (one year).

Carla B. Green, University of Virginia. Cell-Specific Ablation of Circadian Clock Function in the Vertebrate Retina. $29,700. (one year).

Charles M. Grisham, University of Virginia. Structure and Mechanism of Adenylyl Cyclase Toxin. $30,000. (one year).

Tai Liang Guo, Virginia Commonwealth University. Genistein Modulation of the Immune Responses in Mice. $15,000. (one year renewal).

John T. Gupton, University of Richmond. The Application of B-Chloroenones to the Synthesis of Lamellarin G Trimetheyl Ether and Related Alkaloids. $20,000. (one year).

Gregory Hancock, The College of William and Mary. Deducing Uplift Rates and a Detailed Glacial Chronology for the Cordillera Blanca, Peru, using Radionuclides. $17,542. (one year).

Georgia Ann Hammond, Radford University. Molecular and Genetic Characterization of *Cercospora zeae-maydis,* a Fungal Pathogen of Corn. $15,000. (one year renewal).

Brian E. Hanson, Virginia Polytechnic Institute and State University. Catalytic and Stoichiometric Pauson-Khand Reactions as a Potential Route to Novel Monomers for Polymeric Materials Synthesis. $15,000. (one year renewal).

Catherine C. Hedrick, University of Virginia School of Medicine. Glucose-Mediated Regulation of Monocyte Endothelial Interactions in Vascular Disease and Diabetes. $30,000. (one year).

W. Gary Hollis, Roanoke College. Electroactive Multi-metallocenes Assemblies. $30,000. (one year).

Todd A. Houston, Virginia Commonwealth University. Design of Boron-Based Glycosidase Inhibitors. $15,000. (one year renewal).

Helen I'Anson, Washington and Lee University. Metabolic Signals and Onset of Puberty. $9,390. (one year renewal).

Allison Belsches Jablonski, Lynchburg College. Studies of Promoter regulation of HER2: Involvement with Estrogen Receptor Signaling. $25,518. (one year).

David R. Jones, University of Virginia School of Medicine. Role of Bcl-X1 in Chemosensitization of Non-Small Cell Lung Cancer Following the Inactivation of NF-kB. $29,000. (one year).

Mohammed Kalimi, Virginia Commonwealth University/Medical College of Virginia. Protective Effects of Steroids Against Neurotoxin-Induced Hippocampal Cell Death: Cellular and Molecular Mechanisms. $15,000. (one year renewal).

Kenneth R. Kambis, The College of William and Mary. The Effects of Caloric Restriction on Mood States at Sea Level and High Altitude. $10,797. (one year).

Karen Kester, Virginia Commonwealth University. Microsatellite Analysis of Spatial and Temporal Variation. $15,000. (one year renewal).

Eugene B. Kolomeisky, University of Virginia. Theory of Low-Dimensional Bose-Einstein Condensation. $20,000. (one year).

Robert H. Kretsinger and Robert H. Bauerle, University of Virginia. Engineering the Active Site of an Enzyme. $26,270. (one year).

Gary M. Kupfer, University of Virginia School of Medicine. Function of the Fanconi Anemia Protein Complex. $15,000. (one year renewal).

Sophia Lee, Virginia Commonwealth University/Medical College of Virginia. The Role of the Transforming Growth Factor-beta Isoforms in Excisional Wound Healing. $25,925. (one year).

Rong Li, University of Virginia School of Medicine. Mutational Analysis of the BRCT Motif in BRACA1. $15,000. (one year renewal).

Thurman E. Lockhart, Virginia Polytechnic Institute and State University. Effects of Age-Related Changes in Hamstring Activation Rate and Heel Contact Velocity on the Biomechanics of Slips and Falls. $30,000. (one year).

Janos Luka, Eastern Virginia Medical School. Cloning of the Human Herpesvirus-6 (HHV-6) Genome as an Infectious Bacterial Artificial Chromosome: A New Approach to Facilitate Genetic Analysis of Viral Functions. $21,700. (one year). :

Mary C. Mahony and Peter Blackmore, Eastern Virginia Medical School, Jones Institute for Reproductive Medicine. Involvement of the MAP Kinase Signaling Cascade in Sperm Hyperactivated Motility. $30,000. (one year).

Richard T. Marconi, Virginia Commonwealth University/Medical College of Virginia. Identification of Genus Wide Themes in *Borrelial* Pathogenesis: The Role of the Rep Gene Family. $15,000. (one year renewal).

