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Shyamal K. Majumdar, Ph.D.
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Department of Biology
Lafayette College
Easton, PA 18042-1778

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Shyamal K. Majumdar, Ph.D., Editor
Department of Biology
Lafayette College, Easton, PA 18042
Phone: (610) 330-5464
FAX: (610) 330-5705
E-Mail: Majumdas@lafayette.edu

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DISTRIBUTION AND STATUS OF THE FOX SQUIRREL (SCIURUS NIGER) IN PENNSYLVANIA[1]

KATHARINE L. DERGE[2] and MICHAEL A. STEELE[3]

[2]*The Pennsylvania State University*
204 Ferguson Building
University Park, PA 16802

[3]*Department of Biology*
Wilkes University
Wilkes-Barre, PA 18766

ABSTRACT

In Pennsylvania, the presumed range of the eastern fox squirrel (*Sciurus niger vulpinus*) overlaps with the range of the western fox squirrel (*S. n. rufiventer*), but there is almost no documentation of the actual distribution of each subspecies in the state. The Pennsylvania Biological Survey has classified the eastern fox squirrel as "status undetermined" because of a lack of information on its abundance, distribution, and ecology. As a first step towards updating the status of the fox squirrel in Pennsylvania, we report on the results of two state-wide surveys on the distribution of the fox squirrel within Pennsylvania — one completed in 1980 and a second in 1997. We sent written questionnaires to state mammalogists and Pennsylvania Game Commission (PGC) Wildlife Conservation Officers (WCOs), inquiring about the distribution, abundance, appearance, and ecology of the fox squirrels in the state. Distribution data from historic specimens were obtained from six area museums. Respondents from 38 (58%) of the 65 counties for which we received data reported the presence of fox squirrels. Fifty-one (65%) of the respondents recorded fox squirrels from western counties, 23 (29%) from central counties, and 4 (5%) from eastern counties. Fox squirrels in western counties were reported as abundant and widespread in their distribution; whereas those from central and eastern counties appear to comprise small and widely dispersed populations, many of which may have become established in the past 30 years. We conclude that while fox squirrels have increased in numbers and possibly extended their range in some areas of the state, populations within the historic range of *S. n. vulpinus* remain limited in size and distribution. The Delmarva fox squirrel (*S. n. cinereus*) remains extirpated from Pennsylvania. We suggest that *S. n. vulpinus* be designated threatened, but also note the need for additional taxonomic and genetic studies to fully establish the status of *S. niger* in the state.
[J PA Acad Sci 73(2):43-50, 1999]

INTRODUCTION

One of ten recognized subspecies of the fox squirrel in North America (Hall 1981, Koprowski 1994), the eastern fox squirrel (*Sciurus niger vulpinus*) ranges from central Pennsylvania south to Virginia and west to central West Virginia (Hall 1981). The subspecies is considered to have been declining in abundance for the past few decades (Flyger and Lustig 1976). In Pennsylvania, the presumed range of the eastern subspecies overlaps with the range of the western fox squirrel (*S. n. rufiventer*), but to date, virtually no data on the distribution of either subspecies in the state are available (Zegers 1985, Merritt 1987). Between 1920 and 1950, the western subspecies was introduced to most of the state, but most of the introductions appear to have been unsuccessful (Richmond and Rosland 1949, Grimm and

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Roberts 1950, Gifford and Whitebread 1951, Roslund 1951, Roberts and Early 1952, Doutt et al. 1977). Other introductions have been carried out until as recently as the mid-1980's. Some of these introductions may have influenced existing populations of the eastern subspecies and potentially disrupted the genetic distinction between the two subspecies (Zegers 1985). A third subspecies of fox squirrel, the Delmarva fox squirrel (*S. n. cinereus*), was extirpated from southeastern Pennsylvania by the early 1900's and has been on the federal endangered species list since 1967 (Poole 1944, World Wildlife Fund 1991). An attempt to reintroduce Delmarva fox squirrels to Chester County in 1987 was considered unsuccessful (Dunn 1989), although there have been recent reliable reports of fox squirrels in the Chester County area approximately 6-8 years after the introduction (M. A. Steele, pers. obs.)

The taxonomic distinction between the eastern and western fox squirrels is based primarily on a few historic museum specimens and natural history notes (e.g., Poole 1944). The eastern fox squirrel reportedly appeared similar to a gray squirrel (*Sciurus carolinensis*), but was grizzled with yellow or orange on the back and sides and had a white belly (Bangs 1896, Poole 1944, Barkalow 1954). The western fox squirrel is mostly ferruginous, with an orange or yellow belly, but even in 1944 considerable overlap in coloration was reported for the two subspecies (Poole 1944). A taxonomic re-examination of the distinction between the two appears to be necessary (Zegers 1985).

Since our survey of the distribution of the eastern fox squirrel in 1980, sightings of fox squirrels in additional counties have increased over the past 15 years, indicating a possible range expansion within the state. The fox squirrel shows a natural propensity to expand its range and abundance when land use practices favor suitable landscapes and habitats (e.g., mature forest woodlots interspersed with agricultural crops or forested riparian corridors; Flyger and Lustig 1976). Few records on the former abundance or distribution of the fox squirrel are available from the Mid-Atlantic region, but changing land-use patterns, habitat destruction, and overhunting in the early- and mid-1900s probably eliminated the fox squirrel from much of its former range (Bakken 1952, Flyger and Lustig 1976).

The Pennsylvania Biological Survey has classified the eastern fox squirrel as "status undetermined" because of a lack of information on its abundance, distribution, and ecology (Zegers 1985). An update to this classification grouped the eastern and western subspecies together and recommended "status rare" as well as stated that there is no firm evidence of the Delmarva fox squirrel's historic occurrence in the state (Kirkland and Krim 1990). As the next step towards updating the status of the fox squirrel in Pennsylvania, we report on the results of two statewide surveys of the distribution of the fox squirrel—one conducted in 1980 and the second in 1997.

MATERIALS AND METHODS

1980 Survey

In spring 1980, one of us (M. A. Steele) mailed questionnaires to 43 Pennsylvania Game Commission (PGC) Wildlife Conservation Officers (WCOs; called Game Protectors in 1980) of 15 counties within the approximate range of the eastern fox squirrel. A cover letter explaining the purpose of the questionnaire was enclosed. Questions were aimed at estimating the location, number and size of fox squirrel populations in the respondent's jurisdiction and describing habitat characteristics of fox squirrels. Questions were primarily yes/no or multiple choice. For example, a list of potential habitat characteristics was provided for respondents to check all that applied to fox squirrels in their jurisdiction.

1997 Survey

In August 1997 we repeated the survey by mailing questionnaires to state mammalogists and to regional directors of the six PGC regional offices for distribution to the WCOs in each region of the state. We enclosed a cover letter explaining the purpose of the questionnaire.

The 1997 questionnaire included the same questions asked on the 1980 questionnaire as well as questions aimed at documenting the history of the populations and appearance of fox squirrels. All questions were again yes/no or multiple choice. We additionally asked the respondents to mark the distribution of known fox squirrel populations on a county map. After the questionnaires were returned, we arbitrarily divided the state into three regions for the purposes of reporting results (Fig. 1).

Museum Records

We gathered a list of existing fox squirrel specimens collected in Pennsylvania from area museum collections. Museums records were compiled from the Carnegie Museum of Natural History, the United States National Museum of Natural History, the Academy of Natural Sciences of Philadelphia, the Reading Public Museum and Art Gallery, the Pennsylvania State University School of Forest Resources Vertebrate Museum, and the Shippensburg University Vertebrate Museum. We visited the latter three museums to examine the physical appearance of each specimen and determine subspecific status.

RESULTS

1980 Survey

Thirty-seven questionnaires were returned; 12 respondents reported populations of eastern fox squirrels and three reported introduced populations of western fox squirrels. Eastern fox squirrels were reported in only six of the fifteen counties: Blair, Bedford, Franklin, Perry, Juniata, and Cumberland Counties (Fig. 2). Recent introductions of western fox squirrels were reported in Dauphin, York, and Lebanon Counties.

The majority of respondents reporting fox squirrels (67%) estimated less than five distinct populations of fox squirrels were in their jurisdiction (Table 1). The average size of populations was considered less than five individuals for 40% of responses and 6-15 individuals for 40% of responses. Six of 15 (40%) respondents considered fox squirrel populations stable in their county; four (27%) were considered unstable.

Fox squirrel habitat was described as occurring primarily in rural areas (87% of respondents) with cornfields adjacent to woods (87%). Hunting of squirrels was considered common (87%), and populations of gray squirrels were reported nearby by 87% of respondents.

1997 Survey

There were approximately 20-25 WCOs in each of the six regions in 1997. Some respondents returned more than one questionnaire if they were reporting results for more than one county. We received 78 questionnaires reporting the presence of fox squirrels and 60 reporting the absence of fox squirrels. We received no information from two counties (Sullivan and Cameron). Respondents from 38 (58%) of the remaining 65 counties reported the presence of fox squirrels (Fig. 2). Fifty-one (65%) of the positive respondents recorded fox squirrels from western counties, 23 (29%) from central counties, and 4 (5%) from eastern counties (Fig. 1). Specific locations of fox squirrel populations were provided by only some of the respondents, particularly those from central and eastern counties (Fig. 2).

Most respondents were unable to state which of the three subspecies of fox squirrel they observed. This appeared primarily to be caused by a lack of knowledge of the subspecific designations and not by a confusion with the appearance of the squirrels. Nevertheless, few trends were apparent from the question regarding the appearance of the fox squirrels (Table 2). White-, yellow-, and orange-bellied fox squirrels were reported in all three sections of the state.

Respondents from western counties generally reported abundant populations of fox squirrels of indeterminate number (Table 3). Seventeen of 27 (63%) respondents from central and eastern counties estimated less than five distinct populations of fox squirrels in their jurisdiction, numbering less than 10 individuals each (55% of respondents from central and eastern counties). Though the majority of respondents from central and eastern counties were unsure of the history of these fox squirrel populations, 12 of 27 (37%) reported that the populations had been present for less than 30 years. Populations of fox squirrels in all counties were considered stable or increasing over the last 15 years (91% of responses). Two of the four (50%) respondents from eastern counties considered the fox squirrel populations to be declining.

The majority of respondents listed fragmented landscapes with woodlots as the most common habitat characteristic of fox squirrels (76%, Table 4). Few (11%) respondents listed fox squirrels occurring in continuous

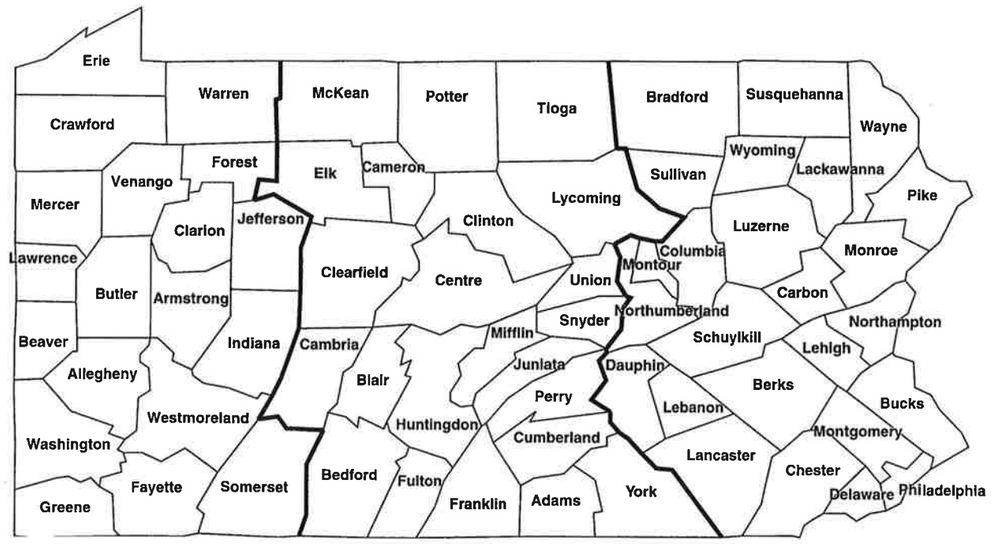

FIGURE 1. Dividing lines separating sections of Pennsylvania arbitrarily labeled "western," "central," and "eastern" for purposes of organizing questionnaire responses by region for a survey of the distribution of the fox squirrel (*Sciurus niger*) in 1997.

forest. Forest types reported were most commonly oak (*Quercus* spp.)-hickory (*Carya* spp.) (74%), with little understory vegetation (60%). Most squirrels were said to occur in rural areas (76%), though some were reported in urban areas (31%), primarily in western counties. Agricultural fields, both crops and pasture, were reported commonly adjacent to fox squirrel habitat (67% and 50%, respectively). Only 19-24% of respondents knew fox squirrels to inhabit riparian or floodplain areas. More than half (54%) of respondents considered hunting of fox squirrels to be common in their counties; whereas 27% considered hunting pressure to be minimal. Gray squirrels are known to occur in the same areas as fox squirrels according to 81% of respondents. Few of the habitat characteristic responses differed among the three regions.

The presence of fox squirrels was not reported in two of the counties for which squirrels were reported in the 1980 survey (Bradford and Lebanon); however, squirrels in Lebanon county had been recently introduced populations (Fig. 2). Respondents to the 1997 survey reported the presence of fox squirrels in two of the counties for which respondents in 1980 reported no fox squirrels (Cambria and Lycoming).

Museum Records

We obtained documentation of 100 specimens of fox squirrels from Pennsylvania, including 6 identified as *Sciurus niger cinereus*, 5 *S. n. vulpinus*, and 89 *S. n. rufiventer.* Collections dated from the mid-1800s to 1982. Delmarva fox squirrel specimens were from Chester, Dauphin, and Lancaster counties; eastern fox squirrel specimens were from Berks, Centre, Cumberland, and Franklin counties; and western fox squirrel specimens were from counties in all regions, with the largest number of specimens originating from western counties (Fig. 2).

DISCUSSION

The current distribution of the fox squirrel in Pennsylvania is more extensive than has been previously documented, but populations appear to be absent from southeastern and northeastern counties. The fox squirrel in the western half of the state is reportedly abundant and widespread, inhabiting a variety of habitats, including urban areas. In the central portion of the state, fox squirrels are most common in small, widely dispersed populations primarily in rural woodlots. Individual populations of fox squirrels are additionally located in a few eastern counties where they were not previously reported.

A low number of individuals in a fox squirrel population is not uncommon. Population densities of fox squirrels range from 0.05-5.0 squirrels per ha (Merritt

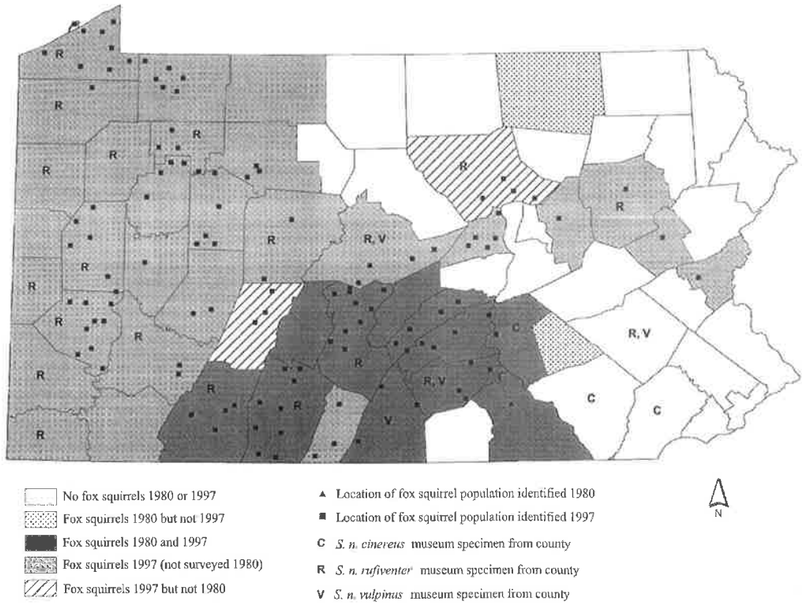

FIGURE 2. Pennsylvania counties in which questionnaire respondents reported the presence of fox squirrels (*Sciurus niger*) in 1980 or 1997, locations of fox squirrel populations identified by respondents, and counties from which museum specimens originated. No survey data were available for Cameron or Sullivan counties.

TABLE 1. Responses to questionnaire regarding the population demographics of fox squirrels from all respondents reporting fox squirrels in 1980 and 1997 and by region in 1997.

