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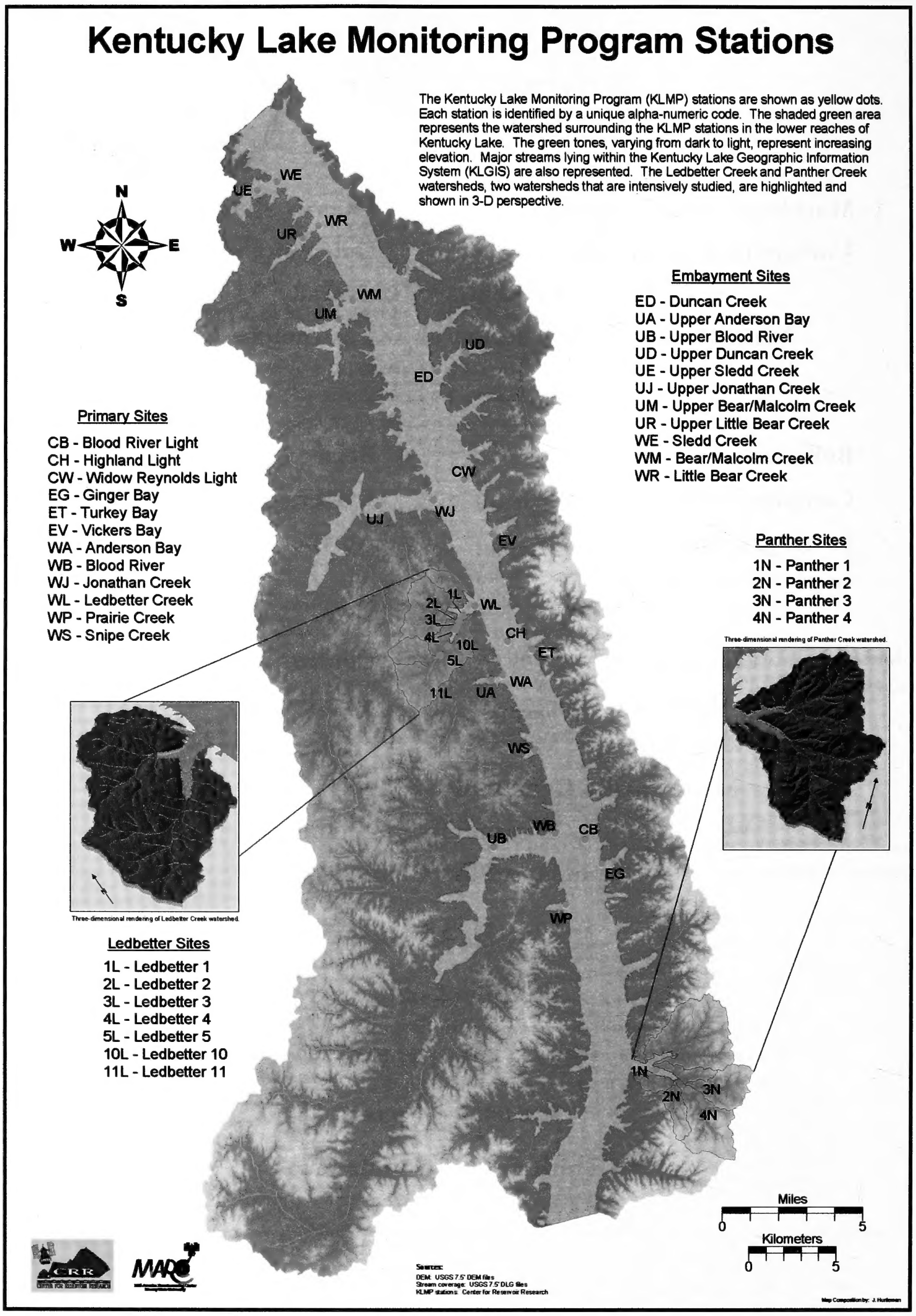

Figure 1. Map of CRR monitoring sites on Kentucky Lake. Primary sites are sampled on 16 and 32 day intervals. Ledbetter sites are in an agricultural/ rural watershed. Panther sites are in a forested watershed in the Land Between the Lakes National Recreation Area. See Table 2 for listings of monitoring parameters at each site.

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The Center for Reservoir Research over Its First Twenty Years with Special Reference to the Long-term Monitoring Program

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ABSTRACT

The Center for Reservoir Research (CRR) at Murray State University is nearing its 20th year. A series of papers on CRR research and education were presented at the 2006 meeting of the Ohio River Basin Consortium for Research and Education, and a selection of these is included in this issue of the Journal of the Kentucky Academy of Science. A major component of CRR is the Long-term Monitoring Program on Kentucky Lake and the resulting database. The database contains suites of physicochemical and biological data that have been collected primarily on 16 or 32 day cycles since 1988. These data are available to students and scientists through the CRR website.

KEY WORDS: Reservoir, Kentucky Lake, long-term monitoring, database, water chemistry.

INTRODUCTION

Murray State University's Center for Reservoir Research (CRR) is now entering its 20th year of operation. To celebrate the anniversary, CRR hosted the annual meeting of the Ohio River Basin Consortium for Research and Education (ORBCRE) in October 2006 on the Murray State campus. The meeting provided faculty and graduate students the opportunity to highlight the breadth of CRR research activities from geographic information systems to chemistry to aquatic ecology. Several of the ORBCRE presentations along with other CRR contributions are included in this issue of the Journal of the Kentucky Academy of Science. The present paper provides an overview of CRR since 1987 with emphasis on the long-term monitoring program. Our goal is to make scientists aware of the long-term database, the parameters available, and how the database may be accessed. Data such as these seem particularly relevant in light of understanding long-term environmental change from regional to global scales.

The Center for Reservoir Research (CRR), originally known as the "Center of Excellence for Ecosystem Studies," was established at Murray State University in 1987 by the Kentucky Council on Higher Education through a statewide competition for Centers of Excellence in Research and Teaching. The other three Centers are at the University of Kentucky and the University of Louisville and cover areas of human health, education, and computational science. The CRR proposal effort was led by Joe King (Chair of Biological Sciences), Vaughn Vandergrift (Chair of Chemistry), Neil Weber (Director of the Mid-America Remote sensing Center), and Gary Boggess (Dean of the College of Science). The mission of the Murray State Center was and continues to be to provide support for education, research, and long-term studies of reservoir ecosystems. Included in a separate proposal was the establishment of an endowment,

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which resulted in the hiring of Murray State's first endowed chair, G. Richard Marzolf.

Three research facilities comprise the base for the research and educational activities: the Chemical Analysis Laboratory (CAL), the Hancock Biological Station (HBS), and the Mid-America Remote sensing Center (MARC). Each facility functions in response to individual or team needs in conducting components of the CRR program and serves as an educational focus for graduate and undergraduate studies in water and associated watershed sciences. CRR presently has a participating faculty and staff of 11 and employs a number of postdoctoral research associates and graduate and undergraduate students.

Over its 20 year history, CRR associated faculty, staff, and students have accumulated close to 200 publications, 4 Doctoral and 65 Masters theses, nearly 300 meeting presentations, and 115 grants. Detailed listings for each can be found at www.murraystate.edu/crr. The Center is a member of the Association of Ecosystem Research Centers (AERC), the Organization of Biological Field Stations (OBFS), the Consortium of Universities for the Advancement of Hydrologic Science (CUAHSI), the Ohio River Basin Consortium for Research and Education (ORBCRE), and the International Association for Great Lakes Research. CRR presently is involved in four RFIs for the National Ecological Observatory Network (NEON) and the Global Lake Ecological Observatory Network (GLEON).

Among the significant milestones in the history of CRR has been the establishment of a long-term monitoring program and database for Kentucky Lake and its watersheds (discussed further below) and a number of large grants that have provided additional facilities or established research and education directions (Table 1). The majority of these grants have been multidisciplinary and have involved co-principal investigators from a number of universities. The Water Science and Engineering in River Impoundments grant (1988) brought reservoir scientists from around the country to the Biological Station at the beginning of the research and monitoring effort, establishing a national presence for CRR and giving guidance for research and establishment of the monitoring program. Facilities grants in 1993, 1998, and 2002 (Table 1) greatly increased the capacity for research and education by providing housing, new research buildings, renovation of laboratories, research equipment, postdoctoral fellows, and new staff. Major grants from DOE/EPSCoR in 1996 and NSF in 1999 established a strong research program on Kentucky Lake, while an NSF/EPA grant in 1996 established research and monitoring on contrasting agricultural and forested tributaries to Kentucky Lake. The 1999 NSF/CRUI grant was important in focusing on undergraduate research within the Center. The EPA EPSCoR Program grant in 2001, along with a number of similar grants not shown, established a recognized environmental toxicology program. The FIRST grant in 1998, although small, was part of a larger national program to utilize field stations as a way to engage college-level faculty in field ecology. FIRST evolved into FIRST II in 2001, a much larger effort encompassing both science and mathematics.

Table 1. Milestone grants received by faculty associated with the Center for Reservoir Research.

Grant	Year of funding	Funding agency
Water Science and Engineering in River Impoundments (workshop).	1988	NSF
New Visiting Scientist Facilities at Hancock Biological Station.	1993	NSF
Transport Accumulation and Utilization of Organic Carbon in Large Reservoir Systems	1996	DOE/EPSCoR
A Comparison of Agricultural vs. Forested Basins: Carbon and Nutrient Cycling within the Hyporheic Ecotone of Streams.	1996	NSF/EPA
Faculty Institutes to Reform Science Teaching (FIRST) Program.	1998	NSF
Glasshouse/Mesocosm Facilities for Hancock Biological Station.	1998	NSF
Biogeochemical and Ecological Processes within a Reservoir Littoral Zone (CRUI).	1999	NSF
Kentucky EPA EPSCoR Program.	2001	EPA/EPSCoR
The Center for Watershed Environments.	2002	NSF/EPSCoR

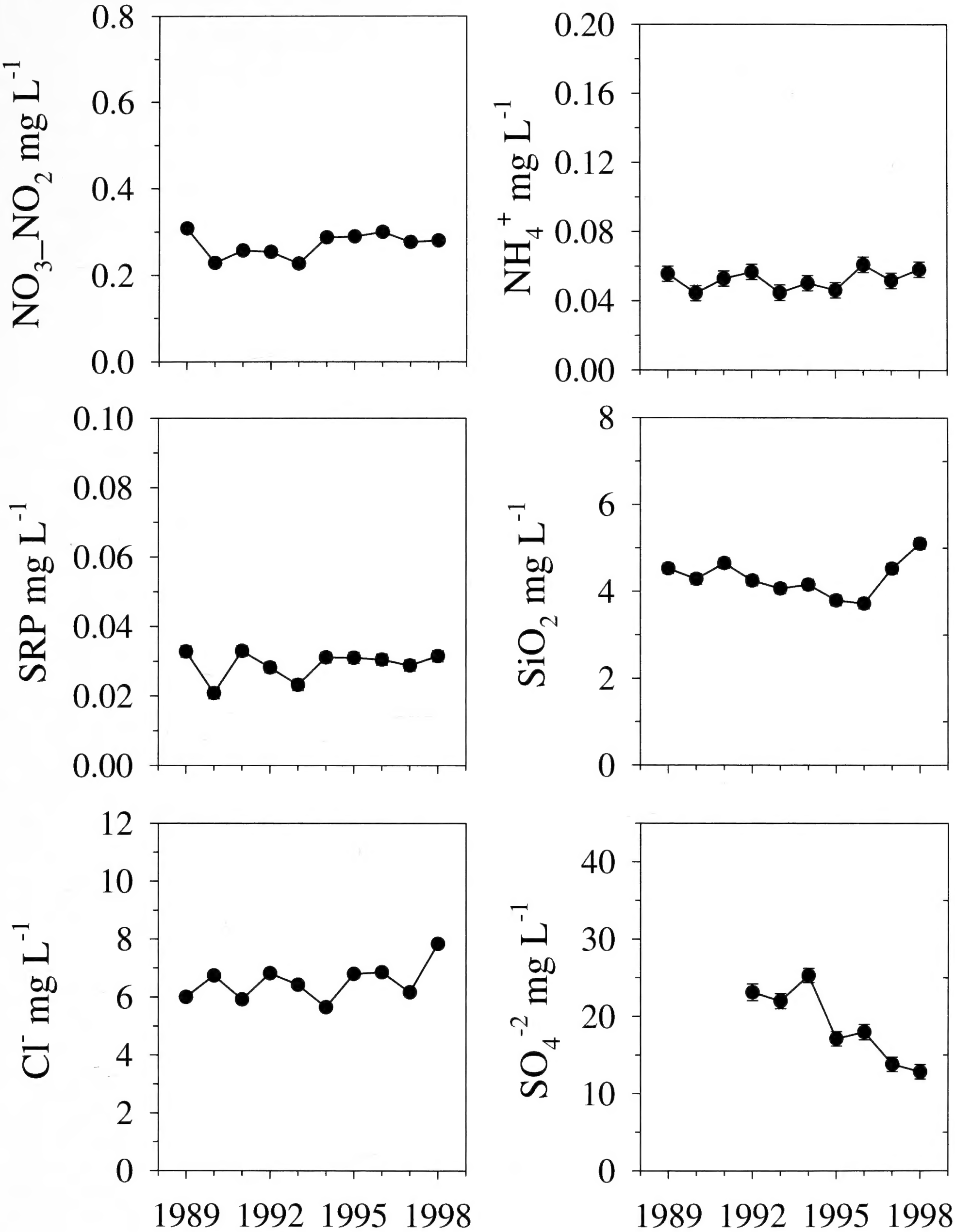

Figure 2. Annual means (±1 SE) for nutrients from mainstem sites CB, CH, and CW over a 10 year period (after Yurista et al. 2004).

LONG-TERM MONITORING AND DATA INFRASTRUCTURE

Crucial to the success of any ecological or environmental center in understanding the relevant ecosystem(s) is the ability to store, maintain, and retrieve the data. The Kentucky Lake Monitoring Program (KLMP) was begun through the efforts of G. Richard Marzolf in July 1988 (White 1990). The program was designed to 1) determine the limnological status at any point in time, 2) determine long-term changes in chemistry and biology, and 3) provide correlative data for experimental work. The monitoring program consists of a suite of

Table 2. Long-term monitoring parameters for Kentucky Lake and Ledbetter and Panther creeks. Site locations given in Figure 1.

Parameter	Sites sampled	Sample interval	Began collecting
Biological Parameters			
Primary Production (^{14}C) Phytoplankton**	All KLMP sites except stream sites, 1N, 1L, and 3L	16 days Apr–Nov, 32 days Dec–Mar	Jul 1988*
Zooplankton	All KLMP sites except stream sites and 1N	16 days Apr–Nov, 32 days Dec–Mar	Jul 1988*
Parameters Related to GIS Data			
Drainage Basin Name Embayment Volume at Summer Pool Surface Area of Basin	All KLMP sites	n/a	n/a
Physiochemical Parameters			
Alkalinity Ammonia Nitrogen Nitrate + Nitrite Nitrogen Soluble Reactive Phosphorus	All KLMP sites	16 days Apr–Nov, 32 days Dec–Mar for all sites except stream sites and 1N; 32 days for stream sites and 1N	Jul 1988*
The following are collected as 1-m vertical profiles at all KLMP lake sites and at two depths at KY Lake remote site Conductivity Dissolved Oxygen Oxidation Reduction Potential pH Turbidity*** Water Temperature	All KLMP sites and KY Lake remote site	16 days Apr–Nov, 32 days Dec–Mar for all sites except stream sites and 1N; 32 days for stream sites and 1N; 15 minutes for the remote site	Jul 1988* for KLMP, Feb 2005 for remote site
Chlorophyll *a*	All KLMP sites and KY Lake remote site	16 days Apr–Nov, 32 days Dec–Mar for all sites except stream sites and 1N; 32 days for stream sites and 1N; 15 minutes for the remote site	
Chloride	All KLMP sites	16 days Apr–Nov, 32 days Dec–Mar for all sites except stream sites and 1N; 32 days for stream sites and 1N	Jan 1989*
Dissolved Organic Carbon	All KLMP sites	32 days	Nov 1995* for streams and 1N, Jan 1996 for 2L, Mar 2000 for all others
Light Intensity and Penetration	All KLMP sites except stream sites and 1N	16 days Apr–Nov, 32 days Dec–Mar	Jul 1988*
Secchi Depth	All KLMP sites except stream sites	16 days Apr–Nov, 32 days Dec–Mar for all sites except 1N; 32 days for 1N	Jul 1988*
Silicon Dioxide	All KLMP sites	16 days Apr–Nov, 32 days Dec–Mar for all sites except stream sites and 1N; 32 days for stream sites and 1N	Jan 1989*
Sulfate	All KLMP sites	16 days Apr–Nov, 32 days Dec–Mar for all sites except stream sites and 1N; 32 days for stream sites and 1N	Apr 1992*
Suspended Solids Total Solids	Stream sites	32 days	Nov 1996

Table 2. Continued.