Kurt McCammon and Paul Ratz, Eastern Virginia Medical School. Calcium Regulation of the Detrusor Contractions. $15,000. (one year renewal).

Karen McGrady, University of Richmond. Synthesis and Characterization of Novel Polymer Electrolytes. $13,733. (one year).

Michael A. McVoy, Virginia Commonwealth University/Medical College of Virginia. Class I Down-Regulation by Human Herpesvirus 6 and Guinea Pig Cytomegalovirus. $15,000. (one year renewal).

Jonathan D. Monroe, James Madison University. Structure-Function Studies on Apoplastic a-Glucosidases of *Arabidiopsis thaliana.* $15,000. (one year renewal).

John R. Morris, Virginia Polytechnic Institute and State University. Molecular Beam Studies of Interfacial Reactions Using Functionalized Self Assenbled Microlayers. $15,000. (one year renewal).

Timothy J. Newman, University of Virginia. Localization Transitions in Directed Polymer Systems (and their relation to biological phenomena). $21,500. (one year).

Linda L. Phillips and Thomas Reeves. Virginia Commonwealth University/Medical College of Virginia. The Role of Dopaminergic Systems in Recovery Following Traumatic Brain Surgery. $10,000. (one year renewal).

Sergio Oehninger and Mahmood Morshedi, Eastern Virginia Medical School. Identification, Characterization of Cellular Pathways and Prevention of Apoptosis in Spermatozoa of Infertile Men. $30,000. (one year).

Brent E. Owens, The College of William and Mary. Geochronology and Geochemistry of the State Farm Gneiss: Oldest Precambrian Crust in the Goochland Terrane, Piedmont Province, Virginia?. $13,900. (one year renewal).

Mark A. Palmer, Virginia Commonwealth University. Examination of the Dendritic to Granular Transition During Solidification. $30,000. (one year).

Manish S. Patankar and Frank A. Lattanzio, Eastern Virginia Medical School. Structural and Functional Analysis of the Ovarian Tumor Marker CA 125. $29,800. (one year).

K. Kevin Pfister, University of Virginia. Mechanism of Adenovirus Transport Along Microtubles. $27,105. (one year).

Gregory M. Plunkett, Virginia Commonwealth University. Interpreting Biogeographic Patterns in Araliacea. $15,000. (one year renewal).

David L. Popham, Virginia Polytechnic Institute and State University. Protein Interactions During Peptidoglycan Wall Synthesis in *Bacillus subtilis.* $30,000. (one year).

John S. Poutsma, The College of William and Mary. Computational Studies of the Structure and Energetics on Non-Protein Amino Acids. $25,000. (one year).

Raina S. Robeva, Bessie H. Kirkwood, Tim Loboschefski, Sweet Briar College. Electroencephalographic and Psychometric Differences Between Female College Students With and Without Attention Deficit/Hyperactivity Disorder. $15,000. (one year renewal).

Sarah C. Rutan, Virginia Commonwealth University. Characterization of Chemical Dynamics in Complex Systems. $15,000. (one year renewal).

Ravi F. Saraf, Virginia Polytechnic Institute and State University. Interfacial and External Field Effects on Self-Assembled and Ultra Thin Polymer films. $15,000. (one year renewal).

Mohamadi A. Sarkar, Virginia Commonwealth University. Development of a Biosensor by Immobilizing Human Recombinant Oxidative Enzymes in a Lipid Bilayer on Gold Electroplated with Silver. $29,260. (one year).

Heidi Scrable, University of Virginia School of Medicine. Molecular Basis of Hippocampal Learning. $15,000. (one year renewal).

Rita Shiang, Virginia Commonwealth University/Medical College of Virginia. *In vivo* and *in vitro* Models of Treacher Collins Syndrome. $20,000. (one year).

Merry J. Sleigh and Geoffrey F. Birchard, George Mason University. Physiological Effects of Prenatal Sensory Stimulation in Bobwhite Quail. $9,875. (one year).

Stanton Q. Smith, Virginia Military Institute. A Synthetic Study of the Rhopaladins: A Newly Discovered Bis-Indole Alkaloid. $17,400. (one year).

Albert T. Sneden, Virginia Commonwealth University. New Phenylpropanoid Glycosides from Virginia *POLYGONUM* Species. $15,000 (one year renewal). :

Daniel E. Sonenshine and Wayne Hayes, Old Dominion University. Immune Dysfunction in Ticks: Why Certain Ticks Transmit *Borrelia Burgdorferi* and Other Disease-Causing Microbes. $15,000. (one year renewal).

