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Contents

Journal Information 54

BIOLOGY: The Decline of Native Brook Trout (*Salvelinus fontinalis*) Populations Along the Upper West Branch of the Susquehanna River: Canaries Outside the Coal Mine 55
Jeffrey E. Lovich and Robert E. Lovich

BIOLOGY: Temporal Effects on Detection of Eastern Chipmunks (*Tamias striatus*) in Forested Landscapes 61
Carolyn G. Mahan and Richard H. Yahner

BIOLOGY: Metal Ion Uptake and Storage by Brain Membranes 65
Ray H. Crist, Gerald D. Hess, Alan M. Gagnon, and DeLanson R. Crist

BIOLOGY: Comparison of Hypercapnia and DNP on Respiration in Cats 69
E.M. Adams and N.S. Cherniack

BIOLOGY: The Diet of Percina Caprodes (*Rafinesque*), (*Pisces: Percidae*) in Small Stream in Pennsylvania 75
Kelly L. Bryan, Martin J. Gutowski, Joseph P. Slusark and Jay R. Stauffer, Jr.

BIOLOGY: Some Fungal Toxins Can Affect the Percent Germination and Germ Tube Elongation of *Rhizomucor miehei* Sporangiospores 80
James J. Deploey, Charles E. O'Rear and Gerald C. Llewellyn

BIOLOGY: The Use of Limestone for Phosphorus Precipitation in a Hypereutrophic Pond 83
Sean M. Furjanic and Thomas E. Murray

GEOGRAPHY: Retirement and Migration: The Case of Pike County, Pennsylvania 88
George A. Schnell

EDITORIAL POLICY AND FORMAT 94

PAS HOME PAGE 95

PAS BOOKS 96

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(Continued to inside back cover)

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E.M. Adams and N.S. Cherniack

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George A. Schnell

EDITORIAL POLICY AND FORMAT 94

PAS HOME PAGE 95

PAS BOOKS 96

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THE DECLINE OF NATIVE BROOK TROUT *(SALVELINUS FONTINALIS)* POPULATIONS ALONG THE UPPER WEST BRANCH OF THE SUSQUEHANNA RIVER: CANARIES OUTSIDE THE COAL MINE[1]

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ABSTRACT

Native brook trout (*Salvelinus fontinalis*) are widely distributed in suitable habitat in Pennsylvania. Over 200 km of streams in the upper West Branch of the Susquehanna River drainage system were surveyed periodically from 1981-1995 for the presence of brook trout. Brook trout were found in 68% of the stream distance surveyed, including 66% of the first order stream segments. Higher order streams were more likely to be occupied by brook trout and had proportionately more stream distance of brook trout habitat than lower order streams. Few other fish species were collected during the survey. The primary cause of brook trout extirpation in the area is acid mine drainage from surrounding bituminous coal mines which occupy significant portions of the regional landscape. Atmospheric deposition of strong acids in these poorly buffered streams has undoubtedly contributed to the extirpation of brook trout populations. In a subset of streams that did not contain brook trout, pH ranged from 3.8-4.7. Unpolluted headwater streams provide important refugia for native fish populations for recolonizing the polluted but recovering West Branch of the Susquehanna River and its major tributaries.
[J PA Acad Sci 70(2):55-60, 1996]

[1]Submitted for publication 19 October 1995; accepted 7 February 1996.

INTRODUCTION

The brook trout (*Salvelinus fontinalis*) is the only native stream salmonid in Pennsylvania (Cooper, 1983). Although the species is a popular game fish and has been stocked worldwide (McAfee, 1966), native brook trout populations have experienced significant declines in portions of the eastern United States as a result of habitat destruction (Lovich, 1984) and possible negative interactions with non-native species such as rainbow trout (*Onchorynchus mykiss*; Schmitt et al., 1993). Recent research suggests that their sensitivity to small changes in habitat quality makes them potentially susceptible to global and regional changes including acid deposition from precipitation (Sharpe et al., 1984, 1987; Webb et al., 1989) and global warming (Meisner, 1990). In recognition of the declining stocks of this species, their importance as an ecological indicator species (Jenkins, 1979; Camuto, 1988) and their intrinsic value (Knuth, 1994), some states have instituted surveys to determine their surviving range (Mohn and Bugas, 1980; Bugas and Mohn, 1981; Pennsylvania Fish and Boat Commission, pers. comm.).

While Pennsylvania still supports widespread and healthy native brook trout populations (Pennsylvania Fish and Boat Commission, pers. comm.) some parts of the state have experienced significant declines of the fishery due to habitat degradation and destruction. In particular, acid mine drainage has had devastating effects on fish populations in the West Branch of the Susquehanna River and its associated tributaries (Cooper and Wagner, 1973; Hocutt and Drawas, 1975). The objective of our paper is to present the results of an

inventory of native trout populations in a portion of the upper West Branch of the Susquehanna River watershed.

MATERIALS AND METHODS

Streams were periodically surveyed for the presence or absence of brook trout in portions of northeastern Indiana County, southwestern Clearfield County and extreme southeastern Jefferson County, Pennsylvania (Figure 1) between 1981 and 1995. Data reported in this paper are based on observations taken from streams draining into the upper West Branch of the Susquehanna River (WBSR) from its confluence with Shryock Run downstream to the mouth of Whisky Run. Beaver Run, a large state stocked stream with a self-sustaining population of brook trout, was excluded unless noted otherwise. Cush Creek and Sawmill Run are the only other state stocked streams in the study area. We also included streams draining into Cush Creek, a major tributary of the WBSR, downstream from the mouth of the West Branch of Cush Creek (this stream is near the towns of Gipsy and Hooverhurst, outside the boundaries of Figure 1) to its confluence with the WBSR. The upper portion of Cush Creek excluded in this survey has a drainage area of approximately 29 km^2. The study area contained a total of 208.75 km of streams with a drainage area of approximately 180 km^2. The drainage area of named tributaries of the WBSR in the study area ranges from 2.31-55.44 km^2 with a mean of 16.66 km^2 (U.S. Geological Survey, 1989).

Stream lengths were determined by averaging the results of three passes with a map wheel on U.S. Geological Survey 7.5 minute quad sheets showing permanent and intermittent streams. Stream names follow those shown on the quad sheets. All streams, including unnamed streams, are numbered and referenced in Table 1 and Figure 1. Stream order designation follows Strahler (1957).

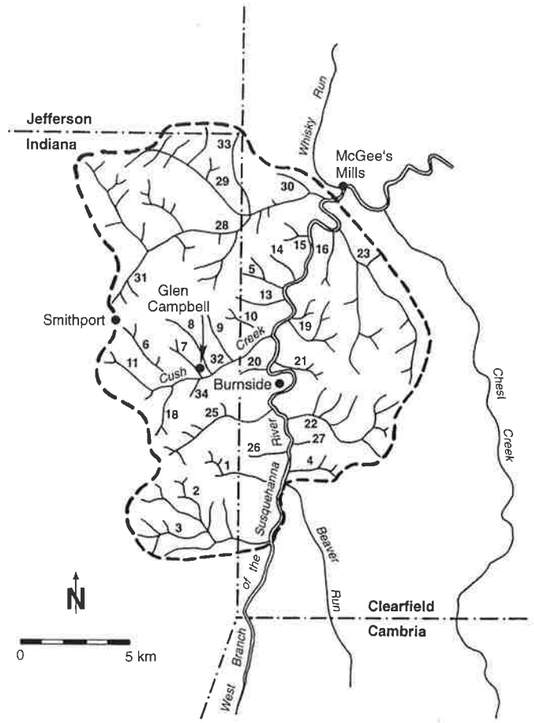

FIGURE 1. Map of the study area surveyed for the presence of native brook trout. Stream numbers follow Table 1. Map modified from Higbee (1965).

TABLE 1. List of stream units surveyed for the presence of native brook trout. Local names of streams are noted with footnotes. Stream numbering is for convenience and matches that of Figure 1. Stream lengths (km) include all tributaries shown on 7.5 minute maps. Latitude and longitude, demarcating the mouth of the stream, are given in degrees-minutes-seconds. Not all streams are independent: some are tributaries of other stream units named in the table. Streams that contained brook trout during the course of the study are marked with an asterisk. Stream numbers 12, 17 and 24 were not included in this study.

Stream	Stream #	Order	Length	Latitude	Longitude
Boiling Spring Run*	1	2	5.18	40-46-28	78-47-05
Shryock Run*	2	3	12.79	40-45-12	78-47-22
Powell Run*	3	2	8.80	40-45-20	78-48-31
Patchin Run*	4	2	3.35	40-46-55	78-46-48
Martin Run*	5	2	4.88	40-50-57	78-47-09
Horton Run	6	1	3.70	40-48-48	78-50-09
Unnamed[1]*	7	1	2.14	40-48-48	78-48-28
Unnamed[2]	8	2	6.41	40-49-01	78-49-06
Unnamed[3]	9	2	3.96	40-49-11	78-48-31
Unnamed[4]*	10	1	2.64	40-49-43	78-47-37
Unnamed[5]*	11	1	2.74	40-48-34	78-50-51
Unnamed[6]	13	1	2.12	40-50-41	78-47-07
Unnamed[7]*	14	1	2.14	40-51-05	78-46-47
Unnamed[8]*	15	2	3.35	40-52-04	78-46-19
North Run*	16	2	2.74	40-52-07	78-45-30
Unnamed	18	2	2.74	40-48-35	78-50-26
Unnamed[9]*	19	2	6.55	40-50-20	78-46-42
Unnamed	20	2	2.43	40-49-09	78-47-29
Unnamed[10]*	21	2	3.04	40-49-22	78-47-19
Sawmill Run*	22	2	14.53	40-47-54	78-46-59
Deer Run*	23	3	20.12	40-52-13	78-45-19
Rock Run*	25	3	11.89	40-48-10	78-47-12
Unnamed*	26	1	1.22	40-47-14	78-47-06
Unnamed	27	1	0.61	40-47-27	78-46-40
S. Branch Bear Run[11]	28	4	38.45	40-52-50	78-45-48
Bear Run[12]	29	3	18.28	40-52-35	78-48-32
Unnamed*	30	2	4.57	40-53-02	78-46-22
Unnamed[13]	31	2	3.96	40-51-47	78-50-56
Cush Creek*	32	3	6.71[14]	40-49-51	78-47-22
Unnamed[15]*	33	2	5.18	40-52-38	78-48-16
Unnamed	34	1	1.53	40-48-49	78-49-32

1. Glen Campbell Run
2. Brady Run
3. Urey Run
4. "Eugus Run"
5. Logan Run
6. Sportsman's Run
7. North School Run
8. Sam McGee Run
9. Elk Lick Run
10. Cherry Run
11. Bear Run
12. Hillman Creek
13. Mud Bridge Run
14. Only part of the stream was surveyed. Refer to text for details.
15. Murry Run

Fish were collected with a Coffelt backpack electroshocker using direct current following the techniques of Lovich (1992), under permits from the Pennsylvania Fish Commission. A small number of voucher specimens are housed at the University of Georgia Museum of Natural History (Appendix). Other streams were surveyed by licensed angling and occasionally by visual observation. Our estimates of stream distances occupied by brook trout are liberal in that we include entire segments of streams known to have brook trout unless we determined that some portions or tributaries did not have brook trout. Although brook trout were rarely found at the source of streams in the study area, many were occupied far into their headwaters making this a reasonable assumption (Cooper, 1983).

Limited data were collected on the pH of selected streams in the study area from 1979-1980.

RESULTS

Brook trout were found in 142 km (68%) of the stream sections surveyed. Relative to lower order streams, higher order streams contained greater proportions of occupied brook trout habitat as measured by linear distance (Table 2). Higher order streams were also more likely to have brook trout present than were lower order streams. Sixty-six percent of all first order stream segments surveyed contained brook trout compared with 83% of all third order streams (Table 3). However, data were too sparse, particularly for fourth order streams, to test the association between stream order and brook trout presence/absence using contingency table analysis without violating statistical assumptions. Using data summarized by U.S. Geological Survey (1989) in conjunction with our survey data, an estimated 159 km^2 (88%) of the drainage area of our study area is occupied by brook trout (including Beaver Run). Streams as small as 1.22 km contained thriving populations of brook trout.

Other fish species collected during the course of the survey included; *Rhinichthys atratulus, Salmo trutta, Semotilus atromaculatus,* and *Semotilus corporalis*. All species have previously been recorded from the Susquehanna River drainage system (Stauffer et al., 1982).

One stream with brook trout (Bear Run, Table 1) had pH values ranging from 5.0-5.6. Three streams without brook trout (all part of the South Branch of Bear Run, Table 1) had pH values ranging from 3.8-4.7.

DISCUSSION

Brook trout populations in the Allegheny Plateau region of Pennsylvania have been subjected to severe anthropogenic modification of the landscape and associated changes in habitat quality for centuries. Although brook trout populations are resilient in the face of natural cataclysms such as extreme natural flooding (Hoopes, 1975), they do not fare well in the face of wholesale conversion of landscapes and associated watersheds.

The first major regional impacts occurred during the extensive logging operations of the late 19th and early 20th centuries (Whitney, 1990). Clearcutting caused increased stream temperatures, increased siltation, unnaturally high floods (Tonkin, 1958), and other conditions that are detrimental to the health of brook trout populations (McAfee, 1966; Mohn, 1995). Later, extensive extraction of bituminous coal developed in the region with surface mining surpassing deep mining in production by 1977 (Phelps, 1983). Approximately 9.8% of the bituminous region of Clearfield County has been strip mined, followed by 2.5% and less than 1% of Indiana and Jefferson Counties, respectively (Schnell and Monmonier, 1983).

One of the negative outcomes of many past and present coal mining operations in Pennsylvania is the production of acid mine drainage and its attendant deleterious effects on stream organisms. Brook trout tolerate pH values from 4.1-9.5 (McAfee, 1966) but do very poorly in streams with a pH of less than 5.0 due to associated decreases in blood pH and the ability of blood to transport oxygen (Packer and Dunson, 1970). Their sensitivity to acid toxicity is heritable (Falk and Dunson, 1977) and generally decreases with age: survival of hatching, freshly fertilized and eyed eggs decreases dramatically at pH values of 5.2 or less, and survival of swim-up fry or yolk-sac fry decreases at pH values between 4.0-4.4 (Ingersoll et al., 1990). In addition, precipitation of $Fe(OH)_3$ in acid mine drainage causes decreased oxygen during formation and covers the gills, body surfaces and eggs of

TABLE 2. Summary statistics for stream lengths sampled during brook trout survey. Figures are in kilometers and percentages of row totals are in parentheses.

	Trout	
Stream order	Present	Absent
First	80.13 (66%)	40.44 (33%)
Second	41.39 (73%)	15.53 (27%)
Third	21.32 (80%)	5.17 (20%)
Fourth	0	4.81 (100%)
Total	142.85 (68%)	65.9 (32%)

TABLE 3. Frequency of brook trout presence/absence according to stream order. Row percentages are in parentheses.

	Trout	
Stream order	Present	Absent
First	20 (66%)	10 (33%)
Second	15 (68%)	7 (32%)
Third	5 (83%)	1 (17%)
Fourth	0	1 (100%)

fish living in the stream (Kimmel, 1983). During our survey brook trout were absent from all streams containing obvious $FE(OH)_3$ precipitate except for stream #11 (Table 1).

Pennsylvania has 4,800 km of streams polluted by daily mine drainage (Kimmel, 1983) including more than 780 km of streams in the WBSR basin (Kinney, 1964). Unfortunately, streams in the WBSR do not flow over extensive deposits of carbonate-bearing rocks and thus have little buffering capacity (Kimmel, 1983). As a result of the extreme degradation of many of the streams in the WBSR basin due to acid mine drainage, fish and invertebrate populations are depauperate or absent (Cooper and Wagner, 1973; Hocutt and Drawas, 1975) and this was confirmed during our survey. The surveys of Cooper and Wagner (1973) found many streams in the WBSR with no fish and pH values less than 4.5. In addition, they reported that brook trout were never observed in streams with pH values less than 5.0.

Even streams that are far removed from direct human activities are affected by atmospheric deposition of acids. Sharpe et al. (1987) surveyed 61 headwater streams on Pennsylvania's Laurel Hill and found that 12 (20%) did not contain trout and only 33 (54%) contained viable trout populations. Fish were absent in 10 (16%) of the streams surveyed. Streams without fish had lower pH and alkalinity, and higher dissolved aluminum relative to streams with fish. Watersheds buffered by limestone derived soils always contained trout. Data on acid runoff indicated severe episodic acidification following snowmelt and rain (Sharpe et al., 1984). Acid precipitation affects an estimated 3,236 km of stocked trout streams in Pennsylvania (Carline et al., 1992).

The principal factors governing the sensitivity of a stream ecosystem to acidification are the size of the drainage area and its buffering capacity. Since the dilution capacity of a stream system is largely dependent on quantity of flow, larger watersheds are less susceptible to acidification than small watersheds (Kimmel, 1983). Also, Carline et al. (1992) noted that because of atmospheric deposition of acids, Pennsylvania trout streams had lower pH values in their upstream reaches than they did downstream. These observations are consistent with our finding that higher order streams in the study area were more likely to have brook trout populations and more occupied habitat than lower order streams.