Parameter	Sites sampled	Sample interval	Began collecting
Total Dissolved Nitrogen Total Dissolved Phosphorus Total Nitrogen Total Phosphorus	All KLMP sites	32 days	Mar 1994*
Miscellaneous Parameters			
Air Temperature	Hancock Biological Station (HBS)	15 minutes	Feb 2005
Stream Discharge	All stream sites	16 days Apr–Nov, 32 days Dec–Mar for 5L, 32 days year-round for others	Mar 1990 for 5L, Nov 1995 for 2N and 3N, Dec 1995 for 10L and 11L, Jan 1996 for 4N
Dam Discharge	Kentucky and Pickwick Lakes	Daily	Jan 1988
Hydraulic Retention Time	Kentucky Lake	Daily	Jan 1988
Precipitation	Land Between the Lakes (LBL) and HBS	Daily for LBL, 15 minutes for HBS	Apr 1999 for LBL, Feb 2005 for HBS
Reservoir Elevation	Kentucky Lake at dam HBS	Daily 15 minutes for HBS	Jan 1988 Feb 2005 for HBS
Water Depth at Site	All KLMP sites except stream sites and 1N	16 days Apr–Nov, 32 days Dec–Mar	Jul 1988*
Wind direction	All KLMP sites except stream sites and 1N, HBS	16 days Apr–Nov, 32 days Dec–Mar for Kentucky Lake sites, 15 minutes for HBS	Jul 1988* for Kentucky Lake sites, Feb 2005 for HBS
Wind Speed	All KLMP sites except stream sites and 1N, HBS	16 days Apr–Nov, 32 days Dec–Mar for Kentucky Lake sites, 15 minutes for HBS	Jul 1988* for Kentucky Lake sites, Feb 2005 for HBS

* Sites 1L, 2L, 3L, 4L, 5L collection began Mar 1990.
Sites 1N, 2N, 3N collection began Nov 1995.
Sites 10L, 11L collection began Dec 1995.
Site 4N collection began Jan 1996.

** Archived but algae not identified.

*** KLMP lake sites collected at two depths through 1992, then as 1-m vertical profiles.

physical measurements, water chemistry parameters, primary production, phytoplankton, and zooplankton data (Table 2).

Initially 12 sites were sampled during each sampling event, or "cruise," including three each in the main channel, small east side embayments, small west side embayments, and larger west side embayments (Primary Sites of Figure 1). Cruises have occurred every 16 days in the months of April through November and every 32 days during winter months. January 2007 marked the 370th cruise. The 16-day interval coincides with the Landsat satellite flyover schedule. Monitoring data, coupled with the Kentucky Lake Geographic Information System (KLGIS) (MARC Associates 1998), form the bulk of the Kentucky Lake Database. Additional sites and parameters were added in the 1990s to more closely monitor inputs and conditions in Ledbetter Creek (agricultural) and Panther Creek (forested) and their embayments (Figure 1). Several of these sites are monitored only on a 32-day interval, and sites in Upper Blood River and Jonathan Creek are monitored only on clear days when satellite imagery might prove most useful. Other Kentucky Lake sites (Figure 1) have been added as interests arose but are not included in the regular on-going monitoring program. Additional Kentucky Lake water quality and weather data are collected from remote sensors located near HBS.

Other datasets are available for the Clarks River and Little River watersheds as well as from various specific research programs. CRR now jointly operates the National Atmospheric and Deposition Program site KY 99 with the Tennessee Valley Authority. Data on precipitation amount, frequency, and chemical composition are collected for this site located in

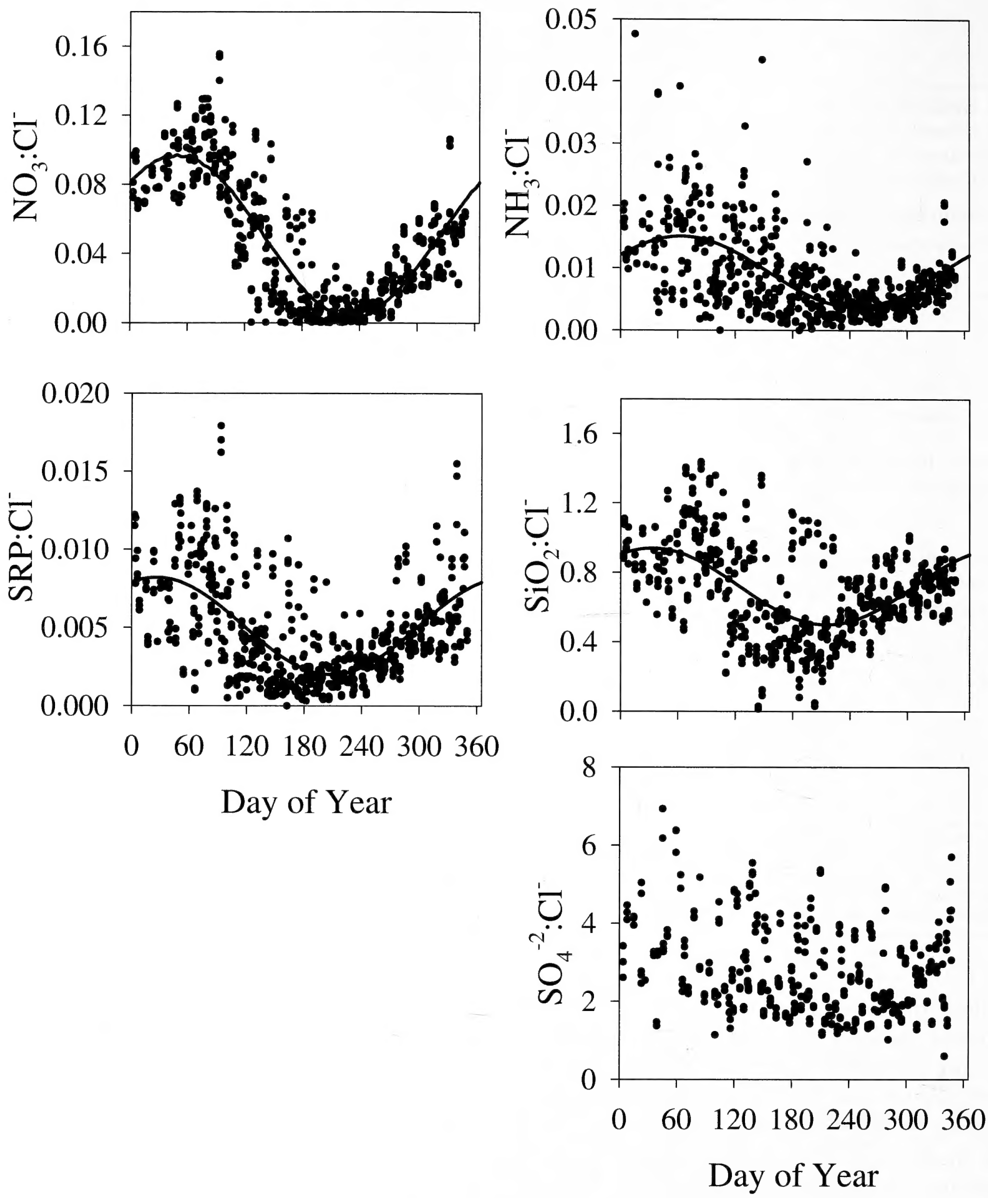

Figure 3. Daily trends in nutrient:chloride ratios from mainstem sites CB, CH, and CW over a 10 year period (after Yurista et al. 2004)

the northern end of the Land Between the Lakes.

As the long-term monitoring program approaches its third decade of existence and data continue to be collected, more people have become aware of the availability of the data, and the database is becoming more frequently and heavily mined. Early on, Lira et al. (1990, 1992) and Marzolf et al. (1991) used ^{14}C primary production data to develop probabilistic models and examine spatial variability. More recently Bukaveckas et al. (2002) used components of the database to investigate regulation of autotrophy and heterotrophy in Kentucky Lake. Balci et al. (2005) utilized the database for background information for a study of *Chironomus major* secondary production and life history.

The zooplankton database has been used to document long-term patterns in cladocerans, including changes following the introduction of the exotic *Daphnia lumholtzi* (Schram and Marzolf 1993, Yurista et al. 2001). Rotifer populations have been examined by Albritton and White (2004, 2006), and Williamson and White (2007) used three years of the database to look at seasonal variability in the copepod community.

One of the more extensive uses of the database was by Yurista et al. (2004), who examined physicochemical patterns over a 10-year period (Figures 2, 3). The data revealed that some chemical ratios had very consistent annual cycles, e.g., nitrate : chloride, while others may be highly variable, e.g., sulfate : chloride. Nitrate/nitrite concentrations remained fairly constant over the 10-year period, while the levels of sulfate have declined noticeably. The decline in sulfate, which was clearly demonstrated in the data, has been attributed to a reduction in the use of high sulfur coal by power plants (Yurista et al. 2004) and. Yurista et al. (2002) also described differences in particulate organic carbon patterns in the main stem and embayments utilizing a number of measurements from the database.

USING THE DATABASE

Presently, the data are available to the public by request via CRR's website (www.murraystate.edu/crr). For the bulk of the KLMP data, there is a form that may be filled out and submitted online. The request is then automatically sent to a data manager who fills it and sends the data to the requesting person. Future plans for the KLMP data include direct access using an interactive form. Visitors to the CRR website will be able to submit data requests and have the results returned to their browsers.

A similar mode of access already exists for the data from the remote water quality and weather sensors. Visitors to the website see the most recent measurements displayed, usually those taken within the last half hour. Visitors can easily retrieve archived data by following the instructions posted alongside the display. Anyone requesting these or other data from CRR must agree to our Data Use Policies and License, regardless of how the request is honored.

ACKNOWLEDGMENTS

We thank the hundreds of faculty, researchers, and students who have participated in Center research and educational activities over the past 20 years. Special thanks are given to Jane Benson and Matt Williamson for assistance with the parameter list of Table 2.

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Groundwater Flow and Reservoir Management in a Tributary Watershed along Kentucky Lake

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ABSTRACT

Understanding groundwater flow in tributary watersheds is important for evaluating water and solute storage and inputs into reservoirs. We delineated groundwater flow at various spatial and temporal scales within the watershed of Ledbetter Creek, a third-order tributary of the Tennessee River (impounded to create Kentucky Lake) in western Kentucky. We monitored hydraulic heads in wells (primarily in the upper watershed) and piezometers (in the lower watershed) and measured the flow of a spring along the embayment where the creek enters the reservoir. Manual measurements were made at least quarterly from July 1999 to March 2002 and were made annually each April from 2002 through 2006. From May 2000 to March 2002, hydraulic heads were recorded continuously in selected piezometers. At the watershed scale, groundwater flow followed the topography, with discharge occurring along the creek and in the embayment. Hydraulic heads in piezometers responded to large storms over periods of hours to days. Longer-term fluctuations in hydraulic head reflect reservoir management in the embayment (stage increased in early spring and decreased in late summer) and seasonal variability elsewhere in the watershed.

KEY WORDS: Ledbetter Creek, Tennessee River, bank storage, seepage, impoundment

INTRODUCTION

Despite the proliferation of research on groundwater/surface-water interactions during the past two decades (as reviewed by Winter et al. 1998; Jones and Mulholland 2000; Bencala 2005; and others) and the development of reservoirs as a dominant feature of the North American landscape during the 20th century, there have been relatively few studies of groundwater/reservoir interactions. Reservoirs have characteristics of both streams and lakes (Thornton et al., 1990; Winter et al. 1998; Wetzel 2001). As in the case of undammed streams, bank storage occurs when reservoir stage is raised and the hydraulic gradient is reversed, i.e., lateral infiltration is induced and the water table rises (Cady 1941; Coffin 1970; Simons and Rorabaugh 1971). A rise in stage also results in vertical infiltration of surface water. In effect, the zone in which infiltrating surface water and groundwater mix adjacent to a reservoir varies between hyporheic (the mixing zone adjoining and beneath a stream) and hypolentic (the mixing zone adjoining and underlying a lake) (Aseltyne et al. 2006). These zones can affect solute exports from groundwater to surface water and aquatic biological activity (White 1993; Winter 2001; Bencala 2005; Aseltyne et al. 2006).

One topic that has received limited attention is the extent of spatial and temporal changes in groundwater flow resulting from reservoir-stage manipulation. Changes in groundwater flow may be more pronounced adjacent to reservoirs than to undammed streams, for which the durations of stage rises are commonly shorter. In modeling flow where a creek enters a reservoir in the foothills of the California Coast Range, Rains et al. (2004) found that a "groundwater backwater effect. . . extends to portions of the terrace, but the most pronounced effects occur on the delta" of the creek. Aseltyne et al. (2006) ex-

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amined infiltration where Ledbetter Creek enters Kentucky Lake, the terminal reservoir on the Tennessee River, in western Kentucky. Using "peepers" (multi-chamber passive-diffusion samplers) to track Cl^- and stable isotopes of H_2O, Aseltyne et al. (2006) showed that the depth of surface-water infiltration below the creek channel increased by 4 to 8 cm following a 0.65-m reservoir-stage rise over a 10-day period. Following release of water from bank storage into a reservoir, the rate of hydraulic-head change in the aquifer should decrease with distance and time (Guo 1997).

This paper expands on previous studies by delineating spatial and temporal patterns in groundwater flow both at the scale of a tributary watershed to a reservoir and in detail at the outlet of the watershed. In particular, this work complements that of Aseltyne et al. (2006) by providing hydraulic data from the same study area.

STUDY AREA SETTING

Kentucky Lake, impounded in 1944 by the Tennessee Valley Authority, is the farthest downstream and largest of nearly fifty reservoirs on the Tennessee/Cumberland River system. Typical of mainstem impoundments on the Tennessee River, it is narrow with a distinct deep main channel and a shallow floodplain that includes the drowned mouths of numerous small tributaries. Ledbetter Creek is a third-order perennial stream that drains a watershed of 24 km^2 in Calloway and Marshall counties, Kentucky (White et al. 2007) (Figure 1). The mouth of the Ledbetter Creek embayment is at Tennessee River mile 42.5, 68.4 km upstream of Kentucky Dam. Land cover in the watershed consists primarily of forests, fields, and rural development. Land surface elevations range from ~158 m above mean sea level (msl) along the watershed divide to ~109 m msl along the shoreline of the embayment. Similar to the site in the California Coast Range studied by Rains et al. (2004), impoundment of Kentucky Lake resulted in the formation of a delta, where Ledbetter Creek splits into multiple distributary channels at the head of the embayment.

The region is located along the northeastern margin of the Gulf Coastal Plain physiographic province, which is marked by rolling land between incised valleys (Fenneman 1938). Coastal Plain strata consist of unconsolidated Cretaceous to Holocene sediments, which dip gently to west and south toward the Mississippi River, overlying Mississippian bedrock. Units exposed in the Ledbetter Creek watershed include silicified Mississippian limestone (Ft. Payne Formation), Upper Cretaceous clays, silts, and sands (the McNairy Formation), Pliocene to Pleistocene clays, silts, sands, and gravels (the Continental Deposits), loess on uplands, and alluvium in valleys (Olive 1965). The surficial (water-table) aquifer lies within the Ft. Payne Formation, the McNairy Formation (except where absent in the northeastern part of the watershed), and alluvium (Figure 2). Regional groundwater flow in the study area is toward the Tennessee River (Morgan 1965).

The climate of the northern Gulf Coastal Plain is humid and temperate (continental), with moderately cold winters, warm summers, and no distinct wet or dry season (Davis et al. 1973; Humphrey et al. 1973). The closest meteorological stations with long-term records to the Ledbetter Creek watershed are Paducah, Kentucky (~66 km west-northwest), and Princeton, Kentucky (~49 km north-northeast). For the years 1972–2005, average air temperatures were 14°C at Paducah and 15°C