Dorothy Spangenburg and Frank Lattanzio, Eastern Virginia Medical School. Thyroxine Hormone in Metamorphosing Jellyfish. $15,000. (one year renewal).

Robert F. Spencer, Virginia Commonwealth University/Medical College of Virginia. Auditory Neuropathy in the Jaundiced Gunn Rat. $28,400. (one year).

Wayne M. Stalick, George Mason University. A Mild Versatile Synthesis of Functionalized γ-Carbolines Derivatives. $30,000. (one year).

David W. Sukow, Washington and Lee University. High-Frequency Oscilliations from a Semiconductor Laser with Two Delayed Optical Feedbacks. $30,000. (one year).

Douglas R. Taylor, University of Virginia. Using the Y-Chromosome Microsatellites in *Silene latifolia* to Study the Origin and Spread of Agriculture in Europe. $30,000. (one year).

Uwe Tauber, Virginia Polytechnic Institute and State University. Scaling and Universality in Non-Equilibrium Systems. $29,502. (one year).

Gary C. Tepper, Virginia Commonwealth University. Development of a Microwave-Based Gamma Radiation Detector. $14,400. (one year renewal).

Bruce J. Turner, Virginia Polytechnic Institute and State University. Immunogenitic Variation in Natural Populations of a Homozygous Clonal Vertebrate. $15,000. (one year renewal).

Kenneth E. Van Ness, Washington and Lee University. Viscous Behavior of Miscible Polymeric Blends. $15,000. (one year renewal).

Stephen P. Watton, Virginia Commonwealth University. Immobilization of Transition Metal Complexes in Porous Silicate Media. $15,000. (one year renewal).

Ian G. Welsford, James Madison University. Biochemistry of Novel Antibacterial Substances Isolated from Gastropod Integument. $30,000. (one year).

Robert E. Welsh, The College of William and Mary. A Multi-Modality Gamma-Ray Imaging Device for Biological Research on Small Animals. $29,850. (one year).

Debra L. Wohl, University of Richmond. The Importance of Functionally Redundant Species on an Ecosystem Process. $26,960. (one year).

H. T. Wright, Virginia Commonwealth University/Medical College of Virginia. Alzheimer's Amyloid Peptide Interactions with Serpins and Cell Surface Receptors. $15,000. (one year renewal).

Jeffrey S. Young, University of Virginia School of Medicine. Role of Nuclear Factor-Kappa B in Post Trauma Acute Lung Dysfunction. $29,935. (one year).

HELMUT WAKEHAM
V.A.S. FELLOW DIES AT AGE 85

Virginia Academy of Science Fellow and long time supporter, Helmut Richard Rea Wakeham, died October 18, at his residence in Cedarfield, Richmond. Dr. Wakeham was born in Hamburg, Germany and moved with his parents to the U.S. as a teenager.

Active in the Boy Scouts throughout his life, he earned his Eagle Scout Badge in 1933. According to his son, Dr. Stuart G. Wakeham, "Wake" exhibited those leadership qualities that imbue Scouting's highest rank throughout his life, shaping his interests in the Boy Scouts, education of youth, and his cultural interests. He served as director and president of the Boy Scouts Robert E. Lee Council from 1973 to 1975. In the 1970's, he helped develop the Camp Brady Saunders.

Wakeham attained Bachelor of Arts and Master of Arts degrees from the University of Nebraska and, a Doctorate of Philosophy in physical chemistry from the University of California , Berkley. He held research positions with Standard Oil Co. of California, the US Department of Agriculture in New Orleans, the Institute of Textile Technology, Charlottesville, the Textile Research Institute in Princeton, N.J., and was Director of the Ahmedabad Industry Textile Research Association in India from 1956-1958. During his tenure at Standard Oil Co., Dr. Wakeham worked to develop a practical synthetic rubber to aid the World War II effort.

In 1958 Dr. Wakeham came to Richmond to head the Research and Development Department of Philip Morris Co. Later he became Vice-President of Science and Technology for that corporation. Being keenly interested in the education and encouragement of scientific achievement of young people, he was instrumental in securing financial support for the Virginia Junior Academy of Science. For many years, Philip Morris Co. supported the VJAS student paper awards in all of the VJAS sections at the time of the annual meetings.