Judging from the low diversity of native fish species we encountered during our survey it appears that acid mine drainage and acid precipitation have had a profound effect on many of the species in the area. In most of the streams we surveyed, brook trout appeared to be the only fish present. Extensive surveys of Pennsylvania fish by Cooper and Wagner (1973) showed that brook trout were found at lower pH values than any of the other species we observed during our survey, suggesting that they persist when other species cannot. Alternative or synergistic explanations for low fish diversity include the infertile nature of many Pennsylvania trout streams (Cooper and Scherer, 1967; Hoopes, 1975) and the strong role brook trout play as piscivores (McAfee, 1966).

In other streams brook trout were absent in spite of stream conditions that looked superficially suitable for their survival (clean water, no obvious mine drainage, and forested watershed). Populations may have been extirpated from these streams due to atmospheric deposition of acids, stochastic events or acid mine drainage from old coal extraction activities. Some of these streams are isolated from other brook trout populations due to the fact that they drain into larger streams that are heavily polluted and do not support fish. When recolonization potential is zero, suitable streams should be evaluated for restocking with native brook trout and other native species from adjacent drainages.

The Pennsylvania Fish and Boat Commission, Division of Fisheries and Management completed a statewide inventory of trout populations. Surveys in Cush Creek during 1986-87 found significant improvements in water quality during the preceding 10 years despite extensive coal mining operations in the drainage system (Hollender and Wilberding, 1987). Brook trout biomass in the upper portion of the stream was estimated at 36.8 kg/ha including stocked fish. Based on the results of their survey they recommended reclassification of the upper stream from a cold water fishery to a high quality cold water fishery.

Similar surveys were conducted by the state in the South Branch of Bear Run and Bear Run in 1979 (Pennsylvania Fish and Boat Commission, file reports) and their results are largely congruent with ours. Not surprisingly, no trout were found in the lower South Branch of Bear Run due to intense acid mine drainage. As in our survey, no trout were collected in the upper tributaries of the South Branch of Bear Run but several *Semotilus atromaculatus* and nine families of invertebrates were present suggesting that some tributaries are suitable for restocking with brook trout. The Pennsylvania Fish and Boat Commission recommended Bear Run for continued management as a wild trout fishery. Our data suggest that this is one of the largest most pristine native trout fisheries in the study area and we recommend that the watershed be stringently protected.

Although Pennsylvania has thriving brook trout populations in many stream systems, some regions, such as our study area, have been severely depleted of their native trout stocks. However, small tributaries remain throughout the drainage that collectively support diverse populations of fish species, including brook trout, that are sensitive to high acidity (Cooper and Wagner, 1973). Thus, these streams are vital sources for natural restocking of native fish in the severely degraded but recovering upper West Branch of the Susquehanna River and its major tributaries (Cooper and Wagner, 1973; Hocutt and Drawas, 1975; Pennsylvania Fish and Boat Commission, file reports).

APPENDIX. List of fish collected and preserved during brook trout surveys along the upper West Branch of the Susquehanna River. Specimens are housed at the University of Georgia Museum of Natural History. Catalog numbers marked as "in part" are lot cataloged with other species from the same locality. Catalog numbers followed by numerals in parentheses indicate the number of specimens of the same species in the lot. Stream names follow those in Table 1.

Species	Catalog #	County	Stream	Collection Date
Rhinichthys atratulus	2843 (in part)	Indiana	Stream #33	26 Nov, 1981
Salmo trutta	2844 (in part)	Indiana	Stream #31	27 Nov, 1981
Salvelinus	2829-2830	Clearfield	Rock Run	2 Aug, 1981
fontinalis	2831 (2)	Clearfield	Sawmill Run	2 Aug, 1981
	2832	Clearfield	Stream #19	8 Aug, 1981
	2833 (3)	Clearfield	Martin Run	24 Jun, 1981
	2835 (2)	Clearfield	Stream #21	5 Sep, 1981
	2836 (in part)	Indiana	Stream #10	5 Sep, 1981
	2837 (3)	Clearfield	Stream #14	6 Sep, 1981
	2838 (3)	Clearfield	Stream #15	6 Sep, 1981
	2841 (3)	Indiana	Bear Run	26 Nov, 1981
	2843 (3, in part)	Indiana	Stream #33	26 Nov, 1981
Semotilus	2836 (in part)	Indiana	Stream #10	5 Sep, 1981
atromaculatus	2844 (in part)	Indiana	Stream #31	27 Nov, 1981

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TEMPORAL EFFECTS ON DETECTION OF EASTERN CHIPMUNKS *(TAMIAS STRIATUS)* IN FORESTED LANDSCAPES[1]

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ABSTRACT

We evaluated the effects of time of day and seasonal period on detection of eastern chipmunks (*Tamias striatus*) in a managed (clearcut) forest type and an unmanaged (uncut) forest type in central Pennsylvania. Eastern chipmunks are an important species to monitor in order to assess the effects of forest management because they have relatively high densities, are diurnal and vocal, and occur in a variety of habitat types. Two, 3.2 km long transects were traversed in each forest type during four seasonal periods in 1992 and 1993. All chipmunks detected within 35 m of an observer were noted, and detection mode (visual or auditory), time of day, and seasonal period was examined using a four-way test-of-independence. Of 647 chipmunks detected, most were detected by audition (90%) rather than visually (10%) in both forest types ($P < 0.001$). More chipmunks were detected in the managed forest type (56%) than in the unmanaged forest type (44%) ($P < 0.004$). Chipmunks were detected more often during early autumn compared to other seasonal periods ($P < 0.001$), perhaps because all ages and sex classes of chipmunks are active aboveground gathering a winter supply of mast at this time. Therefore, we suggest that counts of eastern chipmunks will be most cost- and labor-efficient when conducted during early autumn in both managed and unmanaged forest types.

[J PA Acad Sci 70(2):61-64, 1996]

INTRODUCTION

An even-aged system of forest clearcutting changes vegetative structure and species composition, which can have a major influence on forest small mammal communities (Krull 1970, Sullivan 1979, Yahner 1992, 1993). The eastern chipmunk (*Tamias striatus*) is an important mammal species to assess the effects of forest management for at least three reasons. First, chipmunks provide an adequate sample size for detecting effects of forest management, because they have relatively higher densities and smaller home ranges than larger sciurids, such as gray squirrels (*Sciurus carolinensis*) (Elliott 1978, Thompson 1978). Second, chipmunks are readily detected because they are diurnal and quite vocal (Yahner 1978*a*). Third, the eastern chipmunk is a habitat generalist, occupying mature forests, clearcut forests, farmland woodlots, and residential areas (Krull 1970, Hendersen et al. 1985, Yahner 1987).

Eastern chipmunks have been inventoried using mark-recapture techniques and time-area counts (Yahner 1982, Bowers et al. 1990). Mark-recapture techniques are important for collecting demographic data, but like time-area counts, are labor-intensive and are not useful for large areas (Healy and Welsh 1992). A line-transect technique, however, is a cost- and time-efficient method to inventory wildlife populations in large areas (Burnham et al. 1980, Healy and Welsh 1992). Our objectives were to use a line-transect technique to (1) compare detection of eastern chipmunks between a managed and an unmanaged forest type in central Pennsylvania, and to (2) evaluate the effects of time of day and seasonal period on detection of eastern chipmunks. Results of our study will be useful in developing cost-and labor-efficient techniques for inventorying and monitoring eastern chipmunk populations (after Rollfinke and Yahner 1990).

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MATERIALS AND METHODS

Our study was conducted at the Barrens Grouse Habit Management Area (HMA), which is a 1166 ha portion of State Game Lands 176, Centre County, Pennsylvania (Yahner 1992, 1993). The Barrens Grouse HMA consists of an unmanaged and a managed forest type of similar size. The unmanaged forest type has not been clearcut or disturbed within the last 70 years. The managed forest type consists of a checkerboard pattern of 136 contiguous, 4 ha blocks that are managed via an even-aged system of forest clearcutting. Each block is divided into 4, 1 ha stands. Sixty-six of the 136 blocks each contain two stands of different age that were clearcut during winter 1976-1977 and 1985-1987, respectively. An additional stand was clearcut during winter 1980-1981 in the remaining 70 blocks.

The unmanaged and managed forest types were each traversed by four, pre-existing, parallel, north-south transects 3.2 km long and 0.4 km apart. These pre-existing transects in the managed forest type were established along the boundaries of blocks (Yahner 1992, 1993); thus, transects were adjacent to stands of different age in proportion to their availability. On a given day, one transect in each forest type was walked at 1.5 km/h by a trained observer during three times of day: morning (within 3 h after sunrise), midday (1000-1400 h), or evening (within 3 h before sunset). Using this method, two observers could complete a count (four transects and three times of day) in six days during each of four seasonal periods: early autumn (10-24 September 1992), late autumn (10-24 October 1992), early spring (10-24 May 1993), and late spring (10-24 June 1993). We selected these times of the year to correspond to the seasonal periods when chipmunks are active above-ground. In addition, early spring coincides with pre-dispersal of the spring litter, late spring with dispersal of the spring litter, early autumn with dispersal of the summer litter and intensive larder hoarding of mast, and late autumn with continued larder hoarding and entrance of adults into torpor (Elliott 1978, Yahner 1978*b*, Yahner and Svendsen 1978). We did not conduct counts during midsummer and winter because above-ground activity of chipmunks is dramatically reduced during these seasonal periods (Brenner and Lyle 1975, Yahner 1977, Wrazen 1980).

All chipmunks detected within a 35 m strip centered on an observer were noted, and mode of detection (visual or auditory), forest type (managed vs. unmanaged), and time of day and seasonal period were recorded. A distance of 35 m was selected because we could see and hear chipmunks within this distance in both forest types. Counts were not conducted during rain or when wind velocity exceeded 24 km/h because chipmunk activity was reduced during these weather conditions (Forbes 1966).

We examined dependency of detection mode on forest type and time of day and seasonal period using a 4-way test-of-independence (Sokal and Rohlf 1981, Dixon 1990). Likelihood ratios were used to determine interactions of detection mode with the three other factors (forest type, time of day, seasonal period), using log-linear models (Sokal and Rohlf 1981, Dixon 1990). If detection of chipmunks was dependent on a factor with three or more levels, we used *a posteriori* *G*-tests-of-independence about the cell of interest.

RESULTS

We detected 647 chipmunks during the two years (Table 1). More chipmunks were detected by audition (90%) than visually (10%) in both forest types. Detection (by audition or visually combined) of chipmunks was dependent on forest type, with more chipmunks detected in the managed forest type (56%) than in the unmanaged forest type (44%). Detection of chipmunks also was dependent on time of day. Based on all detections combined, more chipmunks than expected (40%) were detected during evenings (G = 5.50, 1 df, $P < 0.025$) and fewer chipmunks than expected (26%) were detected during morning counts (G = 15.65, 1 df, $P < 0.001$). In addition, detection of chipmunks varied among times of year. More chipmunks than expected (49%) were detected during early autumn (G = 176.3, 1 df, $P < 0.001$) but fewer than expected were detected during early spring (4%) or late spring (18%) (G = 7.2, 1 df, $P < 0.01$). Detection of chipmunks was dependent on time of day, seasonal period, and forest type when considered concurrently (G = 26.63, 6 df, $P < 0.001$). We detected significantly more chipmunks during early autumn in the managed forest type compared to the unmanaged forest type regardless of time of day. In addition, more chipmunks were detected than expected during evening counts in late autumn in both forest types (G = 11.86, 1 df, $P < 0.001$).

TABLE 1. Number of chipmunks detected in an unmanaged and a managed forest type in central Pennsylvania in relation to detection mode and time of day and seasonal period, 1992-1993.

Detection Mode[a]	Forest Type[b]	Time of Day[c]	Seasonal Period[d] Early Fall	Late Fall	Early Spring	Late Spring	Total
Visual	Unmanaged	Morning	0	0	1	3	4
		Midday	3	5	0	1	9
		Evening	3	3	0	6	12
		Total	6	8	1	10	25
	Managed	Morning	9	0	0	3	12
		Midday	1	1	4	6	12
		Evening	1	7	1	5	14
		Total	11	8	5	14	38
Auditory	Unmanaged	Morning	24	6	4	28	62
		Midday	42	27	12	17	98
		Evening	37	44	2	19	102
		Total	103	77	18	64	262
	Managed	Morning	74	2	2	10	88
		Midday	62	21	2	16	101
		Evening	64	64	1	4	133
		Total	200	87	5	30	322
		Grand Total					647

[a]Significant difference between detection modes; G = 483.80, 1 df, $P < 0.001$.
[b]Significant difference between forest types; G = 8.25, 1 df, $P < 0.004$.
[c]Significant difference among times of day; G = 21.44, 2 df, $P < 0.001$.
[d]Significant difference among seasonal periods; G = 301.00, 3 df, $P < 0.001$.

DISCUSSION

We detected more chipmunks by audition than visually probably because most were vocalizing in response to presence and movement of a human observer (see Yahner 1978*a*). Chip-trills (Yahner 1978*a*), for instance, were given often when an investigator came within a few meters of a chipmunk. The relatively high numbers of chipmunks detected in the managed compared to the unmanaged forest type corresponds with the findings of Krull (1970), who also detected higher numbers of chipmunks in a clearcut forest than in an uncut forest in New York. However, we caution that higher relative abundances of chipmunks may not reflect higher habitat quality in the managed forest type compared to the unmanaged forest type (after Van Horne 1983). The managed forest type, for instance, has a lower density of mature, mast-producing trees (Yahner and Grimm 1984). Therefore, chipmunks in the managed forest type may have to remain active aboveground later in the season in order to secure a winter cache of mast comparable to that stored by chipmunks in the unmanaged forest type. These extended periods of activity may be reflected in a higher detection of chipmunks in the managed forest type.

We noted more chipmunks in early autumn than during other seasonal periods, which corresponds to the timing of autumnal larder hoarding and dispersal of individuals from summer litters from natal sites (Elliott 1978, Yahner 1978*b*, Yahner and Svendsen 1978). By late autumn, most adult chipmunks have entered winter torpor (Elliott 1978, Yahner 1978b). Conversely, young-of-the-year chipmunks may remain active later in the season to gain weight before entering torpor (Yahner and Svendsen 1978). In addition, because chipmunks have physiological intolerance to low ambient temperatures (Wang and Hudson 1971), they may prefer to forage during evenings in the late autumn period when temperatures were higher ($\bar{x}$ = 25.8°C ± 1.5) than other times of day (morning: $\bar{x}$ = 13.6°C ± 1.75; midday: $\bar{x}$ = 18.3°C ± 2.5) at the Barrens Grouse HMA (Mahan, unpubl. data).

In addition, ambient temperature may affect chipmunk activity during early spring. Yahner and Svendsen (1978) noted variability in the timing of spring emergence from winter torpor in relation to weather conditions. During a colder spring, emergence from winter torpor was delayed compared to a warmer spring. This variability in emergence dates may make monitoring of chipmunks less efficient during early spring compared to other times of year.

An accurate inventory and monitoring of wildlife populations are important for assessing biological diversity, monitoring relative changes in wildlife population abundance among landscapes, and enhancing the effectiveness of resource management programs on public and private lands (National Research Council 1992). Based on our findings, eastern chipmunks can be efficiently inventoried and monitored using a line-transect technique in managed and unmanaged forest types. We recommend that counts of eastern chipmunks in managed or unmanaged forest types be conducted during early autumn, because all age and sex classes are very active above ground, foraging and caching mast for winter use. In late autumn, counts should be restricted to evening hours.

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METAL ION UPTAKE AND STORAGE BY BRAIN MEMBRANES[1]

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ABSTRACT

The process by which metal ions are taken up and stored in brain tissue was investigated. When Pb^{2+} is added to porcine brain membranes, Mg^{2+}, K^{+}, Na^{+}, Ca^{2+}, and H^{+} are released. In more controlled experiments with membranes pre-treated with Sr^{2+}, addition of the physiologically active metals Li^{+}, Pb^{2+}, Cd^{2+}, Zn^{2+}, and Ca^{2+} released Sr^{2+} and H^{+} in an ion exchange process as demonstrated by measuring concentrations of bound and released species. Values of equilibrium constants for metals displacing Sr^{2+} (K_{ex}) reflected binding strengths of the metals. The ion exchange constant for Pb^{2+} displacing protons (K_{ex}^{H})was found to depend on pH.
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Brain cells, like all animal cells, are known to contain a significant amount of metal content, primarily in the form of various electrolytes such as Na^{+}, K^{+}, Mg^{2+} and Ca^{2+} ions (Guroff, 1980). Other metals, Zn^{2+}, Cu^{2+}, Mn^{2+}, and Fe^{3+} occur to a lesser extent. Physiological effects of metals include the crucial role of Na+ and K+ fluxes in nerve impulse generation. Also, Pb^{2+} exposure may lead to abnormal transport of fluid electrolytes across the blood brain barrier resulting in brain edema (Lajtha, 1982). One effect of Li^{+}, useful in manic depressive therapy, may involve interaction with sodium transport and the sodium pump (Guroff, 1980) in addition to its selective inhibition of inositol 1-phosphatase in brain tissue (Fisher, 1987).