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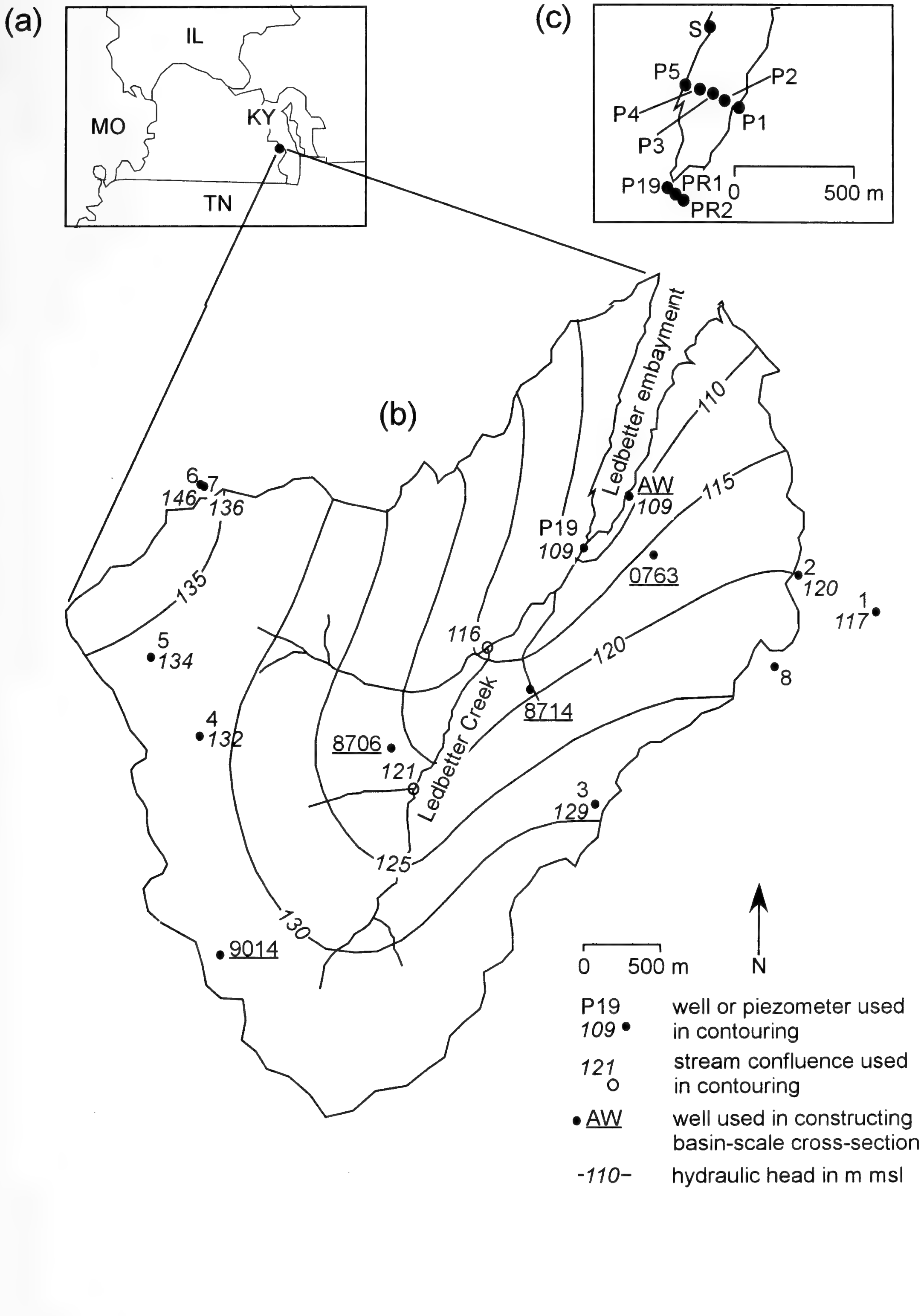
Figure 1. (a) Inset showing location of study area (modified from Aseltyne et al. 2006). (b) Locations of selected wells and equipotentials in the Ledbetter Creek watershed. Wells 1–8 were domestic wells for which water levels were measured; four-digit wells were those for which drillers' logs were available in the state water-well database; AW was the artesian monitoring well; P19 was a floodplain piezometer. Italicized numbers represent hydraulic heads used for contouring, based on 22 April 2006 water-level measurements and elevations of stream confluences taken from the Hico 1:24,000 topographic map. Note that the reservoir stage in the Ledbetter Creek embayment on 22 April 2006 was 109 m msl. Underlined wells were used in constructing the cross-section shown in Figure 2. (c) Inset of Ledbetter Creek floodplain and embayment showing locations of piezometers (prefixed with P) and spring (S). PR2, PR1, and P19 were floodplain piezometers; P1–P5 were piezometer nests in the embayment.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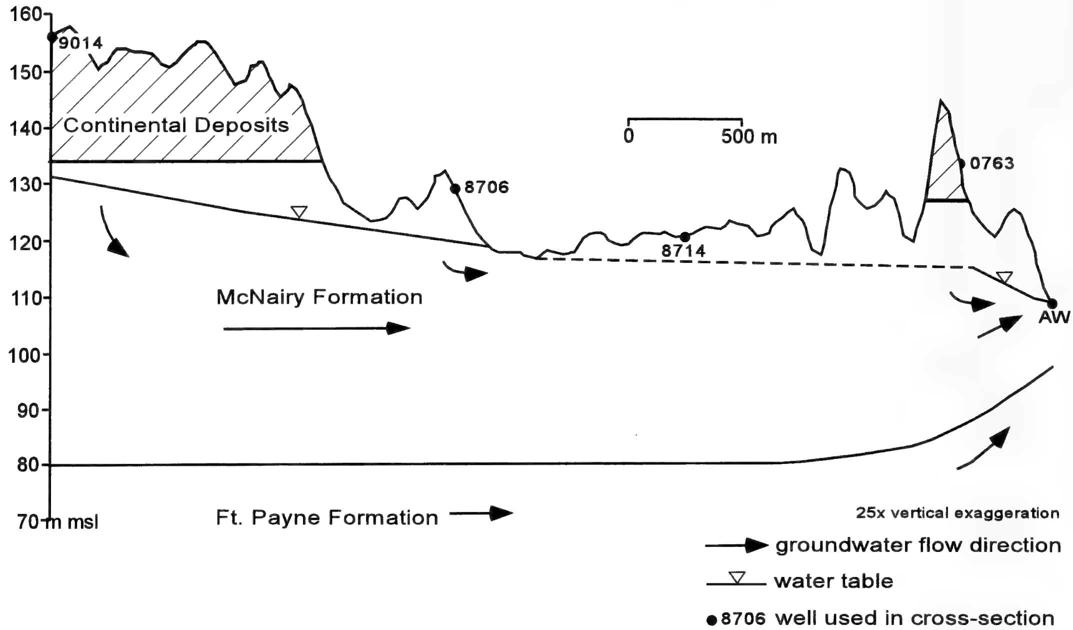

Figure 2. Hydrostratigraphic cross-section within the Ledbetter Creek watershed. Land-surface elevations were taken from the Hico 1:24,000 topographic map using National Geographic TOPO! software. Hydraulic heads were extrapolated from Figure 1(b); wells used in constructing the cross-section are underlined on Figure 1b. Note that groundwater flow was inferred in the plane of the cross-section except between Ledbetter Creek and well 0763; in that area (including well 8714), the line of section was approximately perpendicular to the direction of groundwater flow.

at Princeton, and annual precipitation averaged 123.5 cm at Paducah and 130.7 cm at Princeton (UKAWC 2006). Since 2000, the National Atmospheric Deposition Program/National Trends Network (NADP/NTN) has maintained a monitoring station (KY99) in the Land Between the Lakes National Recreation Area, ~20 km north of the Ledbetter Creek watershed. Annual precipitation at KY99 from 2000 through 2005 averaged 118.4 cm (Illinois State Water Survey 2006). As of 1997, estimated annual average precipitation was 134.8 cm and estimated annual average pan evaporation was 125.0 cm for Calloway County (UKAWC 2006). Monthly estimates of pan evaporation exceeded monthly estimates of precipitation for May through October (UKAWC 2006).

METHODS

In this study, we collected data from domestic wells in the upper reaches of the watershed, piezometers in the floodplain, piezometers and a monitoring well in the embayment (the seasonally inundated mouth of the watershed, which includes the delta), and a perennial spring flowing into the embayment. During March 2000, Hancock Biological Station staff conducted a survey of domestic wells in and adjoining the watershed. We selected 8 of 35 inventoried wells (Figure 1b, Table 1) for long-term water-level monitoring based primarily on accessibility, including both landowner permission and ability to take measurements without becoming entangled in submersible-pump wiring or water-supply pipe. Wells 1, 2, and 4 through 8 had 2-ft (0.6-m) diameter concrete culvert pipe as casing because the driller used a bucket auger to excavate gravels. Well 3 consisted of PVC pipe without a pump installed; it and wells 1 and 6 were not in household use at the time of the study.

Two different sets of piezometers were used (Figure 1c, Table 1). A transect of three individual piezometers across the floodplain of Ledbetter Creek, each completed to 3 m below ground level (bgl), was installed in April 1999. A transect of five piezometer nests across the embayment, each containing two to

Table 1. Land-surface (LS) elevations for wells and piezometers (prefixed P), total depths for wells and piezometers, and screened depths (referenced to approximate midpoint of screened interval) for monitoring well and piezometers. Locations are shown in Figure 1.

ID	LS elevation (m)	Total depth (m)	Screened depth (m)
Well 1	137	29.3	
Well 2	151	36.3	
Well 3	155	42.7	
Well 4	151	25.0	
Well 5	155	27.4	
Well 6	156	12.2	
Well 7	157	29.9	
Well 8	149	48.8	
Monitoring well	109	12.2	11.4
PR2	110.4	3.0	3
PR1	110.4	3.0	3
P19	110.4	3.0	3
P1-2	108.8	1.2	0.6
P1-5	108.8	1.5	1.5
P2-2	108.7	1.5	0.6
P2-5	108.7	1.6	1.5
P2-10	108.7	3.0	3
P3-2	108.8	1.5	0.6
P3-5	108.8	1.8	1.5
P3-9	108.8	2.7	3
P4-2	108.7	1.5	0.6
P4-5	108.7	2.7	1.5
P4-10	108.7	3.5	3
P5-2	108.6	1.2	0.6
P5-5	108.6	2.3	1.5

three piezometers completed to depths of 0.6 to 3 m bgl, was installed in December 1999, when the reservoir was at seasonal low-stand. Piezometers consisted of sections of 4-inch nominal (10.2 cm actual ID) PVC pipe that were slotted or perforated along an interval of ~ 0.3 m, capped at the bottom, and placed in holes drilled with a gasoline-powered solid-stem auger. Piezometers in the embayment were identified first by the nest in which each was located and then by the depth of the slotted or perforated interval in feet; e.g., P1–2 was located in nest 1 and screened at a depth of 2 ft (0.6 m) bgl. A monitoring well also was installed in June 2000 along the east side of the embayment (AW in Figure 1b). This well consisted of 2-inch nominal (5.3-cm actual ID) PVC pipe in a hole hollow-stem augered to a depth of 12.2 m bgl (within the upper rubble zone of the Ft. Payne Formation). The bottom 1.5 m of the well was screened.

We measured depths to water in domestic wells and piezometers using a 300-ft (91-m) electric tape graduated in 0.01-ft (0.003-m) increments. At elevated reservoir stage, we used a folding rule (also graduated in 0.01-ft increments) or measuring tape graduated in 1/16-inch (0.0016-m) increments to measure surface-water levels below the top of the casing for embayment piezometers. Water levels were measured manually beginning June 2000 in domestic wells, July 1999 in floodplain piezometers, and January 2000 in embayment piezometers. The measurements continued monthly to quarterly through April 2002 (with two sets of data for piezometers in July 2000) and annually each April thereafter through 2006. Depth to water or water level was converted to hydraulic head by adding land-surface elevation and subtracting casing height. Land-surface elevations were estimated for wells using the 1:24,000 Hico topographic map, which is accurate to within 5 ft (1.5 m) and were surveyed for piezometers using a GPS unit with an accuracy of 0.2 m. We used a folding rule or measuring tape to measure casing heights for domestic wells and embayment piezometers. In addition, pressure transducers connected to digital dataloggers were installed in piezometer nests P1 and P5 in January 2000. Pressure readings were recorded hourly until March 2002.

We measured discharge of the spring (S in Figure 1c) where it spilled over a ledge ~0.3 m high, ~10 m laterally from the orifice. We used a bucket, stopwatch, and 2-l cylinder graduated in increments of 20 ml. Discharge was measured monthly to quarterly from March 2000 through April 2002 and annually thereafter through April 2006. Beginning in May 2000, discharge measurements were made at least three times in succession and averaged.

RESULTS AND DISCUSSION

Hydraulic Heads at the Watershed Scale

Wells 1, 2, 6, 7, and 8 were determined to be just outside the watershed based on the divide inferred from the topographic map (although wells 2, 6, and 7 were within 100 m of the divide). The map of Morgan (1965) indicates that surface-water divides generally coincide with divides in the surficial aquifer in the area. Well 6 was perched, as indicated by ~9 to 12 m shallower depths to water than

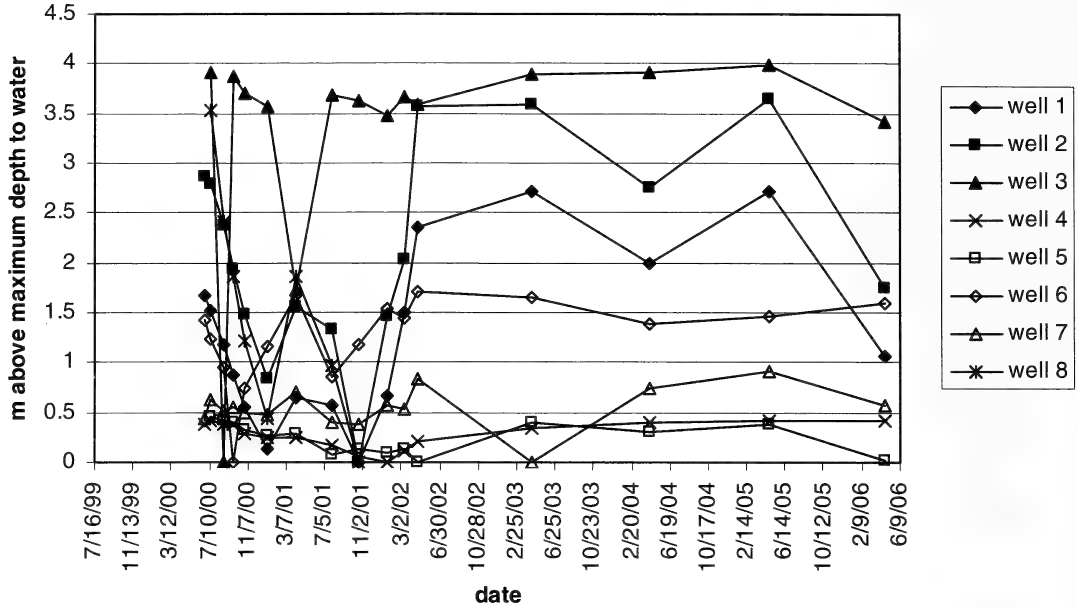

Figure 3. Depths to water in domestic wells monitored from 22 June 2000 through 22 April 2006, normalized against the maximum depth to water in each well. Note that monitoring of well 8 was discontinued after October 2001 at the landowner's request.

those in the adjoining deeper well 7 located ~10 m away and by the sound of water flowing down the casing of well 7. Equipotential lines were contoured assuming (1) symmetry across the watershed divide, (2) all wells were completed in the surficial aquifer (excluding well 6), and (3) equipotentials were a subdued reflection of watershed topography, with hydraulic heads equal to land-surface elevations along the main stem of the creek (Figure 1b). Consequently, as inferred theoretically by Tóth (1963), groundwater was expected to flow away from the watershed divide and converge toward Ledbetter Creek and the embayment (Figure 2). Groundwater discharge in the lower watershed was corroborated by lateral hydraulic gradients in the floodplain piezometers (see below) by the existence of the spring along the embayment and by the observation that the monitoring well along the embayment flowed slowly. Because the top of casing in this well was flush with the concrete pad, the height of artesian rise above land surface was not measured. Therefore, we assumed conservatively that hydraulic head for the monitoring well equaled the land-surface elevation. This last assumption should not significantly affect the equipotential lines shown in Figure 1 nor, given the depth of the monitoring well, should it affect the delineation of groundwater flow directions within the embayment sediments.

Temporal plots of depths to water in domestic wells, normalized relative to the maximum depth to water in each well (Figure 3), showed broad seasonal and annual trends. In terms of seasonal variations from June 2000 through April 2002, water levels tended to peak in spring to summer, decrease to minimum values in fall to winter, then rebound. This behavior agreed with that observed in a previous study of groundwater flow in the 48-km^2 watershed of Bayou Creek, a second-order perennial stream near Paducah, Kentucky (Fryar et al. 2000). The water-level trends were consistent with groundwater recharge occurring from late autumn through early spring, when precipitation exceeded evapotranspiration as approximated by pan evaporation estimates. At the annual scale between April 2001 and April 2006, depths to water varied within 0.91 m on the west side of the watershed (wells 4–7) and varied by 2.07 to 2.29 m on the east side of the watershed (wells 1–3), with depths to water in wells 1 and 2 tracking each other. Annual-scale fluctuations

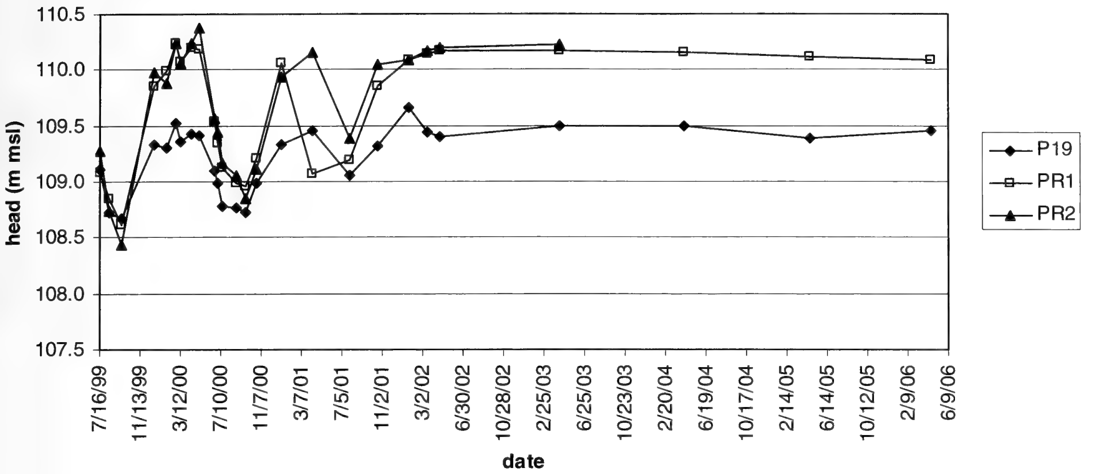

Figure 4. Manually-measured hydraulic heads in floodplain piezometers as a function of time from 16 July 1999 through 22 April 2006. Note that monitoring of PR2 was discontinued after April 2003 because the piezometer could no longer be located.

in hydraulic head were less than seasonal fluctuations; excluding the August 2000 reading for well 3, which was anomalous and suspect, the maximum hydraulic-head fluctuation was 3.59 m (for well 2). It should be noted that water levels in domestic wells other than wells 1, 3, and 6 could have been affected by pumping, although no pumping was observed during measurements.

Hydraulic Heads in the Floodplain and Embayment

Hydraulic heads were typically higher in piezometer PR2 than in PR1 and higher in PR1 than in P19, which indicated groundwater flow toward Ledbetter Creek in the floodplain (Figure 4). Occasional reversals in hydraulic gradient appear to have coincided with the formation of a groundwater ridge between PR2, which was adjacent to an ephemeral channel, and PR1. As observed by Fryar et al. (2000) for Bayou Creek near Paducah, Kentucky, reversals also could have occurred from temporary increases in stream stage following rainfall in the Ledbetter Creek watershed or from seasonal water-table declines that led the stream to become temporarily losing in late summer. In particular, the higher hydraulic head for P19 relative to PR1 on 7 April 2001 could have resulted from rainfall during several days preceding the measurement. Rainfall of 2.8 cm was recorded at Princeton on 3 and 4 April (UKAWC 2006). By contrast, the gradient reversal from P19 to PR1 to PR2 on 17 September 1999 followed a month in which only 0.69 cm of rainfall was recorded at Princeton. In terms of seasonal trends, hydraulic heads in the floodplain piezometers tended to be lowest in late summer or early autumn and rebound to maximum levels in late winter or early spring before falling again (Figure 4). Hydraulic heads varied by as much as 1.95 m for PR2 between September 1999 and May 2000. At an annual scale, hydraulic heads measured each April between 2000 and 2006 tended to be relatively constant, with values varying by only 0.13 m for PR2 and 0.10 m for P19.