In 1980, The Academy recognised Dr. Wakeham's contributions to science and to the Academy, making him a Fellow of The Academy.

Dr. Wakeham's many contributions to Virginia's cultural community include President of the Richmond Symphony from 1979 to 1981 and Chairman, 1981- 1983. He lead efforts to rennovate the "old" Lowes Theater in downtown Richmond to become The Carpenter Center for Performing Arts. He served alternately as president and chairman of that organization from 1980 to 1987. He received the Angel Award of the International Society of Performing Arts, and was Director of the American Symphony Orchestra League.

Dr. Wakeham was instrumental in securing the "old" Broad Street Rail Station as the home of The Science Museum of Virginia. He served as the SMV Foundation President and Director from 1974 to 1977.

Dr. Wakeham was a Fellow of the American Institute of Chemists, receiving the Distinguished Chemist Award in Virginia and was Cultural Laurate in Science and Technology of the State of Virginia.

Besides his son, Dr. Start Wakeham, "Wake" is survived by a brother, Richard Wakeham (Grand Prairie,TX) , and two daughters, Susan Kosiur of Orcas Island, Wash. , Rosemary Wakeham of Rolla, MO, and three grandchildren.

Abstracts from the VAS 2001 Annual Meeting Held at James Madison University

These abstracts were submitted on time but were lost because of e-mail access problems experienced by the editor during June, 2001, .

DEVELOPMENT OF TDI NANOSCALE STRUCTURES. D. Pestov, N. Levit & G. Tepper, Department of Chemical Engineering Virginia Commonwealth University, Richmond, VA 23284. In this work we combine a spray on technique known as Rapid Expansion of Supercritical Solution (RESS) and gas phase crosslinking techniques to develop a new nanoscale composite material. By RESS we obtained about 1 micron diameter particles of poly (dimethylsiloxane), bis (12-hydroxystearate) terminated. These particles were then exposed to 2,4-toluyne diisocyanate (TDI) saturated vapor at room temperature. After this reaction a composite material was obtained. It consists of cross-linked initial siloxane and a new nanoscale needle like TDI polymer. It is found that the TDI polymer needles initiate from surface imperfections. Scanning Electron and Atomic Force microscopy was used for imaging. Based on FT-IR data and microscope-imaging, a scheme for the TDI polymerization reaction is proposed. The composite material has a high surface/volume ratio and potential for use in sensor applications. This work was funded by the EPA, National Center for Environmental Research and Quality Assurance.

SCREENING OF LOW-COST ADSORBENTS FOR LEAD REMOVAL. Kelly Payne & Tarek Abdel-Fattah, Dept. of Biol., Chem.& Env. Sci., Christopher Newport University, Newport News, VA 23606. Batch adsorption kinetic and isotherm studies were conducted to compare and evaluate different types of adsorbents for lead removal from aqueous media. Adsorbent materials such as activated carbon (Calgon Carbon Filtrasorb 300 and 400) and naturally occurring zeolites (clinoptilolite and chabazite) were selected because of their relative low cost. Molecular sieves (13X and 5A) were selected because they provide a basis for comparison with previous studies and represent well-characterized materials. The relative rate for lead adsorption was: 13X modified chabazite clinoptilolite 5A activated carbon modified clinoptilolite. Modeling lead adsorption by different adsorbents using the Langmuir and Freundlich isotherm was used. Molecular sieves (13X and 5A) and activated carbon fit the Langmuir isotherm while naturally occurring zeolites fit the Freundlich isotherm expression.

STUDY OF LOW-COST ADSORBENTS FOR CADMIUM REMOVAL FROM AQUEOUS MEDIA. Mirela Boghea & Tarek Abdel-Fattah, Dept. of Biol., Chem.& Env. Sci., Christopher Newport University, Newport News, VA 23606. The objective of this study concerns the screening of low-cost adsorbents for cadmium removal from aqueous media. Seven different materials were selected in order to perform the experiment including: naturally occurring zeolites (Clinoptilolite and Chabazite), modified zeolites, synthetic zeolites (13X and 5A) and activated carbon (Calgon Filtrasorb 300). These absorbents were investigated to determine their capacity for cadmium removal from aqueous media by batch adsorption. The data showed that all adsorbents have affinity for cadmium removal from drinking water. Molecular sieves

13X, 5A and clinoptilolite have shown to remove more then 95% of cadmium from aqueous media. However, Chabazite removed 55% of cadmium from aqueous media. Langmuir and Freundlich isotherm expressions were used for comparing the affinity of each adsorbent for cadmium and determine the adsorbents' capacity for cadmium removal from aqueous media.