The present work addresses the question of how metal ions are taken up and stored in brain tissue. Metal ions can bind to proteins in the brain and to brain lipids which occur mostly in cellular membranes. The lipid content of the brain is unusually high, amounting to 33% by dry weight of human gray matter and 55% of white matter (Siegel, 1987). Various anionic groups present in brain lipid material can provide binding sites for metals, such as phosphate in the phospholipids (plasmalogens, cephalins, sphingomyelin) (Guroff, 1980), sulfate in sulfatides (Guroff, 1980), and carboxylate in gangliosides (sialic acid residues) (Siegel, 1987).

Metal ions can bind to brain material in essentially two ways: (1) ion exchange, with release of an existing metal ion from an anionic site or H^{+} from an acid group, and (2) complexation at neutral sites. These two models have been discussed in detail for metal-algae (Crist et al., 1994; Crist et al., 1992a) and amine-algae systems (Crist et al., 1992b). The ion exchange model can be illustrated by equations 1a and 1b in which Zn^{2+} displaces Ca^{2+} or H^{+} from monovalent anionic sites X and where the solid phase material is denoted by parenthesis. This model leads to equations 1c and 1d for ion exchange and proton displacement, respectively. The symbols K_{ex} and K_{ex}^{H} represent these ion exchange constants for metal-metal and for metal-proton exchange. It is to be noted that they are dimensionless, enabling direct comparisons. The complexation model can be illustrated by equation 2a in which Pb^{2+} binds to neutral site Y. This model leads to the Langmuir equation 2b,

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ion exchange

$$Zn^{2+} + (CaX_2) \overset{K_{ex}}{\leftrightarrows} (ZnX_2) + Ca^{2+} \quad (1a)$$

$$Pb^{2+} + 2\,(HX) \overset{K_{ex}^{H}}{\leftrightarrows} (PbX_2) + 2H^{+} \quad (1b)$$

$$K_{ex} = \frac{[Ca^{2+}]\,(ZnX_2)}{[Zn^{2+}]\,(CaX_2)} \quad (1c)$$

$$K_{ex}^{H} = \frac{[H^{+}]^2\,(PbX_2)}{[Pb^{2+}]\,(HX)^2} \quad (1d)$$

binding with no displacement

$$Pb^{2+} + (Y) \overset{K_L}{\leftrightarrows} (PbY)^{2+} \quad (2a)$$

$$\frac{[Pb^{2+}]}{y} = \frac{[Pb^{2+}]}{y_{max}} + \frac{1}{K_L y_{max}} \quad (2b)$$

another form of the Scatchard equation (Marshall, 1978), where y is the amount of Pb bound (PbX_2) and y_{max} the maximum value when $[Pb^{2+}]$ is very high and all sites are occupied.

The present study was carried out specifically to determine whether or not metals *displace* other metals and/or protons on binding to brain tissue. This is an important question, since researchers frequently follow a simpler approach measuring only how much metal is bound and plot the data by a Scatchard plot neglecting the released species. A resulting curved plot is then interpreted as a system with multiple types of binding sites, but in fact the system may be one of ion exchange with only one type of site (Crist et al., 1994b).

EXPERIMENTAL PROCEDURES

Porcine brains were obtained from a local abattoir. Cells were fractured by freezing followed by treatment with a Thomas Tissue Grinder having a tight pestle. Six distilled water extractions, each with centrifuging for 15 min at 16,000 x *g*, gave a low-electrolyte content pellet which was used in all experiments. Light microscopic examination of material from the pellet revealed the presence of cellular fragments but few if any intact cells. Although the authors recognize the importance of a clear definition of the brain cell preparation, such analysis was not needed for this preliminary study. It is likely that the preparation studied contained primarily of fragments of membranes derived from neurons and from glial cells. This would include the myelin as well as membranes derived from intracellular organelles. It is assumed that most DNA and chromosomal proteins were eliminated as a result of the 16,000 x *g* centrifugation process. Likewise, it is assumed that most intracelluar enzymes and electrolytes were removed in the course of washing the cell fragments. Remaining metals were assumed to be bonded to anion sites, enabling metal exchange processes to occur both with metals and protons.

The amount and type of bound metals on these pellets were determined by proton displacement. A suspension of cell fragments was divided into aliquots to each of which was added HNO_3 to obtain a given pH in the range 4.5-6.5.

Metal ion uptake (all metals provided as nitrate salts) on porcine brain tissue was studied in a simplified system in which naturally occurring metals and protons were replaced with Sr^{2+}. A pellet suspension was treated with 0.005 M Sr^{2+}. A suspension using pellets was made 0.005 M in Sr and allowed to stand for an hour to exchange Sr for the contained metals with Ca the more strongly bonded being reduced some 20%. The sample was washed 4-5 times to remove excess Sr. This material was used to study the nature and extent of ion exchange with Ca, Cd, and Zn. For this 50 mg of material, suspended and in 10 mL H_2O, was treated in separate experiments with 0.2, 0.4, 0.7, 1.0 mL of 0.01 Zn, for example, the pH being held constant at 6.0 with 0.01 M LiOH. Li added, up to 0.2 mL, is weakly bonded (see Table 2) and would have little effect on the result. Zn and Sr in the aqueous medium and in the sample were obtained. For this the suspension was centrifuged, giving the liquid for AA analysis. The pellet was treated with 10 mL of 3M HNO_3, for 10 min to displace the sorbed metals and the liquid after centrifugation analyzed for Zn, Sr by AA. The dry weight of stock material used throughout was obtained by heating about 50 mg on a microscope slide at 80° C for some 15 min to a constant weight, being careful that no decomposition occurred. If HNO_3 treatment caused some oxidation, this would in no way affect the analysis.

For proton displacements, a pellet, 50 mg, was suspended in 10 mL H_20 and brought to pH 4.5, 5.0, or 5.5. To this was then added successively 0.2, 0.4, 0.7, 1.0 mL of 0.01 M Pb. The pH was held constant with 0.01 M LiOH, which also gave the amount of proton displacement.

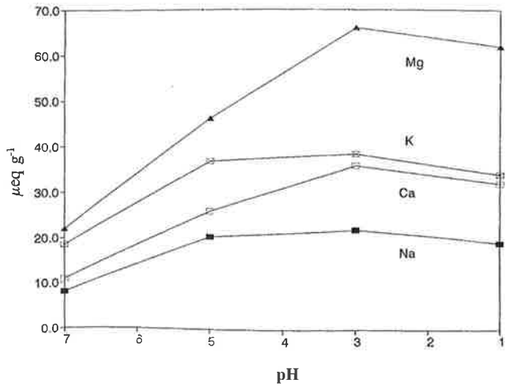

FIGURE 1. Proton displacement of metals from brain membranes.

RESULTS

Analysis of solutions showed that naturally occurring metals were displaced on acidification as shown in Fig. 1. The total amount of metals displaced at pH 1 was about 170 microequivalents per gram on a dry weight basis (μeq g^{-1}), with amounts decreasing in the order Mg^{2+}, K^+, Ca^{2+}, Na^+. Presumably these metals were retained on anion sites and were available for cation exchange.

For uptake of Zn^{2+} on Sr^{2+}-exchanged membranes, ion exchange constants were calculated by equation 1c and are given in Table 1. Values for K_{ex} are essentially constant over a wide range of aqueous concentrations and bound metal. Other metals were investigated for their ability to displace Sr^{2+} from brain membrane, and these results are given in Table 2.

Protons are also released when metal ions bind to the brain membranes. In order to calculate K_{ex}^{H} by equation 1d the amount of (HX) sites must be known. This value can be obtained by difference from the equation $(HX) = (HX)_{total} - [H^+]_{displaced}$. The value of $(HX)_{total}$ can be obtained in two ways, as illustrated by experiments with Pb^{2+}. (1) A 0.01 M Pb^{2+} solution was added successively to a membrane suspension, and H^+ released was back-titrated with 0.01 M LiOH to hold pH constant as shown in Figure 2; a plot of LiOH vs Pb^{2+} added gave a saturation value of 89 μeg g^{-1} taken as $(HX)_{total}$. (2) Increasing amounts of Pb^{2+} were added and the $[H^+]$ released taken as the amount of (PbX_2) bound at what had been (HX) sites. The $[H^+]$ released thus equals y in equation 2b for Pb^{2+} bound at such sites, and y_{max} by this analytical method equals $(HX)_{total}$. Rearrangement of equation 2b predicts that a plot of $[Pb^{2+}]/y$ vs. $[Pb^{2+}]$ would be linear with slope $1/y_{max}$;as shown in Figure 3, this plot is linear with slope giving $y_{max} = (HX)_{total} = 90.9$ μeq g^{-1}, comparable to the titration value for $(HX)_{total}$. It should be noted that though the actual system is one of ion exchange (equation 1b), the adsorption equation is useful as an

TABLE 1. Ion exchange constants K_{ex} for displacement of Sr^{2+} in the reaction $Zn^{2+} + (SrX_2) \leftrightarrows (ZnX_2) + Sr^{2+}$ at pH 6.5.

Experiment	1	2	3	4
Initial Zn				
$[Zn^{2+}]$, meq L^{-1}	0.40	0.80	1.40	2.00
Equilibrium concentrations				
$[Sr^{2+}]$, meq L^{-1}	0.183	0.227	0.306	0.320
$[Zn^{2+}]$, meq L^{-1}	0.0382	0.151	0.376	0.704
(ZnX_2), μeg g^{-1}	80.	146	280	260
(SrX_2), μeq g^{-1}	90.	60.	44	36
K_{ex} values[a]	4.2	3.6	3.8	3.2
		Ave. 3.7±0.3		

[a]Calculated from $K_{ex} = [Sr^{2+}]\,(ZnX_2)/[Zn^{2+}](SrX_2)$. Triplicate data averaged for each K listed. The separate experiments contained 50 mg dry weight of membrane fragments in 10 mL H_2O with Zn added to give indicated initial Zn concentrations.

TABLE 2. Ion exchange constants[a] for displacement of Sr^{2+} and H^+ from brain membranes by various metals.

metal added	pH	K_{ex}	K_{ex}^{H}, x 10^3
Li	6.5	0.0061±0.0004	
Ca	6.5	0.69±0.02	
Zn	6.3	3.7±0.3	
Cd	6.2	5.7±0.4	
Pb	4.5		4.3±1.5
Pb	5.0		3.0±0.3
Pb	5.5		0.58±0.01

[a]Triplicate data averaged for each K listed. See Table 1 for K_{ex} data for Zn.

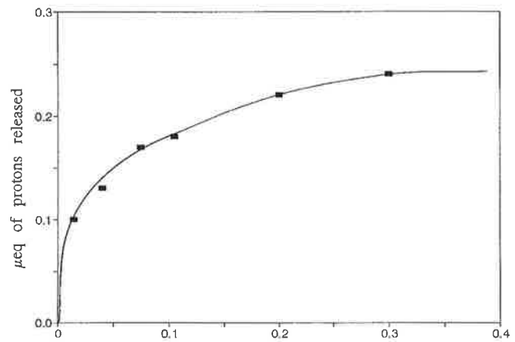

FIGURE 2. Proton displacement from membranes by Pb^{2+}. This method gives $(HX)_{total} = 89$ μeq g^{-1}.

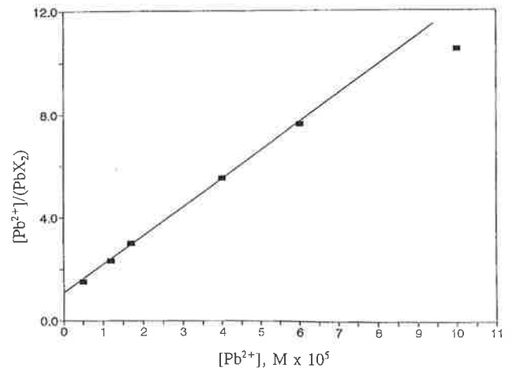

FIGURE 3. Binding of Pb^{2+} by brain membranes plotted according to Eq. 2b where $y = (PbX_2) = [H^+]$ released. The reciprocal of the slope gives $(PbX_2)_{max} = (HX)_{total} = 91$ μeq g^{-1}. This value is in good agreement with that of 89 μeq g^{-1} from Figure 2. See text for use of this adsorption equation in the present case.

empirical representation of the data *at high concentrations* (Crist et el., 1994b) such as in the present case.

Values of K_{ex}^{H} are given in Table 2 for Pb^{2+} at pH 4.5, 5.0 and 5.5 This range is determined by precipitation of Pb^{2+} at pH > 5.5 and by difficulty of measuring H^+ displacement at lower values.

DISCUSSION

Preliminary results showed that Pb^{2+} bound to brain membranes releasing both H^+ and metals (Ca^{2+}, Mg^{2+}, K^+, Na^+).This result indicates ion exchange, but the many metals present made mathematical simplification of the system necessary. This was done by replacing metals and H^+ by Sr^{2+}.

Uptake and storage of metal ions studied occurs by the ion exchange process, as shown by the constant values for calculated K_{ex} in Table 1.The K_{ex} constants for Cd^{2+}, Zn^{2+}, Ca^{2+}, and Li^+ decrease with values of 5.79, 3.66, 0.69, and 0.0061, respectively. For Pb K_{ex} was too difficult to determine, since at a pH which avoids precipitation the bound Sr^{2+} metal content is too low.

Lead, and to a lesser extent the other metals, displaced protons from brain membranes also in an ion exchange process. Values of K_{ex}^{H} for Pb were calculated by equation 1d and found to depend on pH. As the medium became more basic (pH change from 4.5 to 5.5), K_{ex}^{H} decreased sevenfold from $4.3x10^{-3}$ to $0.58x10^{-3}$. An explanation for this result is that (HX) represents a range of acid strengths. At low pH the stronger acids are still present in acid form,and such protons are easier to displace thereby giving larger K_{ex}^{H}. At higher pH these acids have ionized and only weaker acids are still in the acid form. It is more difficult for metals to displace such protons, since they are more strongly covalently bound in (HX) sites. A similar explanation was given for the 10^5 decrease in K_{ex}^{H} for Cd displacing protons from peat moss as the pH changed from 4 to 7 (Crist et al., 1996).

The present data show that toxic heavy metals readily replace the naturally occurring metals present in membranes. Interaction with heavy metals provided as complex ions was not examined, though this would be important in relation to their toxicity. From the present study it seems reasonable to conclude that the predominant mechanism of metal ion uptake and storage by brain membranes is one of ion exchange.

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COMPARISON OF HYPERCAPNIA AND DNP ON RESPIRATION IN CATS[1]

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ABSTRACT

This study compared the respiratory response to DNP (2,4-dinitrophenol) to that produced by CO_2 inhalation. The respiratory responses in spontaneously breathing anesthetized intact cats (n=18) were determined from changes in breathing pattern and $\dot{V}_e/CO_2$ slope. Breathing pattern was determined from respiratory rate (f), tidal volume (V_t), respiratory cycle timing (T_i and T_e), mean inspiratory airflow rate (V_t/T_i), and inspiratory duty cycle (T_i/T_{tot}). The results of this study indicate that while both DNP and CO_2 stimulate $\dot{V}_e$ (minute ventilation) through increases in both f and V_t the DNP stimulation was associated with a higher f and lower V_t when compared to equivalent $\dot{V}_e$ increases produced by CO_2. Both stimuli produced similar changes in V_t/T_i and V_t/T_{tot}. DNP produced a significant leftward shift, but not a change in slope, of the $\dot{V}_e/CO_2$ relationship. The $\dot{V}_e$ responses to the combined stimulation of DNP and CO_2 were additive. Peripheral chemodenervation (n=5) did not alter the $\dot{V}_e$ response to either DNP or CO_2. It is concluded that: 1)DNP and CO_2 increase respiration through different breathing patterns, 2) DNP does not alter the gain of the $\dot{V}_e/CO_2$ response, and 3) the interaction between DNP and CO_2 is additive.

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INTRODUCTION

Respiratory stimulation has long been associated with increased metabolic rate ($\dot{V}_{CO2}$). This coupling between minute ventilation ($\dot{V}_e$) and $\dot{V}_{CO2}$ is an important factor in CO_2 homeostasis and has been observed in a variety of metabolic conditions. For example, during light-to-moderate exercise the changes in $\dot{V}_{CO2}$ are tracked by $\dot{V}_e$ so that arterial P_{CO2} remains unchanged or decreases from pre-exercise values. In addition to exercise, $\dot{V}_e$ has been shown to follow $\dot{V}_{CO2}$ changes in non-exercise states such as feeding (Rodriguez et al., 1985), semi-starvation (Doekel et al., 1976), Luft's syndrome, a metabolic disease due to uncoupling of phosphorylation, (Edelman, Santiago and Cohn, 1975), thyrotoxicosis (Zwillich et al., 1978) and high altitude acclimatization (Huang et al., 1984). In addition, 2,4-dinitrophenol (DNP), an uncoupler of mitochondrial oxidative phosphorylation, has been used for the study of the respiratory consequences of increased CO_2 production (Adams, Swanson and Weil, 1982; Bailen and Horvath, 1959; Levine, 1979; Millhorn, Eldridge and Waldrop, 1982; Prabhakar et al., 1989).