Hydraulic heads for piezometers in the Ledbetter Creek embayment varied spatially, but the most pronounced variations coincided with temporal changes in reservoir stage. The Tennessee Valley Authority (TVA) typically raises the level of Kentucky Lake 1.5 m in March and lowers it 1.5 m over 3 months beginning in August (Aseltyne et al. 2006), as shown for the period December 1999–April 2002 (Figure 5), which encompassed most of our monthly to quarterly monitoring. Vertical hydraulic gradients varied among piezometer nests: at nest P1, hydraulic heads in both piezometers were within 0.01 m (i.e., the vertical hydraulic gradient was negligible) for 18 of 22 measurements between January 2000 and

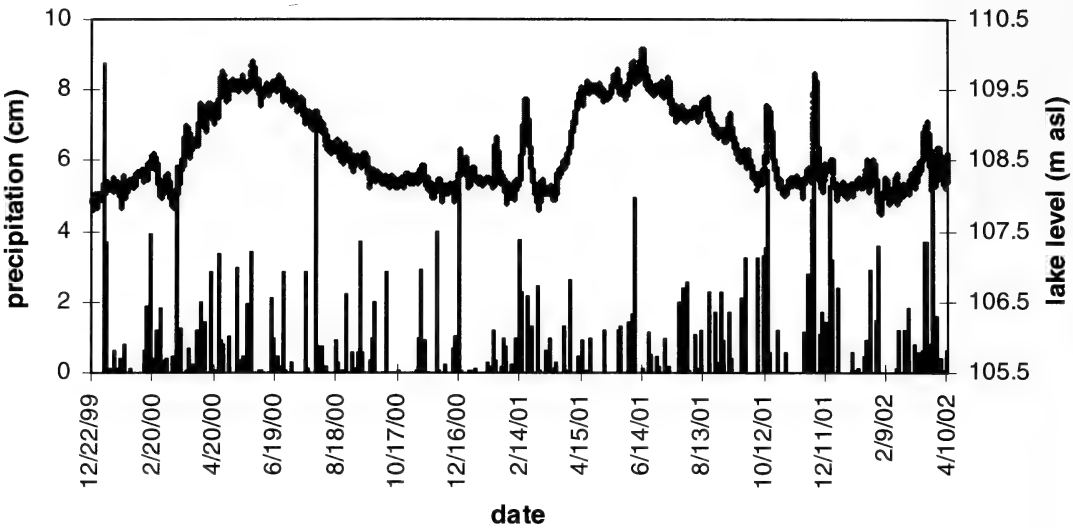

Figure 5. Daily precipitation at Princeton, Kentucky (UKAWC 2006) and hourly reservoir stage at Kentucky Dam (TVA, unpubl. data) for 22 December 1999 through 10 April 2002.

April 2006. Moreover, manually-measured reservoir stage was within 0.01 m of hydraulic heads in nest P1 piezometers for 9 of 11 measurements at summer pool between 2000 and 2006. In contrast, at nest 2, hydraulic head for P2–10 was always greater than for the shallower P2 piezometers and was always higher than manually-measured reservoir stage (Figure 6). Vertical hydraulic gradients varied in direction at nests P3 and P4. At nest P5, hydraulic head for the deeper piezometer (P5–5) was typically greater than for the shallower piezometer and higher than manually-measured reservoir stage except when the stage was being raised in April.

Hydrostratigraphic cross-sections illustrate differences in hydraulic-head distributions across the embayment between summer and winter. Because of imprecision in surveying, values of manually-measured reservoir stage, which should have been virtually identical for a given date, varied by as much as 0.5 m among piezometer nests. Therefore, the stage value measured at each nest on 22 June 2000

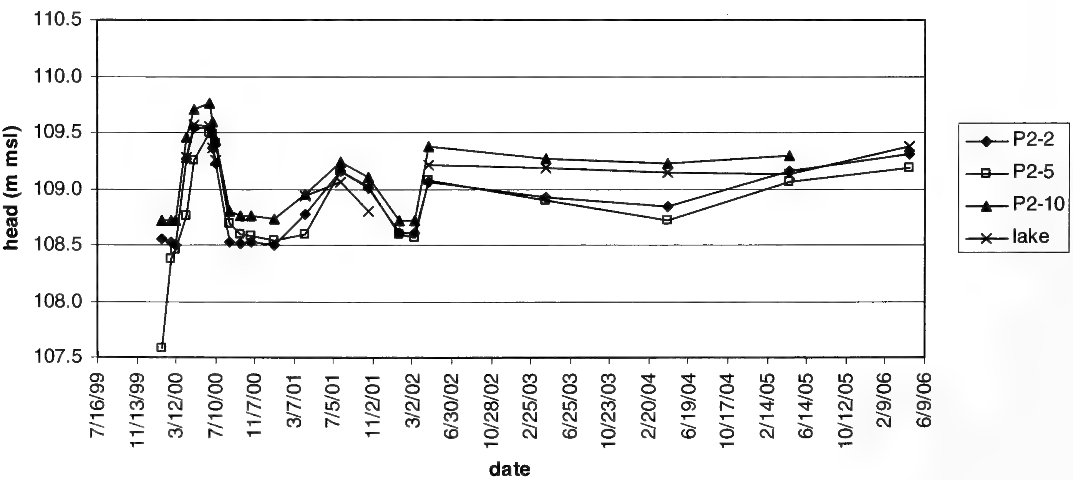

Figure 6. Manually-measured hydraulic heads and summer-pool reservoir stage at nest P2 for 28 January 2000 through 22 April 2006.

was adjusted to the simultaneous value at Kentucky Dam after correcting for the approximate slope of the reservoir surface (following Thompson 2002). Hydraulic heads likewise were adjusted for piezometers in each nest on 22 June 2000 and 7 January 2001, then contoured (Figures 7a–b). In June, when the reservoir was at summer pool, the hydraulic gradient was typically downward from the surface to 0.6 m depth and upward from 3 m depth to 1.5 m depth (Figure 7a). In January when the piezometers were not in standing water, the hydraulic gradient beneath the embayment was upward except in the top 1.5 m of the profile at nests P1, P3, and P4 (Figure 7b).

For piezometers equipped with transducers and dataloggers, raw transducer readings were plotted against hydraulic heads measured manually at approximately the same time (within a period of several hours). Beginning with 7 May 2000 data for P1–2 and beginning with 22 June 2000 data for P1–5, P5–2, and P5–5, plots of measured heads versus pressures were strongly linear ($r^2 = 0.987$ to 0.999, $n = 10$ to 12 for each piezometer). We therefore used the regression equations obtained to convert raw transducer readings to hydraulic heads for nests P1 and P5 starting with the aforementioned dates. We suspect that prior non-linear responses resulted from errors in setting or recording the depths at which the transducers were suspended. Because we were comparing hydraulic heads within a given piezometer nest, rather than between nests, we did not attempt to correct continuous data (or the manually-measured data shown in Figure 6) for imprecision in surveying.

As with manual measurements, continuous data showed that hydraulic heads at nests P1 and P5 tracked reservoir stage, rising in March and falling in August. Continuous data also indicated, however, that water levels in the embayment piezometers responded to storms over shorter time scales. In seven instances between December 2000 and December 2001, five for nest P1 (Figure 8) and five for nest P5 (Figure 9), hydraulic head in piezometers rose at least 0.4 m before falling again over a period of several days to weeks. In five of these instances, precipitation of at least 9.3 cm was recorded at Princeton, Kentucky, within a week prior to the start of the hydraulic-head rise (UKAWC 2006). Four of the instances when piezometer water levels rose at least 0.4 m coincided with even greater stage rise at Kentucky Dam during winter pool. This indicated that the piezometers may have responded to temporary back-flooding in the embayment resulting from regional rainfall in addition to local precipitation. Comparing responses among piezometers for episodes in January 2001, February 2001, and November 2001, hydraulic-head rises were greatest for P5–2, were approximately equal for P1–2 and P1–5 and were smallest for P5–5 (Figures 8, 9). Coincidentally, the timing of hydraulic-head peaks was nearly simultaneous (within 5 hours) for P1–2, P1–5, and P5–2. In contrast, the peak for P5–5 lagged behind the peak for P5–2 by 2 to 4 days, except for an instance in June 2001 (during summer pool) when both piezometers peaked simultaneously. Temporary reversals in hydraulic gradient from upward to downward at nest P5 during reservoir low-stand were thus evident. Differences in the responses at nest 1 and nest 5 may have been a consequence of stratigraphic heterogeneities. Nest P1 was within ~10 m of one of the main distributary channels of Ledbetter Creek, which had relatively coarse, permeable bed sediments, whereas nest P5 was emplaced in mud at the edge of the valley.

Spring Discharge along the Embayment

Discharge for the spring along the embayment showed both seasonal and annual-scale variability. Flow rates peaked in April and decreased to minimum values in autumn or early winter before rebounding (Figure 10). The seasonality was similar to that observed for springs along Little Bayou Creek near Paducah, Kentucky (LaSage 2004), and for hydraulic heads in domestic wells and floodplain piezometers in this watershed. Measured flow rates ranged from 0.0858 L/s on 20 October 2001 to 1.95 L/s on 12 April 2003; these values probably underestimated actual discharge because of the likelihood that not all flow was captured in the bucket. Values measured each April between 2000 and 2006 varied within a factor of 2.5 and decreased progressively from 2003 to 2006. However, April flow rates appear not to have varied systematically either with short-term precipitation (during the pre-

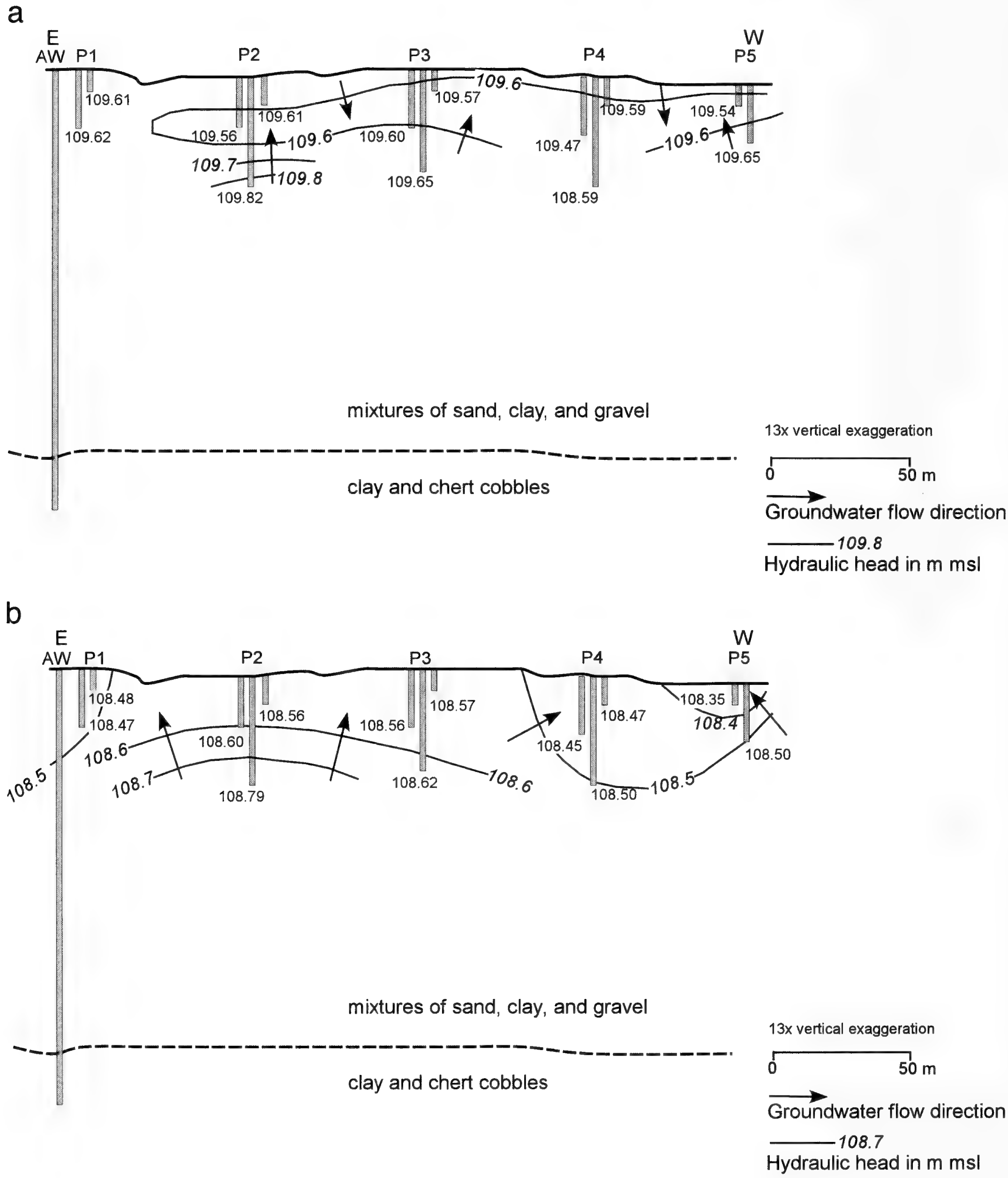

Figure 7. (a) Generalized hydrostratigraphic cross-section across Ledbetter Creek embayment for 22 June 2000 (see Figure 1(c) for map of piezometer locations). Piezometer diameters are not shown to scale; reservoir stage (109.6 m msl) and piezometer stick-ups above land surface are not depicted. Hydraulic heads for each piezometer are in m msl. Note that the hydraulic head for piezometer 4–10 had not yet equilibrated following drilling, so it was not contoured. (b) Generalized hydrostratigraphic cross-section across Ledbetter Creek embayment for 7 January 2001 (see Figure 1(c) for map of piezometer locations). Piezometer diameters are not shown to scale and piezometer stick-ups above land surface are not depicted. Hydraulic heads for each piezometer are in m msl.

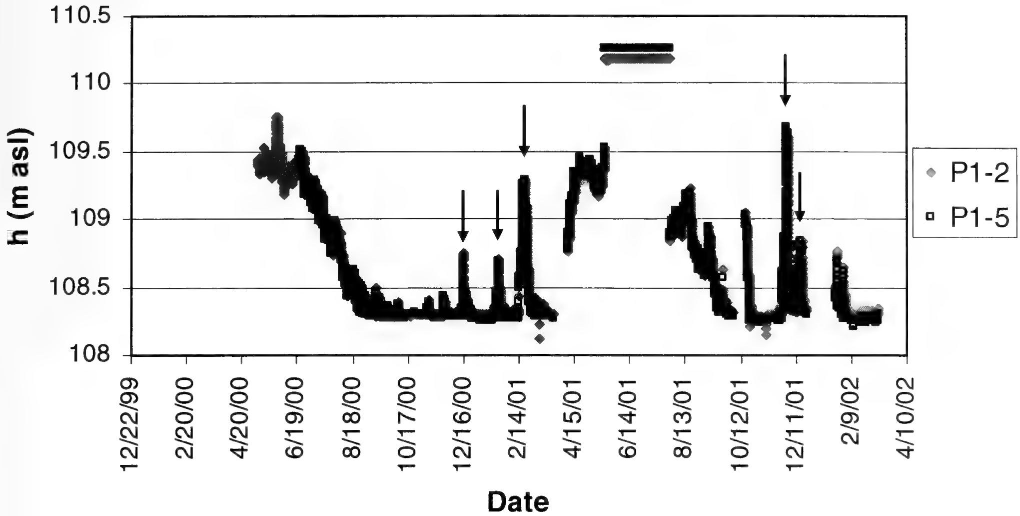

Figure 8. Continuous hydraulic heads for nest P1 (from 7 May 2000 through 10 April 2002 for piezometer P1–2 and from 22 June 2000 through 10 April 2002 for piezometer P1–5). Instances of hydraulic head rises ≥ 0.4 m (noted in text) are marked with arrows. Occasional data gaps resulted from datalogger battery failures or loose wiring connections. Flat-line intervals from 22 May through 28 July 2001 resulted from water levels rising beyond the dynamic range of the transducers.

ceding week) or longer-term precipitation (during the preceding 6 months) recorded at Princeton (UKAWC 2006).

SUMMARY AND CONCLUSIONS

Using manual, monthly to annual measurements of hydraulic head and spring flow over a period of 6 years, as well as continuous, automatic measurements of hydraulic head over a period of 22 months, we documented that groundwater flow within the Ledbetter Creek watershed tended to converge toward the creek and its embayment. Furthermore, we showed that hydraulic heads and spring flow

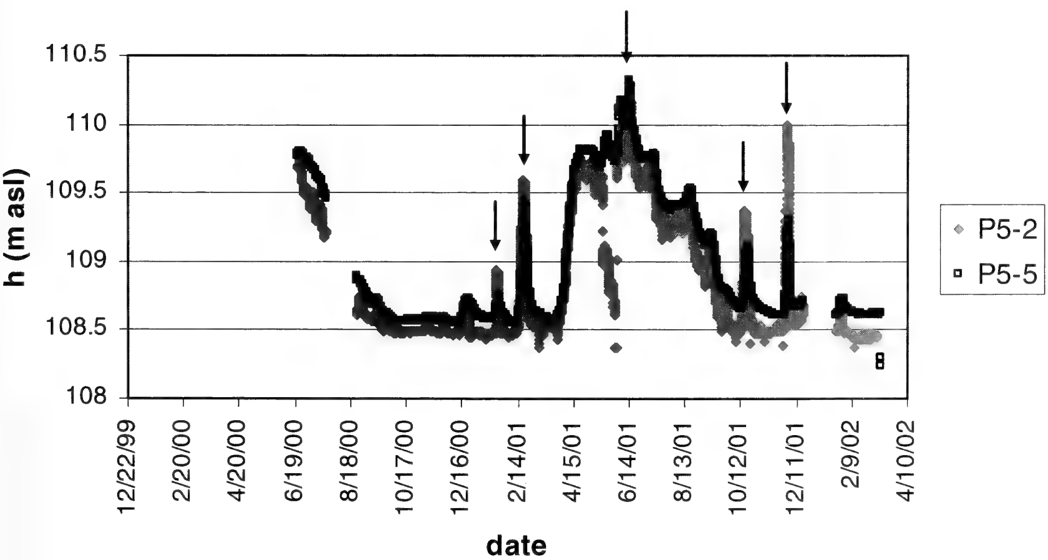

Figure 9. Continuous hydraulic heads for nest P5 from 22 June 2000 through 10 April 2002. Instances of hydraulic head rises ≥ 0.4 m (noted in text) are marked with arrows. Occasional data gaps resulted from datalogger battery failures or loose wiring connections. May 2001 data for P5–2 are suspect and may reflect an equipment malfunction.