UPTAKE OF IRON BY LOW-COST ADSORBENTS. John Hitt & Tarek Abdel-Fattah, Dept. of Biol., Chem.& Env. Sci., Christopher Newport University, Newport News, VA 23606. High levels of fluoride in drinking water are a cause of fluorosis that is characterized by bone deterioration and tooth caries. Activated carbon, naturally occurring zeolites, and synthetic molecular sieves are promising adsorbents for drinking water purification. These adsorbents were modified with Iron to increase their capacity for removal of fluoride from water. The Iron(II) to Iron(III) ratio introduced to the adsorbents was then determined using the spectrophotometric method. At equilibrium, the ratio of Iron(II) to Iron(III) that is introduced to the adsorbents was less than one, except in the case of clinoptilolite. The amount of Iron(II) introduced to the surface of chabazite, activated carbon, and the synthetic molecular sieves (13X and 5A) correlates with the amount of fluoride removed using the modified adsorbents. Adsorbent modified with Iron(II) has a higher capacity for fluoride removal than Iron(III).

MERLOT- ORGANIZED PEER REVIEW OF WEB-BASED RESOURCES TO HELP FACULTY ENHANCE TEACHING AND LEARNING IN TWELVE EDUCATIONAL DISCIPLINES. Martin. D. Zahn, Dept. of Biology, Thomas Nelson Community College. The Multimedia Educational Resource for Learning and Online Teaching (MERLOT) is a web-based resource of online materials in twelve academic disciplines. Discipline teams conduct peer reviews of materials in the collection using a process modeled on the peer review of scholarship; discipline team members are drawn from 23 MERLOT Partner Organizations across the United States and Canada. Reviews are published on the MERLOT web site. Faculty from the Virginia Community College System participate in discipline team activities and the peer review process. This project helps faculty easily identify quality educational materials for use in online courses or as supplemental assignments. Faculty can locate appropriate materials through a browsing function by discipline or subject area within each discipline or an advanced search and sort capability; searches can be by author, title, date, or peer ratings. The project also hopes to increase peer recognition for faculty producing online materials. Faculty may choose to become members of MERLOT at no cost; membership allows faculty to recommend new sites, provide user comments on sites, and post assignments they develop to effectively use a site.

DEVELOPING PACING GUIDES FOR SCIENCE ON BLOCK SCHEDULES. Alvin M. Pettus, James Madison University, Harrisonburg, Va. and Myron E. Blosser, Harrisonburg City Schools, Harrisonburg, Va. Science teachers can use pacing guides to help make sure the instruction they provide is current, relevant, and experiential. The information and concepts in science change so rapidly that science teachers need to plan for keeping course content up-to-date. Students learn best when they are provided with experiences that allow them to understand the applications and real life contexts of the skills and concepts taught. During the presentation, examples of current, relevant, and experiential instructional activities were provided. Examples of pacing guides that can be used to ensure covering all desired content, aligning instruction with desired outcomes and assessments, and making instruction current, relevant, and experiential were presented. Useful resources for developing guides were identified. All teachers who will participate in providing the science instruction, should be involved in the development of the pacing guides.

DEVELOPING EFFECTIVE FIELD TRIP ACTIVITIES FOR PUBLIC SCHOOL CHILDREN: THE OTTER PROGRAM AT THE CLAYTOR NATURE STUDY CENTER. Woody McKenzie and Judy Strang, Lynchburg College, Lynchburg, Va. And Angela M. Arigoni-Mesfioui, Randolph-Macon Woman's College, Lynchburg, Va. During the first year of a two-year initiative at Lynchburg College, funded by the Jessie Ball Du Pont foundation, an environmental education outreach program was implemented. Eleven field trips involving local area public schools engaged children at the Claytor Nature Study Center in Bedford County. The field trip activities involved physical/experiential learning, water-based investigations, and preparatory/follow-up sessions with classroom teachers. Learning was evaluated by a variety of qualitative assessments, using rubrics specific to assignments and mainly occurred during follow-up sessions. The most successful field trips resulted from collaboration with a concerned and involved teacher and included appropriate adult/teacher ratios, involvement in water-based activities, preparatory and follow-up workshops, well-formulated assessment plans, and enthusiastic teachers willing to devote extra time for pre and post trip lessons.

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