These observations raise several questions regarding respiratory drive during episodes of elevated $\dot{V}_{CO2}$. For example, are the changes in respiratory pattern associated with increased $\dot{V}_{CO2}$ the same as those associated with elevated arterial CO_2? Is this metabolic hyperpnea the result of an enhanced CO_2 chemosensitivity? It was hypothesized that DNP stimulates respiration through mechanisms different from those underlying CO_2 chemosensitivity and therefore should not alter respiratory responses to CO_2 inhalation. The present study was designed to test this hypothesis by comparing ventilation and the respiratory response to CO_2 inhalation before and after DNP infusion. Metabolic and respiratory responses to DNP and CO_2 were assessed through changes in breathing pattern and the ventilatory response to CO_2 inhalation. The role of peripheral chemoreceptors in CO_2

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or DNP-stimulated ventilation was investigated by chemodenervation in five animals. The data indicate that the pattern of breathing produced by DNP stimulation differs from than that produced by CO_2 inhalation and that the DNP-related differences are not associated with alterations in CO_2 chemosensitivity.

METHODS

1. Surgical preparation

Data were collected from 18 intact and 5 chemodenervated adult cats (mean weight = 2.2 kg) anesthetized with alpha-chloralose (50 mg/kg, I.P.). After tracheostomy, all animals spontaneously breathed 100% O_2 through a one-way valve (Hans Rudolf, dead space=1.6ml). End-tidal P_{CO_2} was sampled through a small catheter placed in the lower trachea and measured with an infrared CO_2 analyzer (Beckman LB-2). Arterial blood pressure was monitored via a femoral arterial catheter also used for infusing DNP. A femoral vein was cannulated for administration of fluids or anesthetic as needed. Arterial blood samples (0.8 ml) were routinely obtained in duplicate and the P_{O_2}, P_{CO_2} and pH measured using a blood gas analyzer (Corning Model 175). Because of the potential thermogenic effects of DNP administration, great care was taken to prevent changes in core body temperature and its effect on respiration. Therefore, core temperature was carefully monitored using an esphageal temperature probe placed in a midthoracid position and maintained at 38°C with a water-circulating thermal blanket. Data were not included in this study if core body temperature changed during the course of the experiment.

2. Data Collection

CO_2 production (ml CO_2/min/kg) obtained by timed collections of mixed expired gas in a bag. The collected gas volume was measured using a Collin's small animal (11) spirometer and corrected to STPD was used as an index of metabolic rate. Respiratory airflow was measured using a Fleisch pneumotachograph and Validyne differential pressure transducer. V_t was obtained by electrically integrating the inspiratory and expiratory airflows. Inspiratory (T_i), expiratory (T_e), and total respiratory cycle (T_{tot}) times and respiratory rate (f) were measured from a strip chart recorder (Electronics for Medicine, Inc). Minute ventilation ($\dot{V}_e$) was calculated from f and V_t. Mean inspiratory airflow rate (V_t/T_i) and inspiratory duty cycle (T_i/T_{tot}) were also calculated.

3. Experimental protocol

Control airflow, end-tidal P_{CO_2}, and arterial blood gases, pH, and pressure were obtained from 18 intact animals. Two control CO_2 responses were recorded while the animal rebreathed from a bag containing an initial mixture of 7% CO_2 and 93% O_2. After recovery to baseline ventilation, a 4 mg/kg dose of DNP (Sigma) was administered as a 2 mg/ml solution adjusted to a pH of 7.4 with sodium bicarbonate. The DNP solution was infused over an 8-10 minute period into the abdominal aorta via the femoral arterial catheter. This slow infusion into a region of high blood flow provides for rapid dilution of DNP prior to reaching the tissues. After DNP infusion $\dot{V}_e$ and $\dot{V}_{CO_2}$ were determined at 5 minute intervals until both variables remained unchanged for 15 minutes while breathing 100% O_2. Baseline airflow and arterial blood gas and pH data were then recorded. This protocol was followed in the 5 animals in which the glossopharyngeal and vagus nerves had been sectioned bilaterally.

4. Data analysis

The respiratory, metabolic, and cardiovascular data obtained in each experiment were analyzed by comparing the data values obtained before and after reaching a post-DNP-infusion steady-state. In order to assess differences in breathing pattern and timing between DNP-stimulation and CO_2-stimulation, equivalent levels of $\dot{V}_e$ were used for comparison. Statistical significance between pre- and post-DNP data in each cat were determined by paired t-test. Differences were considered statistically significant if $P<0.05$. Statistical analysis software (Statgraphics, STSC) was used to do all statistical analysis. Results are expressed as mean ± SD.

RESULTS

1. Effect of DNP on metabolic rate and resting ventilation.

Figures la-d indicate the time course and magnitude of the metabolic, respiratory, arterial P_{CO_2}, and mean arterial blood pressure responses to single 4 mg/kg infusions of DNP in 18 neurally intact and spontaneously breathing animals. As indicated in Figs. la and lb the $\dot{V}_{CO_2}$ and respiratory responses to DNP were nearly fully developed within twenty minutes; all animals in this study attained respiratory and metabolic steady-state within 30 mins. Because of the parallel rise in metabolism and ventilation, the arterial P_{CO_2} at 30 mins (41 ± 3 torr) was not significantly different from pre-DNP control values (44 ± 4 torr; Fig. lc). Mean arterial blood pressure was not significantly altered by DNP (Fig. ld). In five animals, the respiratory and metabolic effects of DNP administration were observed for 90 mins; if additional DNP was not administered, both $\dot{V}_e$ and $\dot{V}_{CO_2}$ began to return toward control values. Carotid sinus nerve section (n=5) had no effect on either the magnitude or time course of the respiratory and metabolic responses or arterial blood gas levels.

The control $\dot{V}_{CO_2}$, 8±2ml CO2/min/kg (STPD), was associated with an average $\dot{V}_e$ of 245 ± 55 ml/min/kg and

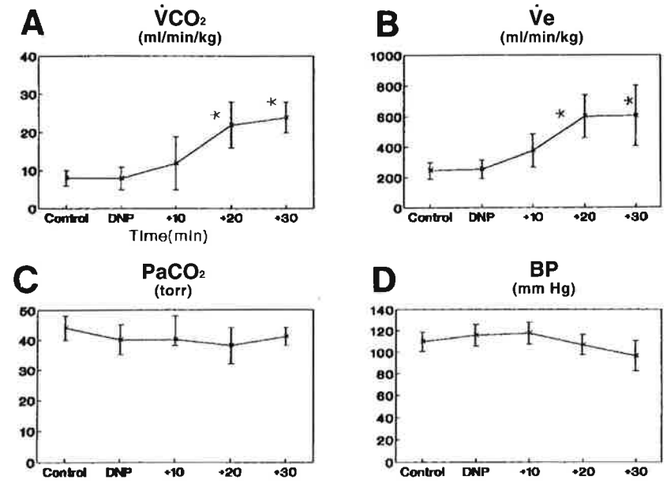

FIGURE I. The time course and magnitude of the DNP effects on (a) CO_2 production, (b) minute ventilation, (c) arterial P_{CO_2}, and (d) mean arterial blood pressure. Each data point represents the averaged (±SEM) value obtained from all neurally intact and spontaneously breathing cats (n=18). (*indicates P<.05).

arterial P_{CO_2} of 44 ± 4 torr. Following the infusion of DNP (4mg/kg), $\dot{V}_e$ and V_{CO_2} increased to 602 ± 198 ml/min/kg ($P<0.003$) and 23.6 ± 3.5 ml CO_2/min/kg ($P<0.001$), respectively. Because the average control $\dot{V}_e/\dot{V}_{CO_2}$ (31 ± 6 ml CO_2/min/kg) was not statistically different from that observed after DNP infusion (26 ± 6 ml/min/kg), the animals remained nearly isocapnic at the time both $\dot{V}_e$ and $\dot{V}_{CO_2}$ had attained steady-state (Fig. 2).

The $\dot{V}_e/\dot{V}_{CO_2}$ relationship became uncoupled in 5 cats when additional doses of DNP were administered (total cumulative dose ≥6mg/kg). Under these conditions $\dot{V}_e$ continued to increase after $\dot{V}_{CO_2}$ had reached a steady-state value, resulting in an average decrease in arterial P_{CO_2} of 12 ± 6 torr. This hyperventilation was always associated with elevations in core body temperature (>39°C). The data collected from hyperthermic animals are not included in these results.

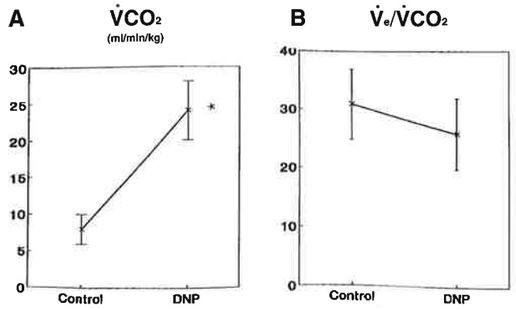

FIGURE 2. The effect of DNP on minute ventilation and arterial P_{CO_2}. Figure 2A shows the mean $\dot{V}_e$(n=18) obtained before, and 30 minutes after, the infusion of 4 mg/kg DNP ($P<0.05$)(2A). Figure 2B indicates that the average value of the arterial P_{CO_2} collected before, and 30 minutes after DNP infusion was not different ($P<0.05$).

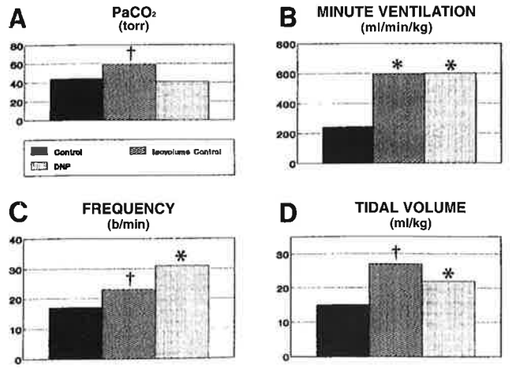

FIGURE 3. Comparison of DNP and CO_2 stimulation on (3A) arterial P_{CO_2}, (3B) minute ventilation, (3C) respiratory frequency, and (3D) tidal volume. For analysis of CO_2-and DNP-stimulated breathing patterns in this figure and Fig. 4, levels of CO_2-stimulated minute ventilation equivalent (isovolume) to steady-state DNP ventilation were compared (3B). In this figure and Fig. 4, the solid bars indicate control data, the hatched bars indicate data collected during CO_2 inhalation (for isovolume comparisons), and the stippled bars indicate data collected 30 minutes after DNP infusion. *indicates statistically significant differences ($P<0.05$) between control and experimental conditions (both CO_2 inhalation and DNP); dagger indicates significant differences between CO_2 and DNP-stimulated responses.

2. Breathing pattern changes produced by DNP and CO_2 inhalation.

In order to compare the effects of DNP and CO_2 on the breathing pattern, equivalent levels of respiration after DNP infusion (602 ± 233 ml/min/kg) and during CO_2 stimulation (599 ± 267 ml/min/kg; $PaCO_2$=59 ± 4 torr) were compared. Changes in respiratory volumes and timing produced by either DNP infusion or CO_2 inhalation in neurally intact cats are shown in Figs. 3 and 4. As seen in Fig. 3 both CO_2 and DNP increased $\dot{V}_e$ through increases in both V_t and f. However, at equivalent levels of ventilation, DNP stimulation generally resulted in higher f (31 ± 6 b/min) and lower V_t (22 ± 7 ml/kg) than CO_2 inhalation (f=23 ± 1 b/min, V_t=27 ± 3ml/kg).

Figure 4 shows that at equivalent levels of ventilation, DNP infusion and CO_2 inhalation produced similar values for V_t/T_i and inspiratory duty cycle (T_i/T_{tot}). V_t/T_i rose from 6.9 ± 2.3 ml/sec/kg during control to 25 ± 10 ml/sec/kg after DNP infusion and 26 ± 3 ml/sec/kg during CO_2 rebreathing. The increases in V_t/T_i were due to a similar decreases in T_i produced by DNP (-31%) and CO_2 (-25%). Although T_i was decreased significantly (P<.01) by both DNP and CO_2, parallel decreases in T_{tot} (-37% and -31% respectively) prevented T_i/T_{tot} from being changed. T_e was significantly (P<.005) shortened by both DNP (-36%) and CO_2 inhalation (-28%),

3. Effect of DNP on the hypercapnic ventilatory response.

The effect of DNP on the hypercapnic ventilatory response was measured in 7 intact and 5 peripherally chemodenervated cats. The experiments in intact animals, summarized in Fig. 5, indicated that the gain of the CO_2 response was not altered by DNP, although there was a slight, but statistically insignificant, increase in the slope.

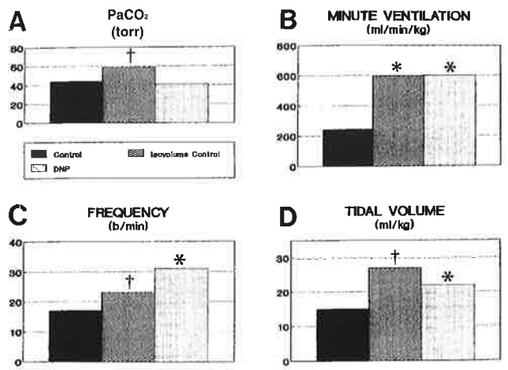

FIGURE 4. Comparison of the effects of DNP infusion and CO_2 inhalation on mean inspiratory airflow, inspiratory and expiratory times, and inspiratory duty cycle. The bar legends are the same as in Fig. 3.

On the other hand, the value of the CO_2-axis intercept was significantly decreased from 34 torr to 22 torr (P<.05). Peripheral chemodenervation did not alter either the slope or threshold of the CO_2 response.

The additive nature of DNP and CO_2 stimulation to minute ventilation is illustrated in Fig. 6. The vertical arrows from Point (a) to Point (b) and Point (c) to Point (d) represent the V_e response to CO_2 before and after DNP infusion, respectively. The increase in $\dot{V}_e$ from Point (a) to Point (c) represents DNP stimulation alone, while Point (d) is the $\dot{V}_e$ response to the combined stimulation of DNP and CO_2.

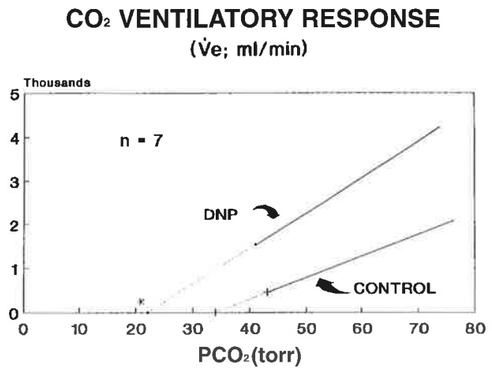

FIGURE 5. The effect of DNP on the hypercapnic ventilatory response. The lower line represents the control ventilatory response to rebreathing CO_2; the upper line is the ventilatory response from the same animals after administration of 4 mg/kg DNP. The slopes of the two response lines were not statistically different, but the decrease in CO_2 axis intercept after DNP infusion was different from control values (P<.05).

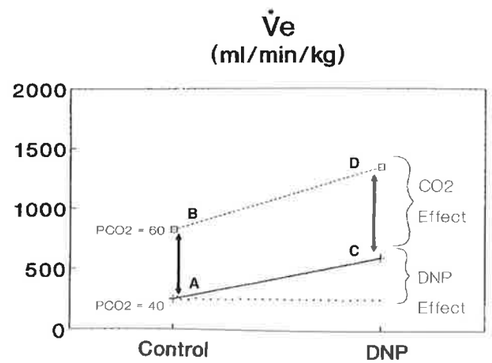

FIGURE 6. This illustration compares the individual and combined effects of DNP and CO_2 stimulation of minute ventilation ($\dot{V}_e$). The vertical arrow from Point (a) to Point (b) is the ventilatory response to CO_2 inhalation alone. The solid line from Point (a) to Point (c) is the isocapnic response to DNP infusion. The dashed line from Point (b) to Point (d) represents the ventilatory response to CO_2 after DNP infusion. Point (d) is the ventilatory response to the combined stimulation of DNP and CO_2. The combined effects of CO_2 and DNP on minute ventilation show an additive interaction.

DISCUSSION

This study was designed to compare the respiratory response to DNP with that to CO_2 inhalation in the anesthetized, spontaneously breathing cat. DNP has been used previously to study the relationship between tissue $\dot{V}_{CO_2}$ and respiratory control mechanisms in the dog (Bailen and Horvath, 1959; Levine, 1979; Levine and Huckabee, 1975; Liang and Hood, 1976; Williams et al., 1958), the cat (Adams, Swanson and Weil, 1982; Millhorn, Eldridge and Waldrop, 1982; Prabhakar et al., 1989), and duck (Geiser et al., 1989). Liang and Hood (1973) and Ramsey (1959), using dogs, and Geiser et al., (1989) using ducks, have noted that many of the cardiorespiratory effects of systemic DNP administration are similar to those observed during exercise.