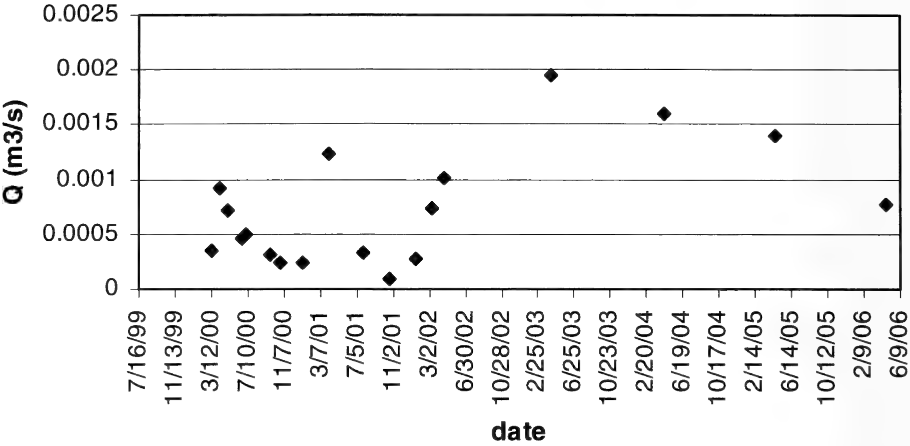

Figure 10. Discharge for spring (S) along embayment from 13 March 2000 through 22 April 2006.

rates fluctuated over various time scales. Above the embayment, including the Ledbetter Creek floodplain and the valley slope where the spring discharges, hydraulic heads and spring flow varied seasonally, probably in response to groundwater recharge, as noted elsewhere in the region. In the floodplain, hydraulic heads also varied over shorter time scales, probably as a result of precipitation and flooding. Interannual variability in hydraulic heads was less than seasonal variability. In embayment sediments, hydraulic heads fluctuated with precipitation over periods of hours to weeks, but the most pronounced changes were in response to reservoir-stage manipulation. The effects of such manipulation on groundwater flow were spatially localized. Reservoir-stage rise resulted in temporary reversals of hydraulic gradients from upward to downward to depths of 1.5 m in parts of the embayment, but Kentucky Lake management did not appear to affect the floodplain piezometers and the spring, for which land-surface elevations are within 2 m of the land surface in the embayment.

The results of this study are generally consistent with the limited number of prior studies on groundwater-reservoir interactions. In particular, as observed by Rains et al. (2004) for the East Park Reservoir in California, hydraulic-gradient reversals in the Ledbetter Creek watershed were most pronounced beneath the delta where the creek enters the reservoir. The depth of surface-water infiltration resulting from reservoir-stage rise may only be a few tens of centimeters, as indicated by stable-isotope and chloride analyses of porewater at piezometer nest P1 (Aseltyne et al. 2006). However, this study has shown hydraulic gradient reversals propagating to greater depths at other sites in the Ledbetter Creek embayment. Such reversals are likely to perturb solute distributions in sediments below the boundary of the hyporheic-hypolentic zone.

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Nutrient Uptake and Retention Patterns in Two Streams with Contrasting Watershed Landuse

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ABSTRACT

We estimated the uptake lengths (S_w) of NO_3-N and PO_4-P using the nutrient enrichment method to assess their transport dynamics in Panther Creek (forested stream) and Ledbetter Creek (agricultural stream). The agricultural stream had higher ambient NO_3-N than the forested stream, while PO_4-P was higher in the forested stream than agricultural stream. Although we expected shorter S_w estimates in the forested stream based on the ambient nutrient concentrations and higher metabolism, the agricultural stream (Ledbetter) had shorter S_w (372 m for N and 327 and 242 m for P) than estimates from Panther Creek (753 and 870 m for N; 827 m for P). Nutrient uptake patterns in these streams may reflect the combined effects of biological activities and hydrologic characteristics. Ledbetter Creek contained several large pools and backwater areas that allowed longer residence and contact time for amended nutrients than Panther Creek. It was also assumed that Ledbetter had a potential for higher abiotic phosphorus adsorption and microbial denitrification due to the presence of large pools and finer substrate composition along the study reach.

KEYWORDS: streams, Kentucky, nitrogen, phosphorus, nutrient retention, watershed landuse

INTRODUCTION

Nutrient dynamics in stream ecosystems are described in terms of spiraling length, which is the longitudinal travel distance for a nutrient atom as it completes a cycle through dissolved and particulate forms (Newbold et al. 1981; Stream Solute Workshop 1990; Newbold 1992). The spiraling length is composed of uptake length (downstream transport in inorganic form in water column) and turnover length (distance traveled as a molecule in biota until regeneration). Uptake length constitutes the majority of the spiraling length, thus it can serve as a measure of total spiraling length in stream ecosystems under base flow (Newbold et al. 1981; Mulholland et al. 1985). Nutrient uptake length is a useful index of stream ecosystem functions because it integrates retention processes occurring along a flow path, including longitudinal and vertical connections to subsurface interstices (Munn and Meyer 1988; Stream Solute Workshop 1990; Valett et al. 1996). Uptake length is an indicator of stream nutrient retention efficiency, and a stream with short nutrient uptake length can be considered as efficient in utilizing nutrients.

Nutrient uptake patterns are influenced by various biotic and abiotic processes occurring in the surrounding watershed and within the stream channel. Watershed lithology, geomorphology, and vegetation coverage influence discharge regime, stream water chemistry, and nutrient supply and may influence the limiting conditions and uptake patterns of nutrients (Munn and Meyer 1990; Martí and Sabater 1996; Valett et al. 1996). Watershed landuse patterns also may influence the hydrologic regime and nutrient dynamics in receiving streams. Landuse changes such as urbanization or agricultural development and tree removal from the watershed increase sediment and nutrient loading into streams that eventually alter the stream benthic habitats and biotic characteristics (e.g., Aumen et al. 1990; Haggard et al. 2001; Grimm et al. 2005; Bernot et al. 2006). Nutrient uptake data also can be used as a tool for assessing the functional characteristics of stream ecosystems (Grimm et al. 2005; Bukaveckas 2007).

The goal of this study was to characterize patterns of NO_3-N and PO_4-P uptake in two streams with contrasting landuse patterns and parent geology. Streams used in this study have different hydrology, channel geomorphology, and water chemistry stemming main-

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Table 1. Watershed and long-term chemical characteristics of South Fork Panther Creek and Ledbetter Creek. Channel slopes were estimated using a 1:50,000 topographic map. Water chemistry data are obtained from the 3-yr averages during 2003–2005. Data acquired from the Center for Reservoir Research, Murray State University, Murray Kentucky.

	Panther Creek	Ledbetter Creek
Watershed area (ha)	830	1103
Area forested (%)	96	55
Chanel slope (%)	0.62	0.49
NO_3-N (mg/liter)	0.035 (0.017–0.049)	0.208 (0.003–0.540)
PO_4-P (mg/liter)	0.032 (0.012–0.136)	0.009 (0.03–0.086)

ly from the bedrock lithology and watershed landuse, while experiencing similar climatic environments due to their geographic proximity. We postulated that such contrasting watershed and stream characteristics would result in different nutrient uptake patterns in streams.

STUDY SITES

The South Fork Panther Creek (36.51°N, 87.98°W) is located on the east side of the Land Between the Lakes (LBL) in Stewart County, Tennessee. Ledbetter Creek (36.74°N, 88.15°W) is on the west side of Kentucky Lake in Calloway County, Kentucky. Both streams are spring-fed and enter into Kentucky Lake as 3rd order streams. They are shaded by deciduous riparian vegetation throughout the growing season (April to October) with beds composed mainly of cobble and gravel. While they have similar basin areas and experience similar weather patterns due to the geographic proximity, they showed very different chemical and hydrological characteristics mainly due to the watershed landuse and bedrock geology (Table 1).

Panther Creek basin is more than 95% second growth oak-hickory forest with small patches of grassland (MARC 1999). The geology is composed mainly of limestone and chert, derived from the St. Louis and Salem Limestone, Warsaw Limestone, and Port Payne Formations (Marcher 1967). Ledbetter Creek has ~55% second growth oak-hickory forest in various stages of succession and ~45% agriculture/rural development areas (MARC 1999) (Table 1). Its geology is composed mainly of noncalcareous silt, sand, clay, gravel, and chert derived from the continental deposits, Loess, Fort Payne, and Clayton and McNairy formations (Olive 1965).

Long-term (year 2003–2005, n = 27) monitoring data indicate different nutrient concentrations in two study streams (Table 1). Panther had higher PO_4-P concentration (0.032 mg/l) than Ledbetter Creek (0.009 mg/l), while NO_3-N was higher in Ledbetter Creek (0.208 mg/L) than Panther Creek (0.035 mg/l).

METHODS

Nutrient Enrichment Experiments

We conducted two short-term (3.0 hrs on July and 0.5 hrs on August) tracer addition experiments during summer 2006 to compare nutrient uptake. Lengths of the experimental reach were 149 m and 168 m in Panther and Ledbetter Creek, respectively. We amended nutrients NO_3-N and PO_4-P in forms of $NaNO_3$ and NaH_2PO_4 and a conservative tracer, Cl as NaCl, during stable flows. Solutions containing the tracers were injected into streams at constant rates using a DC powered QB pump (Fluid Metering, Inc., New York). Duration and flow rate of solute injections were adjusted depending on discharge to achieve the nutrient concentrations clearly above (>20 μg/l) the background concentrations during samplings. We continuously monitored and recorded the specific conductivity of stream water at 3 locations along the study reach to monitor the arrival of injected solutes. Three grab samples were taken at the solute concentration plateau or peak with 125 ml Nalgene bottles at each monitoring station and stored on ice. Samples were filtered through Whatman GF/C filters (1.0 μm) upon returning to the laboratory and frozen until analysis. Samples were analyzed for NO_3-N, PO_4-P, and Cl using a Lachat Flow Injection Analyzer (Lachat Instrument, Milwaukee, WI) following the Quick Chem methods.

Hydrologic Characteristics

Instantaneous discharge (Q, l/s) was measured using the velocity-area method and an electromagnetic flow meter (Flo-Mate 2000, Marsh-McBirney, Inc., Frederick, Maryland) following each tracer release experiment. We estimated the nominal travel time (T_n, s) as the time required for tracer (measured as conductivity) to reach half of its plateau concentration or to pass half of the total added tracer at the most upstream and downstream sampling stations. The average water velocity (v, m/s) was determined from T_n and distance between the two sampling stations (m).

Nutrient Uptake Parameters

Uptake length (S_w, m) is the average distance traveled by a nutrient molecule in dissolved form before being removed from the water column by benthic biotic and abiotic processes (Newbold et al. 1981). It was calculated as the inverse of nutrient uptake coefficient (k, m^{-1}), which was calculated from the regression of background-corrected nutrient/conservative tracer (Cl) concentration ratios with distance downstream from the solute injection point (x, m) using the equation

$$\ln(R_n) = \ln(R_o) - kx,$$

where, R_0 and R_n are the nutrients/Cl concentrations corrected for background at the most upstream sampling location and any downstream locations, respectively. Linear regression analyses were performed using the Microsoft Excel 2003.

RESULTS

Physicochemical Parameters

The two study streams showed different physical and chemical characteristics during the experiments (Table 2). Panther had almost 2× higher discharge and velocity than Ledbetter. Ambient NO_3-N concentrations were higher in Ledbetter Creek, while Panther Creek had higher PO_4-P during the tracer experiments. Water temperature was slightly higher in Ledbetter than Panther on both dates.

Nutrient Retention

Overall, uptake lengths of NO_3-N and PO_4-P were shorter in Ledbetter than Panther (Table 2). Two experiments with different release durations (3 and 0.5 hrs) showed the similar uptake lengths in both streams (Table 2). Comparisons of nutrient uptake parameters based only on those experiments resulted in statistically significant k regression ($P < 0.05$, see method). In July, both P and N data resulted in significant regressions at Ledbetter, while the regression to estimate k for PO_4-P was not significant at Panther Creek (Figure 1). The S_w were 372 m and 327 m for NO_3-N and PO_4-P, respectively, at Ledbetter Creek (Figure 2). Panther Creek had S_w of 753 m for NO_3-N.

In August, nutrient retention was higher in Ledbetter than Panther (Table 2). The regressions to estimate k were significant except for the S_w of NO_3-N in Ledbetter Creek (Figure 1). The S_w were 870 m and 827 m for NO_3-N and PO_4-P, respectively (Figure 2) at Panther Creek. At Ledbetter Creek, S_w for PO_4-P was 242 m.

Table 2. Hydrological and chemical characteristics during the nutrient retention experiments and nutrient uptake parameters in Panther Creek and Ledbetter Creek.

Parameters	Panther Creek	Ledbetter Creek
July		
Water temperature (°C)	18.9	20.6
Q (liter/s)	52	30
v (cm/s)	8.7	3.4
Ambient NO_3-N (mg/liter)	0.050	0.133
Ambient PO_4-P (mg/liter)	0.018	0.007
Ambient N/P	2.78	19.0
NO_3-N S_w (m)	753	372
PO_4-P S_w (m)	—	327
August		
Water temperature (°C)	18.2	21.8
Q (liter/s)	57	28
v (cm/s)	8.4	3.9
Ambient NO_3-N (mg/liter)	0.041	0.082
Ambient PO_4-P (mg/liter)	0.029	0.06
Ambient N/P	1.41	13.67
NO_3-N S_w (m)	870	—
PO_4-P S_w (m)	827	242

DISCUSSION

The uptake lengths of P and N were shorter in Ledbetter Creek, an agricultural stream, than in Panther Creek, a forested stream. This can be attributed to various biotic and abiotic processes related to nutrient and stream water

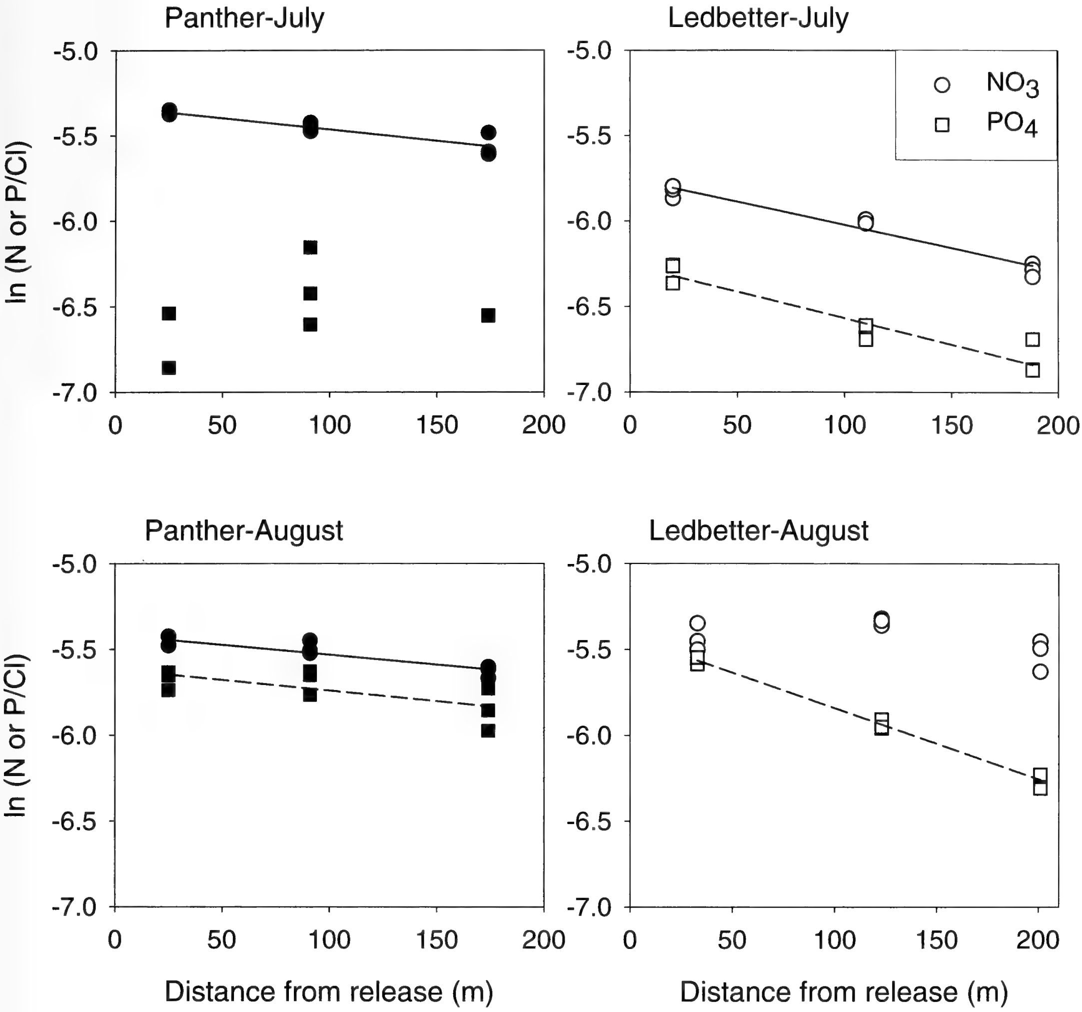

Figure 1. Nutrient/conservative trace ratio along the study reach during nutrient retention experiments. Regression lines in graph indicate the retention coefficient estimates were significant.

transport and retention dynamics. The two streams are geographically close and thus experience a similar climatic regime. However, they have different watershed lithology, land-use and channel geomorphology that influence discharge regime and nutrient dynamics. Differences in these physico-chemical characteristics may account for the different nutrient retention characteristics observed in this study (Munn and Meyer 1990; D'Angelo and Webster 1991; Martí and Sabater 1996; Valett et al. 1996; Webster et al. 2000; Hall et al. 2002; Bukaveckas 2007).