Question and Response	Number of Responses (Percent of Total)				
	1980 Survey	1997 Survey	Western Counties 1997	Central Counties 1997	Eastern Counties 1997
Number of respondents reporting fox squirrels[a]	15	78	51(65%)	23(30%)	4(5%)
Origin of populations:					
Relict populations	12(80%)	62 (79%)	40 (78%)	20 (87%)	2 (50%)
Introduced populations	3 (20%)	3 (4%)	2 (4%)	0 (0%)	1 (25%)
Unknown origin	0 (0%)	13 (15%)	9 (18%)	3 (13%)	1 (25%)
Present for how many years?					
0-15	N/A	12 (15%)	5 (10%)	6 (26%)	1 (25%)
16-30	N/A	5 (6%)	2 (4%)	2 (9%)	1 (25%)
31-50	N/A	1 (1%)	1 (2%)	0 (0%)	0 (0%)
>50	N/A	19 (24%)	17 (33%)	2 (9%)	0 (0%)
Unknown	N/A	41 (52%)	26 (51%)	13 (56%)	2 (50%)
How many populations?					
1	0(0%)	7 (9%)	3 (6%)	1 (4%)	3 (75%)
2-5	10 (67%)	21 (27%)	8 (16%)	12 (52%)	1 (25%)
6-10	1 (7%)	10 (13%)	6 (12%)	4 (17%)	0 (0%)
11-15	1 (7%)	4 (5%)	4 (8%)	0 (0%)	0 (0%)
Unknown or abundant	3 (19%)	36 (46%)	30 (59%)	6 (26%)	0 (0%)
#individuals per population?					
1-5	6 (40%)	15 (19%)	8 (16%)	4 (17%)	3 (75%)
6-10	4 (27%)	23 (29%)	15 (29%)	8 (35%)	0 (0%)
11-15	2 (13%)	4 (5%)	2 (4%)	2 (9%)	0 (0%)
16-20	0 (0%)	6 (8%)	2 (4%)	4 (17%)	0 (0%)
Unknown or abundant	3 (20%)	30 (39%)	24 (5%)	5 (22%)	1 (25%)
Status of populations:					
Stable over past 15 yr	6 (40%)	36 (36%)	27 (53%)	8 (35%)	1 (25%)
Increasing over past 15 yr	N/A	27 (35%)	14 (27%)	13 (56%)	0 (0%)
Declining over past 15 yr	4 (27%)	6 (8%)	4 (8%)	0 (0%)	2 (75%)
Unknown	5 (33%)	9 (11%)	6 (12%)	2 (9%)	1 (25%)

[a]Percents in this row are calculated as percent of all responses; in all other rows they are percent of column totals. All values were calculated using only the respondents who reported the presence of fox squirrels.

1987, Weigl et al. 1989). Nixon et al. (1984) suggested that low fox squirrel densities were a result of the largely asocial behavior of the species. For example, as compared with gray squirrels, fox squirrels maintain larger home ranges (Merritt 1987), are more cursorial (Dueser et al. 1988), more solitary (Koprowski 1996), and less tolerant of conspecifics (Brown and Batzli 1985b).

We were unable to determine the precise distribution of each subspecies of fox squirrel; however, *S. n. vulpinus* appears to be limited to roughly six counties in the south-central portion of the state. Squirrels fitting the description of both the western and the eastern subspecies have been observed together in populations in these counties (K. L. Derge and M. A. Steele, pers. obs.) Color variation in the eastern fox squirrel has been reported as early as 1944 (Poole 1944) and may have increased since the mid-1900s when western fox squirrels were introduced across the state (Doutt et al. 1977) The continued overlap of the

TABLE 2. Responses to questionnaire regarding characteristics of fox squirrel habitat from all respondents reporting in 1980 and 1997 and by region in 1997

	Number of Responses (Percent of Total)[a]				
Response	1980 Survey	1997 Survey	Western Counties 1997	Central Counties 1997	Eastern Counties 1997
Number of respondents reporting fox squirrels[b]	15	78	51(65%)	23(30%)	4(5%)
Heavily wooded	7(47%)	38(49%)	19(37%)	18(78%)	1(25%)
Sparsely wooded	6(40%)	28(36%)	23(45%)	3(13%)	2(50%)
Fragmented with woodlots	N/A	59(76%)	43(84%)	14(61%)	2(50%)
Continuous forest	N/A	9(11%)	6(12%)	2(9%)	1(25%)
Oak-hickory forest type	N/A	58(74%)	35(69%)	19(83%)	4(100%)
Northern hardwood forest	N/A	28(36%)	22(43%)	4(17%)	2(50%)
Hemlock/white pine forest	N/A	1(1%)	1(2%)	0(0%)	0(0%)
Little understory	6(40%)	47(60%)	33(65%)	11(48%)	3(75%)
Heavy understory	4(27%)	14(18%)	13(25%)	1(4%)	0(0%)
In an urban area	1(7%)	24(31%)	23(45%)	0(0%)	1(25%)
In a rural area	13(87%)	59(76%)	41(80%)	15(65%)	3(75%)
Cropfields nearby	13(87%)	52(67%)	35(69%)	15(65%)	2(50%)
Pasture or grass fields nearby	6(40%)	39(50%)	29(57%)	8(35%)	2(50%)
Riparian area	N/A	19(24%)	10(20%)	6(26%)	3(75%)
In a flood plain	N/A	15(19%)	9(18%)	4(17%)	2(50%)
Hunting of squirrels common	13(87%)	42(54%)	30(59%)	12(52%)	0(0%)
Little hunting pressure	1(7%)	21(27%)	12(23%)	5(22%)	4(100%)
Population of gray squirrels nearby	13(87%)	63(81%)	41(80%)	19(83%)	3(75%)

[a]More than one response was possible from each respondent; therefore, column total percents are greater than 100%.

[b]Percents in this row are calculated as percent of all responses; in all other rows they are percent of column totals. All values were calculated using only the respondents who reported the presence of fox squirrels.

range of the two subspecies and the range of coloration among the two suggest that a closer examination of the subspecies designation is in order. We specifically recommend that a genetic examination of eastern and western fox squirrels be conducted, comparing them to populations of each in West Virginia and Ohio, respectively, as well as to Delmarva fox squirrels in the east. Considerable genetic material for the Delmarva subspecies has already been collected (Moncrief 1998).

The Delmarva fox squirrel remains extirpated from Pennsylvania. Previous examinations of the status of the Delmarva fox squirrel have failed to provide evidence of the historic presence of the subspecies in the state beyond historic natural history notes fitting the description of the squirrel (Kirkland and Krim 1990). Poole (1944) referred to the existence of 10 specimens of the Delmarva fox squirrel from the state. We found that at least two of these specimens still exist in addition to four others not mentioned by Poole (1944); thus offering proof of the former presence of Delmarva fox squirrels in Pennsylvania.

Preferred habitats of fox squirrels in the Midwest are characterized by mature forest woodlots with an open understory, proximity to an agricultural or park-like edge, and proximity to a stream (Flyger and Lustig 1976, Flyger and Gates 1982, Drake and Brenner 1995). Fox squirrel ecology, however, can potentially vary greatly with region and subspecies (Weigl et al. 1998). Fox squirrels in Pennsylvania appear to inhabit fragmented landscapes with mature forest woodlots and agricultural fields nearby.

The preference of fox squirrels for mature forest stands has been attributed to their decline in some parts of the East (Taylor 1973, Dueser et al. 1988). For example, heavy logging in Pennsylvania in the late 19th and early 20th centuries devastated mature forests in the state. However, by the mid-1900's and continuing to the present, the amount of forest land bordering rural areas in Pennsylvania has been increasing, largely from reversion of agricultural lands to forest (Powell and Considine 1982). The continued growth and maturation of these forest stands, coupled with the introductions

conducted by the PGC, could help explain the increase in fox squirrel populations in the central and eastern portions of the state.

Gray squirrels appear to be sympatric with fox squirrels throughout their range in Pennsylvania. Investigations of interactions between the two species have found little evidence of direct interference interspecific competition (Brown and Batzli 1985a, Armitage and Harris 1982, but see Steele 1988), although the two species exploit nearly identical resources and occupy overlapping niches in many populations (Koprowski 1994, Edwards et al. 1998). Studies of habitat use by the two species in Pennsylvania have shown subtle differences in habitat use among sympatric populations (Drake and Brenner 1995, Derge 1997).

Hunting of squirrels is another factor that may affect populations of fox squirrels. Squirrels rank with rabbits as the most popular game species in Pennsylvania; the harvest of gray, red, and fox squirrels was estimated at 1.4 million in 1996 (Diefenbach 1997). Flyger and Gates (1982) cite hunting as a major influence on populations of fox squirrels. Survey respondents in central and eastern counties, where fox squirrel populations are small, considered hunting pressure on fox squirrels to be minimal. Fox squirrel populations in western Pennsylvania are probably large enough to sustain the annual harvest.

We conclude that over the last few decades, *S. niger* has increased in numbers in several areas of the state and has extended its range to now include counties not previously occupied by the species. We disagree with the Kirkland and Krim (1990) determination of rare status for both *S. n. rufiventer* and *S. n. vulpinus*. While additional field, museum, and genetic research is needed to further establish the status of the fox squirrel in eastern Pennsylvania, we found that current populations within the historic range of *S. n. vulpinus* and *S. n. cinereus* are disjunct, fragmented, and at risk of further decline. We suggest that *S. n. vulpinus* should be considered for state threatened status and that *S. n. rufiventer* be considered secure.

Field research is needed to determine if, in fact, *S. n. cinereus* currently exists in Pennsylvania and to confirm recent reports of Delmarva fox squirrels in northern Delaware before additional reintroductions are considered for this area (K. Giavenga, pers. comm.). We argue that reintroduction efforts be performed only in sites that are adjacent to, or connected to, areas currently occupied by the subspecies. Reintroductions to sites that are disjunct from existing populations may result in significant founder effect, a higher probability of local extinction, and virtually no possibility for dispersal to and from existing populations.

TABLE 3. Responses to questionnaire regarding appearance of fox squirrels from all respondents reporting fox squirrels by region in 1997.

	Number of Responses (Percent of Total)[a]			
Response	All Respondents	Western Counties	Central Counties	Eastern Counties
Number of respondents reporting fox squirrels[b]	78	51(65%)	23(30%)	4(5%)
Rust-colored overall	29(37%)	23(45%)	6(26%)	0(0%)
Back grizzled with gray	31(40%)	22(43%)	8(35%)	1(25%)
Steel gray-colored overall	4(5%)	2(4%)	2(9%)	0(0%)
Orange wash in tail	55(70%)	36(70%)	16(69%)	3(75%)
Orange wash on side	50(64%)	30(59%)	16(69%)	4(100%)
Orange belly	42(54%)	28(55%)	13(56%)	1(25%)
Yellow belly	17(22%)	13(25%)	3(13%)	1(25%)
White belly	10(13%)	6(12%)	2(9%)	2(50%)

[a]More than one response was possible from each respondent; therefore, column total percents are greater than 100%.

[b]Percents in this row are calculated as percent of all responses; in all other rows they are percent of column totals. All values were calculated using only the respondents who reported the presence of fox squirrels.

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THE SEASONAL AND SPATIAL DISTRIBUTION OF SELECTED ORTHOPTERA (ACRIDIDAE, TETRIGIDAE, AND TRIDACTYLIDAE) FROM SERPENTINE BARRENS IN SOUTHEASTERN PENNSYLVANIA[1]

JOSEPH K. SHELDON, CURTIS A. HARRINGTON, LANCE S. EDWARDS and PETER L. SWARTZENTRUBER

Messiah College,
Department of Natural Science,
Grantham, Pennsylvania 17027

ABSTRACT

A three-year survey was conducted from 1996 to 1998 on the spatial and seasonal distributions of three families of Orthoptera (Acrididae, Tetrigidae, and Tridactylidae) in Nottingham and Goat Hill Serpentine Barrens in Chester County, Pennsylvania. A total of twenty species of acridids representing three subfamilies were collected at these two sites, including five species of Gomphocerinae, six species of Oedipodinae, and nine species of Melanoplinae. *Syrbula admirabilis* and *Eritettix simplex* dominated the gomphocerines, while *Trachyrhachys kiowa* and *Dissosteira carolina* were the most abundant oedipodines. *Melanoplus femur-rubrum* outnumbered all other species of Melanoplinae except within the riparian area at Goat Hill where *Melanoplus gracilis* was the dominant acridid. *Melanoplus confusus* appears to be a rare species in Pennsylvania. It has only been reported from serpentine barren habitats (Goat Hill Barren and Pink Hill Barren) and one undefined site in western Pennsylvania. Five species of Tetrigidae and one species of Tridactylidae were also recorded during the study. A note on the Acrididae of Pink Hill Barren is included.
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INTRODUCTION

Serpentine ecosystems are globally widespread, but of small total area, and generally characterized by plant communities of unusual composition (Brooks 1987). Research has focused on the geology, soils, and ecosystem structure and function (Brooks 1987, Kruckeberg 1951, Miller 1977, Proctor and Woodell 1975, Turitzin 1978, and Whittaker 1954). Within the United States, serpentine ecosystems are largely restricted to two bands of ultramafic ("ma" for magnesium and "f" for iron) rocks stretching from California to British Columbia on the west and from Alabama to northern Quebec on the east (Brooks 1987). The ultramafic rock known as serpentinite forms at fracture zones beneath the ocean floor where hot sea water interacts with certain minerals (see Latham 1993 for additional discussion and references). The soils derived from serpentine rock tend to be infertile, dry, and contain high levels of toxic heavy metals. These combine to form what is referred to as the "serpentine factor" (Brooks 1987). According to Latham (1993), serpentine soils typically have very low levels of phosphorus, nitrogen, calcium, and potassium- minerals required by plants in greatest quantity. The soils are high in magnesium and iron (hence the name "ultramafic") and, in addition, they often contain high concentrations of nickel, chromium, and cobalt. The latter three metals are known to be toxic in high concentrations to many plants. Serpentine species must not only tolerate the toxic heavy metals, but they also must cope with the low levels of calcium and high magnesium concentrations. Latham suggests that tolerance of the serpentine barrens plant species to the

unusually high ratio of magnesium to calcium may be the determining factor that defines the floral community of the barrens (Latham 1993). The high concentrations of chromium resulted in commercial exploitation and degradation of the Pennsylvania and Maryland barrens during the first part of this century. In addition, the green-colored serpentine rock was highly valued as a building stone for construction in Philadelphia and Baltimore during the nineteenth and early twentieth century (Latham 1993). The scientific community today recognizes serpentine barrens as a unique, rare ecosystem that requires protection.

Serpentine soils support unusual and/or unique plant communities with many rare, endangered, and disjunct species. The bedrock surrounding the Pennsylvania and Maryland serpentine outcrops is typically schist or gneiss that supports a mesic, nearly closed canopy deciduous forest. In contrast, the typical serpentine barrens landscape is a prairie or savanna with scattered trees (Latham 1993). The vegetation that is present at a particular site will depend on the nature and intensity of the most recent disturbance (fires are frequent); the stage of successional development; availability of moisture; and the mineral content of the bedrock (Latham 1993). The serpentine barrens of Pennsylvania and Maryland are typified by a mosaic of vegetation types varying from areas of open grassland to closed canopy forests of *Pinus rigida* Mill., *Quercus stellata* Wang., and *Quercus marilandica* Muenchh. The latter has become more common due to fire suppression. The forest undergrowth is often a dense, nearly impenetrable tangle of *Smilax rotundifolia* L. and S. *glauca Walt.*

Although serpentine plant communities have been well studied, little research has been directed toward the animal populations (Brooks 1987). The few animal studies that have been conducted indicate that, at least in insects, an unusual composition of animals may also exist. Johnson, Keith, and Ehrlich (1968) report that some colonies of the butterfly *Euphydryas editha* Boisduval appear to be restricted to areas of serpentine soil. Shapiro (1981) found that in California the larvae of the pierid butterfly *Pieris sisymbrii* Boisduval feeds preferentially on the serpentine-soil mustard *Streptanthus breweri* Gray. Wild (1975) conducted an extensive study on the relationship of termites with serpentine soils in Zimbabwe.

The state line barrens form a string of seven sites along the Maryland and Pennsylvania border and constitute the largest area (approximately 850 ha) of serpentine vegetation in eastern North America (Latham 1993). Nottingham Barren lies within Nottingham County Park, Chester County, and is Pennsylvania's largest relatively undisturbed barren. The adjacent Goat Hill Barren (two km to the west) is slightly smaller. It is a state forest natural area and is managed in conjunction with The Nature Conservancy. Together they comprise approximately 400 ha (Latham 1993).

The eastern North American serpentine barrens "are ranked high in priority for biodiversity conservation because they are rich in rare species of both plants and animals, including several that are globally rare and in danger of extinction. The barrens rate high in importance also because they comprise one of the rarest and most unusual sets of natural communities in the eastern North American temperate forest region" (Latham, 1993, p. 61). Nottingham and Goat Hill Barrens have been extensively surveyed by The Nature Conservancy for rare plants and Lepidoptera. Sixteen species of plants listed as rare or threatened have been recorded from Goat Hill including the rare glade spurge and four listed plant species have been found within Nottingham Barren. The survey of the Lepidoptera has recorded several state rare species including the buck moth, *Hemileuca maia* (Drury). Some species present in the 1980's now appear to be absent or sub-viable. According to Dr. Dale F. Schweitzer of The Nature Conservancy (personal communication), *Erynnis martialis* (Scudder) has probably been eradicated by deer from both Goat Hill and Nottingham. Deer appear also to be responsible for the loss of *Erastria coloraria* (Fabricius) from Goat Hill and *Callophrys irus* (Godart) from Nottingham. The latter is now probably sub-viable in Goat Hill. Several other Lepidoptera species have also disappeared from the two barrens for unknown reasons. Nottingham Barren is also known as a birding hot spot and has been officially designated by Pennsylvania as an Important Bird Area.

This purpose of this study was to determine the faunal composition of the Orthoptera families Acrididae (short-horned grasshoppers), Tetrigidae (pygmy grasshoppers), and Tridactylidae (pygmy mole crickets) within these two serpentine barrens. We examined both the spatial and seasonal distribution of the species present and sought to determine whether any of the species represent disjunct populations and/or are rare, threatened, or endangered. It was hypothesized that Orthoptera species not characteristic of the eastern deciduous forest might be found in association with the unique plant community. It is hoped that the information provided by the study will buttress the conservation efforts for serpentine barrens with Pennsylvania and beyond.