Data from the present study indicate that in the absence of temperature changes systemic administration of DNP in the cat leads to parallel changes in both $\dot{V}_e$ and $\dot{V}_{CO_2}$. Thus, the $\dot{V}_e/\dot{V}_{CO_2}$ rate and arterial P_{CO_2} remained unchanged by arterial DNP infusion. Previous studies have reported either poor $\dot{V}_e/\dot{V}_{CO_2}$ coupling (Levine, 1979; Millhorn, Eldridge and Waldrop, 1982; Williams et al. 1958) or nearly isocapnic respiratory responses (Geiser et al., 1984; Levine and Huckabee, 1975; Laing and Hood, 1976; Laing and Hood 1973; Prabhakar et al, 1989). One study using DNP reported that following an initial isocapnic response in cats, ventilation continued to rise after $\dot{V}_{CO_2}$ reached a steady-state, resulting in arterial hypocapnia (Millhorn, Eldridge and Waldrop, 1982). Similar studies using salicylates have reported that ventilation increases in excess of metabolism resulting in arterial hypocapnia (Millhorn, Eldridge and Waldrop, 1982, 1983). Their failure to observe isocapnia at constant body temperature may have resulted from elevated brain temperature related to the higher doses of DNP administered (5-20 mg/kg). We observed dissociations between ventilation and metabolism only in those instances where core body temperature rose above control values. Although the primary effect of DNP is to uncouple oxidative phosphorylation, at doses higher than 4 mg/kg DNP there may be an increase in core body temperature (Levine 1979; Laing and Hood, 1973; Ramsey, 1959; Williams et al., 1958).

Minute ventilation changes produced by either DNP or CO_2, resulted from increases in both tidal volume and respiratory rate. There was, however, a stimulus-dependent difference in the relative contribution of these two ventilatory factors. The respiratory rate increase was greater with DNP than with CO_2 inhalation at similar ventilatory levels. Isocapnic frequency and tidal responses to DNP in the present study are comparable to those reported in both the duck (Geiser, et al., 1984) and the dog (Laing and Hood, 1976). However, when analyzed in terms of mean inspiratory flow rate (V_t/T_i) and inspiratory duty cycle (T_i/T_{tot}) there was no difference in the breathing patterns produced by either DNP or CO_2 inhalation. Similar results have been reported when comparing the effect of CO_2 inhalation with the effects of exercise on the breathing pattern in man (Askanazi, et al., 1979).

The relationship between elevated $\dot{V}_{CO_2}$ and respiratory CO_2 chemosensitivity ($\dot{V}_e/P_{CO_2}$) has been the subject of many studies. Because the stimulation of respiration by DNP in this study was not associated with a significant change in the slope of the $\dot{V}_e/P_{CO_2}$ response, it is unlikely that the DNP stimulation of ventilation is due to increased $\dot{V}_e/P_{CO_2}$. There was, however, a significant decrease in the CO_2 intercept. Studies in man have indicated that there may be a increase in the slope of the $\dot{V}_e/P_{CO_2}$ response associated with exercise (Weil et al, 1972) or feeding (Rodriquez et al., 1985). However, others have reported no change in $\dot{V}_e/P_{CO_2}$ response in patients with Luft's syndrome (Edelman, Santiago and Cohn, 1975). Studies in dogs have shown either enhanced (Williams et al., 1958) or attenuated (Levine and Huckabee, 1975) $\dot{V}_e/P_{CO_2}$ responses to CO_2 after DNP administration. The multiplicity of species, experimental conditions, and findings makes it difficult to draw conclusions concerning the relationship between metabolic rate and CO_2 chemosensitivity.

The mechanism(s) of the DNP stimulation of $\dot{V}_e$ have not been identified, although both central and peripheral components have been implicated. The importance of central respiratory structures in the DNP response has been demonstrated in recent experiments in which $AgNO_3$ applied to the ventral medullary surface blocked the respiratory response to DNP but not the respiratory response to stimulation of the mesencephalic locomotor area, carotid sinus nerve, or somatic afferents (Prabhakar et al., 1989). The CO_2 inhalation data from vagotomized and carotid sinus denervated animals in the present study suggest that if DNP acts on structures at or near the ventral medullary surface they are probably those responsible for central CO_2 chemosensitivity. The present data are consistent with the hypothesis that part of the DNP-induced hypernea may be the result of direct action on central neural structures. This hypothesis is supported by experiments in which salicylate, another metabolic stimulant, increased $\dot{V}_e$ without raising whole body $\dot{V}_{CO_2}$ when introduced into the lateral cerebral ventricles (Millhorn, Eldridge and Waldrop, 1983).

The contribution of peripheral afferents to the DNP hyperpnea may also be a significant factor. Levine and Huckabee (1975) and Levine (1979), using cross-perfused dogs, reported that the majority (89%) of the overall ventilatory response to DNP in their studies originated from non-carotid body somatic inputs. Ramsey (1959) reported that perfusion of vascularly isolated but neurally intact hind limbs with DNP leads to a hyperpnea that is abolished by section of the afferent nerves, a view supported by Liang and Hood (1976) but refuted by

Bailen and Horvath (1959). The presence of tissue metabolism receptors has been further supported by studies demonstrating increases in $\dot{V}_e$ produced by intra-aortic infusions of sodium cyanide in carotid and aortic denervated dogs whose heads were isolated from the peripheral circulation (Levine, 1975).

The data in this and other studies are consistent with the hypothesis that DNP acts directly on both central and peripheral structures to increase respiratory drive. If this direct effect were associated with neural metabolism it is possible that neural activity could be altered in proportion to CO_2 production in other tissues.

In summary, the present study in cats indicates that: (1) DNP-stimulated ventilation increased in proportion to metabolic CO_2 production, (2) the pattern of breathing was stimulus-dependent (3) the gain of the hypercapnic ventilatory response was not altered, and (4) the interaction between DNP stimulation and the CO_2 drive was additive.

ACKNOWLEDGMENT

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THE DIET OF *PERCINA CAPRODES (RAFINESQUE), (PISCES: PERCIDAE)* IN SMALL STREAM IN PENNSYLVANIA[1]

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ABSTRACT

Stomach contents of 112 logperch, *Percina caprodes* (Rafinesque), were studied from Woodcock Creek upstream of Woodcock Creek Lake near Blooming Valley in Crawford County, Pennsylvania. Six collections were made from November 1990 through October 1991. Chironomids were the dominate prey item found in the stomach contents of the logperch. Simuliids, tipulids and ephemeropterans were predominately utilized in November and trichopterans in May and October. No significant difference ($p>0.05$) existed in the feeding habits of logperch based on sex or age class. Oligochaete worms comprised the bulk of the benthos sampled on all collection dates; however, they were absent in the stomach contents of logperch.
[J PA Acad Sci 70(2):75-79, 1996]

INTRODUCTION

Percina caprodes (Rafinesque), logperch, is one of the most widely distributed of all darters (Scott and Crossman, 1973) and occupies the Ohio River basin and much of the upper St. Lawrence basin. It is native or introduced in some Atlantic drainages from the Potomac to the Hudson. The logperch inhabits a variety of environments and is found in warm streams to large rivers and lakes. It often populates reservoirs, which is unusual among darters (Kuehne and Barbour, 1983). The species is uncommon in small streams, which lack chutes and strong riffles (Kuehne and Barbour, 1983). In Pennsylvania, *P.c. caprodes,* Ohio logperch, is restricted to the Mississippi Valley and Great Lakes drainages. It has been found spawning near the mouth of small tributaries to Lake Erie (Cooper, 1983). Cooper (1983) reports that it is not a swift water species and can tolerate wide environmental conditions.

Logperch are visual feeders; feeding during daylight hours. Keast and Webb (1966) report that its feeding behavior consists of a series of short, forward searching movements with intervening rests on the bottom. It uses a rounded knob on its snout to overturn leaves and small stones to expose potential food. When a prey is discovered it is secured with a sudden dart forward; the ventro-terminal mouth snapping shut. Emery (1973) reported that at night logperch seek cover and become inactive. The unique feeding behavior of *P. caprodes* and other logperches allows it to exploit food resources not available to other darters.

The feeding habits of *P. caprodes* have been reported by Turner (1921), Dobie (1959), Mullan et al. (1968), Thomas (1970), Murray and Tarter (1979) and others. Most of these studies, however, were restricted to feeding behaviors of logperch collected from reservoirs, lakes, or large streams and also did not compare the presence of aquatic insects in the stomach versus that available in the environment. The main objective of our study was to determine the diet of *P. caprodes* collected from a small stream and to compare diets of different age classes and sexes.

MATERIALS AND METHODS

Six collections were made in Woodcock Creek upstream of Woodcock Creek Lake in Crawford County, Pennsylvania, near Blooming Valley from November 1990 until October 1991. At this site, Woodcock Creek is approximately 5-10m wide and is heavily wooded on both sides. The substrate is mostly rubble, cobble, and gravel in riffle areas and sand and silt in pools. Water depth in the area studied ranged seasonally from 0.25m to 1.5m.

Logperch were collected with a 1.5 x 3.0m, 3.2-mm

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mesh seine for approximately 1 hour during daylight hours. Specimens were placed in a 10% formalin solution and within a few days transferred to 50% isopropyl alcohol and deposited in The Pennsylvania State University Fish Museum. Standard lengths of preserved specimens were measured to the nearest (mm) using dial calipers after the stomachs had been removed (Hubbs and Lagler, 1958). After removal of stomach contents, each logperch was weighed and measured to the nearest (g) using a digitized scale. Stomach analysis involved dissection and inspection of the entire digestive tract. Stomach contents of 112 logperch were identified under a stereomicroscope to the lowest practical taxon and enumerated. Specimens were sexed based on the presence or absence of modified scales on the breast. Presence of scales indicating males and absence indicating females. Age classes of logperch were determined from scale examination. Several scales were taken from the side of the body below the lateral line at a point midway between the first and second dorsal fins (Steinmetz and Muller, 1991), placed in a drop of water and annuli were counted using a stereomicroscope. Specimens were grouped into 0+, 1+ and 2+ age classes.

After each fish collection, benthic samples were collected with a D-frame kick net (0.9mm mesh) as described by Frost et al. (1970). The substrate was disturbed directly upstream of the net; nine randomly chosen samples (20 sec each) were taken and pooled. Macroinvertebrates were fixed in 50% isopropyl alcohol with a small amount of formalin and were identified, using stereomicroscope, to the lowest practical taxon according to Peckarsky (1990). The diet of logperch was characterized by combining data from all stomachs on each collection date. Strauss' Linear Index (L) was used to compare the prey items of the logperch with those available in the environment:

$$L = r_i - p_i;$$

where r_i and p_i, are the relative abundances or proportions of prey item *i* in the gut and habitat (Strauss, 1979). The index ranges from -1 to +1; with positive values indicating preference and negative values indicating avoidance or inaccessibility. A selection value of 0 indicates that a prey item was consumed in the same proportion as it occurred in the environment. A two sample t-test was used to determine if significant differences ($p < 0.05$) in feeding habits existed among age classes and between sexes.

RESULTS

Average standard length (SL) of logperch was 69.6 mm ±10.7, with a mean weight of 3.5 g ± 1.7. Mean SL of males was 71.1 mm ± 9.6, with an average weight of 3.6 g ± 1.7. Female mean SL was 68.3 mm ± 11.4, with a mean weight of 3.4 g ± 1.8. Mean SL of age class 0+ logperch was 58.2 mm ± 8.2, mean weight of 2.0 g ± 0.87, age class 1+ averaged 72.5 mm ± 6.2, mean weight 3.9 g ± 1.3, and age class 2+, 81.1 mm ± 8.3, with a mean weight of 5.4 g ± 2.3.

In the benthos collections (n=9), a total of 7,034 macroinvertebrates were identified. Oligochaetes were the most numerous taxa (10-60%) found in the benthos on all collection dates (Figure 1). Heptageniids (mayflies)

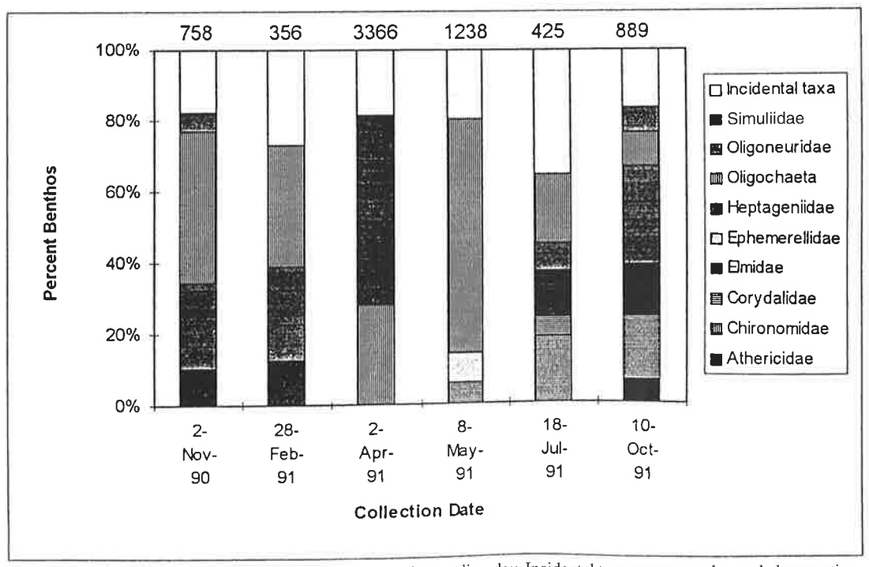

FIGURE 1. Plot of taxa proportions collected from the benthos on each sampling day. Incidental taxa represents the pooled proportions of taxa that did not comprise at least 5% of a sample on at least one day. Numbers above the bar indicate the number of macroinvertebrates collected on each date.

comprised 5-28% and elmids (beetles) comprised 10-14% of the benthic samples. Chironomids constituted 5-18% of the benthic samples collected in April thru October. Simuliids (blackflies) were only found in the April collection, comprising 45% of the benthic samples.

A total of 1,264 food items were identified from stomach contents of logperch. Numerically, chironomids dominated the food throughout the year (35-91%) (Table 1); except on 2 November 1990 (Figure 2). In November, logperch fed primarily on elmids, simuliids and tipulids (craneflies). There was no food found in the stomachs of logperch (n=19) collected in February. The number of macroinvertebrates (n=356) collected in February benthos samples was not significantly different ($p > 0.05$) than macroinvertebrates collected in July (n=425). Chironomids comprised 44-91% of the prey items consumed by all three age classes in April thru July. In May, chironomids (44%) and hydroptilids, (caddisflies), (45%) were consumed in relatively equal proportions. In October, logperch consumed a variety of organisms with the bulk of the diet consisting of chironomids (35%) and limpets (24%).

Strauss' Linear Index indicated that logperch showed a preference for chironomids on April and July collection dates. In November, logperch showed a preference for simuliids (L=0.14) and tipulids (L=0.24) compared to chironomids (L=0.01). In May, Strauss' Linear Index value was slightly higher for hydroptilids than chironomids and in October, limpets were slightly favored to chironomids. Oligochaete worms, although found in the benthic samples, in abundance had a negative food selection index. The food selection index indicated that logperch either avoided the worms or they were inaccessible to predation. Both males and females primarily consumed dipterans (chironomids), ephemeropterans and trichopterans, with chironomids comprised over 30% of the diet on all collection dates except November. Strauss' Linear Index showed that both males and females preferred the same prey organisms on all collection dates except May. In May, males preferred hydroptilids (Trichoptera) and females preferred chironomids (Diptera). A two sample t-test indicated no significant difference ($p > 0.05$) in the feeding habits of logperch based on sex.

TABLE 1 Percentages of various food items found in the stomachs of Age Class 0+, 1+, and 2+ logperch collected at Woodcock Creek, Pennsylvania. (** = food items composing <0.01% of stomach contents)

	Collection Date														
	1990						1991								
	NOVEMBER			APRIL			MAY			JULY			OCTOBER		
Age Class (sample size)	0 (5)	1 (7)	2 (2)	0 (0)	1 (9)	2 (4)	0 (0)	1 (9)	2 (2)	0 (2)	1 (8)	2 (1)	0 (0)	1 (3)	2 (4)
Diptera															
Chironomidae					0.76	0.44		0.48	0.18	0.93	0.92	0.86		0.08	0.45
Simuliidae		0.16			0.11	0.48		**			0.01				0.03
Tipulidae	0.17	0.27			0.01			**		0.04	0.04				
Ephemeroptera															
Unidentified to Family		0.02	0.25		0.01	0.04		**	0.05	0.02					0.03
Baetidae	0.17				0.01			**	0.02		**				
Empididae								0.02							
Emphemerellidae											**				
Heptageniidae		0.12	0.5					**				0.14			0.03
Leptophleidae								0.01							0.03
Oligoneuridae		0.08												0.17	0.03
Trichoptera															
Unidentified to Family		0.02						0.01							
Hydroptilidae								0.44	0.5		**				
Hydrospychidae		0.03			0.03	0.04									0.03
Polycentropodidae	0.17	0.08							0.02		0.01			0.42	
Psychomidae					0.03										
Hydracarina															0.06
Amphipoda		0.02													
Anisoptera											**				
Annelidae															
Oligochaeta	0.17														
Coleoptera															
Unidentified to Family		0.02													
Elmidae	0.17	0.16									**			0.08	0.06
Plecoptera								**							
Perlidae	0.17		0.25												
Fish Eggs								**	0.25						
Limpet		0.02												0.25	0.24

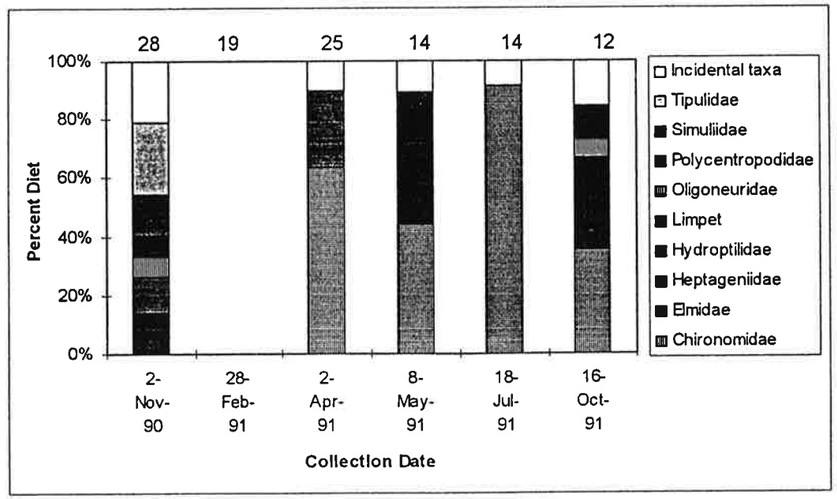

FIGURE 2. Plot of taxa proportions collected from logperch stomachs pooled for each sampling day. Incidental taxa represents the pooled proportions of taxa that did not comprise at least 5% of a sample on at least one day. Numbers above the bar indicate the number of logperch collected on each date.