Uptake length (S_w) estimates based on the concentration changes of nutrients are integrated measures of the hydrologic and biological effects along the study reach (Stream Solute Workshop 1990). Uptake length is influenced by the hydrological and biological factors occurring in various compartments in stream. Hydrologic retention of nutrients occurs when surface water enters into slow flowing transient storages and the residence time of water is increased (D'Angelo et al. 1993; Mulholland et al. 1997; Butturini and Sabater 1999; Hall et al. 2002). Pools have higher nutrient retention capacity than riffles (Munn and Meyer 1990). In Ledbetter Creek, surface storage structures occur as pools, where stream flow is reduced. These pools also ac-

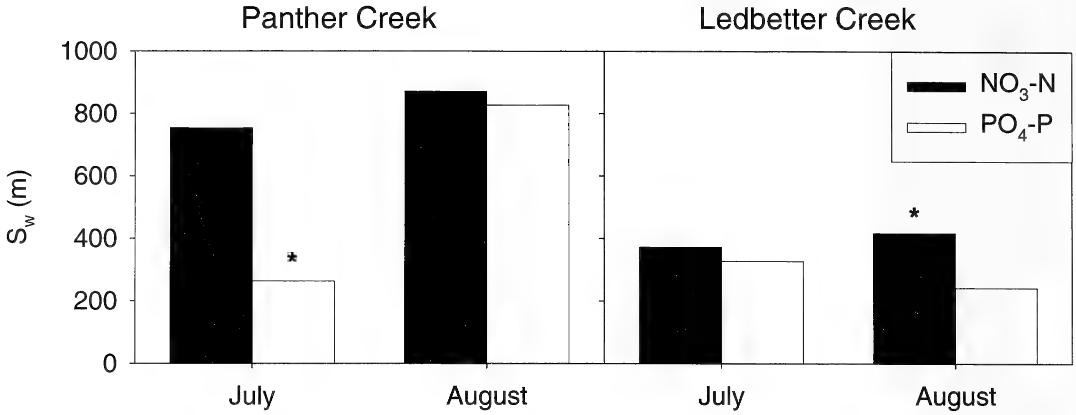

Figure 2. Nutrient uptake lengths estimated in Panther Creek and Ledbetter Creek. * indicates the uptake distance was estimated using data from 2 most downstream sampling stations.

cumulate large amounts of organic debris. Lower stream discharge and velocity have allowed longer contact time between stream water and sediment for biological and physical retention of nutrients in Ledbetter. Similar negative correlations between discharge and velocity against nutrient retention have been observed in previous studies (D'Angelo and Webster 1991; Valett et al. 1996; Simon & Benfield 2002; Bukaveckas 2007).

Retention of P reflected potential biotic and abiotic differences between the two streams. The higher ambient phosphorus concentration and low N : P ratio could partially explain the lower P uptake in Panther compared with Ledbetter Creek. In Ledbetter Creek, phosphorus could be a limiting nutrient due to its low background PO_4-P concentrations (0.007 and 0.006 mg/l). On the other hand, background phosphorus concentrations in Panther Creek (0.018 and 0.029 mg/l) were close to the biological saturation. These phosphorus concentrations reflect their limiting condition in Ledbetter, while they were not limiting in Panther (Mulholland et al. 1990; Newbold 1992). The physiological requirement of biota in stream sediment is explained by using the atomic N/P ratio (Redfield 1958). The N/P ratio was much higher in Ledbetter (19.0 and 13.67) than Panther Creek (2.78 and 1.41). Abiotic adsorption to sediments represents the principle component of P retention in streams (Hill 1982; Reddy et al. 1996; Meals et al. 1999). Meyer (1979) observed that coarse sandy sediments had lower phosphorus sorption capacity than silty sediments in Bear Brook, New Hampshire. Ledbetter Creek contained larger areas of slowing flowing pools with such find sediments likely to have higher adsorption of phosphorus than panther Creek.

Shorter uptake length of N in the agricultural stream than the forested stream was especially unexpected, because Ledbetter had higher ambient NO_3-N content and lower metabolism rates (GPP and CR) than Panther Creek (H. Jin, unpubl. data). In addition to the hydrologic retention mentioned above, instream denitrification, likely occurring in streams with high nitrate concentrations, may explain the higher N retention in Ledbetter Creek than Panther Creek (Böhlke et al. 2004; Royer et al. 2004). Sanuders and Kalff (2001) also suggested that denitrification could be the primary mechanism of nitrogen retention in aquatic systems. Denitrification is an anaerobic microbial process and occurs at high rates in stream sediments containing high organic contents such as in pools and backwaters (Burns 1998; Jacinthe et al. 1998; Martin et al. 2001). Deep (>1 m) pools and backwaters constituted a majority of habitat in Ledbetter Creek where stream flow was reduced and where there was much accumulation of organic debris.

Although we expected greater nutrient retention in the forested stream due to its higher metabolism, the nutrient uptake distances were shorter in the agricultural stream. These results can be attributed to various biotic and abiotic factors: hydrology, ambient nutrient concentrations, adsorption and other unmeasured biological activities such as denitrification. A detailed understanding of nutrient dynamics in streams would require more information on both hydrologic and biotic structure and functions. Further detailed studies focusing on these different compartments should help to explain overall ecosystem processes in Ledbetter Creek and Panther Creek.

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Benthic Algae Taxa (Exclusive of Diatoms) of the Little River Basin, Western Kentucky, 2000–2003

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ABSTRACT

The Little River is a highly disturbed system, heavily impacted by non-point source pollution from agricultural runoff in the form of excessive siltation, nitrogen and phosphorus, and organic pollution. Sixty-seven taxa of non-diatom benthic algae were documented for 16 sites in the Little River basin of western Kentucky during four sampling periods in 2000 and 2003. Algal taxa most often encountered included members of the Cyanophyta: *Oscillatoria lutea* (15 of 16 sites), *O. subbrevis* (13 of 16 sites), and *Schizothrix calcicola* (15 of 16 sites). Chlorophyta taxa most often encountered included *Oedogonium* sp. and *Rhizoclonium hieroglyphicum,* both at 10 of 16 sites. No trends were found between the algal taxa and areas of nutrient enrichment in the Little River basin. Because little is known of the benthic algal flora in the Little River, this report represents information complementary to that published previously on the benthic diatom taxa found at the same sites during the same study period.

KEY WORDS: "soft" benthic algae, water quality, pollution, streams, agricultural, Kentucky, North America

INTRODUCTION

Algae have been used as water quality indicators for over a century. Although most of the attention and effort has focused on diatoms (Bacillariophyta) as environmental indicators (Patrick and Reimer 1966, 1975; Lowe 197; Dixit et al. 1992; Stoermer and Smol 1999; Mezor et al. 2006), the non-diatom, "soft" algae have been shown to give further insights into habitat characteristics and water quality of lotic ecosystems as well (Prescott 1951; Palmer 1977; VanLandingham 1982; Rott 1991; Wehr and Sheath 2003). Benthic algal community composition reflects changes in nutrient and organic pollution inputs resulting from human activities.

The Little River basin drains a variety of land-uses; however, non-point source agricultural runoff is the primary contributor of pollutants (KDOW 1996; KWRRI 1999). While several references exist for algae in the southeastern U.S. (e.g., Dillard 1990, 1991, 1993, 2000), relatively few publications describe the composition of algal communities of western Kentucky streams and rivers. The purpose of this paper is to document the non-diatom benthic algal taxa found at 16 sites in the Little River basin during four sampling surveys in 2000 and 2003. This report complements the diatom taxa list of Hendricks et al. (2006) in the Little River basin.

STUDY SITES

The study sites in the Little River basin were characterized by Hendricks et al. (2006). Briefly, the Little River is located in the Lower Cumberland basin in western Kentucky and drains approximately 1190 km^2 (KDOW 1996). Geology of the basin is karst limestone, sandstone, and shale. Numerous springs provide the Little River with a relatively constant baseflow, and substrates are dominated by gravel, sand, silt, and bedrock. Land-use is primarily agricultural, but there also is extensive urban/suburban development around the towns of Hopkinsville and Cadiz. Little riparian vegetation exists in the basin to act as buffer strips for runoff.

Sixteen sites (Figure 1) were sampled for algae as part of a larger assessment of habitat, biological, and chemical conditions (White et

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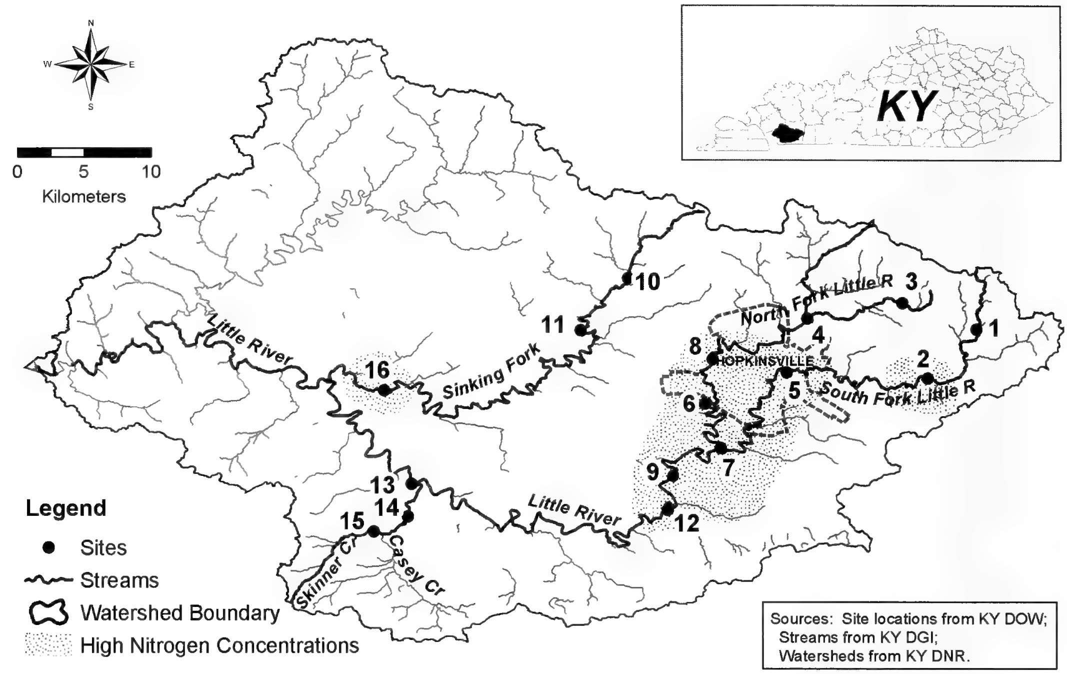

Figure 1. Study reaches in the Little River Basin. Stippled areas represent sites where NO_3+NO_2-N concentrations were highest in the basin (3.6–4.7 mg/l). Sites 6–9 and 12 around Hopkinsville were where PO_4-P concentrations were high (> 0.1 mg/l). Modified from Hendricks et al. (2006).

al. 2001; Hendricks et al. 2006) in the Little River. All sampling took place in spring (May or June) and summer (early September) of 2000 and 2003. NO_3+NO_2-N concentrations were highest in many areas of the basin during the study period, ranging from 3.6–4.7 mg/l (Figure 1). Sites 6–9 and 12 near or downstream from Hopkinsville were relatively high in PO_4-P as well with concentrations ranging from 0.10–0.37 mg/l. Other physicochemical conditions have been presented in detail in a previous publication (see Hendricks et al. 2006) and, therefore, are not included here.

MATERIALS AND METHODS

Composite, qualitative algal samples were collected at all sites from all major habitats and substrate types including riffles, pools, runs, rock, sand, and woody debris. Standard collection and identification methods used have been described in KDOW (2002). All samples were collected from natural substrates when stream flow was normal to low. Algae were sampled using a micro-spatula for scraping substrates and/or a turkey baster for sucking material from substrates. Algae were placed in 60-ml Nalgene™ bottles as a composite sample from each site, preserved in 2% gluteraldehyde, and refrigerated until processed.

The non-diatom, soft-bodied algae were subsampled from the preserved, composite sample, mounted on pre-cleaned microscope slides, identified to the lowest possible taxon (genus and species where possible) using standard taxonomic keys (Drouet 1981; Komárek and Fott 1983; Prescott 1951; Wehr and Sheath 2003; Whitford and Schumacher 1984), and counted. A minimum of 3 slides was created and counted for each sample. Some species were reported as being of uncertain identity but were identified to genus and included on the checklist. These taxa, noted as sp. 1, 2, 3, etc., were consistently recognized as taxonomic units and were included in the total number of taxa.

Fixed slides, preserved samples, bench sheets, and field shields were archived at Hancock Biological Station.

RESULTS AND DISCUSSION

Sixty-seven taxa representing five phyla of benthic "soft" algae were identified from the Little River basin in 2000 and 2003 (Table 1). Twenty-three taxa belonged to the Chlorophyta, 37 taxa to the Cyanophyta, 5 taxa to the Rhodophyta, and one taxon each to the Euglenophyta and the Cryptophyta. Sixteen taxa identified to genus only were noted; their species were unknown or of uncertain identity. Some species, such as those in the genera *Closterium* and *Gonium,* are known to occur but have not yet been described; hence the notation sp. 1.

Ten taxa (15% of total) were found during both 2000 and 2003. Thirty-two taxa (50% of total) were found only in 2000 while 23 taxa (35% of total) were found only in 2003. Site 12 had the fewest taxa (8), while site 3 had the highest (27).

Soft, benthic algal taxa most often encountered included members of the Cyanophyta: *Oscillatoria lutea* (15/16), *O. subbrevis* (13/16), and *Schizothrix calcicola* (15/16). *O. lutea* is tolerant of high salinities (Cloern and Dufford 2005). *O. subbrevis* is commonly found in sewage treatment ponds (Haughey 1969) and, therefore, is highly tolerant of organic pollution. *Schizothrix* species are also tolerant of saline conditions (Komárek et al. 2003). Cyanophyta in general are known to reside under highly eutrophic conditions, such as those found in the Little River, and were fairly evenly distributed throughout the basin.

Two members of the Chlorophyta, *Oedogonium* sp. and *Rhizoclonium hieroglyphicum,* were commonly encountered at 10 of 16 sites. *R. hieroglyphicum* is the most common of the 5 freshwater *Rhizoclonium* species found in North America and lives on turtles (John 2003). *Mougeotia* sp. was another chlorophyte found fairly commonly throughout the basin.

Two phyla, the Euglenophyta and the Cryptophyta, were represented by one taxon each, *Euglena* sp. and *Cryptomonas* sp., respectively. *Euglena* species are known to be tolerant of organic pollution (Lackey 1968) and reside in aquatic systems surrounded by agriculture (Rosowski 2003).

In a previous study carried out on the Little River in 1988 (KDOW 1996) five phyla and 42 taxa were represented: Chlorophyta (24), Chrysophyta (2), Cyanophyta (11), Euglenophyta (3), and Rhodophyta (2). Notable differences between the two studies were that no chrysophytes were found in the present study, and no cryptophytes were found in the 1988 study. Further, the Euglenophyta in the 1988 study were represented by *Phacus* and *Traechelomonas*; only *Euglena* was found in this study. Euglenophytes in general are known to tolerate organic pollution and eutrophic conditions.

The Rhodophyta (red algae) were represented by different taxa in the two studies. It should be noted that *Batrachospermum* was found during the 1988 study, and although it was observed in the field and collected during the present study, it did not appear in any subsamples. *Batrachospermum* has been included in Table 1 because of our field observations. A total of 16 taxa was shared between the two studies, and when both studies were combined, a total of 77 taxa of soft-bodied algae now have been recorded for the Little River basin since 1988. Notably, many more cyanophytes were found during this study than in 1988 (37 vs. 11, respectively).

We have no explanation for the discrepancies in taxa held in common between the two sampling years of this study, 2000 and 2003, or between the present study and that of 1988 (KDOW 1996). Perhaps the sample composites made in the present study were more representative of the total flora than those in 1988. Results of the 1988 study were based on only one sampling survey, whereas the present study was based on two sampling periods (two seasons) from two years with the results combined (Table 1). Thus, the greater diversity presented here should not be too surprising. Similarly, many more diatoms were found in 2000 and 2003 than in the 1988 study (Hendricks et al. 2006).

CONCLUSIONS

The Little River is a highly disturbed system, receiving excessive agricultural runoff and some urban/suburban inputs in the form of nutrients (N and P), organics, pesticides, and silt. The canopy has been opened up throughout much of the basin with little riparian vegetation. With high nutrient loading and higher light intensity reaching these disturbed habitats, algal growth may be stimulat-

Table 1. Non-diatom algal taxa found in the Little River basin by site. Occurrences are noted from 2000 (x/) and 2003 (/x) or both (x/x). Sites are ordered from upstream to downstream in respective reaches and correspond with Figure 1.