MATERIAL AND METHODS

The study took place during (1996-1998) with fieldwork conducted from late April through late October. Sampling was done approximately every two weeks for four to six hours per day with one or two people collecting. Approximately equal amounts of time were spent at Goat Hill and Nottingham. Both upland and riparian areas were surveyed. The upland sites included prairie dominated by the grasses *Schizachyrium scoparius* (Michx.), *Andropogon gerardii* Vitman., and *Sporobolus heterolepis* A. Gray; savannah with *Juniperus virginiana* L. and *Pinus rigida* joining the prairie species; and oak-pine woodlands dominated by *Quercus stellata*, *Q. marilandica,* and *Pinus rigida* with an understory of *Smilax rotundifolia* and *S. glauca*. A wide variety of herbaceous species dominated the riparian stream edges along with trees including *Alnus serrulata* (Ait.) Willd., *Salix fragilis* L., and *S. nigra* Marsh. Sampling was conducted in the prairie, savanna, woodlands, and in the creekside riparian zone. Samples in Nottingham were taken near the dirt road that passes through the park. Goat Hill samples were from the vicinity of the power line that passes through the barren. Approximately equal time was spent in the riparian and upland sites. Upland collecting was primarily in the prairie and savanna communities because of the difficulty of coping with the dense greenbriar (*Smilax*) and the apparent lack of acridid, tridactylid, and tetrigid species within the woodland areas. Although both barrens are currently dominated by woodland (More than 60% in Nottingham according to an unpublished report), efforts (primarily controlled burning) are underway in Nottingham to reverse the trend of forest expansion. Aerial insect nets were used to capture the specimens. Collecting techniques consisted of slowly pacing throughout the area and capturing every individual observed. Most specimens were immediately placed into ethyl acetate killing jars, however a few specimens were kept alive in plastic bags for subsequent photography. Approximately 500 Acrididae, 60 Tetrigidae and 20 Tridactylidae were collected during the study.

Collected material was taken to Messiah College, Grantham Pa., for curation and identification. Species of the subfamilies Gomphocerinae and Oedipodinae were identified using the keys in Otte 1981, 1984. Helfer, 1953 was an aid in the identification of the Melanoplinae. Dr. Otte subsequently confirmed all identifications of Acrididae. The species of Tetrigidae were identified using keys in Rehn and Grant, 1961. Tridactylidae were identified by the description and keys in Blatchley 1920 and the descriptions in Arnett 1985.

Three categories were used to describe the abundance of the Acrididae. A=abundant: numerous (>10) individuals observed on most collecting trips during the peak seasonal activity period with no effort made to collect all individuals seen. C=common: only a few individuals observed per collecting trip during the species peak seasonal activity period with an effort made to collect all individuals that were observed. U=uncommon: fewer than 10 individuals observed/collected throughout the three-year study.

RESULTS

Acrididae: Observations were made on the spatial and seasonal distribution of the 20 species of Acrididae recorded from Nottingham and Goat Hill Barrens. Three subfamilies are represented among the species collected from the two sites (see Table 1). These include five species of Gomphocerinae (the slant-faced grasshoppers), six species of Oedipodinae (band-winged grasshoppers), and nine species of Melanoplinae (spur-throated grasshoppers).

The seasonal activity period of the Acrididae ranged from early spring to late fall. Six of the species were present as adults during spring (April–June). *Eritettix simplex* (Scudder), *Arphia sulphurea* (Fabricius) and *Chortophaga viridifasciata* (De Geer) were present as early as April. Two *Melanoplus* species, M. *confusus* (Scudder) and *M. sanguinipes* (Fabricius) were first collected in May. The latter persisted through October. Two species were collected only during mid-summer–*Chloealtis conspersa* (Harris) and *Orphulella speciosa* (Scudder). The remaining species, with one exception, were present from mid summer through fall. The exception was two specimens of *M. punctulatus* (Scudder) which were collected by Barb Barton of The Nature Conservancy at a bait station on November 7, 1996.

Most species preferred the upland habitat (See Table 2). Only *Melanoplus gracilis* (Bruner) was collected exclusively within the riparian zone at Goat Hill where its brilliant green color was cryptic against the background of the vegetation on which it rested. It was never encountered resting on the ground. Several other species were also encountered within the riparian zone, but in

TABLE 1. Seasonal distribution for Acrididae collected in the Goat Hill and Nottingham Serpentine Barrens.

TAXA	MONTH							
Gomphocerinae	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Eritettix simplex	X	X	X	X				
Chloealtis conspersa			X	X	X			
Orphulella speciosa				X	X			
Syrbula admirabilis				X	X	X	X	
Dichromorpha viridis					X	X	X	
Oedopedinae								
Arphia sulphurea	X	X	X					
Chortophaga viridifasciata	X	X		X				
Trachyrhachys kiowa				X	X	X	X	
Hippiscus ocelote				X	X	X	X	
Dissosteira carolina				X	X	X	X	
Encoptolophus sordidus					X	X	X	
Melanoplinae: *Melanoplus*								
Melanoplus confusus		X						
Melanoplus sanguinipes		X		X		X	X	
Melanoplus bivittatus				X	X	X		
Melanoplus keeleri				X	X		X	
Melanoplus femur-rubrum					X	X	X	
Melanoplus differentialis					X	X	X	
Melanoplus gracilis					X	X	X	
Melanoplus scudderi							X	
Melanoplus punctulatus								X

each case they also were recorded from the upland habitat where they were more abundant.

Three species could best be described as geophiles preferring areas of dry open ground. They were seldom if ever seen on vegetation and would fly readily when approached. *Arphia sulphurea* (Fabricius), *Dissosteira carolina* (Linnaeus), and *Trachyrhachys kiowa* (Thomas) all have background colors that are highly cryptic against the rock-littered serpentine soil.

Unlike the ground dwellers, several acridids appear to be associated with serpentine vegetation and typically were not encountered on the exposed soil surface. *Eritettix simplex* (Scudder) and *Syrbula admirabilis* (Uhler) were frequently found on the clumps of grass growing within the prairie and savanna areas of the barrens.

Dichromorpha viridis (Scudder) and *Chortophaga viridifasciata* (DeGeer) were present in two color forms. The green form appeared to show a distinct preference for resting high on green vegetation. It was not clear, however, whether the gray-brown forms were preferentially selective toward a darker substrate. The other species were either present in such low numbers that generalization regarding substrate preference could not be made, or no preference was observed other than the generalization that they tended to associate more with vegetation than with the xeric bare open areas.

Several species were abundant during their seasonally active period (See Table 2). As defined above, these were species that could be reliably caught in significant number on most any visit during their active season. It should be pointed out that abundance in grasshoppers shows wide annual variation. Some species may be extremely abundant one year and virtually absent the next. During our study, the most abundant species were *Eritettix simplex, Syrbula admirabilis, Arphia suphurea, Trachyrhachys kiowa, Hippiscus ocelote* (Saussure), and *Melanoplus femur-rubrum* (De Geer).

Species occurring in low numbers (less than ten individuals collected during the three-year period) included *Chloealtis conspersa* (Harris), *Orphulella speciosa* (Scudder), *Dichromorpha viridis*, *Melanoplus confusus* (Scudder), *M. sanguinipes* (Fabricius), *M. scudderi* (Uhler), and *M. punctulatus* (Scudder). These species were considered uncommon.

TABLE 2. Spatial distribution and abundance for Acrididae collected at Nottingham and Goat Hill Serpentine Barrens. A=abundant: numerous (>10) individuals observed during the species' peak seasonal activity period with no effort made to collect all individuals seen; C=common: only a few individuals observed during the species' peak seasonal activity period with all individuals observed collected; and U=uncommon: fewer than 10 individuals observed during the 3-year study.

TAXA	HABITAT PREFERENCE		
Gomphocerinae	**GH upland**	**N upland**	**GH riparian**
Eritettix simplex	A	A	U
Chloealtis conspersa	U	U	
Orphulella speciosa	U	U	
Syrbula admirabilis	A	A	U
Dichromorpha viridis	U	U	U
Oedopedinae			
Arphia sulphurea	A	A	
Chortophaga viridifasciata	C	C	
Trachyrhachys kiowa	A	A	U
Hippiscus ocelote	A	U	U
Dissosteira carolina	A	A	
Encoptolophus sordidus	U	C	U
Melanoplinae: *Melanoplus*			
Melanoplus confusus	U		
Melanoplus sanguinipes	U	U	
Melanoplus bivittatus	C	C	U
Melanoplus keeleri	C	U	
Melanoplus femur-rubrum	A	A	C
Melanoplus differentialis	U		U
Melanoplus gracilis			C
Melanoplus scudderi	U		
Melanoplus punctulatus	U		

Tetrigidae: Five species of Tetrigidae were identified from Nottingham and Goat Hill barrens. Their distribution was predominately in wet areas adjacent to streams, seeps, and temporary ponds following heavy rain where they rested on the wet soil. No specimens were observed on vegetation. The adult seasonal distribution (see Table 3) peaked in the spring and again in the fall with low densities recorded during the summer. None of the species were observed in large numbers. *Tetrix ornata* (Say) was observed only at Nottingham; the other four species were observed at both sites.

Tridactylidae: One species of tridactylid, *Ellipes minuta* (Scudder), was recorded from the barrens (Table 3). It was observed at Goat Hill in May 1998 where it was collected from the area of wet soil along the creek margin running under the power line and from adjacent wet seep areas. The species is quite small (4 to 5 mm) and hard to visually census. Specimens were collected by sweeping an aerial net over the mud and catching the previously unseen tridactylids as they flew.

TABLE 3. Seasonal distribution of Tetrigidae and Tridactylidae of Nottingham and Goat Hill Serpentine Barrens

TAXA	MONTH						
Tetrigidae	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Nomotettix cristatus (Scudder)	X	X			X		
Tetrix ornata (Say)		X				X	
Tetrix arenosa Burmeister	X	X				X	
Paratettix cuculatus (Burmeister)		X	X				
Tettigidea lateralis (Say)	X	X	X		X	X	
Tridactylidae							
Ellipes minuta (Scudder)		X					

Note on Pink Hill Barren species: The results of a previously unpublished study by Jodi G. Sheldon conducted on the Orthoptera of Pink Hill Serpentine Barren, Tyler Arboretum, Delaware County, PA in 1992 recorded five species of Acrididae. The species were *Arphia suphurea, Chloealtis conspersa, Chortophaga virdifaciata, Dissosteira carolina*, and *Melanoplus bivittatus.* Pink Hill is a relatively small, largely undisturbed barren with only a few acres of open grassland.

DISCUSSION

Our primary goal of the study was accomplished — to determine the species composition, adult seasonality (phenology), and habitat preference of the Acrididae, Tetrigidae, and Tridactylidae of Nottingham and Goat Hill Barrens. A sizable number of acridids (20 species), tetrigids (5 species) and tridactylids (1 species) was recorded over the three-year study period. Our results on the seasonal distribution concur with those reported by Otte 1981, 1984 for the Oedopedinae and Gomphocerinae species; by Blatchley 1920 for the Melanoplinae; by Rehn 1961 for the Tetrigidae; and by Blatchley 1920 for the Tridactylidae. Early spring species overwinter as late instar nymphs or adults. A single brood is typical for most species at the latitude of Pennsylvania. Readers wishing additional information on the details of the seasonal life history including the number of generations per year should consult the above references. The number of broods per year is unknown for some species.

Our second goal – to determine whether any of the species are represented by highly disjunct populations and whether any species are considered to be rare, threatened, or endangered – was not fully accomplished in this study. Grasshoppers are not currently monitored by The Nature Conservancy, the Pennsylvania Natural Diversity Inventory (PNDI), or the U.S. Fish and Wildlife Service because of the lack of general information regarding their status. Although The Academy of Natural Sciences of Philadelphia houses the largest Orthoptera collection in the world, no effort has been yet made to do a systematic survey of the Orthoptera of Pennsylvania. Dr. Dan Otte's three-volume work on the grasshoppers of North America is nearing completion with two of the three volumes already published. This work, however, is on a continental scale and does not adequately address local distribution and abundance. One cannot rely on museum specimens alone to understand current distributions. Museum collections tend to overemphasize areas near major institutions of taxonomic research and under represent remote areas. At best, the specimens may present an accurate description of the distribution of species when they were collected, but may not accurately depict the present range. For example, most of Pennsylvania's forests were logged during the 19th and early part of the 20th century creating a much more open habitat than exists today (Smith 1989). With the logging, the fires that often followed, and the subsequent farming activity, the resulting disturbed open habitat would have been conducive to the invasion and establishment of Orthoptera species more typical of the grasslands to the west. Thus, some species collected during this period may have been undergoing range expansions and would have served as reference material for the distribution maps in Dr. Otte's three-volume work on the grasshoppers of North America. The re-growth of Pennsylvania's forests to their current state which covers 58% of the landscape (Smith 1989) has significantly changed the nature of the landscape compared to that of 100 years ago leaving serpentine barrens, for example, as vestigial remnants of the more open landscape of the past. Thus, there is good reason to expect that species that experienced range expansion following the logging era may, because of the re-growth of Pennsylvania's forests, occupy reduced and/or disjunct ranges today. Relict disjunct populations may also be present in some of the northern Pennsylvania barrens and balds left behind following the retreat of the last continental glacier. We currently do not have sufficient knowledge to answer these questions which will require a fine grain analysis (county by county) of the museum specimens, their date of collection, plus extensive additional collecting to fill in the gaps.

Although many of the species encountered during this study appear to have wide distributions within the eastern United States, at least two species seem to be of regional interest. *Melanoplus confusus*, which occurs from Maine to North Carolina and west to British Columbia and Arizona (Blatchley 1920), appears to be rare within Pennsylvania. Although it is dense in the Great Plains, it is recorded in Pennsylvania only from Sumerset County in the west and Philadelphia (or perhaps Delaware County) in the east (Newton and Gurney 1956). The latter record is difficult to determine from the small map. In addition to these published records and our specimens from Goat Hill barren, two specimens of *M. confusus* are present in the collection at The Academy of Natural Sciences of Philadelphia from Pink Hill Serpentine Barren just west of Philadelphia. The distribution/abundance of *M. punctulatus* also is poorly known perhaps due to its inconspicuousness and its late season activity. Although it also has a wide range (Maine to Georgia and west to Minnesota and Texas (Blatchley 1920), it is seldom encountered. Blatchley (1920) reports that the species is predominately an autumn insect and may be found even after the heavy frosts of late October and November. It is more common in wooded areas than fields and often rests on the trunks and branches of trees where it is very difficult to see.

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INHIBITION OF TRANSCRIPTIONAL INITIATION AND ELONGATION OF THE BACTERIOPHAGE T_7 EARLY PROMOTER REGION BY OLIGONUCLEOTIDE TRIPLE HELIX FORMATION[1]

CELIA ROSS and STEVEN L. BROITMAN

Department of Biology
West Chester University
West Chester, PA 19383

ABSTRACT

We have targeted a naturally-occurring triplex-forming region on the Bacteriophage T_7 genome early promoter A_3 initiation site. By utilizing in vitro conditions that transcriptionally initiate at either the A_1 or A_3 start site, we investigate the ability of a synthetic triplex-forming oligonucleotide to inhibit either in vitro transcriptional initiation or elongation. Formation of triplex along this target is observed to significantly inhibit both initiation and elongation, with greater inhibition observed at the level of transcriptional initiation.
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INTRODUCTION

Since their discovery over 30 years ago (Rich and Davies, 1956; Felsenfeld et al., 1957), nucleic acid triplex structures have been a subject of considerable study. Many principals governing their structural geometry and thermodynamic stability have been established, and many studies have shed additional insight into the influence of factors such as sequence specificity, nearest-neighbor sequence, target size, ionic strength, etc. (for reviews see Cheng and Pettitt, 1992; Frank-Kamenetskii and Mirkin, 1995; Broitman, 1995).

Recently, attention has focused on a possible biological role of triplexes as potential inhibitors of gene transcription (for example see Helene, 1991). Initial experiments originally demonstrated the ability of synthetic polynucleotide triplexes to inhibit *in vitro* transcription from synthetic poly(A)•poly(T) duplex templates (Morgan and Wells, 1968). Later work demonstrated the potential for triplex inhibition of unique-sequence biological genes *in vitro* (Cooney et al., 1988), and *in vivo* (Postel et al., 1991; Grigoriev et al., 1993). Most strategies have targeted purine-rich duplex tracts along transcriptional control regions where triplex formation may interrupt transcriptional initiation. An obvious limitation of this approach is the availability of naturally occurring purine-rich target sequences specifically located at transcriptional initiation sites. However, if triplex structures formed along genomic structural regions are able to derail already initiated elongating transcription complexes, then the repertoire of potential genes subject to this regulation would be greatly increased. Previous studies have addressed this possibility; in some systems inhibition of transcriptional initiation but not elongation was reported (Maher, 1992), whereas in others (Young et al., 1991; Rando et al., 1994) inhibition of both initiation and elongation was observed.

To further address this possibility we have established a model system utilizing *in vitro* transcription from the bacteriophage T_7 early promoter. This region contains three transcriptional initiation sites, A_1, A_2, and A_3, each of which may be utilized during biological infection. Previously, we identified a purine-rich triplex binding target overlapping the A_3 initiation site, and reported transcriptional inhibition due to triplex formation along this region (Ross et al., 1992). In this investigation, we utilize specific dinucleotides to form stable *E. coli* RNA Polymerase transcription initiation complexes at either the A_1 or A_3 initiation site, followed by rifampicin treatment which inhibits subsequent transcriptional initiation but not elongation. With this system we have been able to investigate the effects of triplex formation on transcriptional initiation (by initiating at the A_3 position) versus elongation (by initiating at A_1).