Ephemeropterans were utilized heavily in November, whereas trichopterans (hydroptilids and polycentropodids) were consumed in May and October. Food was only found in the stomach of age class 0 logperch collected in November and July. As a result, we are unable to speculate on dietary shifts in maturing logperch. A two sample t-test indicated no significant difference ($p > 0.05$) in feeding habits of logperch based on age class. Age class 1 logperch consumed more total prey items on all collection dates except October 1991.

DISCUSSION

Chironomids were the predominant prey found in the stomachs of logperch throughout the study. They were, however found in the benthos samples in low proportions. Chironomids may have been under estimated in the environment because they demonstrate distinct preference for certain types of substrate, creating 'patchy' or microdistrubutional patterns. Because of their clumped distribution they may have missed in benthos samples, thus causing low abundance estimations. Dipterans (simuliids and tipulids) and ephemeropterans were predominantly utilized in November, and trichopterans in May and October. Oligochaetes comprised the bulk of the benthos samples on all collection dates however, they were not found in the stomach contents of logperch. The lack of feeding seen in February is possibly due to the inability of the logperch, because of reduced metabolism at low temperatures, to forage effectively to obtain prey and not scarcity of prey items. Reduced rates of feeding at low temperatures have been observed in many fishes (Keast, 1968; Mathur, 1973; Weddle, 1992).

Percina caprodes is a benthic invertivore feeding primarily on insect larvae (Turner, 1921; Mullen et al., 1968; Keast and Webb, 1968, and Murray and Tarter, 1979). A diet of small, bottom-dwelling organisms correlates with the position, diameter, and structure of the small, ventro-terminal mouth (Keast and Webb, 1996). Turner (1921) noted specimens collected from Lake Erie and inland lakes fed primarily on small crustaceans with amphipods and insect larvae increasing in the diet as the logperch matured. Logperch collected from streams, however, ate small crustaceans constantly, but in small proportion with Diptera and Ephemeroptera constituting most of the diet (Turner, 1921). Turner (1921) suggested that *P. caprodes* in its very early stages is a surface feeder becoming a bottom feeder as it matures. The results of our study are consistent with the results of previous studies.

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SOME FUNGAL TOXINS CAN AFFECT THE PERCENT GERMINATION AND GERM TUBE ELONGATION OF *RHIZOMUCOR MIEHEI* SPORANGIOSPORES[1]

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ABSTRACT

Studies were done to determine the effect of five fungal toxins (helvolic acid, patulin, penicillic acid, rubratoxin B and secalonic acid) on the germ tube elongation and percent germination of sporangiospores from *Rhizomucor miehei*. Only patulin and secalonic acid caused a statistically significant decrease in percent spore germination. All five toxins had a statistically significant effect on germ tubes, either stimulating or inhibiting their elongation.
[J PA Acad Sci 70(2):80-82, 1996]

INTRODUCTION

Both mesophilic and thermophilic fungi are common inhabitants of decaying vegetation and the compost used as a substrate for producing mushrooms. Mesophilic species of *Aspergillus, Penicillium, Trichoderma, Gliocladium,* and other fungi producing exotoxins are commonly found in such substrates (Fergus 1964) along with a number of species of thermophilic actinomycetes and fungi including *Rhizomucor pusillus* (Lindt) Schipper (Fergus 1964) and *R. miehei* (Cooney & Emerson) Schipper (Cooney and Emerson 1964). Since sporangiospores are the principal means used by *R. pusillus* for propagation, they should be exposed to the exotoxins produced by mesophilic microbes in the earlier stages of composting. Only two studies are known dealing with the effect of fungal toxins on the germination of spores from thermophilic fungi (Deploey et al. 1993; Thakre and Johri 1973). The report by Thakre and Johri (1973) involved a study of the effect of 3 antibiotics (aureofungin, amphotericin B and hamycin) on the germination of spores from *Aspergillus fumigatus* Fresenius, *Humicola lanuginosa* (Griff. & Maubl.) Bunce, *Rhizopus* sp. and *Sporotrichum thermophile* Apinus. The study by Deploey et al. (1993) centered on the effect of 5 fungal toxins on the germination and germ tube elongation of mitospores from *Dactylomyces thermophilus* Apinus and *R. pusillus.* Neither of these two reports included a study of the effect of fungal toxins on the germination of *R. miehei* sporangiospores. Since *R. miehei* plays a role in the decomposition of vegetation and the retting of guayule shrub (Cooney and Emerson, 1964) used as a source of latex, we decided to determine how five fungal toxins affect both the percent germination of sporangiospores from *R. miehei* and the elongation of their germ tubes.

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MATERIALS AND METHODS

Rhizomucor miehei (Fergus E-21) was obtained from the Department of Biology, Pennsylvania State University. It was cultured at 40°C for 3 days in tubes containing sterile, slanted, yeast extract-glucose (YG) agar (yeast extract, 5g; glucose, 10g; agar, 20g; distilled water, 1L). Washed suspensions of *R. miehei* spores were prepared from these cultures (Deploey 1985), and the concentration was adjusted to 500,000 spores/ml by adding sterile glucose-basal (GB) liquid medium (Deploey and Fergus 1975).

The five toxins used in this study were helvolic acid, patulin, penicillic acid, rubratoxin B, and secalonic acid. Three concentrations of each toxin were prepared (50, 25, 10 µg/ml). The GB liquid medium containing spores and the solutions containing the different concentrations of each toxin were prepared at double the strength stated. This was done so that when equal volumes of the spore suspension/GB medium (1ml of each) and a toxin solution were mixed, they diluted each other to the desired concentration (50, 25, 10 µg/ml). Fifteen sterile test tubes (5 toxins, 3 conc.) were prepared by adding equal volumes of the liquid GB medium/spore suspension and the appropriate concentration of a toxin. Two different controls were prepared. One control contained equal volumes of the liquid GB medium/spore mixture and 0.8% sterile, aqueous acetone solution to simulate the concentration of acetone used to dissolve four of the toxins (helvolic acid, penicillic acid, rubratoxin B, secalonic acid). The second control contained equal volumes of the liquid GB medium/spore mixture and sterile distilled water. All of the tubes were sealed with sterile parafilm and incubated in a water bath at 45°C for six hr. Percent germination was determined by examining 100-300 spores (450X) from each tube. The average germ tube length of a minimum of 12 randomly selected spores was determined. Experiments were repeated three times. All materials used were of reagent grade. The toxins were obtained from the Sigma Chemical Co., St. Louis, MO.

RESULTS AND DISCUSSION

Two of the toxins, patulin and secalonic acid, caused a statistically significant decrease in percent germination of *Rhizomucor miehei* sporangiospores (Table 2), but secalonic acid was the most effective (Table 1) causing a

TABLE 1. Effect of fungal toxins on the percent spore germination and germ tube elongation of *Rhizomucor miehei* sporangiospores incubated in liquid glucose basal medium at 45° C for 6 hr.

Toxin Conc. µg/ml[b,c]	Helvolic Acid		Patulin[a]		Penicillic Acid		Rubratoxin B		Secalonic Acid	
	% Germ	Germ Tube Length µm	% Germ	Germ Tube Length µm	% Germ	Germ Tube Length µm	% Germ	Germ Tube Length µm	% Germ	Germ Tube Length µm
10	83.8	81.5	76.9	36.8	84.4	55.2	89.1	139.7	2.1	1.2
25	80.7	77.6	49.1	28.1	82.4	50.0	83.8	77.3	2.3	1.5
50	88.3	89.0	8.0	8.1	37.6	13.7	59.0	27.9	9.6	1.8

[a]Dissolved in aqueous solution. Remaining toxins dissolved in aqueous acetone solution.
[b]Control (GB only) - Percent germination - 85.7, germ tube length = 99.8 µm.
[c]Control (GB plus acetone) - Percent germination = 74.8; germ tube length = 30.7 µm.

TABLE 2. Statistical analysis (anovar, Duncan's multiple range) of the effect of fungal toxins on the percent spore germination and germ tube elongation of *Rhizomucor miehei* sporangiospores incubated in liquid glucose basal medium at 45°C for 6 hr.

	Helvolic Acid		Patulin		Penicillic Acid		Rubratoxin B		Secalonic Acid	
	% Germ	Germ Tube Length µm	% Germ	Germ Tube Length µm	% Germ	Germ Tube Length µm	% Germ	Germ Tube Length µm	% Germ	Germ Tube Length µm
Anovar P =	0.3266[a]	0.0185[b]	0.004[b]	0.0364[b]	0.1388[a]	0.0698[b]	0.1716[a]	0.0156[b]	0.0001[b]	0.0271[b]
Statistically Significant		0 & 10[c]	0 & 50	0 & 10		0 & 50		0 & 10	0 & 10	0 & 10
Pairs		0 & 25	10 & 50	0 & 25		10 & 50		10 & 50	0 & 25	0 & 25
(Duncan's Multiple Range)		0 & 50	25 & 50	0 & 50		25 & 50			0 & 50	0 & 50

[a]No statistically significant difference between the control spores (0 µg toxin/ml) and the spores incubated in the toxin solution.
[b]A statistically significant difference was detected between the control spores and spores incubated in solutions containing toxins or between the spores incubated in the solutions containing different concentrations of toxins.
[c]Toxin concentrations (µg/ml).

virtual cessation of germination when compared to the controls. Also, the decrease in percent spore germination caused by patulin was dose-related occurring only at the 50 µg/ml level, while percent germination was significantly decreased (Table 2) when the spores were incubated in all three concentrations of secalonic acid.

The effect of patulin on percent germination is also clearly species specific as evidenced in a report by Deploey et al. (1993) where the percent germination of sporangiospores from *R. pusillus* was reduced but conidia from *Dactylomyces thermophilus* were unaffected. Similar results were also obtained by Reiss (1973) who studied the effect of patulin on the germination of spores from *Cladosporium herbarum* Link ex Fries and *Rhizopus nigricans* Ehrenberg, both mesophiles. He reported that germination of conidia from *C. herbarum* was markedly inhibited when incubated for 18 hr in liquid nutrient medium containing 100 µg/ml of patulin, whereas *R. nigricans* spores germinated but produced very short germ tubes compared to the controls.

Helvolic acid, penicillic acid and rubratoxin B (Tables 1 and 2) did not affect the percent germination of spores from *R. miehei* when compared to the controls. However, in the report by Reiss (1972), it was demonstrated that the hyphae of certain fungi are damaged by exposure to 100 µg/ml of rubratoxin B. Of the seven fungi he studied, three species were affected by the toxin. Of these, he noted that the germ tubes of *R. nigricans* were short and of irregular shape and the hyphal branches of *Aspergillus niger* van Tiegham and *A. flavus* Link often "were swollen and much septate or had formed spherical bodies with diameters of 18 to 21 µm." No such abnormalities of cell morphology in the germ tubes of *R. miehei* were noted in this study, but a statistically significant increase in germ tube length occurred (Table 2) when germinating spores were incubated in solutions containing rubratoxin B (10 µg/ml). This same effect was observed (Table 2) when germinating spores were exposed to penicillic acid (50 µg/ml) and helvolic acid (10, 25, 50 µg/ml).

While both patulin and secalonic acid inhibited germ tube elongation (Table 1), secalonic acid proved to be the most potent inhibitor resulting in almost total stoppage of hyphal growth at all three concentrations tested. Germ tube elongation by sporangiospores of the thermophile *R. pusillus* (Deploey et al., 1993) and the mesophile *R. nigricans* (Reiss, 1973) was also reported to lag behind that of the controls when they were incubated in solutions containing patulin at certain concentrations, but elongation of *D. thermophilus* germ tubes (Deploey et al., 1993) was increased as the concentration of patulin decreased.

In related studies, Wadia et al. (1986) found that culture filtrates of various fungi containing undefined metabolites diminished percent spore germination and germ tube elongation of *Pestalotiopsis palmarum* (Cooke) Steyaert, a mesophile. Additionally, the antibiotic aureofungin proved to be effective (Thakre and Johri, 1973) in reducing germ tube length and percent germination of spores from thermotolerent and thermophilic fungi. Furthermore, Thakre and Johri (1973) demonstrated that the addition of nutrients to the culture medium could curtail the effectiveness of aureofungin in reducing germ tube elongation and percent germination of the spores compared to that occurring when a water agar/aureofungin medium was used. No reports could be found in the literature concerning how nutrients influence the effectiveness of fungal toxins to reduce germ tube elongation and percent germination of sporangiospores from *R. miehei.* Furthermore, no reports were found documenting the presence of fungal toxins in compost or decaying vegetation. However, it seems reasonable that fungi, known for producing exotoxins, will produce these exotoxins in their natural habitat. These problems will be the focus of continuing research of the physiology of spores from thermophilic fungi.

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THE USE OF LIMESTONE FOR PHOSPHORUS PRECIPITATION IN A HYPEREUTROPHIC POND[1]

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ABSTRACT

A small, hypereutrophic pond in Harrisburg, Pennsylvania receives nutrient rich inflow from a first order stream subject to agricultural non-point source loading. A limestone filter was designed to remove phosphorus from that inflow stream and therefore decrease phytoplankton production. Total phosphorus, ammonium, nitrate/nitrite, total alkalinity, and phytoplankton biomass were monitored when the inlet was open, closed, and then fitted with a porous steel filter containing limestone chips. Samples were taken in the epilimnion, hypolimnion, inflow, and outflow, on three occasions during the summer of 1995. A significant correlation was found between a decreasing phosphorus concentration and algal counts in the epilimnion after installation of the limestone trap. The filter was successful in significantly reducing total phosphorus concentrations and improving transparency in the pond.
[J PA Acad Sci 70(2):83-87, 1996]

INTRODUCTION

Long term lake management must deal with both the symptoms of eutrophication (i.e., increased algal or macrophyte growth) and the causes of eutrophication (excess nutrient loading). In freshwater systems, phosphorus is typically the limiting nutrient. Increasing the phosphorus concentrations in freshwater results in increasing productivity (Schindler 1974). While in most lakes, point sources of phosphorus have been identified and controlled over the last two decades, non-point sources (NPS) continue to be a problem. Lakes in agricultural and/or urban areas are particularly susceptible to high phosphorus loading from non-point sources (Omerlink 1977). In the Commonwealth of Pennsylvania, Duda (1985) estimated that more than 50% of the lakes were threatened by non-point source pollutants. In many small lakes, with moderate to large drainage basins, eutrophication control must incorporate a strategy for reducing that source of phosphorus.

Historical studies indicate that pre-treatment or dilution of high phosphorus sources to lakes can significantly reduce algal productivity, particularly in lakes with short hydraulic turnover times (Edmondson 1970). Pretreatment is technically challenging or cost prohibitive in many large lakes, however it is a viable alternative for eutrophication management in small lakes with well defined inflows. Pretreatment typically involves phosphorus precipitation. The most commonly used in-lake method for phosphorus precipitation is the addition of chemicals that produce low solubility salts or sorption products. Aluminum, iron, and calcium compounds are most often used because of their affinity to bind phosphorus, relatively low cost, and minimal toxicity to aquatic organisms at most application doses.

Aluminum sulfate (alum) has been used almost exclusively in municipal wastewater treatment and has also been used to a lesser extent in eutrophication control. Alum removes phosphorus both as aluminum phosphate and as a colloidal aluminum hydroxide floc which tightly sorbs phosphorus. While an effective method, alum application has some limitations. The removal of phosphorus by alum is strongly pH dependent and effectiveness decreases below pH5 and above pH7 (Cooke et al. 1993). Adding aluminum ions to softwater lakes can result in a drop in pH that must be buffered by additional salts like sodium aluminate (Smeltzer 1990). In addition, the loss of $Al(OH)_3$ from the water column requires that at least 2x the stoichiometric dose to remove phosphorus must be applied (Snoeyink and Jenkins 1980) Finally, an often overlooked side effect of alum application is the impact of the sulfate moieties on internal phosphorus loading in some lakes (Murray 1995).

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Iron is also effective at phosphorus removal under oxidized conditions when $Fe(OH)_3$ dominates. Iron salts like $FeCl_3$ or $Fe_2(SO_4)_3$ are used in some municipal wastewater treatments plants. However, the presence and stability of $Fe(OH)_3$ is both pH and Eh dependent. Singer (1972) documented the maintenance of phosphorus binding under reducing conditions if ferrous phosphate (vivianite) forms. Under the typical conditions of low Eh and pH encountered in the hypolimnion of stratified lakes however, phosphorus can be released from the iron. That limitation requires additional management strategies, like aeration or sediment oxidation, to be effective.