	South Fork			North Fork				Little River			Sinking Fork			Casey/Skinner		
Sites	1	2	5	3	4	8	6	7	9	12	10	11	16	15	14	13
Taxon																
CHLOROPHYTA																
Ankistrodesmus falcatus var. *mirabilis* (West & West) G. S. West					/x											
Characium pringsheimii A. Braun		x/		x/				x/				x/				x/
C. rostratum Reinhard ex Printz				x/												
Cladophora sp.	/x	/x				x/			x/	x/			x/			x/
Closterium moniliferum Ehrenberg ex Ralfs					/x											
C. sp. 1															x/	
Cylindrocapsa conferta W. West				/x												
Dichotomosiphon tuberosus (A. Braun ex Kützing) A. Ernst											/x					
Eudorina elegans Ehrenberg				x/												
Gonium pectorale O. F. Müller														x/		
Gonium sp.1														x/	x/	
Hydrodictyon reticulatum (L.) Lagerheim					/x											
Mougeotia sp.		x/		x/x					x/		x/	x/	x/	x/	x/	
Oedogonium sp.	x/	x/x		x/x	x/x	x/					/x	x/x		x/x	/x	/x
Pithophora kewensis Wittrock		x/		x/		x/		x/								
Rhizoclonium crassipelitum West & West													/x			
R. hieroglyphicum (C. Agardh) Kützing	x/			x/x	/x				x/		/x	x/x	/x	/x	x/x	/x
R. hookeri Kützing		x/		/x	/x		/x	/x								
Scenedesmus dimorphus (Turpin) Kützing				x/	x/											
S. quadricauda (Turpin) Brébisson				x/			/x									
Spirogyra sp.	/x			x/x	/x											
Stigeoclonium sp.														x/		
Ulothrix sp.								x/								
CRYPTOPHYTA																
Cryptomonas sp.					x/											
CYANOPHYTA																
Anabaena sp.				/x	/x										x/	x/
Aphanocapsa sp.				x/												
Calothrix parietina (Nägeli) Thuret														x/		
C. sp.											/x					
Chamaesiphon incrustans Grunow		x/									x/	x/				
Chroococcus turgidus (Künzing) Nägeli				x/												
Chroococcus sp.								x/		x/		/x				
Coccochloris elabans (Breb.) Drouet and Daily				x/												
Coelosphaerum sp.							/x									
Dactylococcopsis acicularis Lemmermann	/x	/x														
Lyngbya diguetii Gomont		/x		/x		/x										
L. limnetica Lemmermann								/x								
L. nana Tilden	/x															
Merismopedia punctata Meyen	x/		x/	x/		x/					x/	x/		x/		
Nostoc sp.			x/		x/							x/				
Oscillatoria agardhii Gomont									/x						/x	/x
O. amoena (Kützing) Gomont							/x							/x		
O. articulata (Gard.)			/x													
O. limnetica Lemmermann	/x										/x					
O. lutea C. Agardh	x/		x/	x/	x/	x/	x/	x/	x/	x/	x/	x/	x/	x/	x/	x/
O. nigra Vaucher							/x	/x		/x		/x		/x	/x	
O. rubescens De Candolle			/x	/x	/x		/x									
O. splendida Greville											/x					
O. subbrevis Schmidle			/x	/x	/x	/x	/x	/x	/x	/x	/x		/x	/x	/x	/x
O. submembranacea Ardissone & Strafforello ex Gomont							x/									
O. tenuis C. Agardh		/x	/x	/x		/x					/x	/x				

Table 1. Continued.

	South Fork			North Fork				Little River			Sinking Fork			Casey/Skinner		
Sites	1	2	5	3	4	8	6	7	9	12	10	11	16	15	14	13
O. terebriformis C. Agardh	/x															
Oscillatoria sp.		x/	x/			x/										
Poryphyrosiphon animalis (C. Agardh) Drouet										x/						
P. notarisii Kützing ex Gomont						x/										
P. splendidus (Greville) Drouet		x/	x/	x/								x/		x/	x/	
Schizothrix arenaria Berkeley						x/						x/				
S. calcicola (C. Agardh) Gomont	x/	x/	x/	x/	x/	x/	x/	x/	x/	x/	x/		x/	x/	x/	
S. friesii (C. Agardh) Gomont				x/												
S. sp.			x/		x/			x/	x/						x/	
Spirulina subsalsa Örsted				x/												
Stichosiphon sansibaricus Örsted			x/		x/	x/		x/	x/						x/	
EUGLENOPHYTA																
Euglena sp.	x/															
RHODOPHYTA																
**Batrachospermum* sp.																
Lemanea cf. *fluviatilis*		x/	x/				x/		x/	x/			x/			
Lemanea sp.	/x		/x	/x					/x							
Rhotochorton sp.		/x	/x				x/	/x	x/x	x/	x/		x/			
Total Number of Taxa	13	15	15	27	17	13	13	12	13	8	15	13	9	14	14	9

* Encountered in the field and collected, but did not appear in subsamples. May be common throughout the basin.

ed rather than impaired as has been observed in other agriculturally impacted streams (Niyogi et al. 2004). High numbers of diatom taxa were found at sites with higher NO_3+NO_2-N (Hendricks et al. 2006); however, the soft-bodied algae were more evenly distributed throughout the basin and did not exhibit a strong pattern that reflected responses to higher nitrogen and phosphorus inputs.

Further studies of both diatom and non-diatom taxa, in relation to physicochemical conditions, will need to be carried out in order to clarify relationship between the algae and factors controlling their diversity in this lotic ecosystem. The list presented here is a useful documentation of the presence of algal taxa in the Little River basin that could be used in future comparative studies.

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Accumulation of Polychlorinated Biphenyls in Pine Needles Collected from Residential and Industrial Areas in Western Kentucky

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ABSTRACT

One-year-old pine needles collected from residential and industrial locations in Kentucky were analyzed for polychlorinated biphenyls. For comparison, pine needle samples from the highly contaminated Linden Chemicals and Plastics (LCP) Superfund site in Brunswick, Georgia, also were analyzed. PCB concentrations in residential Kentucky ranged from 1.91 ng/g dry wt. to 12 ng/g dry wt. These sites were comparatively less polluted than those from the Superfund site, that had concentrations ranging from 15 ng/g dry wt. to 34 ng/g dry wt. Finger printing of PCB contamination sources was possible using pine needle PCB profiles. PCB-28 and PCB-52, PCB-101, PCB-153 and PCB-138 in pine needles indicated uptake of Aroclors −1016 and −1242, −1254, −1260 in Kentucky sites. Pine needles from the Superfund site and its vicinity contained higher chlorinated PCBs especially, PCB-196, PCB-199, and PCB-206 characteristic of Aroclor 1268. When the toxic equivalencies (TEQs) of PCBs were compared, Kentucky pine needles were only slightly lower (0.03–0.17 pg/g dry wt) than pine needles from the Superfund site (0.24–0.48 pg/g dry wt). Considering the homologue distribution of PCBs in pine needles from Kentucky and the Superfund site, lower chlorinated PCBs seem to have traveled farther than the higher chlorinated PCBs.

KEY WORDS: Pine needles, PCBs, residential, industrial, Superfund site, Aroclor 1268

INTRODUCTION

Global environmental contamination by polychlorinated biphenyls (PCBs) was recognized more than 30 years ago when PCBs were detected in pike from Sweden (Jensen 1966). Since then, numerous studies have reported PCBs in various environmental compartments and the occurrence of PCBs in remote areas, such as the Arctic (AMAP 1998), which provide evidence for the long-range atmospheric transport of these contaminants. PCBs were known for their widespread environmental contamination, bioaccumulation, biomagnification in food chains, and exposures cause toxic effects in wildlife and humans (Loganathan and Kannan 1994). Although the production of PCBs was banned in many developed nations over two and a half decades ago, due to its recalcitrant properties, PCBs continue to cycle among various environmental compartments. Ecosystems located near or at sites are at the greatest risk from exposure to PCBs through air, water, or contaminated soil or fish.

Westernmost Kentucky is endowed with a variety of industries and state-of-the-art agricultural operations. A major industrial area in western Kentucky is the Calvert City Industrial Complex (CCIC) in Marshall County (Kentucky Outlook-2000 1997). These industries manufacture various chemicals including polymers, solvents, gases, heavy metals, etc. Over 9.32 million pounds of annual TRI (Toxic Release Inventory) chemicals were reported in air emissions in Marshall County in 1988 with a steady decrease in release to 3.49 million lbs in 2001 (USEPA 2003). Another industry in westernmost Kentucky is the Paducah Gaseous Diffusion Plant (PGDP) constructed in 1951 as a uranium enrichment facility. Along with other chemicals, PCBs were used in the enrichment process as lubricating agents (Kentucky Outlook-2000 1997).

There are several PCB-contaminated Superfund sites in the United States and these sites were protected to limit dispersal of PCBs. The Linden Chemicals and Plastics

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(LCP) Superfund site in Glynn County, Brunswick, Georgia, had a chlor-alkali plant that was established in 1955 and operated until 1994, when it was designated as a Superfund site. As a result of multi-industrial operations, the site and the adjacent brackish water have been severely contaminated by metals (mercury, lead, chromium and zinc) and organics (PCBs, polycyclic aromatic hydrocarbons [PAHs] and phenolic compounds) (USEPA 1995; Kannan et al. 1997, 1998). Aroclor 1268 is a highly chlorinated PCB formulation that was applied to electrical equipment used in the chlor-alkali process at the LCP site (Kannan et al. 1997, 1998). Our earlier study showed presence of Aroclor 1268 congeners in street dust and soil collected near the Superfund site, suggesting the escape of the PCB beyond the restricted area of the site (Loganathan et al. 1997).

Because semi-volatile organic compounds are known to partition between the vapor phase and lipophillic substances, plant foliage concentrations of organic pollutants have been reported to be good indicators of atmospheric contamination (Calamari et al. 1991). Epicuticular wax of pine needles accumulates organic pollutants from the atmosphere, and pine needle samples have been used for monitoring both local and regional distributions of semi-volatile organic air pollutants and delineating specific sources of the pollutants (Kylin et al. 1994; Tremolada et al. 1996; Hanari et al. 2004; Falandysz et al. 2006; Grimalt and van Drooge 2006; Romanic and Krauthacker 2006; Wyrzykowska et al. 2006). The present study was conducted to examine the possibility of atmospheric contamination using pine needle as a "bioindicator." We proposed that pine needles could be used as a fixed site, regenerative, annual monitoring matrix for the evaluation of local and regional distributions of lipophillic air pollutants (Eriksson et al. 1989; Loganathan et al. 1998). The diffusion of gaseous lipophillic pollutants into the cuticle can be viewed as a chemical partitioning process in which a compound is transferred from the ambient air to the pine needle until equilibrium is reached.

In this paper, we present selected PCB congener data from second year class (one year old) needles of loblolly pine (*Pinus taeda* L.) collected from residential and industrial areas of Kentucky and adjacent to the LCP Superfund site, Georgia. Contamination profiles of PCBs were illustrated on regional basis and the toxic potential of the contaminants in pine needles from residential and industrial sites were described based on toxic equivalencies (TEQ) calculated using World Health Organization's (WHO) toxic equivalent factors (TEFs).

MATERIAL AND METHODS

Sample Site Selection

Pine needle sampling was done in residential and industrial areas in western Kentucky and Brunswick, Georgia (Figure 1, Table 1). The sites R-1, R-2, and R-3 were residential areas in McCracken County. Sites R-1 and R-2 were southeast of Paducah Gaseous Diffusion Plant (PGDP). Site R-3 was centrally located with respect to several industrial sites in western Kentucky. The sampling sites R-4 and R-5 were residential locations near the Clarks River Wildlife Refuge in Marshall County. Sites I-1, I-2, and I-3 were near PGDP that has used PCBs in the past and still contain PCBs in the soil and water near the plant. Site I-1 was north of the plant, while sites I-2 and I-3 were southwest of the plant. Sites I-4 through I-8 were 'downwind' of the CCIC where various chemicals were manufactured. The sampling sites S-1 through S-4 were near the LCP Superfund site in Brunswick, Glynn County, Georgia (Figure 1). The sites selected ranged in distance from the plant from a few meters (e.g., S-1 and S-2) to several kilometers away (e.g., S-3 and S-4) (Table 1).

Sampling

The most common pine species available in western Kentucky are loblolly (*Pinus taeda* L.), white pine (*Pinus strobes* L.), and Virginia pine (*Pinus virginiana* Mill.). At all locations loblolly needles were collected, except at site R-2 where Virginia pine needles were collected. Using a telescoping pruner, a tip of a primary branch was cut from an accessible part of the tree. The pine needles were then wrapped in pre-cleaned aluminum foil and transported to the laboratory in an iced cooler. The age of the needles was determined by growth patterns on the primary branch. The needles were cut using pre-cleaned stainless steel scissors, packed in pre-cleaned alumi-

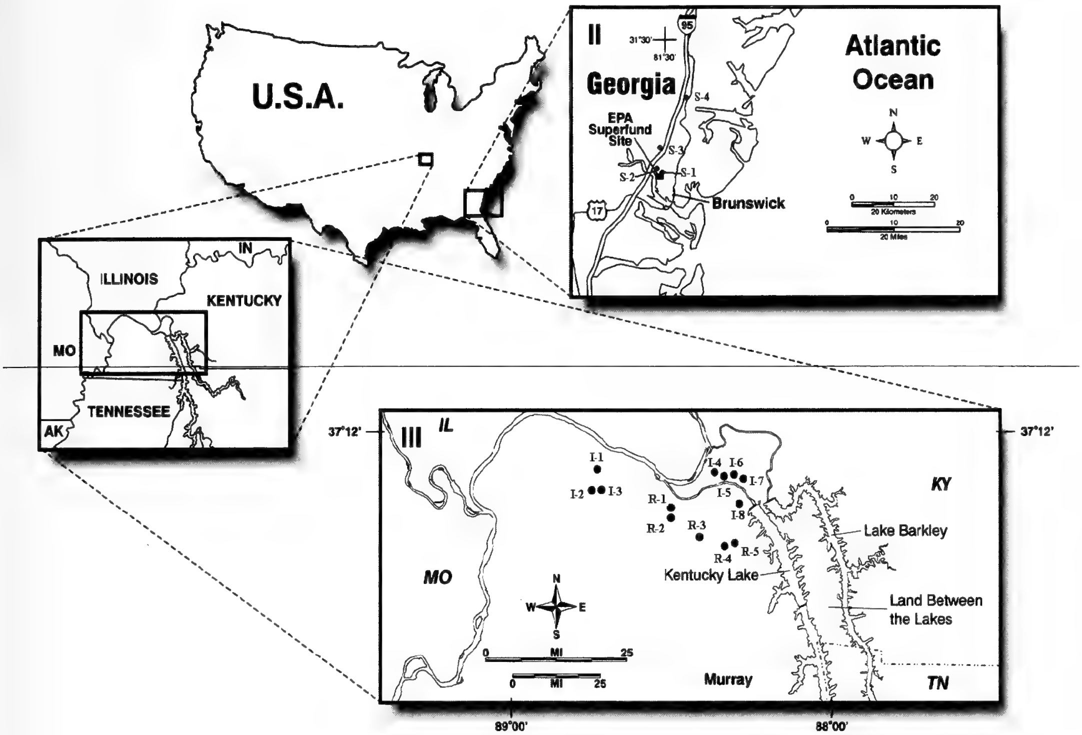

Figure 1. Map showing pine needle sampling locations in western Kentucky and Brunswick, Georgia. R = Residential; I = Industrial; S = LCP Superfund site.

Table 1. Details of pine needle sampling dates and locations.

Sample ID	Location	County, state	Sampling date	Latitude	Longitude
Residential					
R-1	Island Creek	McCracken County, KY	7/5/1999	N 370 03′ 17.0″	W 880 35′ 39.0″
R-2	Island Creek	McCracken County, KY	7/6/1999	N 370 02′ 17.0″	W 880 35′ 35.0″
R-3	East Fork Clarks River	McCracken County, KY	7/6/1999	N 360 57′ 10.0″	W 880 29′ 21.0″
R-4	Oak Vally Road	Marshall County, KY	6/30/1999	N 360 54′ 54.0″	W 880 22′ 10.0″
R-5	Foust Sledd Road	Marshall County, KY	6/30/1999	N 360 54′ 58.0″	W 880 21′ 36.0″
Industrial					
I-1	Ogden Landing Road	McCracken County, KY	3/12/2001	N 370 08′ 09.1″	W 880 48′ 35.8″
I-2	Woodville Road	McCracken County, KY	3/12/2001	N 370 05′ 24.9″	W 880 50′ 49.8″
I-3	Rice Springs Road	McCracken County, KY	3/12/2001	N 370 05′ 24.6″	W 880 50′ 43.9″
I-4	Across from Oliver Road	Livingston County, KY	7/10/2002	N 370 05′ 00.3″	W 880 21′ 00.4″
I-5	East of Bloodworth Road	Livingston County, KY	7/10/2002	N 370 04′ 00.5″	W 880 20′ 01.0″
I-6	Fred Tracey Road	Livingston County, KY	7/10/2002	N 370 04′ 00.1″	W 880 20′ 00.5″
I-7	Bloodworth Road	Livingston County, KY	7/10/2002	N 370 04′ 00.4″	W 880 20′ 00.1″
I-8	Off Hwy 282	Marshall County, KY	7/10/2002	N 370 02′ 00.2″	W 880 19′ 00.7″
Superfund					
S-1	50 ft. from Site[a]	Glynn County, GA	7/24/1997	NA	NA
S-2	200 ft. from Site	Glynn County, GA	7/24/1997	NA	NA
S-3	4 mi. from Site, Left of I-95	Glynn County, GA	7/24/1997	NA	NA
S-4	12 mi. from Site, Right of I-95	Glynn County, GA	7/24/1997	NA	NA

[a] LCP Superfund Site.

Table 2. PCB congener concentrations (ng/g dry wt.) in pine needles collected from residential, industrial areas in western Kentucky and Superfund Site at Brunswick, Georgia.