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MATERIALS AND METHODS

Oligonucleotides

Triplex forming oligonucleotides (TFO's) containing modified nucleotides were synthesized on a Milligen Bio Search 8750 synthesizer utilizing phosphoramidite chemistry, followed by purification on a Waters PPRI reverse phase HPLC (provided by Apollon, Inc., Malvern PA). Purified oligo(C) used as the non-TFO) was obtained commercially from The Midland Reagent Company, Midland Texas.

Formation of Transcriptional Initiation Complexes

50 units of E.coli RNA Polymerase (Pharmacia) was incubated in 0.21 ml initiation buffer (0.15 M NaCl, 1.0 mM Spermine, 0.01 M $MgCl_2$, 0.1 mM EDTA, 0.12 mM DTT, 0.59 mg/ml BSA, 100 units RNasin, 179 µM ATP, 0.04 M Tris pH 7.5) with 50 µg T_7 genomic DNA template (purchased from Sigma), and 1.79 mM dinucleotide (either A_pC for initiation at A_3, or C_pC for initiation at A_1) for 10 minutes at 25°C. After 10 minutes, 5 µg Rifampicin was added, mixtures were allowed to sit for an additional 5 minutes at 25°C, and then placed on ice.

RNA Transcription

Reactions were started by transferring initiation complexes to a 25°C water bath, and then adding the remaining NTP's. Final transcription mixes contained 50 units of *E.coli* RNA Polymerase, 200 µg/ml T_7 Bacteriophage DNA, 0.01 M $MgCl_2$, 0.15 M NaCl, 1.0 mM Spermine, 0.1 mM DTT, 0.5 mg/ml BSA, 0.1 mM EDTA, 150 µM of each rNTP, 1.5 mM dinucleotide, 0.5 - 1.0 µCi[14C]UTP, 100 units RNasin, 5 µg rifampicin, 0.04 M Tris pH 7.5, and deionized H_2O to bring final reaction volumes to 0.25 ml. At indicated time points triplicate 10 µl reaction aliquots were removed, added to 0.05 ml cold salmon sperm DNA (500 µg/ml in 20 mM EDTA), and mixed with ice cold 10% TCA. Radiolabeled RNA precipitates were recovered on Whatman GF/C filter discs, washed repeatedly with cold 10% TCA and 95% ethanol, and counted in a Beckman LS-5801 liquid scintillation counter. Reactions were quantitated on the basis of total nMoles UTP incorporated into RNA by using the average of triplicate points.

Annealing Procedures

In order to promote optimum equilibrium triplex formation template DNA was pre-annealed with each oligonucleotide for 24 hours at room temperature (in 1 mM Spermine, 0.15 M NaCl, 0.01 mM $MgCl_2$, 0.1 mM EDTA, 0.04 M Tris pH 7.2). DNA templates not interacted with oligonucleotide were also subjected to this procedure.

RESULTS AND DISCUSSION

Figure 1a shows the nucleotide sequence of the T_7 early promoter region. Transcriptional initiation sites A_1, A_2, and A_3 are circled, the triplex target region is shown

```
1   TCTCACAGTG  TACGGACCTA  AAGTTCCCCC  ATAGGGGGTA  CCTAAAGCCC  AGCCAATCAC
61  CTAAAGTCAA  CCTTCGGTTG  ACCTTGAGGG  TTCCCTAAGG  GTTGGGGATG  ACCCTTGGGT
121 TTGTCTTTGG  GTGTTACCTT  GAGTGTCTCT  CTGTGTCCCT  ATCTGTTACA  GTCTCCTAAA
181 GTATCCTCCT  AAAGTCACCT  CCTAACGTCC  ATCCTAAAGC  CAACACCTAA  AGCCTACACC
241 TAAAGACCCA  TCAAGTCAAC  GCCTATCTTA  AAGTTTAAAC  ATAAAGACCA  GACCTAAAGA
301 CCAGACCTAA  AGACACTACA  TAAAGACCAG  ACCTAAAGAC  GCCTTGTTGT  TAGCCATAAA
361 GTGATAACCT  TTAATCATTG  TCTTTATTAA  TACAACTCAC  TATAAGGAGA  GACAACTTAA
421 AGAGACTTAA  AAGATTAATT  TAAAATTTAT  CAAAAAGAGT  ATTGACTTAA  AGTCTAACCT
481 ATAGGATACT  TACAGCCATC  GAGAGGGACA  CGGCGAATAG  CCATCCCAAT  CGACACCGGG
541 GTCAACCGGA  TAAGTAGACA  GCCTGATAAG  TCGCACGAAA  AACAGGTATT  GACAACATGA
601 AGTAACATGC  AGTAAGATAC  AAATCGCTAG  GTAACACTAG  CAGCGTCAAC  CGGGCGCACA
661 GTGCCTTCTA  GGTGACTTAA  GCGCACCACG  GCACATAAGG  TGAAACAAAA  CGGTTGACAA
721 CATGAAGTAA  ACACGGTACG  ATGTACCACG  TGAAACGACA  GTGAGTCACC  ACACTGAAAG
781 GTGATGCGGT  CTAACGAAAC  CTGACCTAAG  ACGCTCTTTA  ACAATCTGGT  AAATAGCTCT
841 TGAGTGCATG  ACTAGCGGAT  AACTCAAGGG  TATCGCAAGG  TGCCCTTTAT  GATATTCACT
901 AATAACTGCA  CGAGGTAACA  CAAGATGGCT  ATGTCTAACA  TGACTTACAA  CAACGTTTTC
```

FIGURE 1a. Nucleotide sequence of the T_7 early promoter region. Asterisks (*) indicate nucleotide contacts essential for *E.coli* RNA Polymerase initiation at the A_3 start site. Note that the T_7 early promoter contains three possible transcriptional initiation sites (circled): A_1, A_2, and A_3. The purine-rich triplex binding target is shown in the enclosed box, and the ATG translation signal for the first codon is underlined.

in a box, and the positions with an asterisk depict nucleotides determined to be essential RNA Polymerase contact points for A_3 transcriptional initiation (Siebenlist and Gilbert, 1980). Figure 1b shows the 17 nucleotide TFO previously used to inhibit A_3 transcriptional initiation (Ross et al., 1992) positioned along its triplex target. Note that this triplex forming strategy utilizes 5-Methyl C and 5-Bromo U opposite G and A target residues respectively, configured in the "all pyrimidine parallel orientation" triplex forming motif (Han and Dervin, 1994). These residues have been found to increase triplex stability and extend the pH range for CGC triplet formation to neutrality (Maher et al., 1992; Posvic and Dervin, 1989; Lee et al., 1984). In this system 5 MeC was selected for positioning opposite both C and T pyrimidine target interruptions, and oligo(C) was utilized as the control non-TFO because of the impossibility of it forming triplex along this AT-rich target sequence and at neutral pH (Thiele and Guschlbauer, 1971; Letai, et al., 1988; Yoon, et al., 1992).

Figure 2 demonstrates the effectiveness of the experimental system in eliciting site-specific transcriptional initiation off of naked T_7 templates at either the A_1 or A_3 start site. Initiation complexes were formed at the A_1 or A_3 location through the addition of ATP plus either C_pC or A_pC, respectively. After Rifampicin treatment, elongation of already formed initiation complex was activated by the addition of the remaining NTP's (see methods). As shown in Figure 2, Rifampicin completely inhibits all transcriptional activity if dinucleotide is excluded from initial incubation mixtures. This confirms the requirement for dinucleotide to make pre-formed initiation complex, as well as the complete inhibition of subsequent transcriptional initiation in the presence of Rifampicin. Figure 2 also confirms that addition of either C_pC or A_pC to initial mixtures allows formation of initiation complex which can be subsequently elongated in the presence of Rifampicin. Previous investigations analyzing transcript lengths under similar conditions (Dausse et al., 1976; Minkley and Pribnow, 1973) confirm that C_pC and A_pC form initiation complexes specifically at the A_1 and A_3 start site, respectively. As previously observed (Dausse et al., 1976), A_3 functions as the more efficient transcriptional initiator under these conditions.

Figure 3 shows transcriptional activity in the presence and absence of TFO. Panel (a) represents elongation of transcription complexes initiated along the A_3 start site, whereas panel (b) shows transcription initiated only from A_1. Because the triplex binding target overlaps nucleotides essential for A_3 initiation, panel (a) represents triplex inhibition of transcriptional *initation*. Similarly, because the A_1 transcriptional initiation site is positioned upstream from the triplex target (see figure 1), panel (b) represents triplex inhibition of transcriptional *elongation* of pre-formed initiation complexes. As shown, transcriptional initiation (a), and elongation (b) are both inhibited at all time points in the presence of TFO, relative to transcription in the presence of non-TFO, and versus naked T_7 templates.

In this system greater inhibition of transcriptional initiation is exhibited: ~80% inhibition of transcriptional initiation (incorporation of signal relative to naked DNA at time = 20 minutes) is observed, versus ~ 60% reduction of signal due to inhibition of elongation. We note that incorporated signal from Figure 3b presumably includes truncated transcripts that have been elongationally

```
690  C·G
     C·G
     G·C
     T·A
     G·C
     T·A
     A·T
     T·A bU
     T·A bU
     C·G mC
     C·G mC
     A·T mC
     C·G mC
     T·A bU
     T·A bU
     T·A bU
     G·C mC
     T·A bU
     T·A bU
    *T·A bU
    *T·A bU
    *G·C mC
    *C·G mC
    *C·G  C
    *A·T
     A·T
     C·G
     T·A
     G·C
     T·A
720  T·A
```

FIGURE 1b. Sequence of the triplex-forming oligonucleotide designed to bind along the purine-rich T_7 triplex target region 697-713. bU = 5BrU and mC = 5 MeC.

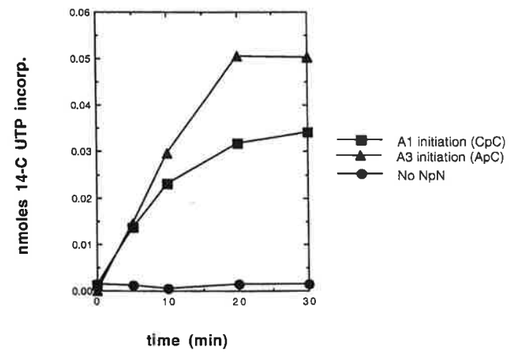

FIGURE 2. Site-specific transcription from the Bacteriophage T_7 early promoter region. Enzyme and genomic DNA were pre-incubated with dinucleotides to form stable transcriptional initiation complexes at initiation site A_1 (squares) or A_3 (triangles). Rifampicin is added to prevent additional initiation of transcription, but elongation of pre-formed initiation complexes can still proceed. Data represent the average of triplicate samples taken at each time point.

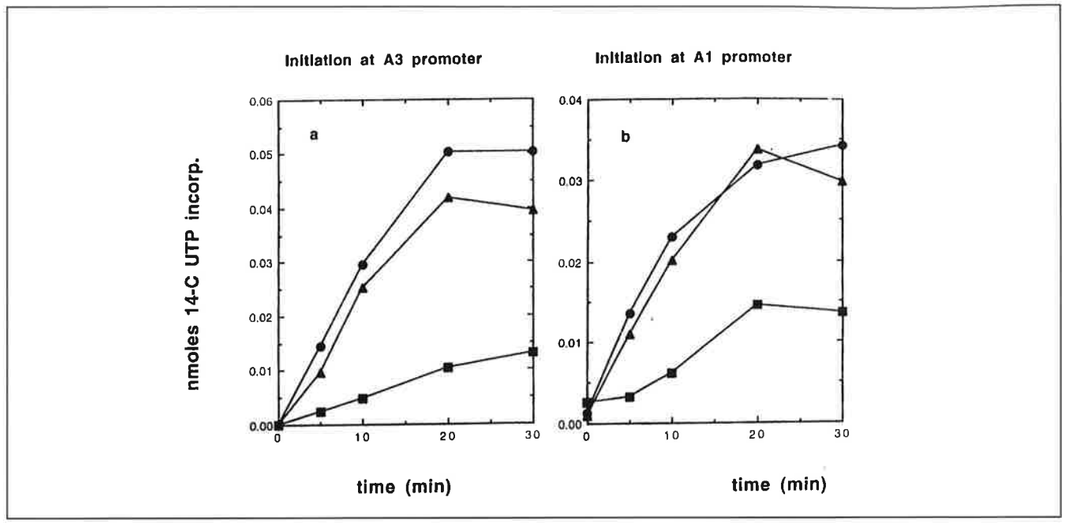

FIGURE 3. Site-specific transcription from the Bacteriophage T_7 early promoter region in the presence of 16 μM triplex-forming oligonucleotide (squares), non-triplex-forming oligo (C14) (triangles), or naked DNA (circles). Each data point represents the average of triplicate samples taken at each time. Average variation from the mean values were 2.3% and 3.7% for (a) and (b) respectively.

(a): Dinucleotide A_pC was added to the pre-incubation mixtures to form initiation complex at the A_3 initiation site to investigate triplex inhibition of transcriptional *initiation.*

(b): Dinucleotide C_pC was added to the pre-incubation mixture to form initiation complex at the A_1 initiation site to investigate triplex inhibition of transcriptional *elongation.*

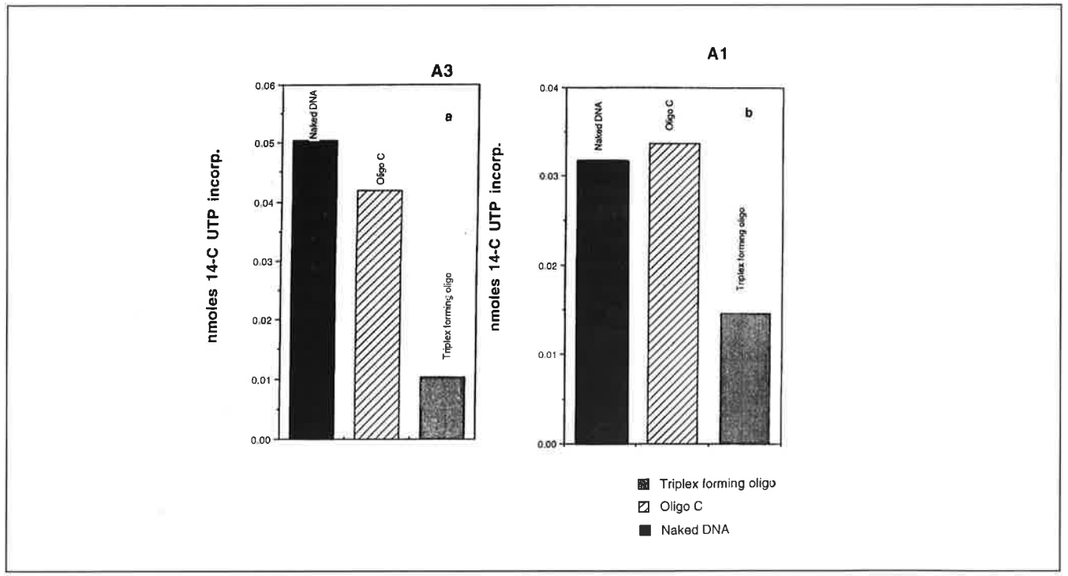

FIGURE 4. Triplex inhibition of *initiation* of transcription from the Bacteriophage T_7 A_3 promoter site (a), versus inhibition of transcriptional *elongation* from the A_1 site (b). Inhibition was calculated by signal incorporated relative to naked DNA (at time = 20 min) from Figure 3.

aborted, as well as longer transcripts formed by transcriptional read-through past the triplex binding region. Thus, it is not possible from these experiments to determine the extent to which elongation was inhibited due to pausing of the transcription complex at the triplex site, followed by later dislocation of triplex and transcriptional read-through, or alternatively from the complete dissociation of elongating transcription complex upon encountering triplex. We note that these results (summarized in Figure 4) are observed at a TFO concentration of 16 μM, which corresponds to a molar ratio of TFO/template of 4000/1, corresponding to a ratio of 1.7/1 on a nucleotide basis.

These results are consistent with some previous findings (Young, et al., 1991; Rando, et al., 1994) which indicate that transcriptional initiation and elongation can both be inhibited by triplex formation; thereby suggesting that genomic targets for triplex inhibition of transcription do not have to be specifically limited to transcriptional control regions. This should significantly expand the potential to locate suitable purine-rich triplex targets that can be utilized to inhibit expression of various genes at the transcriptional level.

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SYNTHESIS AND CHARACTERIZATION OF DINITRONITROSYLBIS (TRIPHENYLPHOSPHINE) RHODIUM, $Rh(NO)(NO_2)_2(PPh_3)_2$[1]

EDWARD RAJASEELAN[2], JEANETTE CRAYMER[2] and ROBERT D. FELTHAM[3]

[2]*Department of Chemistry*
Millersville University
Millersville, PA 17551

[3]*Department of Chemistry*
University of Arizona
Tucson, AZ 85721

ABSTRACT

The missing dinitrite analog $Rh(NO)(NO_2)_2(PPh_3)_2$ of the series $Rh(NO)X_2(PPh_3)_2$ has been synthesized and characterized. This is the first direct derivative of $Rh(NO)Cl_2(PPh_3)_2$. The IR spectrum of $Rh(NO)(NO_2)_2(PPh_3)_2$ shows the presence of both nitro and nitrito groups as does the ^{15}N NMR spectrum of the complex. The ^{31}P NMR spectrum shows the phosphines are magnetically equivalent and trans to each other. The compound crystallizes in a cell approximately isomorphous to $Rh(NO)Cl_2(PPh_3)_2$. Due to severe disorder, the structure was not successfully refined. The partial x-ray diffraction information obtained shows the molecular geometry is that of a distorted square pyramid with a bent nitrosyl in the apical position.