Low pH conditions in the hypolimnion also affect the chemistry of calcium compounds. Calcium carbonate precipitation is a natural regulator of algal production in many hardwater lakes (Otsuki and Wetzel 1972). Calcium carbonate sorbs phosphorus, and at high pH when calcium is in excess, forms hydroxyapatite;

$$5Ca^{2+} + 3PO_4^{3-} + OH^- = Ca_5(PO_4)_3(OH)(s)$$

Unlike both iron and aluminum sorption of phosphorus, hydroxyapatite has its lowest solubility at pH>9.5 (Cooke et al. 1993). The effectiveness of hydroxyapatite precipitation is a function of both nucleation time and the rate of crystal growth (Ferguson et al. 1971; 1973). In field studies, slaked lime ($Ca(OH)_2$)has been shown to be the most effective form of calcium used in phosphorus removal (Murphy et al. 1988). Prepas et al. (1990) documented reductions in particulate organic carbon, chlorophyll *a* and a species switch away from *Aphanizomenon* when lime slurries were applied to Figure Eight Lake, Alberta. Calcium compounds are generally lower in toxicity than aluminum or iron compounds, and should therefore be a useful tool in eutrophication control in small water bodies.

The objective of this study was to assess the effectiveness of a limestone filter in removing phosphorus from the inflow of small, privately owned, hypereutrophic pond. Limestone was selected over calcite or slaked lime due to its greater availability and lower cost to a typical homeowner. The hypothesis of this study was that the limestone filter would remove phosphorus from a nutrient rich inflow, and that precipitation would result in decreased algal production and increased transparency.

MATERIALS & METHODS

Study site

Laurel Pond is a small (Z_{max}=1.5m), artificial, hypereutrophic pond near Harrisburg, PA. It has a volume of 575 m^3 and a surface area of 520 m^2 (Figure 1). A first order stream runs parallel to Laurel Pond, and the pond has inflow and outflow structures to and from the stream. The stream drains primarily agricultural areas to the south and west of the pond. The drainage basin has a slope

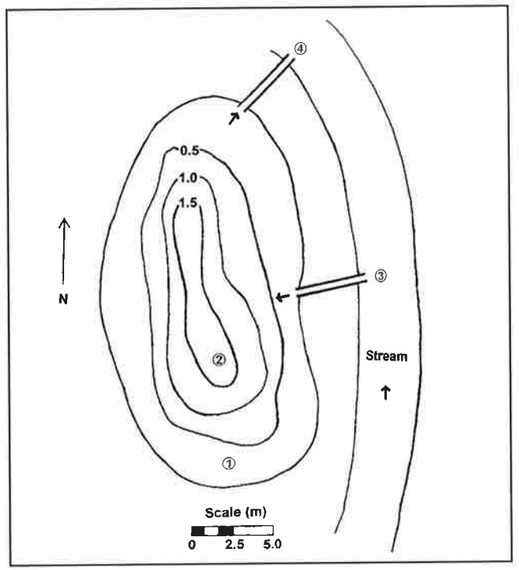

FIGURE 1. Bathymetric Profile of Laurel Pond (m). Samples were taken in the epilimnion (1),hypolimnion (2), inflow (3), and outflow (4).

≥15% on both sides of the stream leading to high storm flows with poor quality runoff. Even during baseflow conditions, nutrient concentrations in the stream are high. One consequence of that poor quality water is late summer blooms of *Anabaena* and *Microcystis* in the pond.

The 10 cm diameter inlet pipe from the stream to Laurel Pond is usually kept open by the property owner unless there is heavy rain. A similar size outlet pipe allows return flow to the stream. The epilimnion (0 to 0.8 m) of Laurel Pond in late summer is characterized by a deep green-brown color with large mats of blue-green algae floating on the surface. The hypolimnion (l-l.5 m) remains oxic during summer stratification. A pilot study conducted in 1994 indicated that when the inlet was closed off from the stream the transparency improved in less than two weeks.

Sampling & Chemical Analyses

Water quality in Laurel Pond was monitored in three stages during a late summer algal bloom in 1995. The first set of samples was collected during baseflow conditions when the inlet pipe was open. A second set was collected after the inlet was closed for one week and a final set was collected after the inlet was open for three weeks and fitted with a limestone filter.

The filter was a cylindrical wire mesh basket (15 cm tall x 11.5 cm diameter) that was filled with approximately 0.5 Kg of limestone chips from a local quarry. The limestone chips had an average mass of 67g (±6g)

and an average surface area of 58 cm^2 ±5 cm^2). The total surface area of the limestone was approximately 1285 cm^2. No attempt was made to quantify the amount of calcium in the limestone.

Sampling was focused on the late summer when previous algal blooms had been observed. On August 14, 1995, water samples were obtained at four sites: the inflow pipe, epilimnion, hypolimnion, and the outflow pipe (Figure l). The inlet was then closed and samples were again collected at the four sites on August 21. The inlet was opened for one week, and on August 28 the limestone filter was attached to the end of the inflow pipe. Samples were obtained for a final time three weeks later, on September 19. On each occasion, dissolved oxygen and temperature were recorded with a YSI Model 51B DO Meter. Secchi disk depths were determined and water samples were collected in acid washed polyethylene bottles. Water samples were kept at 4°C until analysis.

All water samples were subsampled and analyzed in triplicate for total phosphorus, nitrate/nitrite, ammonium, total alkalinity, pH, and total algal cells present. Analysis for total phosphorus (P_{tot}) was accomplished using a modified Molybdate/Ascorbic Acid procedure with a nitric/sulfuric acid digestion(APHA 1995). Nitrate/nitrite was analyzed by cadmium reduction and diazotization (Hach, 1984). Determination of ammonium was performed by direct Nesslerization (Hach 1984). pH data were recorded in the laboratory using a Corning Model 320 pH meter. Standard curves for all analyses were strongly linear (R^2=.99).

In order to assess any impact of the limestone on acid neutralizing capacity, total alkalinity was measured with a Gran titration (Wetzel and Likens 1991). Algal counts were determined using a Sedgwick-Rafter counting cell and 30 random fields of view (Wetzel and Likens, 1991). One way analyses of variance (ANOVAs) and correlation analyses were performed to assess significance of limestone application on nutrient concentrations, algal biomass, and transparency across both sampling times and sites.

RESULTS & DISCUSSION

The nitrate/nitrite analyses indicated a decrease of approximately 280 µg/L in the epilimnion after the pond was closed off from the stream for one week, attesting to the large contribution of nitrogen from the stream prior to the experiment (Figure 2). The limestone filter did not significantly reduce nitrate/nitrite levels in the epilimnion (F(2,6)=0.95, ns). Dissolved nitrates presumably were outcompeted by binding sites on the limestone surface by phosphates, which have a higher affinity for complexing with calcium. The filter did prevent large quantities of organic material from entering the pond (small leaves, detritus, etc), although particulate nitrogen was not determined to test whether total nitrogen in the pond had decreased. No significant flow reduction was measured with the filter in place.

Analysis of ammonium concentrations (Figure 3) revealed that levels in the epilimnion were initially higher than in the stream indicating augmentation from either N_2 fixation or decomposition of autochthonous organic matter. The inlet stream is normally a significant source of NH_4^+ indicated by the epilimnetic decrease (530 µg/L) when the inlet was closed. Hypolimnetic ammonium increases after application of the filter indicate increased respiration of organic matter, although the cause for that increase was not determined. Settling of

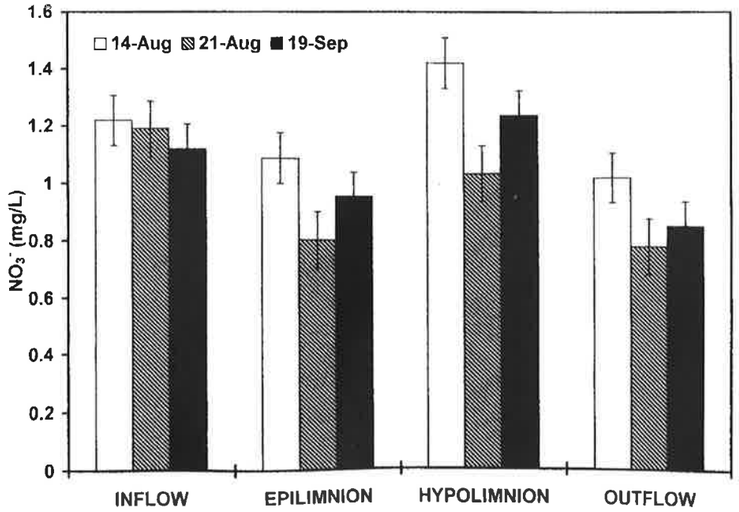

FIGURE 2. Nitrate in Laurel Pond. Nitrate concentrations in the epilimnion were not significantly lower with the limestone filter in place.

algal cells through the water column and subsequent decomposition is one possibility for the increase in NH_4^+.

Total phosphorus concentrations at the inflow pipe also indicate the overall poor quality water in the inflow stream (Figure 4). On August 14, epilimnetic P_{tot} concentrations were 85% of those measured in the stream. After the inlet was closed for a week, epilmnetic P_{tot} concentrations had decreased to approximately sixty-two percent of stream concentrations. During that week, water color changed from green-brown to a light green, implying a decrease in algal growth in the epilimnion. Algal counts decreased over that time period (Table 1).

Temperature and oxygen data indicate that the pond was weakly stratified during the study, but the hypolimnion was not anoxic. This supports the hypothesis that the majority of P in the pond was infiltrating from the stream and internal loading from the sediment was much less of a factor. The lack of strong stratification and short hydraulic turnover time limited internal phosphorus loading.

TABLE 1. Water Column Chemistry in Laurel Pond. Significant correlations were observed between total algal counts and Secchi disk depth (r=0.975) and epilimnetic phosphorus and total algal counts (r=0.953).

Sample Date	Total Algal Counts (cells/mL)	Secchi Depth (m)	Epilimnetic Phosphorus (µg/L)
August 14,1995	255	0.32	165
August 21, 1995	148	0.57	102
September 19, 1995	109	0.74	78

When compared to the open-inlet condition, the limestone filter was successful in significantly reducing total P concentrations in the epilimnion [$F(2,6)=7.93, p<.01$]. A Pearsons correlation between algal counts and Secchi disk depths (measurements of light extinction) revealed a nearly perfect negative relationship between the two parameters (r=-0.975) (Table 1). A significant positive correlation was found between epilmnetic algae and P_{tot} (r= 0.953). That would be expected if phosphorus were the limiting nutrient in the pond. An anonymous reviewer correctly pointed out however, that the correlation would also be expected because P_{tot} includes phosphorus stored in algal biomass. In that case, soluble phosphorus would have been a more useful correlate and should be taken into consideration in future studies.

The analysis of P_{tot} (Figure 4) reveals that as stream concentrations remained high throughout the three sampling dates, total P concentrations in the pond declined more dramatically with the limestone filter in place. Acid neutralizing capacity (mean ± SE=77 ± 3.4 mg $CaCO_3$/L) was not significantly increased by the filter.

The results of this experiment suggest that in small, shallow aquatic ecosystems, the use of a limestone filter on a nutrient rich inflow can reduce the amount of total phosphorus entering receiving water bodies, constraining algal growth, and improving water quality. In this study the source of the nutrients was a stream receiving agricultural NPS loading. For the owners of small lakes and ponds suffering from late season algal blooms, this method offers a low cost, low maintenance tool for phosphorus precipitation and eutrophication management.

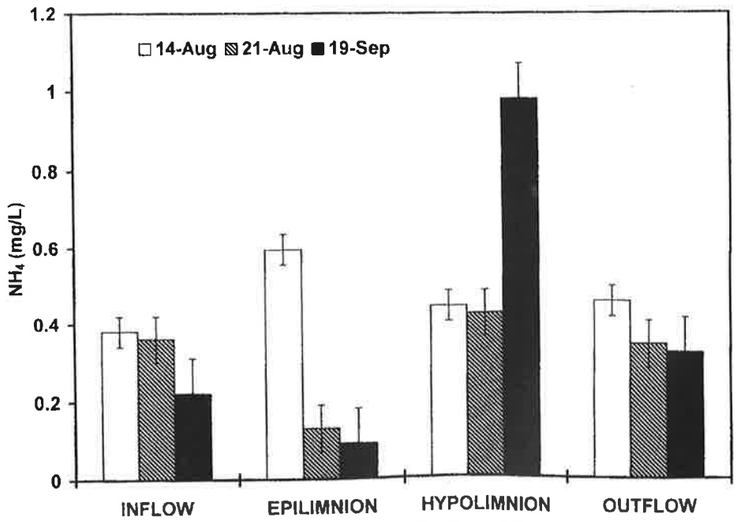

FIGURE 3. Ammonium in Laurel Pond. Epilimnetic ammonium decreased when isolated from the stream, but no further significant decrease occurred with the filter in place.

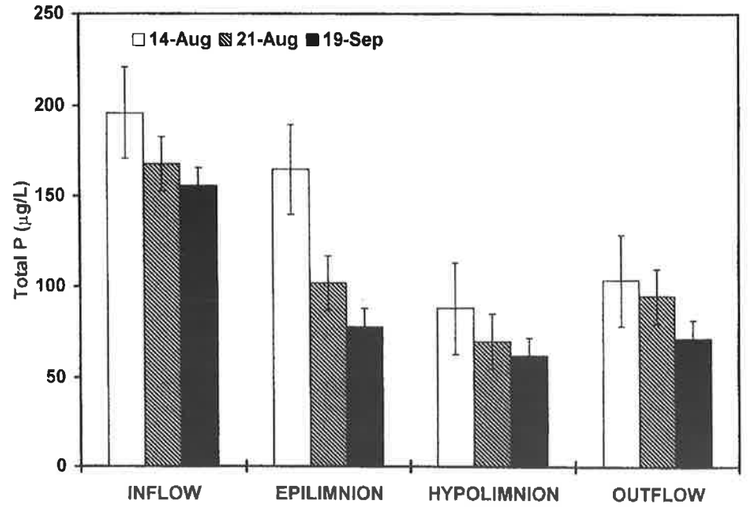

FIGURE 4: Total Phosphorus in Laurel Pond. Epilimnetic phosphorus concentrations were significantly lower with the limestone in place.

ACKNOWLEDGEMENTS

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RETIREMENT AND MIGRATION: THE CASE OF PIKE COUNTY, PENNSYLVANIA[1]

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ABSTRACT

Pennsylvania's aged population (over 65 years), large by national standards, has increased over recent decades in every county. Although rates of change varied widely, one constant in aged growth remains—Pike County. Located in the Delaware Valley/Pocono Plateau, opportunities for recreation and tourism abound and second-home owners are estimated to outnumber full-time residents by almost two to one. These factors practically guarantee continued growth of the aged population of Pike County. Data on retired migrants to Pike County were collected on the basis of age, sex, place of birth, marital status, occupation at the time of retirement as well as place of work, educational attainment, and the like by a survey/ questionnaire. The focus, however, was to determine why these retirees migrated to Pike County. Although responses to that question varied, Pike's rural character, prior knowledge of the county and, most importantly, the prior ownership of a second home in Pike County were well represented in the mix.
[J PA Acad Sci 70(2):88-93, 1996]

INTRODUCTION

Pike County is a phenomenon—the leading county in growth in a state which has languished over the period from 1970 to 1990, and did not grow very much in the earlier decades listed on Table 1. Concerning the aged, Pennsylvania is among the national leaders, just behind Florida in percentage change, 1980 to 1990 — and Pike County has been among the Commonwealth's leaders since 1940, at least. Such vigor has not gone unnoticed. In a study published more than 30 years ago, Simkins found that relatively large concentrations of aged in the state were usually the result of out-migration of younger cohorts, but that Pike County was different, offering as an alternate explanation aged in-migration. Simkins said "Unless Pike County has experienced an unusual population development in the past, the most likely explanation is that the in-migration included a substantial portion of aged retiring to this resort area" (1). Similarly, in work of mine almost as old, aged in-migration was found to contribute to Pike's growth during the 1960s (2). The fact is that aged migration simply was not as common in the '60s in this society. In 1964, Thomlinson described aged migration as a countertrend from the dominance of young adults as migrants. He said that "Recently...the aged have become more numerous, more financially independent, and more willing to pull up stakes and move..." (3). The subject of this paper is aged retirees' migration to Pike County, their demographic and residential characteristics, and their reasons for choosing Pike County

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MATERIALS AND METHODS

The data for this study, in addition to population and economic statistics obtained from official sources, were compiled in 1995 from a questionnaire directed to persons who were 65 or older and had migrated to Pike County after retiring. Over 300 responses were returned involving about 410 individuals (4). The difference caused by some married couples providing answers for both spouses and, for others, getting responses from just one of the couple. The survey was initially placed in the

Newsletter of the Pike County Office for the Aging. It was also distributed at the two centers for the aged in Milford and Lackawaxen. In addition, the presidents of the ten "seniors" clubs were sent questionnaires to be distributed and collected at one of their meetings. All distribution and collection of questionnaires was completed by August 1995. The returns were disaggregated by source and by municipality of residence, and the few duplicates were discarded. This sample is equal to more than 9 percent of the County's population 65 and older. Moreover, of those over 64 in attendance at the club meetings when the survey was taken, some 58 percent among those present were 65 or older, retired, and in-migrants, suggesting how large the in-migrant aged population might be among the aged cohort. Average age of those polled was 73.8 years (73.5 for women and 74.1 for men), and ranged from 65 to 101 years of age. The sample consists of almost 55 percent women and 45 percent men. Over 70 percent of the respondents were married, almost 20 percent widows and 4.5 percent widowers. Moreover, 4.2 percent were divorced (8 women and 9 men), and less than 2.0 percent single (4 women and 3 men).