[J PA Acad Sci 73(2):63-66, 1999]

INTRODUCTION

The NO_2 group is one of the more strongly bound and common ligands for transition metals. Over one hundred nitro complexes, mostly of chromium, cobalt, nickel, copper, zinc, and platinum have been prepared and characterized (Hitchman and Rowbottom 1982). Several nitrosylnitro complexes of cobalt and iron have also been reported (Vallenilla 1985, Ileperuma and Feltham 1977).

One of the novel reactions that the NO_2 ligand undergoes is oxygen atom transfer to other ligands including alkene, carbon monoxide, nitric oxide, and phosphine ligands (Andrews and Kelley 1981, Feltham and Kriege 1979, Kreige-Simondsen et al. 1982, Ugo et al. 1976, Ileperum and Feltham 1976, Tovrog et al. 1979). These oxygen atom transfer reactions result in oxidation of the other ligand and formation of metal nitrosyl complexes [M—N—O] (Andrews and Kelley 1981, Feltham and Kriege 1979, Kreige-Simondsen et al. 1982, Ugo et al. 1976, Ileperum and Feltham 1976, Tovrog et al. 1979). Subsequently, dioxygen can oxidize the resulting nitrosyl ligand back to a nitro ligand (Andrews and Kelley 1981, Feltham and Kriege 1979, Kreige-Simondsen et al. 1982, Ugo et al. 1976, Ileperum and Feltham 1976, Tovrog et al. 1979, Clark et al. 1972). Thus, some nitrogen oxide complexes catalyze the oxidation of various substrates by dioxygen via this series of reactions. Such catalysis has attracted investigation due to the importance of the oxidation of alkenes to epoxides and of alcohols to ketones in industry (Dumas and Bulani 1974).

The discovery of the remarkable catalytic properties of $RhCl(PPh_3)_3$ by Wilkinson has stimulated numerous studies of the chemistry of the lower oxidation state rhodium complexes (Jardine et al. 1965, Osborn et al. 1966). Many analogues of $Rh(NO)Cl_2(PPh_3)_2$ have been prepared by replacing PPh_3 with other Group V ligands, and chloride by other anions (Critchlow and Robinson 1976). The synthesis and characterization of the NO_2 analogue $Rh(NO)(NO_2)_2(PPh_3)_2$ is described below.

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MATERIALS AND METHODS

The reaction of $Rh(NO)Cl_2(PPh_3)_2$ with $NaNO_2$ was carried out under an atmosphere of purified nitrogen using Schlenk techniques (Jolly 1960, Shriver 1969). 99.5% labeled $Na^{15}NO_2$ was purchased from MSD Isotopes, Canada. The elemental analyses were carried out by Atlantic Microlab, Inc., Atlanta, Georgia, and Hoffman Laboratories, Inc., Golden, Colorado. Methanol was freshly distilled from magnesium methoxide. The starting material $Rh(NO)Cl_2(PPh_3)_2$ was synthesized by previously published procedures (Ahmad et al. 1974, Robinson and Uttley 1971). The infrared spectra were obtained by using a Perkin-Elmer Model 983 infrared spectrometer. The ^{31}P and ^{15}N NMR spectra were obtained at -65°C using a Bruker AM 250 spectrometer.

Preparation of $Rh(NO)(NO_2)_2(PPh_3)_2$

$Rh(NO)Cl_2(PPh_3)_2$, 0.36 g (0.50 mmol) was mixed with 0.14 g (2.0 mmol) sodium nitrite in 5.0 mL of methanol. The reaction mixture was stirred for five days and the light brown $Rh(NO)Cl_2(PPh_3)_2$ turned to a bright green color. This bright green solid product was filtered and washed twice with small portions of acetone and was then vacuum dried. The extraction of this green solid with dichloromethane yielded a bright green solution. The bright green solution was loaded onto a column of neutral alumina and eluted with dichloromethane under nitrogen. The eluted bright green solution was evaporated to a volume of about 3.0 mL under vacuum, and about 10.0 mL of petroleum ether was added. The bright green precipitate was removed by filtration and dried under vacuum (0.21g, 56% yield). The compound was recrystallized from dichloromethane/petroleum ether using vapor transfer techniques (Friedman and Ford 1987). Dark bright green crystals were obtained after 3 days. (Found: C, 57.63; H, 4.01; N, 5.47; O, 10.13%. $RhC_{36}H_{30}N_3O_5P_2$ requires C, 57.69; H, 4.03; N, 5.61; O, 10.67% $\delta^{31}P$ (250 Mhz; solvent 10% $CDCl_3/CH_2Cl_2$; standard 85% H_3PO_4) 24.63 [2 P, d, J(RhP) 122.96 Hz, 2 PPh_3].

Preparation of $Rh(NO)(^{15}NO_2)_2(PPh_3)_2$

The preparation was carried out as described above using 0.36 g (0.50 mmol) of $Rh(NO)Cl_2(PPh_3)_2$ and 0.075 g (2.0 mmol) $Na^{15}NO_2$ in 5.0 mL methanol; (0.18 g, 48% yield). $\delta^{15}N$ (250 Mhz; solvent 10% $CDCl_3/CH_2Cl_2$; standard nitromethane) 82.77 [1N, d, J(RhN) 26.02 Hz, $^{15}NO_2$] and 214.47 [1N, s, $O^{15}NO$].

Collection of X-ray Diffraction Data for $Rh(NO)(NO_2)_2(PPh_3)_2$

A green crystal of $Rh(NO)(NO_2)_2(P(C_6H_5)_3)_2$ having approximate dimensions 0.18 x 0.25 x 0.30 mm was mounted on a glass fiber in a random orientation. Preliminary examination and data collection were performed with Mo Kα radiation (λ = 0.71073 Å) on a Syntex $P2_1$ difractometer.

Cell constants and an orientation matrix for data collection were obtained from least-squares refinement, using the setting angles of 25 reflection in the range 20°< 20, 30°. The monoclinic cell parameters and calculated volume are: a = 23.143(1), b = 9.819(1), c = 16.257(1) Å, ß = 115.18(1)°, V = 3343.3 $Å^3$.For Z = 4 and F.W. = 749.51, the calculated density is 1.49 g/cm^3 (d observed = 1.48 g/cm^3). As a check on crystal quality, omega scans of several intense reflections were measured; the width at half-height was 0.25°, indicating good crystal quality. The systematic absence of: h01, 1 = 2n + 1; indicates the space group Cc (#9) or C2/c (#15).

The data were collected at a temperature of 23 ± 1°, using the θ-2θ scan technique. The scan rate varied from 2 to 8°/min. Data were collected to a maximum 2θ of 50.0°.

RESULTS AND DISCUSSION

The title compound was prepared from the reaction of $[Rh(NO)Cl_2(PPh_3)_2]$ with sodium nitrite in methanol. The IR spectrum of $[Rh(NO)(NO_2)_2(PPh_3)_2]$ (Table 1) indicates the presence of two different sets of NO_2 frequencies in the complex (Nakamoto et al. 1958, Thewalt and Marsh 1970, Goodgame and Hitchman 1965, Strouse and Swanson 1971, El-Sayed and Ragsdale 1967). One set of frequencies corresponds to an N-bound nitro ligand while the other set corresponds to an O-bound nitrito ligand. $^{15}NO_2$ labeling produced the shifts in the IR bands expected for N-bound and O-bound ligands (Nakamoto 1986). The absorption band for the nitrito bending mode was obscured by a very strong absorption band of phosphine ligand.

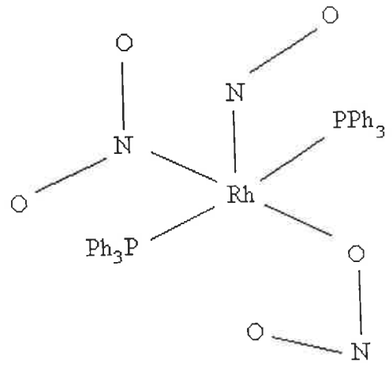

FIGURE 1

The ^{31}P NMR spectrum at of the complex (at –65°C) consists of a doublet at 24.63 ppm relative to 85% H_3PO_4 with a J(RhP) coupling of 122.96 Hz. The presence of only one doublet shows that the phosphine ligands have trans geometry.

The ^{15}N NMR spectrum was measured for $[Rh(NO)(^{15}NO_2)_2(PPh_3)_2]$ (99% ^{15}N enriched) at -65°C in 10% $CDCL_3/CH_2Cl_2$. A sharp singlet at 214.47 ppm and a doublet with a coupling constant $^1J(RhN)$ of 26.02 Hz at 82.77 ppm relative to nitromethane were observed. $Cr(acac)_3$ was added to promote relaxation, but not in sufficient amount to require a susceptibility correction. With 500 scans, a spectrum with excellent signal to noise ratio was obtained. No coupling to the ^{31}P nuclei was observed, nor was the $^2J(Rh^{15}N)$ coupling. The presence of a singlet at 214.47 ppm and a doublet at 82.77 ppm confirmed the presence of the N-bound nitro group and O-bound nitrito group in the complex in good agreement with the infrared data (Mason 1983).

The compound was crystallized in a cell isomorphous to $[Rh(NO)Cl_2(PPh_3)_2]$ (Goldberg et al. 1975). The space group was ambiguous and is either the acentric Cc (#9) or centric C_2/c (#15). In both space groups Z = 4. In the centric spage group a 2-fold axis is imposed upon the Rh-N vector of the NO group. This requires a disordered oxygen position and results in chemically unreasonable distances and angles of the Rh-N-O (nitrosyl) unit. Modeling this with a disordered rigid NO group causes the loss of information in the resulting structure. Another consequence of the 2-fold axis is that the two NO_2 groups must be equivalent. Since IR and ^{15}N NMR spectroscopic evidence indicated different binding modes of this ligand, it must be disordered as well. None of the models of the NO_2 groups which were investigated, refined to chemically reasonable values. Since the nature of these groups is the question of interest, a refinement using rigid group techniques was not attempted. The refinement in the acentric group doesn't require 2-fold symmetry. The nitrosyl group refined to more reasonable values with a Rh-N-O angle of 128(2)° (the Rh-N-O angle in $Rh(NO)Cl_2(PPh_3)_2$ is 124.8°) (Goodgame and Hitchman 1965), unfortunately, the NO_2 groups did not refine in this space group.

The methylene chloride solution of the complex was oxidized to the yellow $Rh(NO_3)_3(PPh_3)_2$ complex, by a slow flow of oxygen. This same complex has been reported earlier (Crithlow and Robinson 1976, Dowerah and Singh 1977) and was obtained by the action of 16 M nitric acid, on benzene solutions of $Rh(H)(CO)(PPh_3)_3$ or $Rh(NO)(NO_3)_2(PPh_3)_2$.

TABLE I. Infrared frequencies (cm^{-1})[a] of $Rh(NO)(NO_2)_2(PPh_3)_2$

	Rh-NO_2			Rh-ONO			RhNO
Compound	νN-O asymm	νN-O symm	δNO_2	νN-O asymm	νN-O symm	δONO	νNO
$Rh(NO)(NO_2)_2(PPh_3)_2$	1401 s	1313 s	818 m	1408 s	1123 m	–	1658 s
$Rh(NO)(^{15}NO_2)_2(PPh_3)_2$	1382 s	1289 s	811 m	1376 s	–	–	1658 s

[a]KBr disc s = strong, m = medium

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GROUND LEVEL AMBIENT OZONE IN ALLEGHENY COUNTY, PA[1]

GEORGE W. LENEY

(formerly Allegheny County Health Department)
5335 Tomfran Drive
Pittsburgh, PA 15236

ABSTRACT

Allegheny County and southwestern Pennsylvania are a borderline case for meeting the currently applicable, 1-hr average ozone air quality standard. Violations occur during episodes that are exceptional events when ozone may build up for periods as long as eight days before reaching exceedance levels. In hot summers the ozone season with hourly maximums over 80 ppb may begin in early May. Tracing of mass trajectories shows that different exceedances can have their origins in entirely different areas, with completely different populations of sources contributing. Normal changes in the daily routine of businesses and individuals cause ozone concentrations that are higher on weekends and holidays than during the week, and may have a larger impact on ozone than regulatory measures that can be implemented to control emissions. There is an urban/valley regime where ozone is scavenged and nitrogen oxides accumulate at night while residual ozone, remains in rural/upland areas. Monitor data shows that most ozone in the Pittsburgh urban area is limited by the amount of available nitrogen oxides during the day, and that there is progressive aging of these oxides during an episode Measurements of VOC (volatile organic compounds) and observed nitrogen oxides shows that high ozone concentrations do not fit typical ozone isopleths predicted by computer modeling and may be limited by the available energy from high temperature and sunlight.

Episodes of high ozone in Allegheny County are best reduced by event controls or strategies that obtain large reductions of emissions for a short period before or during the episode. Efforts of regulatory agencies should be focused on better forecasting of ozone episodes and development of predictors to improve the reliability of forecasts.
[J PA Acad Sci 73(2):67-74, 1999]

INTRODUCTION

Allegheny County has been a borderline case in meeting the currently applicable 1-hr ozone standard since the early 1980's. During normal summers it monitors attainment, but in hot summers, such as 1987, 1988, 1995 and 1999, there are numerous exceedances of the standard which create violations. After recording violations in 1987, the County began studying its monitor data to understand the causes of high ozone, and characteristics that are unique to the area. The 1990 Clean Air Act resulted in the creation of a seven county non-attainment area in southwestern Pennsylvania and the ozone program is now administered by the Pennsylvania Department of Environment Resources. The area met the 1-hr ozone standard in 1996-98, but had violations in 1999. A new three year 8-hr standard established by the U.S. EPA has been remanded by the Courts and has not been implemented. The current attainment status of southwestern Pennsylvania is not defined.

MONITORING NETWORK

Figure I shows the Allegheny County Health Department ozone monitoring network. There are four ozone monitors aligned roughly SW-NE in the direction of prevailing winds. The upwind monitor is at South Fayette. Lawrenceville monitor is immediately downwind of the city of Pittsburgh central business district. Penn Hills is an intermediate downwind monitor, and Harrison Twp./Brackenridge monitor is the downwind

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site. There are nitrogen oxide monitors at Lawrenceville and Flag Plaza in downtown Pittsburgh, and the Department operates additional No_x monitors at South Fayette and Harrison Twp. There are carbon monoxide monitors at the Point and Downtown in Pittsburgh.

ANNUAL OZONE CYCLE

Allegheny County operates its Harrison Twp./Brackenridge ozone monitor on a year round basis for background information. Figure II shows the daily 1-hr maximum ozone at Brackenridge in 1988. The summer was unusually hot, and the period with maximums over 80 ppb (parts per billion) was from May 8 – August 28. There were thirteen days with ozone over the allowable maximum of 124 ppb. Figure II also shows "100 sin h," where h is the vertical angle of the sun at noon. It is a measure of the incident solar radiation which peaks on June 21, about 3-4 weeks before the height of the ozone

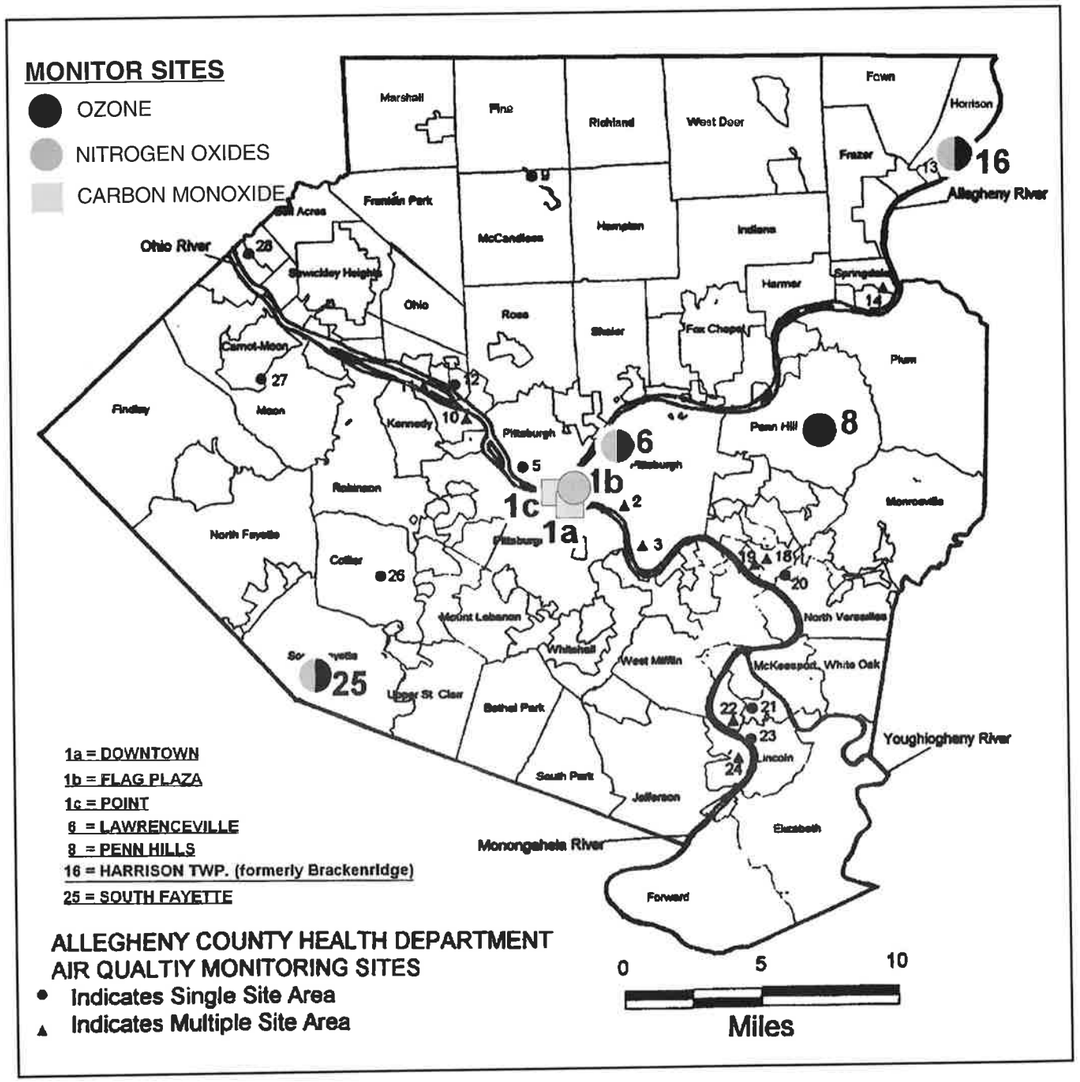

FIGURE I. Monitoring Sites for Ozone and Precursors.

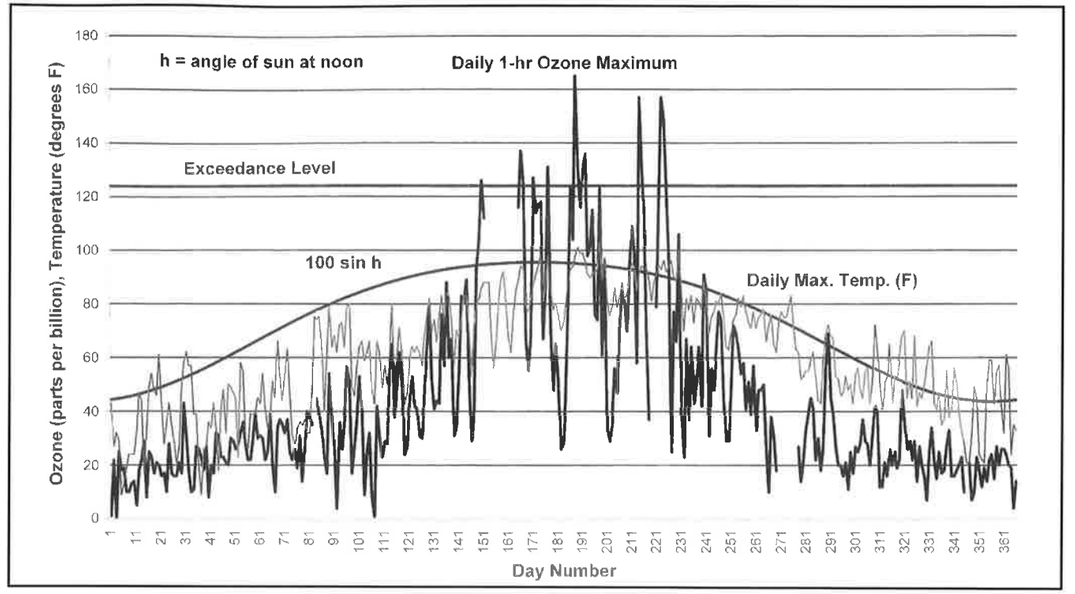

FIGURE II. 1988 1-hr Daily Maximum Ozone at Brackenridge, PA.

season. Maximum daily temperatures recorded at Greater Pittsburgh International Airport are also shown on the figure. They peaked in August, 4-5 weeks after the middle of the ozone season. Curves can be fit to the ozone/temperature relationship showing that ozone peaks increase by about 4 ppb for each one degree increase in maximum temperature.

Both temperature and solar radiation provide energy for the ozone reaction. Most of the time, maximum ozone is limited by the available energy. This conclusion is implicitly recognized by the U.S. EPA in setting the ozone season from April - October. There will not be an exceedance of the 1-hr standard in Allegheny County unless the temperature is at least 85°F and historically, there have been only a few exceedances at temperatures below 90°F. Only about 40% of the days over 90°F experience exceedances. During a normal summer, there are 5-10 ninety degree days, and thus there are only a handful of days each year when controls to reduce emissions will limit the ozone peak. Regulatory efforts must be devoted to those few days because most of the time ozone concentrations are "energy limited". Implementation of the new 8-hr standard will result in a larger number of exceedance days, and is expected to be accompanied by a shift of monitored exceedances toward more rural areas. In this situation, year round or seasonal controls may be required to reduce regional averages, and event controls would still be necessary to reduce the high peaks caused by exceptional events.

DISTRIBUTION OF OZONE PEAKS

Figure III shows the distribution of ozone maximums for Brackenridge/Harrison Twp. in 1988 and 1995. The Brackenridge monitor was relocated about one-half mile and renamed Harrison Twp. in 1989. The curves show the number of days with one-hour ozone maximums in each 10 ppb range during the ozone season. The 1988 Brackenridge data also shows the distribution for January 1 - March 31, and for the full year. The figure also shows the exceedance level of 124 ppb and the "design value" for each year. The "design value" is the fourth highest hourly average at any monitor site in the non-attainment area during a three year period. It is worth noting that the design value was reduced from 149 ppb in 1988 to 133 ppb in 1995. It suggests real progress in reduction of ozone, although it could also be due in part to differences in meteorology between the two years. The design value must be further reduced to 124 ppb to meet the standard. In 1988, the peaks at the upper end of the curve are somewhat normally distributed because there were many hot days and more exceedances. In 1995, there were seven exceedance days, and they were clearly exceptional events that produced a hump on the end of the curve.

SIP (State Implementation Plan) strategies, that require annual or at least seasonal reductions of emissions are "brute force" attempts to shift the entire curve to the left or at least shrink it sufficiently to bring the

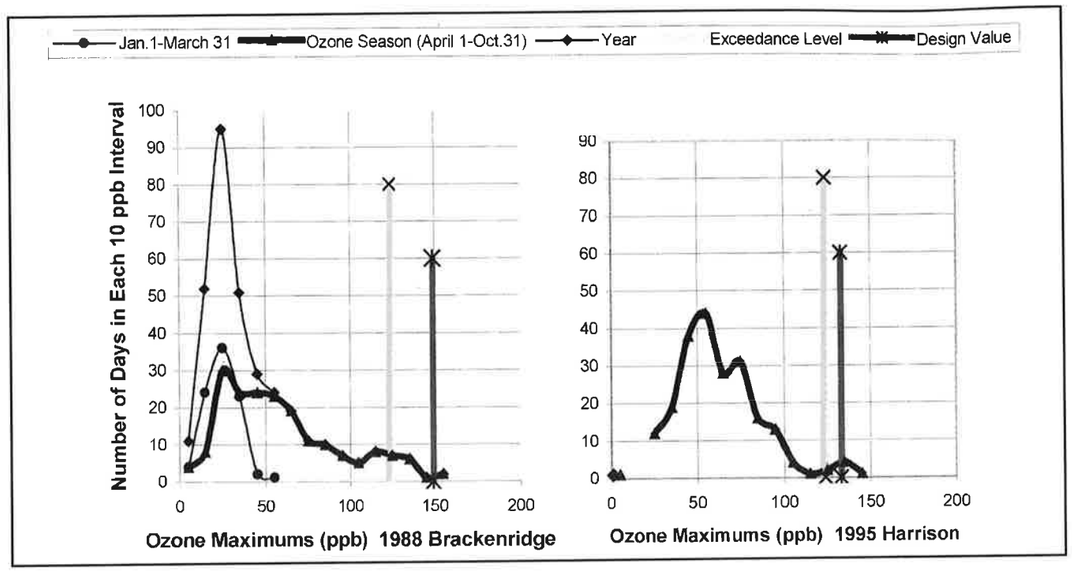

FIGURE III. Distribution of Ozone Maximums at Brackenridge/Harrison Twp., PA.

design value down to the exceedance level. They are effective only when there are large numbers of exceedances and, at the limit, they require drastic emissions reductions that may be impractical to achieve. When an area has reached borderline status as in southwestern Pennsylvania, the remaining exceedances are exceptional events, and are best eliminated by event controls that cut off the end of the curve with large reductions of emissions before and during a high ozone episode. Under an 8-hr. standard, a combination of SIP controls and event controls will be needed to shift the curve to the left while cutting off the tail caused by exceptional events. Because of the large component of transported ozone in southwestern Pennsylvania, it is also essential that ozone concentrations be reduced in upwind areas early in the development of an episode.

OZONE DISTRIBUTIONS BY DAY OF THE WEEK

Ozone maximums also vary according to the day of the week. On weekends and holidays, the morning and evening rush hour traffic is replaced by a more even traffic flow, and many small businesses and plants shut down. A whole new population of sources becomes active in recreational areas, shopping centers, and backyard barbecues. These major shifts in activities are accompanied by significant changes in ozone levels. Table 1 shows the average ozone peak by day of the week at each of the Allegheny County monitor sites in 1988. Sunday was the highest day at all sites except Brackenridge. Thursday was the lowest day. For the group as a whole, Thursdays were 15 ppb lower than the Sunday/Holiday average. This is a larger difference than can be achieved by any reasonable SIP strategy, i.e. normal changes in our daily routine have a larger impact than practical measures that can be implemented to control emissions.

Results for any one year would be strongly influenced by coincidence if a number of events occurred on any particular day. Similar studies of data for 1986, 87, and 1995 show some scatter, but weekends and holidays are

TABLE 1.

OZONE 1-HR MAXIMUM BY DAY OF THE WEEK

	Monitor Site				
Day	SF	LV	PH	BR	Aver.
Sun/Hol.	72.72*	68.67*	61.07*	66.41	67.22*
Monday	65.62	62.50	59.13	67.82	63.77
Tuesday	69.93	66.07	58.96	65.96	65.23
Wednesday	65.22	63.37	56.63	66.62	62.96
Thursday	57.48	52.15	46.59	53.17	52.35
Friday	62.78	55.56	48.38	57.71	56.11
Saturday	67.76	67.31	58.48	69.09*	65.66
Average	65.93	62.23	55.61	63.83	61.90

*-Highest Day Underline -Lowest Day

Notes:
1. Values are average 1-hr daily maximum ozone recorded at the monitor site in ppb during the period April 1 - September 30, 1988.
2. SF = South Fayette, LV = Lawrenceville, PH = Penn Hills, and BR = Brackenridge monitor site in Allegheny County, PA.
3. Sunday/H. totals include 1-hr. maximums for Monday following Memorial Day, 4th of July, and Labor day weekends.

clearly the worst ozone days on average, and Thursday is the best day. There is a significant effect from changing the population of sources between weekdays and weekends, especially the major holiday weekends. This difference must be considered in ozone planning.

AIR MASS TRAJECTORIES

By using average wind speeds and directions, one may trace a parcel of air causing an exceedance backward in time. For example, if there is an exceedance at a monitor, and winds are out of the southwest at 5 mph, one may assume that the parcel of air causing it was about 5 miles southwest an hour earlier. Figure IV shows trajectories for two air masses that caused exceedances in Allegheny County. On July 15, 1995 (black circles), winds were out of the southwest all day. The block of air that caused an exceedance at Harrison Twp. at noon, started south of Wheeling, WV about midnight the previous night. It passed through Pittsburgh in the early morning, spending a relatively short time in the urban area. It probably included a large component of transported ozone.

By contrast, the exceedance of August 2, 1995 at Lawrenceville (open circles) came from a parcel of air that probably originated around Youngstown or Lordstown, OH. It was near Neville Island at midnight on August 1, and wandered aimlessly over Pittsburgh's north side for sixteen hours before ozone was high enough to cause an exceedance at Lawrenceville. It would have had a large component of local emissions.

This method of tracing trajectories is not technically exact, but it does illustrate the point that different exceedances have their origins in entirely different areas and that completely different populations of sources may be contributing. It also does not account for ozone aloft that may be transported from different upwind areas and mixed downward during the day.

OZONE EPISODES

Figure V shows data for the Memorial Day weekend in 1988. It is considered to be the "type specimen" for an ozone episode in Allegheny County. The lower chart shows hourly ozone at Lawrenceville (heavy line), and

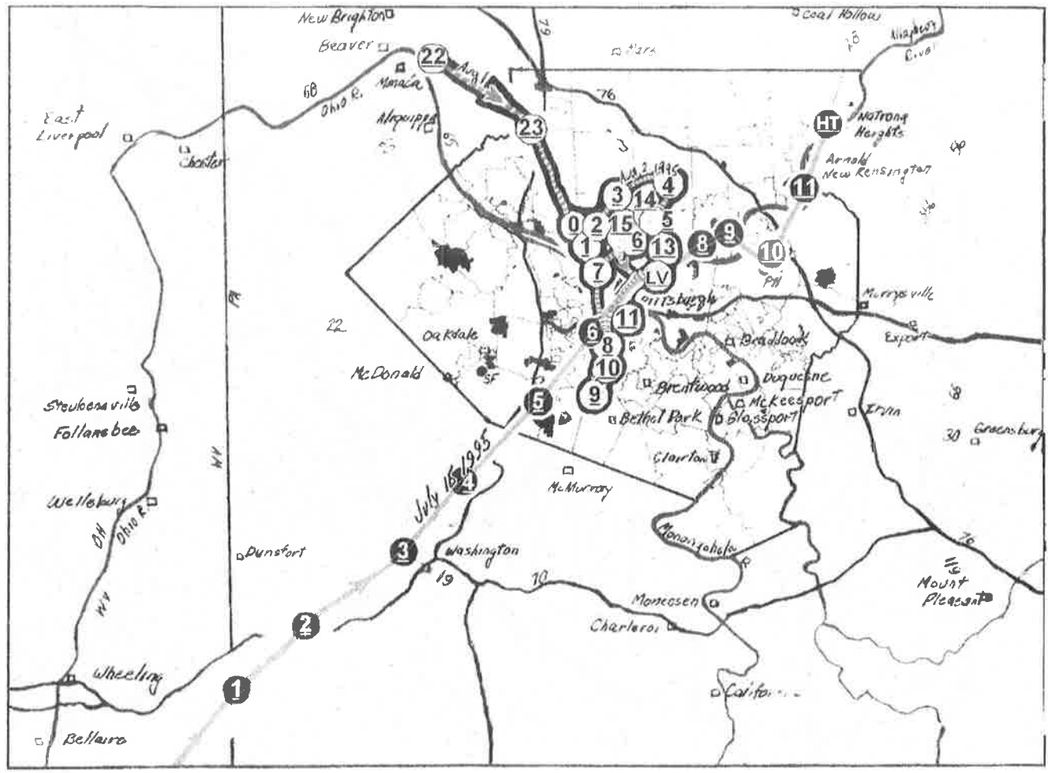

FIGURE IV. Air Mass Trajectories for Exceedances on July 15 and August 2, 1995.

South Fayette (light line) compared to the ozone standard. Ozone built up for four days to a peak on May 29, and then declined to the end of the episode. At Lawrenceville, ozone concentrations increase rapidly after sunrise each morning, and drop to zero, shortly after sunset as ozone that is residual, or is transported into the area, is scavenged by new nitric oxide (NO) emissions. At South Fayette, there are few NO scavengers, and ozone remains high at night, showing only a gradual dispersion.

The upper chart shows nitrogen oxides at Lawrenceville. The monitoring instrument measures NO (lowest light line), and NO_x (heavy line), which by definition is considered to be the sum of NO + NO_2 (nitrogen dioxide). As soon as the ozone has been scavenged in the evening, nitrogen oxides build up rapidly. They disperse somewhat during the night and then build quickly to a sharp peak in the morning. The morning peak is higher than the evening because there is no remaining ozone to be scavenged. When the sun comes up new ozone is created, and nitrogen oxides fall to low, nearly uniform, levels during the day. The nitrogen oxide concentrations decline from day to day as ozone levels build up. The presence of increasing amounts of ozone limits the build up of nitrogen oxides on succeeding days. We have interpreted this data as showing that Lawrenceville ozone is "NO_x limited". The system runs out of NO and NO_2 at the height of the daily ozone peak. To reduce ozone one should limit NO/NO_2 so it is depleted earlier at a lower ozone level. In order for NO_x to remain at constant levels during the day, higher oxidation products such as HNO_3 must be removed from the system as new nitric oxides are added from emissions sources.

We have described the contrasting patterns at Lawrenceville and South Fayette as the "urban/valley" and "rural/upland" regimes. During inversions, the river valley system, extending up the Allegheny and Monongahela, and in the urban and industrial areas down the Ohio River act as a large basin or sink for accumulation of NO_x under the temperature inversion during the night. With residual ozone in the upland rural areas, it provides the base for higher ozone peaks on succeeding days.