As Table 2 indicates, the sample is rather well distributed among the municipalities of Pike County judging by the distribution of the total and aged populations. Most of the greater disparities are explicable—Blooming Grove and Greene townships are the residences of many club members, thus their outsize portion of the sample. Matamoras Borough's aged failed to respond in part because the local club president had a personal problem and missed the meeting on the day the questionnaire was to be distributed. The remainder of the paper focuses on the sample data from the responses to the questionnaire, using reference populations from Pike County and the Commonwealth where appropriate.

Table 1. Pike County and Pennsylvania Population Trends, 1940-1990, Total and Aged Populations

	Pike County				Pennsylvania			
	Total		Aged		Total		Aged	
	Number	%*	Number	%*	Number	%*	Number	%*
Population 1940	7,452	100.0	903	12.1	9,900,180,	100.0	677,468	6.8
Change,1940-1950	973	13.1	226	25.0	597,832	6.0	209,357	30.9
Population 1950	8,425	100.0	1,129	13.4	10,498,012	100.0	886,825	8.4
Change, 1950-1960	733	8.7	248	22.0	821,354	7.8	241,700	27.3
Population 1960	9,158	100.0	1,377	15.0	11,319,366	100.0	1,128.525	10.0
Change, 1960-1970	2,660	29.0	604	43.9	474,543	4.2	143,601	12.7
Population 1970	11,818	100.0	1,981	16.8	11,793,909	100.0	1,272,126	10.8
Change, 1970-1980	6,453	54.6	1,311	66.2	69,986	0.6	258,807	20.3
Population 1980	18,271	100.0	3,292	18.0	11,863,895	100.0	1,530,933	12.9
Change, 1980-1990	9,695	53.1	1,065	32.4	17,748	0.1	298,173	19.5
Population 1990	27,966	100.0	4,357	15.6	11,881,643	100.0	1,829,106	15.4

Source, U.S. Bureau of the Census, *Census of Population*, years indicated.
*The upper percentage for each year is that population as a percentage of the unit's total; the lower figure for each decade is percentage change.

Table 2. Population Distribution by Municipality, Total and Selected Groups

	Total, 1991		Aged, 1990		Aged Sample, 1995	
	Number	%	Number	%	Number	%
Blooming Grove Twp.	2,292	7.3	370	8.5	82	20.1
Delaware Twp.	4,274	13.1	423	9.7	43	10.6
Dingman Twp.	5,318	16.3	494	11.3	47	11.5
Greene Twp.	2,386	7.3	385	8.8	59	14.5
Lackawaxen Twp.	3,214	9.9	518	11.9	21	5.2
Lehman Twp.	4,136	12.9	276	6.3	38	9.3
Matamoras Borough	2,035	6.3	392	9.0	0	0.0
Milford Borough	1,224	3.8	223	5.1	14	3.4
Milford Twp.	1,199	3.7	148	3.4	21	5.2
Palmyra Twp.	2,181	6.7	401	9.2	33	8.1
Porter Twp	191	0.5	36	0.8	1	0.2
Shohola Twp.	1,749	5.4	287	6.6	54	8.4
Westfall Twp.	2,260	6.9	404	9.3	14	3.4
Total	32,560	100.0	4,357	100.0	407	100.0

Source: U.S. Bureau of the Census, Census of Population, years indicated; Pike County Department of Planning; and survey of retired migrants.

RESULTS AND DISCUSSION

To begin, the residence of the aged sample at the time of retirement and migration is quite straight-forward–New York City (the counties of Bronx, Kings, New York Queens, and Richmond listed on Table 3) accounted for 129 persons or almost a third of the sample. Add the suburban counties of Westchester and Rockland as well as Nassau and Suffolk on Long Island, and the percentage swells to almost half of the sample. New Jersey is represented by counties which are almost all in the New York City metropolitan area, but the respondents are mostly from suburban boroughs and townships in these counties (Figure 1). Pennsylvania's counties where Pike migrants originated are strung along the Delaware River from Philadelphia and its suburbs northward to include the Allentown-Bethlehem-Easton area in Lehigh and Northampton counties. Farther north are the Poconos counties neighboring Pike–Monroe and Wayne, both on the Delaware River. Others include Lackawanna (Scranton), Berks, (Reading), Lycoming (Williamsport), and Centre counties (State College). Not surprisingly, all of these save Wayne in Pennsylvania and Sussex in New Jersey are metropolitan. Thus, as Figure 1 clearly illustrates, the vast majority of these aged migrants' origins are either counties with large populations or their suburbs. The others are very close to Pike County. The pattern shown on Figure 1 is broken by the absence of aged in-migrants to Pike from Orange and Sullivan counties, located directly across the Delaware River from Pike County in New York. There is no ready explanation beyond the most plausible one of simply missing them in what is not a very extensive sample.

Place of birth of the sample population is quite different from their residences at the time they migrated to Pike County (Table 4). Although it is not obvious at the county level (except for New York City and Philadelphia), the responses on place of residence at retirement or migration include many metropolitan suburbs, whereas the earlier data on place of birth include many New Jersey cities which have fallen on hard times. These include Newark, Jersey City, Perth Amboy, Passaic, Paterson, and Hoboken. None but Hoboken, the city near the entrance to the Holland Tunnel with a PATH station and which has experienced some redevelopment (gentrification), would make *Money Magazine's* middle ranks of desirable places to live. Place of birth when compared to the immediate residential origins of the sample, thus reflects the evolution of residential desirability–from cities with booming economies based on manufacturing earlier in this century to the suburbs of these and other urban places more recently. Turning to the foreign born, also shown on Table 4, they are mostly German and most of them lived in their port of entry–New York City. The fact that just six Pike County natives were picked up in the sample is not surprising given that Pike's population in 1930 was less than 7,000. At any rate, returning home to Pike County at retirement might have made the change easier given the familiarity

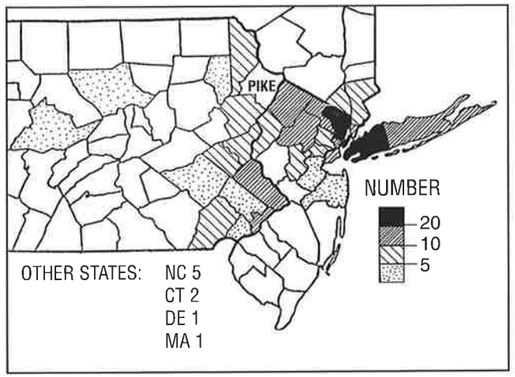

Figure 1. County of residence of retired migrants at time of move to Pike County, Pennsylvania.

Table 3. Residence at Time of Retirement/Migration, Aged Sample, 1995

Pennsylvania		New York		New Jersey		Other States	
County	Number	County	Number	County	Number	State	Number
Philadelphia	18	Queens	36	Bergen	35	North Carolina	5
Bucks	17	New York	34	Passaic	16	Connecticut	2
Lehigh	9	Nassau	29	Hudson	14	Delaware	1
Northampton	7	Kings	26	Morris	13	Massachusetts	1
Chester	5	Bronx	17	Sussex	10		
Monroe	5	Richmond	16	Essex	9		
Wayne	5	Suffolk	14	Union	9		
Lackawanna	4	Westchester	8	Somerset	8		
Montgomery	4	Rockland	5	Middlesex	4		
Centre	3			Warren	2		
Delaware	3			Monmouth	1		
Lycoming	2			Unspecified NJ	1		
Berks	1					n=399	
Total	83 (20.8%)		185 (46.3%)		122 (30.6%)		9 (2.3%)

Source: Survey of retired migrants, 1995.

Table 4. Place of Birth, Aged Sample, 1995

Pennsylvania		New York		New Jersey		Other States		Foreign Born	
County	Number	County	Number	County	Number	State	Number	Country	Number
Philadelphia	31	New York	86	Essex	16	Illinois	7	Germany	14
Lackawanna	15	Kings	47	Hudson	14	Massachusetts	4	England	3
Lehigh	9	Bronx	17	Bergen	9	California	3	Italy	3
Northampton	8	Queens	17	Passaic	9	Ohio	3	Wales	3
Pike	6	Richmond	4	Middlesex	7	Delaware	1	Belgium	1
Wayne	5	Nassau	3	Union	4	Iowa	1	Ethiopia	1
Bucks	4	Erie	1	Morris	2	Kansas	1	Spain	1
Chester	3	Rockland	1	Monmouth	1	Michigan	1	Switzerland	1
Luzerne	3	Westchester	1	Ocean	1	Texas	1		
Berks	2			Somerset	1				
Delaware	2			Sussex	1				
Lebanon	2								
Monroe	2								
Montgomery	2								
Allegheny	1								
Blair	1								
Cambria	1								
Columbia	1								
Dauph in	1								
Lancaster	1								
TOTAL	100 (25.6%)		177 (45.2%)		65 (16.6%)		22 (5.6%)		27 (7.0%)

Source: Survey of retired migrants, 1995.

with the area and the chance that relatives and friends may still be in residence. The ease with which one can visit his former residence to see friends and relatives would seem to make the move less stressful as well.

As Table 5 shows, the sample of retired migrants is better educated than Pike County's population over 16, but not as well as second home owners. More than two-thirds of the sample graduated from high school, and the percentage of college graduates is almost 20 percent, both impressive for a cohort whose average age is almost 74. The second-home owners referred to in Table 5 are a population 25 and older and, although not residents, are obviously well off judging by their multiple home ownership alone. Therefore, their higher educational attainment, when compared to the aged sample, is expected. Pike was inhabited by fewer than 28,000 residents in 1990, but the county planners estimate that almost twice that number resided in second homes. In addition, the same source reported that a great number of Pike's resident labor force works outside the county, many in New York City and environs (5). This is not that far-fetched given the commuter railroad station in Port Jervis, New York (just across the Delaware River from Matamoras, Pennsylvania) is only 2.25 hours from Hoboken, where there is easy access to Manhattan via the Port Authority Trans-Hudson Train (PATH).

Table 6, showing occupation for the sample and the population 16 and over in Pike County, fully supports the notion that the aged in-migrant sample is of higher status than the labor force in residence. For the retired sample, of course, these listings represent their jobs at the time of retirement, and the percentages are quite high for managers/professionals and the technical/sales/ administrative support categories when compared to Pike's totals. This is to be expected since the comparison is of one group of people at the peak of their working lives (many in the aged sample) with another group ranging from youngsters and young adults in entry-level jobs to those near retirement and some, like the sample, occupying responsible, high paying positions. Further, although not counted among the labor force in the occupational data, more than 60 women reported their occupation on the questionnaire as housewife or homemaker. It is doubtful if very many of the younger women, either residents or second-home dwellers in Pike County, would describe themselves as either a housewife or a homemaker. Indeed, it would be surprising if most were not employed full time outside the home–as are more and more American women of all classes.

The underlying reason for migration to *Pike County* in this study is obviously retirement, but the major reason for retirement to *Pike County* is second-home ownership in Pike County. Over 54 percent of the returns indicated second home ownership at the time of migration to Pike County (6). The former is so because the sample is limited to retirees, whereas the latter is so, at least partly, because of Pike's array of residential

Table 5. Educational Attainment, Pike County Population Over 25 and Aged Sample

Attainment (yrs.)	Total	Second Home Owners	Aged Sample
0-11	20.8%	5.1%	12.7%
12	44.3	31.5	67.5
16 or more	14.7	40.7	19.9
			n=403.

Source: Pike County Planning Commission, *Economic Development Needs . . .*, and survey of retired migrants.

Table 6. Occupation, Employed Persons 16 and Older, 1990, and Retired-Migrant Sample, 1995

	Pike Co. Employed 16 and Older, 1990		Retired-Migrant Sample	
	Number	%	Number	%
Managerial and Professional	2,690	21.5	102	30.7
Technical, Sales, and Administrative Support	3,782	30.2	131	39.5
Service	1,860	14.8	23	6.9
Farming, Forestry, and Fishing	270	2.2	3	0.9
Precision Products, Craft, and Repair	2,107	16.8	42	12.7
Operators, Fabricators, and Laborers	1,819	14.5	31	9.3
Total	12,528	100.0	332	100.0

Source: Pike County Planning Commission, Economic Development Needs..., and survey of retired migrants, 1995.

amenities–lower costs (of land, housing, and general living expenses compared to the places of origin of migrants to Pike from New York and New Jersey) and Pike's proximity to a very large metropolitan population.

Table 7, however, which lists what the aged sample members *state as their reasons* for migrating to Pike County, indicates that 39 listed retirement despite the proviso that only retirees complete and return the survey! The attractions ("pulls") to Pike are classed under the headings of amenities, economic, and family, and combine to account for almost 74 percent of the total. Leading reasons are "country living," "I/we like Pike County," and "to be close(er) to relatives." The repulsive reasons ("pushes") that respondents listed were directed toward cities and the desire to flee from their crime, congestion, and crowding. Needless to say, the counties of New York City (save Richmond) and Philadelphia led in this category but many others were mentioned, even places on Long Island and Lackawanna County (Scranton).

Table 7. Reasons for Retired Aged Migrating to Pike Co.

	No.	%
AMENITIES		
(environmental, housing, life style, and recreation)	118	41.1
Country living	39	13.6
Like Pike County	36	12.5
Recreation	14	4.9
Peace and Quiet	13	4.5
Open space/less crowding, congestion	9	3.1
Other	7	2.4
ECONOMIC	48	16.7
Lower cost of living (taxes)	22	7.7
Retired to second home	22	7.7
To afford home ownership	2	0.7
Other	2	0.7
FAMILY	46	16.0
To be close to relatives (especially children)	39	13.6
Husband died	4	1.4
To return home	3	1.0
URBAN CRIME, CONGESTION, CROWDING (escape)	36	12.5
RETIREMENT	39	13.6
	n=287	

Source: Survey of retired migrants.

Responses to the last question, on satisfaction with services, appeared on only about 60 questionnaires (fewer than 20 percent). Most were from city dwellers, especially those from New York City, who complained mostly about the lack of public transportation, distance to health or medical services, and high taxes. They obviously want urban conveniences in a rural, low density county where such services probably must await far more residents to achieve the economies of scale required to support them. If and when that happens, Pike County will doubtless be a far different place than it is today.

CONCLUDING REMARKS

Migration is an adjustment process in response to real or perceived inequalities that establishes flows to desirable places and away from the repulsive. Pike's strong attraction for retired metropolitan populations is based primarily on its rural environment, its proximity to major metropolitan areas, and the array of recreational opportunities that exist. As the population grows, these attractions should diminish as residential densities increase and commercial and other services expand to accommodate the additional residents and the growing visitors and vacationers in the county. Indeed, some of this residential and commercial growth has already occurred in Westfall Township along the Delaware River on Rte. 209 near the lovely Borough of Milford–an attractive residential development not far from a trailer park right along the highway, plazas featuring, among many other stores, Walmart, Kmart, McDonald's, Wendy's, a very large True Value Hardware store, and much more. In addition, the Delaware Valley School District appears to have doubled its building area over

recent years. The peace and quiet sought by so many who have moved here are not as apparent as they once were, given recent population and economic growth. Some residents probably see the home construction and tourist industries as incompatible, a number that certainly would grow should development continue. Growth in numbers of retired migrants seems inevitable given the great number of second home dwellers–a number that now amounts to almost twice the resident population. Moreover, a recent survey of second-home owners indicates that "Although 'plan for retirement' was cited as a reason for home purchase by only 16 percent of the second-home owners, about one-half intend to reside in the County in the future. About sixty-five percent expect to make the move in the next ten years, suggesting that not only retirees will settle in the County, but working families will as well" (7). Further, the county planners projected a resident population of over 65,000 in less than 15 years (2010) and a second-home population of nearly 66,000 in *1994* (8). A recent document calls for recognition of the need for "sensible, environmentally sound economic development to maintain the long-term viability of the community" (9). Local officials have a job to do if the Pike County that is prized for its many amenities is to continue to exist, if somewhat diminished.

ACKNOWLEDGEMENTS

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3. Thomlinson, Ralph. 1965. Population Dynamics. Random House, New York, 227.
4. The number of responses varies, as do the counts of individuals, because of the differences in the number answering each question.
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6. An earlier study in counties in New York's Catskill Mountains reported similar findings regarding second-home ownership. They found "The modal pattern of relocation was for a couple to build or purchase a summer cottage and make improvements in the property over a number of summers prior to retirement." See Lane, William C. and Patricia P. Pine. 1988. Catskill Counties Aging Study. A Study of Long-Time Residents and Relocated Retirees in Four Rural Counties in New York State. Final Report. Conducted under the auspices of the Gerontological Society of America's Postdoctoral Fellowship Program in Applied Gerontology and funded by the Administration on Aging, 15.
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