OZONE ISOPLETHS and VOC/NO_x RATIOS

Atmospheric chemistry for the formation of ozone is complex, and a given ozone concentration may result from many different combinations of NO_x and VOC (VOC is defined as volatile organic compounds that

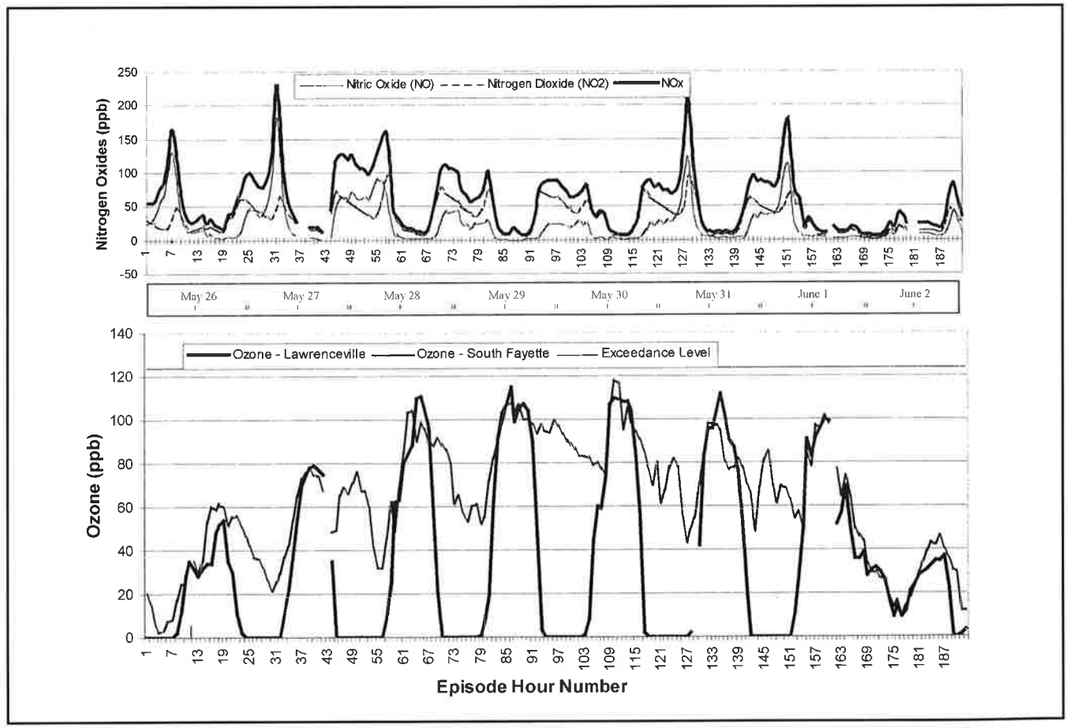

FIGURE V. Time Series Plots of Ozone and Nitrogen Oxides for May 26-June 2, 1988.

participate in ozone reactions). It does not include some organic compounds that U.S. EPA has determined are non-reactive. The principle one is methane. A graph of NO_x versus VOC with ozone "isopleths" connecting points of equal ozone offers a convenient way of illustrating the relationship. The isopleths generally take the form of a series of nested parabolas, with higher ozone values for increasing concentrations of NO_x and VOC. The isopleths represent 1-hr maximum daily ozone peaks, and NO_x and VOC are for early morning averages (6-9:00 a.m.). If normal early morning concentrations can be established for an area, as a starting point on the chart, the isopleths can be used to develop ozone reduction strategies. Any strategy, which shifts the concentration of one or both pollutants to an isopleth with lower ozone should work. The most direct route is normal to theisopleth intersecting the plotted concentration, and may involve both NO_x and VOC changes. However, any direction may be taken to reach a lower ozone isopleth. Sometimes an increase in NO_x or VOC will decrease maximum ozone, and conversely, a reduction of one or both concentrations may cause an increase in ozone. Ozone isopleths are often plotted for computer modeling runs to aid in the selection of strategies.

Figure VI gives an example of an ozone isopleth taken from one of the EPA manuals for EKMA (Empirical Kinetic Modeling Approach) (United States Environmental Protection Agency, July, 1989 - EPA-450/4-899-009a and 012). Isopleths for Lawrenceville, PA for 1991 are superimposed on the diagram. Non-methane organic compounds (NMOC) are plotted along the x-axis in ppmC (parts per million carbon), and NO_x values in ppmv (parts per million by volume) are plotted on the y-axis. NMOC may include minor non-reactive volatile organic compounds that are not VOCs. The example isopleths are nested parabolas for ozone concentrations of 0.06-0.20 ppm in 20 ppb intervals. For VOC and NO_x concentrations that plot in the upper left portion of the diagram, an ozone reduction strategy would call for reductions in NMOC (VOC), and increases in NO_x emissions. A reduction in NO_x would increase the ozone peaks. For concentrations plotting in the lower right portion of the diagram, lowering ozone would require a reduction of NO_x. VOC reduction strategies would have little or no effect.

Isopleths for Lawrenceville were derived from measurements of NMOC on ninety-five cannister samples collected at the monitor site from May 28-September 17, 1991. Cannisters were analyzed by the Maryland Department of the Environment as part of an EPA project. Points shown on the chart are for observed NO_x (ppmv) on sampling days, and measured NMOC (ppmC)

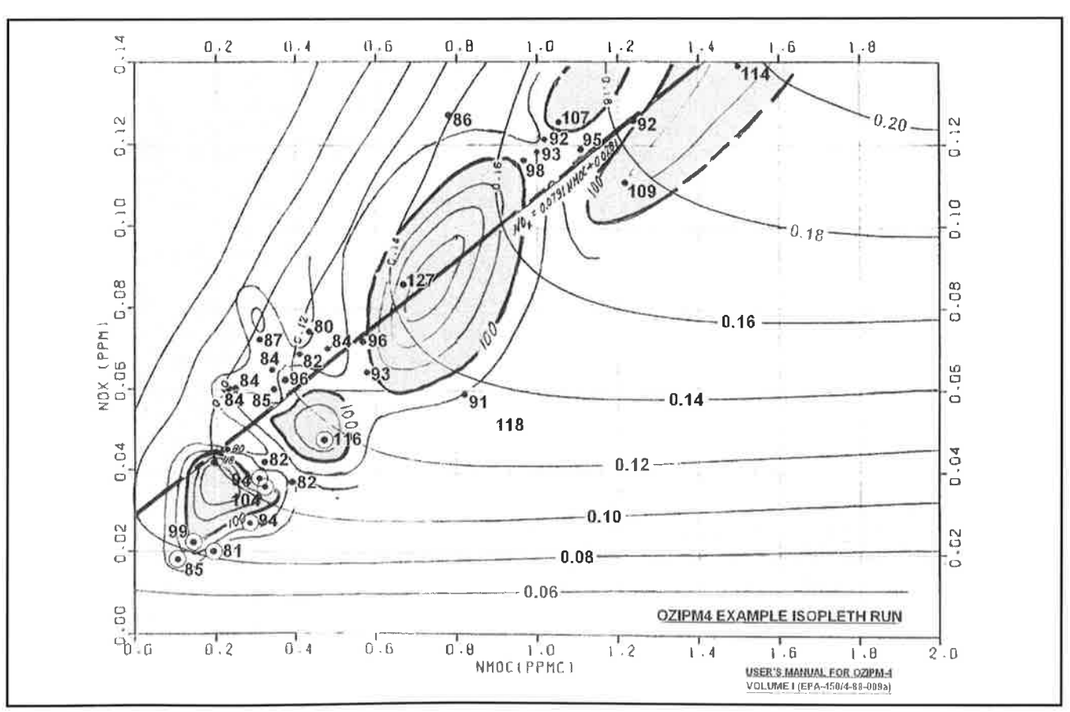

FIGURE VI. Typical Ozone Isopleths and Isopleths for Alleghey County Lawrenceville Monitor in 1991.

during the collection time from 6:00-9:00 am. They are labeled with the observed maximum hourly ozone for the day. Only "high ozone days" with a maximum over 80 ppb were used because ozone on other days is likely to be limited by either the temperature, available sunlight, or meteorological conditions instead of NMOC or NO_x concentrations. Isopleths were drawn from the observed data.

The Lawrenceville isopleths are closely grouped along a diagonal line and do not bear any resemblance to the nested parabolas generated for the AZIPM4 example (OZIPM4 - Ozone Isopleth Plotting With Optional Mechanisms- 4, is an ozone isopleth plotting program for EKMA applications) (United States Environmental Protection Agency, July, 1989 - EPA-450/4-89-009a). At Lawrenceville, there was no increase in ozone maximums for higher early morning concentrations of NMOC and NO_x. The highest maximum of 127 ppb is more or less in the center of the drawing, but 114 ppb was measured at the highest NO_x and NMOC concentrations, and 118 ppb at some of the lowest concentrations.

The fact that NO_x and NMOC increase together suggests that both are abundant, and are being concentrated by weather conditions that accumulate emissions. On a single ozone episode that lasted from July 18-27, 1991 with maximums above 80 ppb, the plot of NMOC/NO_x concentrations moved diagonally across the chart with high ozone at both high and low concentrations of VOC and NO_x precursors. There are only seven days at the lower end of the curve, (open circles), when monitored ozone maximums were comparable to or lower than the values predicted by the isopleths for the measured concentrations. On all other days, ozone values should have been much higher. It would suggest that the theory is wrong as applied to Lawrenceville, or that all other days were limited by the available energy. VOC/NO_x control strategies would only have reduced ozone on the seven days that fit the curves. It calls into question the validity of strategies to reduce NMOC (VOC) or NO_x in order to control ozone at this location on the other days.

A more usable conclusion may be drawn from a study of VOC/NO_x ratios. A regression line has been calculated by the method of least squares for the plotted data and has been shown on the chart. The equation is:

$$NO_x = 0.0791\ NMOC + 0.0281$$

The National Research Council (1991) discussed ozone isopleths in their report and concluded that the "VOC/NO_x ratio is typically about 8:1 along the ridge line of the parabolas" (p.165). Their typical isopleths show that if the VOC/NO_x ratio is higher than 15:1, the area is "NO_x limited". The regression equation for Lawrenceville has a slope of 0.0791, corresponding to a VOC/NO_x ratio of 12.6. It tends to confirm the previous conclusion, from the study of ozone and NO_x data, that the area is NO_x limited and NO_x reductions should be the strategy of choice to lower the ozone peaks.

CONCLUSIONS

Allegheny County and southwestern Pennsylvania are a borderline case for meeting ozone air quality standards. Violations of the 1-hr standard occur during episodes that are exceptional events. These are best eliminated with event controls or strategies that obtain large reductions of emissions for a short period before or during an episode. Implementation of the new 8-hr standard may require a different mix of regulatory (SIP) controls and voluntary event reductions. Efforts of regulatory agencies should be focused on better forecasting of ozone episodes and development of predictors to improve the reliability of the forecasts. Ozone episodes should be considered the same as any other weather related event, like winter storms or hurricanes. Advance warning would allow the public, communities, and industrial sources to prepare for them. With adequate planning, the area can meet the air quality standards and avoid the worst of unhealthful exposure during high ozone episodes.

ACKNOWLEDGEMENTS

The original work on the study of ozone monitor data in Allegheny County was carried out while the author was employed by the Allegheny County Health Department, Air Quality Program as Ozone/Carbon Monoxide Coordinator and Section Head of the Emission Inventory Section. He wishes to thank the Air Quality Program and it's staff for their support, and for making the monitor data available for additional study. The author accepts sole responsibility for the information presented and any conclusions expressed.

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Journal of the Pennsylvania Academy of Science 73(2):75-79, 1999

RESEARCH NOTE

A CONTINUING REDUCTION IN THE NUMBER OF GENERA REPRESENTED IN THE PLANKTON OF HARVEYS LAKE, PENNSYLVANIA[1]

CHARLES B. REIF

Wilkes University
112 North Franklin Street
Wilkes-Barre, PA 18701

ABSTRACT

Oligotrophic Harveys Lake in NE Pennsylvania with 268 hectares of surface and mean depth of 11 meters, following unauthorized introduction of alewife, *Alosa pseudoharengus*, circa 1975-76, has experienced a reduction in planktonic genera represented: 216 in 1984 to 28 in 1998. A large population of legally introduced smelt (*Osmerus mordax*), 1952 has been eliminated. Alewife also have been eating bottom fauna. Chemistry of lake water 1975-1998 has not changed.
[J PA Acad Sci 73(2):75-79, 1999]

INTRODUCTION

Harveys Lake is the largest of more than one hundred natural lakes in northeastern Pennsylvania. The latitude and longitude of the lake are respectively 41° 21′ 45″ and 75° 02′ 30″. It has a surface area of 268 hectares, a maximum depth of 28 meters, and a mean depth of 11 meters. The ratio of drainage area to lake area is 7:1. The surface of the lake fluctuates between 347 and 348 meters above mean sea level. In 1950 the biota of the lake included more than 200 species of plant and animal plankters. Plankters in the genera *Leptodora, Holopedium, Diaptomus, Cyclops,* and *Daphnia* were much in evidence. However, the legal introduction of smelt, *Osmerus mordax*, in 1952, began a change in the plankton, and an unauthorized introduction of alewife, *Alosa pseudoharengus*, possibly in 1975-1976, has radically altered the plankton of Harveys Lake.

[1]Received for publication 27 March 1999; accepted 20 August 1999.

REVIEW OF LITERATURE

For their study of immune sera Cohen and Reif, (1959), were able to collect a solid quart of planktonic crustaceans with one vertical net tow. At that time four species of copepods and twelve species of cladocerans were represented in the plankton. However, when Reif and Tappa (1966) undertook a study of the vertical migration of the daphnids they found that selective predation by the smelt had greatly reduced the number of *Daphnia pulex* and that they were observing the relatively weak migrators, *D. dubia*. Reif (1986), having observed three cycles in which the smelt population rose as the population of *Daphnia pulex* declined, accompanied by an increase in the numbers of *Daphnia dubia* in each cycle, expected a fourth such cycle but then discovered that the alewife were also preying on both species of daphnids. Thus, attention was turned to the literature of alewife in freshwater lakes.

Hall, Cooper and Werner, (1970), were the first to find that alewife ate not only plankton but also ingested specimens of bottom fauna, as have the alewife in Harveys Lake. Kohler and May, 1932, found that in Claytor Lake,

Virginia, where the alewife had greatly altered the limnetic biota, recovery was very good following a die-off of the alewife. Hutchinson, 1971, reported the marked predation of alewife on the plankton in Adirondack lakes. Warshaw, 1972, also observed the deleterious effects of alewife predation on the zooplankton of Lake Wonoaskopomas in Connecticut. Zaret and Kerfoot, 1975, suggested that alewife in Lake Gatun, Panama, preyed upon *Bosmina longirostris* more because of body size than upon visibility in their selection. Gregory, Brown and Dodson, 1983, recognized the changes in feeding habits of young alewife as the proportions of gill rakers altered with growth. Ridgeway, Hurley and Scott, 1990, noted changes in the feeding habits of alewife related to differences in water temperature. Keilty, 1990, found that alewife in the Great Lakes preyed almost exclusively on the European cladoceran, an import, *Bythortephes cederstroemi*. Dodson, 1990, held that complementary feeding niches were more or less constant and based on size selection by the alewife. Bronte, Selgeby, and Curtis, 1991, reported that the alewife population fell and rose according to the available plankton food supply in the Great Lakes and that alewife were adaptable to changing species of prey. Stone and Jessup, 1994, also found alewife in the Atlantic off Nova Scotia to be adaptable and thus able to change their diets as the situation demanded.

MATERIAL AND METHODS

Weekly vertical tow net samples were taken from Harveys Lake, from a depth of 85 feet at the same location on the lake, weekly, from the 10th of June through the 30th of September, 1998. The samples were preserved in glass vials with glutaraldehyde. In the laboratory, a one-cubic-centimeter portion of each sample was placed in a Sedgewick-Rafter counting chamber and the organisms therein identified to genus or species, as could best be done microscopically, and then enumerated. The counts with dates are presented in Tables 1 and 2.

Observations made from the shore of Harveys Lake, on quiet evenings at sunset, revealed, during the summer of 1998, as it had in previous summers, that schools of alewife were busy near the surface of the lake, apparently feeding on emerging diptera. The author's appeal to the

TABLE 1. Enumeration of planktonic algal genera represented in Harveys Lake, Summer of 1998

	6/10	6/17	6/24	7/1	7/8	7/15	7/22	7/29	8/5	8/12	8/19	9/3	9/9	9/23	9/30
Chlorophyta															
Eudorina	7	7	0	0	0	24	30	11	98	0	209	640	1110	10	2
Pandorina	0	3	48	0	0	4	4	10	12	1	110	0	0	4	0
Spondylosum	0	0	0	0	0	0	0	0	91	0	0	0	0	0	0
Staurastrum	0	0	0	0	0	12	10	10	408	20	88	61	40	81	32
Volvox	0	0	0	0	0	2	0	9	0	3	0	2	15	16	5
Chrysophyta															
Dinobryon	2	1	408	21	3	11	19	10	101	152	19	17	102	90	151
Fragillaria	0	0	0	0	0	0	0	0	0	0	5	2	3	10	1000
Bacillariophyta															
Asterionella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10
Tabellaria	3	18	60	24	130	3	4	8	10	10	45	40	15	9	10
Pyrrophyta															
Ceratium	0	0	0	0	0	0	0	0	0	0	0	0	4	1	1
Cyanophyta															
Anabaena	1	33	120	50	30	11	60	5	1	4	17	20	51	102	1000
Coelosphaerium	43	81	1200	81	470	707	201	100	101	209	39	30	60	40	25
Oscillatoria	0	0	0	0	0	0	0	0	0	0	0	0	0	10000	0

TABLE 2. Enumeration of planktonic animal species recognized in Harveys Lake, Summer of 1998

	6/10	6/17	6/24	7/1	7/8	7/15	7/22	7/29	8/5	8/12	8/19	9/3	9/9	9/23	9/30
Rotifera															
Asplanchna priodonta	5	0	3	0	45	44	7	0	10	5	0	7	51	4	21
Conochilis hippocrepis	7	0	0	0	0	0	0	0	0	0	20	0	0	0	0
Kelicottia longispina	19	88	48	240	700	1200	350	910	1010	55	101	60	91	0	0
Keratella cochlearis	4	18	140	14	151	4	99	104	404	75	170	325	240	201	802
Notholca longispina	38	111	221	2	1	2	20	1	602	0	0	0	0	0	0
Polyarthra vulgaris	23	13	121	25	180	80	41	80	303	81	79	310	490	101	40
Tetracerca sp.	1	0	18	2	4	5	4	7	99	2	1	4	4	5	1
Tetramastic opalensis	0	0	0	0	0	0	0	0	0	5	6	20	30	15	0
Trichocerca multirinis	1	8	15	1	5	4	7	5	87	45	20	2	2	5	0
Cladocera															
Bosmina longirostris	15	54	207	110	65	2	35	15	44	40	56	15	60	81	41
Ceriodaphnia quadrangula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Daphnia pulex	0	0	1	0	1	11	0	3	8	3	6	8	60	61	5
Chydorus sphaericus	25	16	56	30	1	183	5	7	3	4	7	4	1	4	2
Copepoda															
Cyclops spp. immature	68	54	138	106	43	134	121	137	141	35	155	93	70	106	22
Nauplii	8	36	2	3	8	18	21	1	40	2	3	30	30	40	16

Pennsylvania Fish Commission for help in netting alewife was not heeded because of the Commission's heavy schedule so that examination of alewifian stomachs was not accomplished for this study as it has been in the past, Reif 1992.

RESULTS

In Table 1 are listed the names of the planktonic algal genera present and the number of individuals counted in one cubic centimeter of plankton sample for each of the dates on which plankton was collected from Harveys Lake during the summer of 1998. Although the alewife apparently are selective in capturing zooplankton they also ingest individuals of the phytoplankton. The population of phytoplankton, in Harveys Lake during the summer of 1998, undoubtedly was dependent on the hours of sunlight and the temperature of the lake's water, as it has been observed in previous summers. Thus, the populational variations of the several phytoplankters, during the summer of 1998, are not as significant, as far as alewifian ingestion is concerned, as are the values given in Table 2, which presents the numbers of the zooplanktonic forms.

The largest zooplankters in Harveys Lake during the summer of 1998 were two species in the genus *Cyclops*. However, fully adult individuals were found so rarely, although numerous immature individuals were present, that they were not identified to species in the counting.

Immature cyclops were easily distinguished from nauplii for enumeration. Thus it seems reasonable that the alewife were indeed selecting the mature individuals of two species of adult *Cyclops* present in Harveys Lake during the summer of 1998.

Another of the author's findings is interesting in that it conflicts with the report of Zaret and Kerfoot, 1975, who found that alewife were selecting individuals of *Bosmina longirostris,* in Lake Gatun, whereas in Harveys Lake during 1998 they were apparently avoiding *B. longirostrias* as well as *Chydorus sphaericus*, the two species being similar in appearance. The larger numbers

of *Daphnia pulex* on 9/9/98 and 9/23/98 may have been fortuitous in that the alewife did not scour that portion of Harveys Lake during those times.

Of more import is the reduction in the numbers of genera and species represented in the plankton in Harveys Lake. In 1983, Reif and Smith studied the desmids of 100 lakes in northeastern Pennsylvania and found eight species of desmids represented in Harveys Lake: *Closterium acerosum, Cosmarium botrytis, C. granatum, C. ornatum, C. rectangulare, Pleurotanium ehrenbergii, P. trabeculare,* and *Spondylosum pulchrum,* none of which was found in Harveys Lake during the summer of 1998. Further, Smith and Reif (1984 and 1990) noted a number of other species which Reif did not find during the summer of 1998, as shown in Table 3.

TABLE 3. Gives the numbers of genera of the major taxa represented in Harveys Lake during the summers of 1984, 1990, and 1998.

Taxa	Numbers of genera represented 1984	1990	1998
Chlorophyta	115	45	5
Euglenophyta	5	1	0
Pyrrophyta	2	3	1
Chrysophyta	5	8	2
Bacillariophyta	44	7	2
Cyanophyta	16	7	3
Rotifera	15	13	9
Cladocera	12	6	4
Copepoda	2	2	2

Table 4 shows that during the last twenty-three years, 1975-1998, the chemistry of Harveys Lake has changed little. Thus the chemical values of the water of Harveys Lake have not changed as radically as have the kinds of organisms composing the plankton of the lake.

TABLE 4. Alkalinity, phosphates, and pH of Harveys Lake

	1975	1982	1998
Total Alkalinity	18	18	16

DISCUSSION

Although detailed studies of the alewife in Harveys Lake are not available, it is certain that they have been present in the lake for more than two decades. Specimens have been taken by angling during the summer months and by netting through the ice in winter. None was larger than five inches. Their stomach contents were examined and the conclusion reached that they have ingested various kinds of plankton as well as benthic organisms. No study has been made of the populations of benthic organisms in Harveys Lake but a marked decline has been noted in the planktonic taxa previously found in the lake. The disappearance of the smelt from Harveys Lake may have been hastened by the introduction of alewife although some blame may also be placed on the netting of smelt during their spawning runs up the tributary streams which flow into Harveys Lake.

The eutrophication of Harveys Lake was lessened following the installation of a sewage system completely around the lake. The productivity of Harveys Lake, which may have become more oligotrophic during the war years, 1939-1946, due to a reduction in resident population around the lake, with numerous septic tanks, had become markedly eutrophic during the sixties and seventies. Thus the functioning of the new sewage system, put into action in 1977, was initiated shortly after the supposed introduction of the alewife and the disappearance of the smelt, which eliminated the competition for food between the smelt and alewife. It is important here that the chemistry of Harveys Lake, although moderately productive, has not changed during the past twenty years. During that period the taxa represented in the plankton as well as the populations of those taxa still present, have been reduced, apparently due to the depredation of the alewife.

In 1967 Reif observed the bloated bodies of many dead alewife on the shore of Lake Michigan, near Holland, MI. The individuals were about 10 inches long, whereas the alewife taken from Harveys Lake on a lure or by net have not exceeded five inches. The difference in size may have been due to a poorer diet or the crowding of individuals in a lake so much smaller than Lake Michigan.

What causes the final collapse or disappearance of freshwater populations of alewife is not known with certainty. Die-offs of freshwater populations of the anadromous alewife appear to be due to the reduction of their freshwater food supply below a subsistence level, but it is usual that the plankton of such a stressed lake does recover.

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ACADEMY HISTORY

THE HISTORY OF THE PENNSYLVANIA ACADEMY OF SCIENCE ON THE 75th ANNIVERSARY, 1924-1999[1]

WILLIAM A. URICCHIO

Professor Emeritus, Carlow College
300 Fox Chapel Road
Pittsburgh, PA 15238

The Pennsylvania Academy of Science was organized on April 18, 1924 in Harrisburg, Pennsylvania. It was an outgrowth of a AAAS meeting in Cincinnati, Ohio in 1923. At that meeting, a group of influential scientists from Pennsylvania with national recognition discussed the feasibility of forming an organization to advance science within our state. A survey was conducted among scientists of the state to determine whether or not there was any interest in a state academy. The response was favorable, and so it was formed. Its aims were to encourage scientific research, promote interest in the teaching of science, and to stimulate the exchange of ideas among those in scientific work, especially in Pennsylvania. In addition, they saw the benefit in forming fellowships with researchers and educators in the various fields of science, as well as an opportunity to disseminate scientific knowledge through publications, and to promote interest in Junior and Senior High School students in scientific investigations. This was an ambitious undertaking.

Of the founders, 21 were from colleges and universities, 14 from state government, 5 from high schools, and 3 from other areas. Although the group was diversified, the predominate members were geologists, geographers, botanists, zoologists, museum representatives, and individuals representing the Departments of Agriculture and Forest and Waters. The first papers were presented at the November meeting of the group in Harrisburg, PA in 1925.

[J PA Acad Sci 73(2): 80-81, 1999]

[1]This paper was presented at the 75th Annual Meeting of the Pennsylvania Academy of Science at Pocono Manor, Pocono Manor, PA on April 10, 1999.

There has been a shift in the scientific interests of those presenting papers at the annual meeting. In the earlier years, there was a higher emphasis of papers on geology and geography, with a scattering of papers on botany, zoology, and agriculture. In 1954, for example, there were 12 papers on geology, 4 on geography, 18 on biology and 3 on medicine, 2 on physics, and 1 on chemistry. Looking at this years' program, one will see a predominance of papers in the biological sciences as well as environmental science and microbiology, with lesser emphasis on geology, geography, physics, and chemistry.

Along with the shift in scientific interest, there has been an increase in the number of papers presented at the annual meetings. In addition, in recent years the program has included a number of poster session presentations. Meanwhile, the growth in membership has declined significantly. In 1954, the total number of members was 723, and presently, we have approximately 574 members. Some of the reduction in membership is due to Biologists of the Commonwealth holding their own meetings, as well as the fact that many of the institutions who participated in the past no longer have an active role.

Another change which has occurred over the years is in those presenting papers. Initially most of the papers were given by professors and researchers, whereas now, a significant portion are given by students. We are saddened by the reduction in paper presentations by established senior faculty and scientists, but we are also encouraged by the significant increase in student participation because it accomplishes our aim to encourage scientific research.

It was not, however, until 1964 that the Academy was incorporated through the Court of Common Pleas, in

Northampton County, PA. The initial registered office of the corporation was Lafayette College.

During the early years of the Academy, two meetings were held each year. The spring meeting, or what we presently refer to as the Annual Meeting, was held on campuses of various colleges or universities, as well as museums. The summer meeting was a field event to a place of botanical, historical, or geographical interest, with members and local experts serving as guides.

Considering the publications, the first volume of the Proceedings was published in 1927. In earlier years, it included a list of officers, minutes of the Executive and Annual Meetings, index of authors, abstracts, completed papers, the program for the Annual Meeting, as well as a list of members. In addition, there were a number of papers which dealt with issues in Pennsylvania such as: "Major Pennsylvania Mine Accidents Caused by Gas Explosion," "Distribution of Population in Pennsylvania," and "Recreation Potential of Upper Susquehanna Basin." In 1989, we changed from publication of the annual *Proceedings*, to the *Journal* which consists of three issues, plus supplements. It has been further improved with the requirement of peer reviews by experts in the field.

In 1943, the Academy began to publish the *Newsletter* which carried current scientific news and activities in the state, as well as meeting information and other items of information concerning the members. The *Newsletter* is published five times a year and sent to the entire membership. In 1984, the Academy initiated book publication on topics important to Pennsylvania and the nation as a whole. At this writing, twenty six books have been published. These books have been used as reference texts for multidisciplinary courses, scientific research, and teaching purposes.

The Pennsylvania Junior Academy of Science, which is a state-wide organization of Jr. and Sr. high school students, was designed to stimulate and promote interest among its members through development of research projects and investigations. The PJAS exists by sanction of the PAS which recognizes the Jr. Academy as an integral part of its organization. The first director was Dr. Karl Oerlein of the State Teacher's College of California, PA, in 1934, who served until 1941. Their meetings were held in conjunction with the PAS, and their growth went from 25 projects to about 2800 projects expected in May of 1999 at Penn State University.

The Sr. Academy has been fortunate to have a long standing award. In 1952, the Darbaker award was established through a bequest of the late L. K. Darbaker who was President of the Academy in 1939. This award is given for work in the biological sciences involving the use of a microscope and related optical equipment. A number of other awards have been established for the Jr. Academy.

Some observations of interest which are noteworthy:

It is interesting to note that we have among us, three members of the Academy who have been presenting papers to the Academy since the 1960's - Drs. Miller, Schnell, and Fried.

Annual meetings in the past were held at college campuses or museums. Now they are held at conference centers. We have gone from two meetings a year, Spring and Summer, to one meeting a year.

Papers in the early years were presented largely by professors and researchers while, presently, the greater number are presented by students, which has fulfilled an important goal of the Academy of promoting and fostering an early interest in science.

While the membership has decreased in recent years, the numbers attending the Annual Meeting have increased since the early 1960's by over 250%.

There have been only two women presidents in the history of the organization: Dr. Phyllis Martin and Sister/Dr. Gabrielle Maze.

Presidents, up until 1964 served the Academy for one year. Since then the term has been for two years.

The number of female participants attending the Annual Meeting has increased by over 500% over the past years.

The brief history covers the first 75 years of the Pennsylvania Academy of Science, yet it cannot begin to recognize the tireless efforts of its founders and supporters over that period of time, many of whom are in the audience tonight. Those people are responsible for helping to develop lifelong interests in science in thousands of students. It is through their dedication that we may find a cure for cancer, the common cold and AIDS, that we may be able to develop life-sustaining communities on other planets, that we may be able to develop products safer for our health and that of our planet. In short, just as other facets of society have prepared for the future, the PAS has played it's scientific role to enable us to more confidently enter the new millennium.

PENNSYLVANIA ACADEMY OF SCIENCE
JOURNAL INFORMATION FOR AUTHORS

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The *Journal* of the Pennsylvania Academy of Science publishes original papers, research notes, commentary, and review articles in the natural, physical, engineering, and social sciences. All papers must discuss the relevance of the data presented and a clear interpretation of its meaning in view of current knowledge of the discipline concerned. Helpful references for the author are: (1) Day, R.A. 1983. How to write a scientific paper. 2nd ed. ISI Press, Philadelphia, xv + 181 pp.; (2) O'Connor, M. and F.P. Woodford. 1976. Writing scientific papers in English, Elsevier, Amsterdam, vii + 108 pp.; (3) MacGregor, A.J. 1979. Graphics simplified; and (4) How to plan and prepare effective charts, graphs, illustrations, and other visual aids, University of Toronto Press, Toronto, 1-64 pp.

Authors are requested to examine recent issues of the *Journal* in order to conform to the general style of the journal. Papers are accepted for consideration at any time, but most articles submitted for publication will have been presented at the spring meeting of the Academy and are to be given to the section chairperson for transmittal to the Editor. Submitted manuscripts are accepted for review with the understanding that the same work has not been submitted for publication or review elsewhere, and that all persons cited as a personal communication have consented to be cited. Authors are billed for page charges to partially defer the costs of publishing. **Page charges are mandatory.**

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All authors are requested to conform to the following:

1. *General Format.* All papers should be typed, double spaced, and on good quality 8.5 x 11-inch (21.5 x 28 cm) bond paper, with 3 cm margins all around. *Do not use single spacing anywhere* (including Literature Cited), and do not use erasable bond paper. Normally, manuscripts will be organized as follows: (1) an unnumbered cover sheet with Title, Authors, their institutions and addresses, and name, address, and telephone number of the author to receive proof, (2) an unnumbered sheet with an Abstract, (3) Introduction, (4) Materials and Methods, (5) Results, (6) Discussion, (7) Acknowledgments, and (8) Literature Cited. All pages of the text, Introduction through the Literature Cited, are to be numbered (top center on right), and the names of authors should appear in the upper right-hand corner of each page. The text should begin in the middle of the first numbered page.

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Darbaker Prize

The Darbaker Prize is a Pennsylvania Academy of Science (PAS) award given for outstanding scholarly contributions which use microscopic techniques and present microscopic illustrations in the reporting of biological research. The award is competitive amongst qualified papers submitted in association with the Academy's annual meeting.

The Pennsylvania Academy of Science established the Darbaker Prize in 1952. Funds for the award are made available through a bequest of the late L.K. Darbaker, 1939 PAS President. Referring to the award, Dr. Darbaker's will states: "Any sum applicable to the Pennsylvania Academy of Science shall be for grant or grants in Microscopical Biology."

To qualify for the Darbaker Prize, a scientist or scientists must: (1) have used microscopy (light, SEM, TEM, or other technologies) in the research they report, (2) submit in proper format a manuscript reporting the results of the completed study for consideration to be published in the *Journal*, (3) specifically state a request to the Editor of the *Journal* to have their manuscript considered for the Darbaker Prize for the current calendar year, and (4) be a member of the Pennsylvania Academy of Science. Darbaker Prize competition manuscripts are expected to be presented and submitted at the PAS annual meeting, but if not, manuscripts will be accepted for consideration within four weeks (28 calendar days) following the last day of the annual meeting. Only manuscripts that have successfully completed the review process and have been accepted for publication in the *Journal* will be eligible for the award.

The Editor of the *Journal* will examine all manuscripts submitted for award consideration to determine the fulfillment of requirements. The editor will then forward the eligible manuscripts with his/her and reviewers recommendations to the PAS President for final decision. The Darbaker Prize recipient or recipients will be informed of their selection by the President of the Academy. Formal public announcement of the Darbaker Prize will be made in the *Journal* at an appropriate time. The individual or individuals awarded the Darbaker Prize will receive a publication grant for page charges required to print their article in the *Journal,* and may or may not receive a monetary award.

For further information contact Shyamal K. Majumdar, Ph.D., **Editor of the *Journal,*** Professor of Biology, Lafayette College, Easton, PA 18042 (610) 330-5464, FAX (610) 330-5705.

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