IUPAC No.	8	18	29	28/50	52	104	44	101	87	154	118	153	105	138	126
R-1	1.7	0.50	0.31	0.67	1.1	0.94	0.60	1.3	0.66	1.1	0.43	0.95	BDL	0.22	0.04
R-2	0.44	BDL	BDL	0.45	0.86	0.89	0.49	1.4	0.55	0.90	0.33	0.38	BDL	0.31	BDL
R-3	0.12	BDL	0.40	0.32	0.95	0.43	0.68	0.93	0.37	0.98	0.26	0.29	0.18	0.19	0.18
R-4	BDL[a]	0.22	BDL	0.22	0.65	0.20	0.41	1.1	0.35	0.98	0.36	0.32	BDL	0.22	0.02
R-5	0.06	BDL	BDL	0.29	BDL	0.26	0.64	1.5	0.54	1.1	0.51	0.39	0.08	0.32	0.16
I-1	0.58	0.35	BDL	1.37	BDL	0.46	0.56	1.9	0.48	0.56	0.39	0.49	0.07	0.56	BDL
I-2	0.53	BDL	BDL	0.20	BDL	0.22	0.30	0.76	0.22	0.35	0.26	0.33	0.09	0.10	BDL
I-3	0.57	0.24	BDL	0.09	0.42	1.6	0.46	1.7	0.43	0.57	0.33	0.34	0.07	0.25	BDL
I-4	0.20	BDL	BDL	0.16	BDL	BDL	BDL	0.63	0.13	0.19	0.11	0.19	BDL	0.09	BDL
I-5	0.26	BDL	BDL	0.05	0.39	BDL	0.22	0.62	0.16	0.20	0.12	0.18	0.04	0.08	BDL
I-6	0.28	0.16	BDL	0.10	BDL	BDL	BDL	0.91	0.19	0.24	0.16	0.23	BDL	0.11	BDL
I-7	0.30	0.18	BDL	0.50	BDL	0.14	BDL	1.2	0.26	0.38	0.22	0.28	0.06	0.15	BDL
I-8	0.32	0.19	BDL	0.55	BDL	0.13	0.33	0.91	0.18	0.26	0.16	0.30	0.05	0.11	BDL
S-1	0.49	BDL	BDL	0.69	BDL	0.47	BDL	2.4	1.2	2.1	0.57	0.80	BDL	0.51	BDL
S-2	0.27	BDL	BDL	0.47	BDL	BDL	0.65	2.5	1.2	2.8	0.57	0.96	BDL	0.75	BDL
S-3	0.98	3.0	BDL	1.2	1.4	BDL	1.2	2.8	1.2	3.3	1.0	1.7	BDL	1.6	BDL
S-4	0.05	1.8	0.27	0.55	0.95	BDL	0.81	1.8	0.76	1.8	0.52	0.82	BDL	0.74	BDL

[a] BDL, Below detection limit.

num foil, and stored below −20°C until analyses were performed.

Sample Analysis

About 20–30 g of pine needles from each sample location were cut into small pieces (2–3 cm) using pre-cleaned stainless steel scissors. The needles were then Soxhlet extracted using 400 ml of a 3:1 v/v ratio of dichloromethane/acetone (pesticide grade, Fisher Scientific/ optima, Fisher Scientific) mixture for 16-h. Internal standards (4,4′-dibromooctafluorobiphenyl (Fisher Scientific) in hexane, 200 ng/20 μl; PCB 103 (Fisher Scientific) in hexane, 200 ng/100 μl) were spiked in to each sample and blank before the Soxhlet extraction. The extract was then concentrated to 10 mL using a Kuderna-Danish (K-D) apparatus. Solvent transfer to hexane was preformed by repeating the K-D concentration twice after adding 100 ml of hexane (optima, Fisher Scientific) each time. The sample extract was then further concentrated to 5 ml using a stream of nitrogen gas to evaporate the solvent. Treatment with sulfuric acid was then preformed to oxidize interfering organic species (e.g., wax which consists mainly of long-chain esters, polyesters, and paraffins, pigments, carbohydrates, alcohols) from the sample. This was preformed by adding an equal volume of concentrated sulfuric acid (trace metal grade, Fisher Scientific) to each extract. The upper hexane layer was carefully removed from the sulfuric acid layer. The hexane layer containing the analytes was then washed with an equal volume of nanopure water to remove trace amount of acid from the hexane. The upper hexane layer was then carefully removed and transferred to another tube. The extract was then evaporated to 5 ml using a gentle stream of nitrogen gas.

Silica-gel column chromatography was carried out to remove interfering organic and polar species and to separate the PCBs from the pesticides. For each sample, 1.5 g of silica gel (Wakogel® S-1, Wako Pure Chemicals Industries, Japan) was activated by heating it at 130°C in an oven for 3 h. After 3 h, the silica gel was immediately added to 20 mL of ultra pure hexane (B&J GC2®, Burdick & Jackson, USA) and packed in a 10 mm ID glass column. A thin layer of anhydrous sodium sulfate salt (certified A.C.S., 10–60 mesh, Fisher Scientific) was then added on top of the silica gel to remove any water that might have been in the sample. PCBs were eluted with 120 ml of ultra pure hexane. This fraction was concentrated using a K-D concentration apparatus to 10 ml followed by nitrogen gas evaporation to 0.1 ml and then used for GC-ECD analysis.

Instrumental Analysis

PCB congeners were analyzed using a Shimadzu Model GC-17A gas chromatograph

Table 2. Extended.

187	202	201	180	200	170	198	199	196	208	195	207	194	206	209	Total
0.05	BDL	0.48	0.12	BDL	0.08	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.84	BDL	12
0.03	BDL	BDL	0.12	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.28	BDL	7.4
0.04	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.22	BDL	6.6
0.02	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.19	BDL	5.2
0.10	BDL	0.05	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.45	BDL	6.5
0.11	BDL	0.17	0.17	BDL	0.06	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.08	BDL	8.4
BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	3.4
BDL	BDL	BDL	0.07	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	7.4
BDL	BDL	0.14	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	1.9
BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	2.3
BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	2.4
BDL	BDL	0.23	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	3.9
BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	3.5
2.0	2.2	0.76	0.84	0.25	0.09	1.2	3.5	3.1	1.7	1.5	0.54	0.72	4.0	0.66	32
2.1	2.4	0.95	0.69	0.35	0.12	2.2	3.3	3.0	1.6	BDL	0.50	0.58	3.8	0.59	32
1.2	2.0	1.2	1.1	0.57	0.31	0.27	2.1	BDL	1.1	0.97	0.40	0.36	2.3	0.50	34
0.38	0.47	0.33	BDL	0.03	0.08	0.1	0.69	BDL	0.33	0.30	0.12	0.14	0.87	0.17	15

(GC) with a Shimadzu Model AOC-17 auto injector. The GC was equipped with a DB-5 (30 m; 0.25 mm ID; 0.25 μm film thickness) capillary column (J&W Scientific, USA) and a ^{63}Ni electron capture detector. The column oven temperature program began with a 1 min. hold time at 90°C then ramped at 10°C/min. until 200°C then ramped at 1°C/min. until 280°C with a hold time of 20 min. The injector and detector temperatures were 280°C and 330°C respectively. Helium (1.1-ml/min.) and nitrogen (28-ml/min.) were used as carrier and makeup gases, respectively.

Forty one PCB congeners were analyzed, and their results were reported individually from all the locations. To determine the retention times of the individual PCB congeners, pure standards were injected into the GC-ECD. Five different concentrations of the standard mixture were injected in order to obtain calibration curves of all the PCB congeners. The mean slope (response factors) and r^2 values were calculated for all the PCB congeners. The PCB congeners were identified in the sample extract by comparing the retention time from the standard mixture and quantified using the response factors.

Quality Assurance and Quality Control

Quality assurance and quality control protocols were followed to evaluate the reliability of the data. The approved method was used to calculate the detection limits (Federal Register 1984). The area of baseline noise over the elution time of each congener was determined from seven injections of the matrix blank spiked with the lowest concentration of the calibration standard. The instrument detection limits (IDL) were three times the standard deviation of the baseline noise divided by the slope of the calibration curve. The quality control used for PCB congener's analysis was a reagent blank to indicate laboratory contamination. The concentration of analytes detected in the reagent blank was less than the method detection limit. A surrogate spike recovery was used to indicate loss of analytes during the analytical procedure. Surrogate spike recoveries were 100 ± 30% of known standard spiked prior to extractions. Also the relative accuracy of the method was determined using Standard Reference Material-1941a in which 100 ± 30% of the known certified material was obtained. Due to the low recovery of both surrogate spike internal standards I-1's, I-2's, and I-3's concentrations were corrected using their PCB-103 recovery. Calibration and calibration verification (five-point calibration with r^2 = 0.99) were checked routinely. Results were compared using Stat View version 5.0.1. An unpaired *t*-test was used to compare the data. The difference between two sets of data was considered statistically significant if the p-values obtained were less than 0.05. This p-value corresponded to the 95% confidence level.

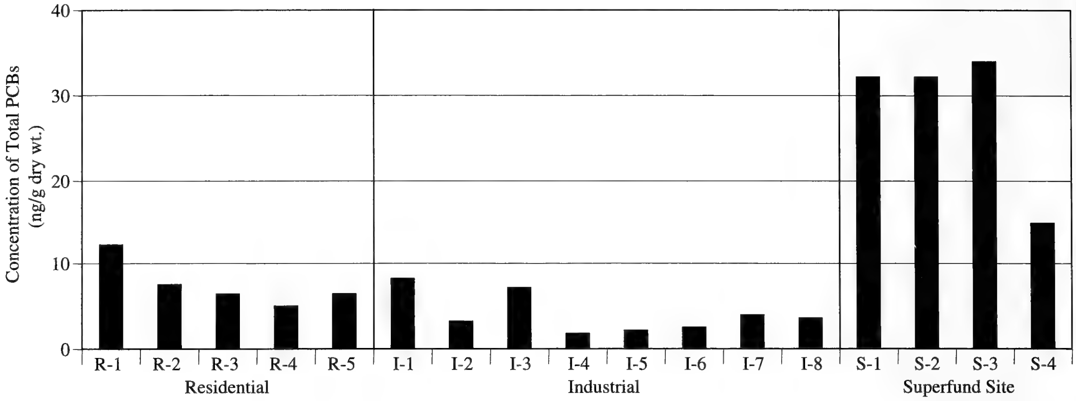

Figure 2. Total PCB concentrations in pine needles collected from residential, industrial and Superfund sites.

RESULTS

Among the various sampling locations in Kentucky, pine needles collected from site R-1 (residential location, Island Creek, McCracken County, KY) exhibited the greatest total PCB concentration, 12 ng/g dry wt. (Tables 1, 2). Among the pine needle samples collected from the industrial areas, one from Livingston County, I-4, displayed the lowest concentration of total PCBs. Industrial locations other than sites I-1 and I-3 (both at PGDP) revealed comparatively lower concentrations of total PCBs than pine needles collected at residential locations. Total PCB concentration in pine needles from the LCP Superfund site in Georgia exhibited highest concentrations (about an order magnitude higher) than all other pine needles from residential and industrial locations in Kentucky (Table 2, Figure 2).

The homologue profile from the residential area exhibited predominantly lower chlorinated congeners (dichloro-pentachlorobiphenyls). Hepta, octa, nonachloro, and decachlorobipheny congeners were barely detected or not detected (Figure 3). Pine needles from industrial locations contained limited number of congeners representing both lower chlorinated and higher chlorinated (hexa-decachlorobiphenyl) congeners (Figure 3). In contrast with the residential and industrial sites, pine needles from the LCP Superfund site contained highest concentrations of lower chlorinated and higher chlorinated homologue groups both qualitatively as well as quantitatively (Figure 3). PCB-18, PCB-52, PCB-153, and PCB-180 were consistently detected in all the samples. Higher chlorinated PCBs (hepta-, octa-, nona-, deca-) congeners were barely detected or below the detection limit in most of the residential pine needle samples from western Kentucky. However, PCB-206 was consistently detected in samples from western Kentucky. Pine needles collected at the LCP Superfund site, site S-3, had the highest total concentration of PCBs of 34 ng/g dry wt., while site S-4 had the lowest concentration of 15 ng/g dry wt. Samples from sites closer to the LCP Superfund site, S-1, S-2, and S-3, had total PCB concentrations more than 2 times that of sample S-4 (Figure 2).

Total PCB concentrations in pine needles decreased with increasing distance from the LCP Superfund site (Figure 4). PCB congener composition in pine needles revealed that PCB-206 was detected (Figure 3) in all samples, and the concentration was relatively higher than other congeners in samples S-1 and S-2 (Table 2). However, in samples S-3 and S-4 congeners PCB-18, PCB-101, and PCB-77 were the dominant congeners while these congeners were comparatively lower or BDL in samples S-1 and S-2 closest to the LCP Superfund site. One non-ortho-chlorine substituted PCB analyte, PCB-154/77, was detected in all samples (Table 2).

DISCUSSION

PCBs were widely used for industrial and in electrical appliances beginning in the 1930s. Although production of most of these pollutants ceased in the 1970s, environmental con-

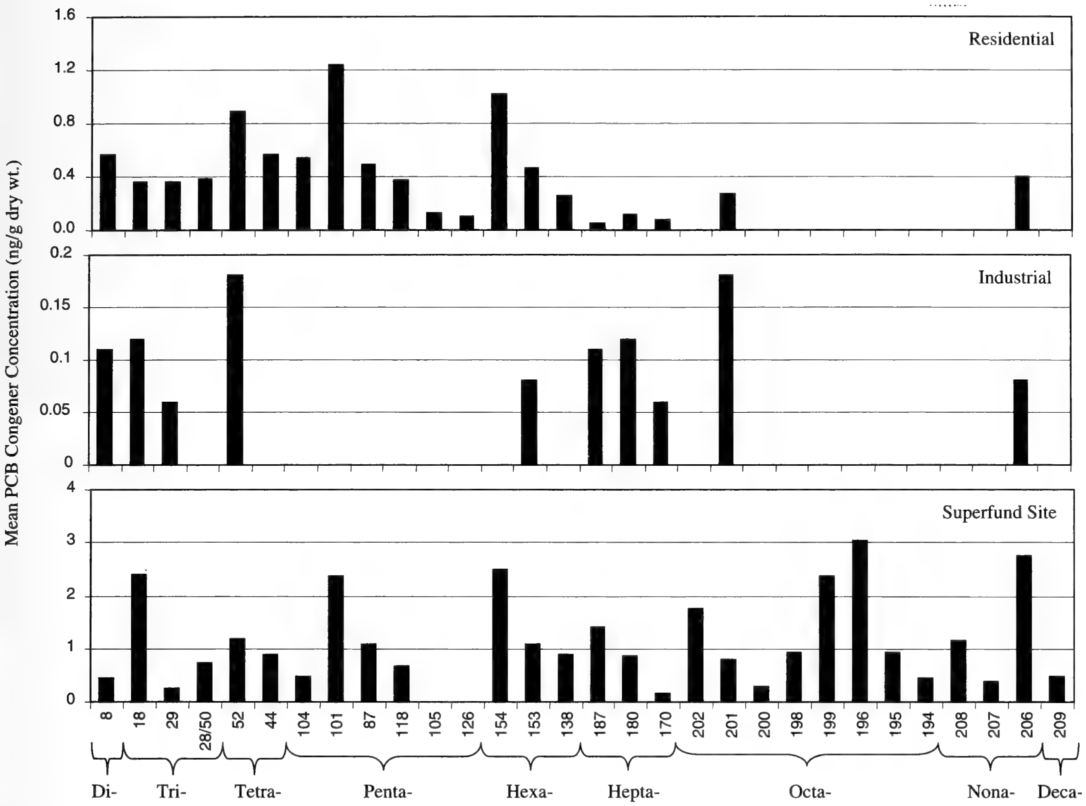

Figure 3. Mean PCB congener concentrations in pine needle collected from residential, industrial and LCP Superfund site.

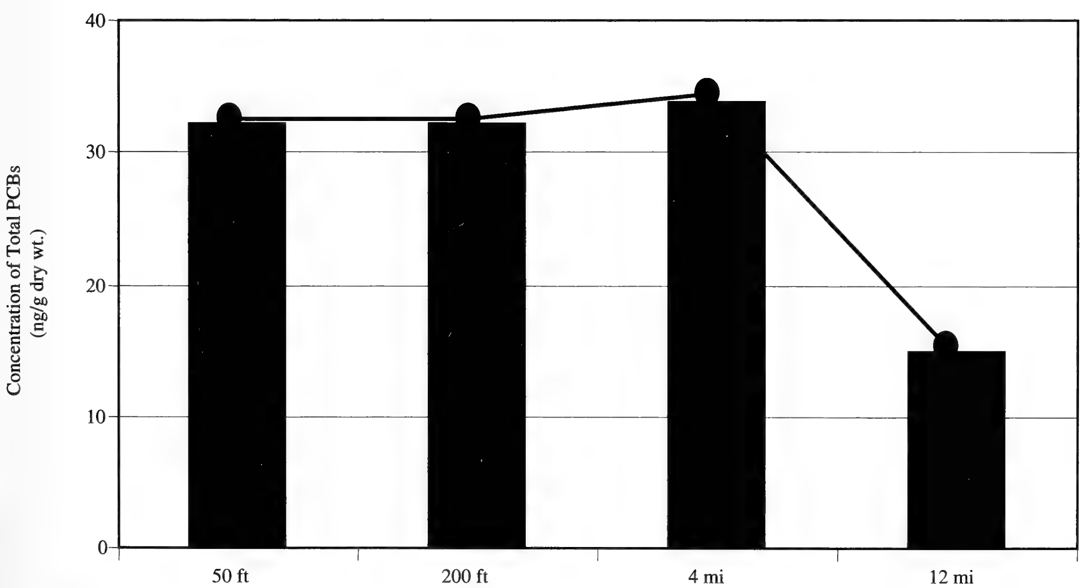

Figure 4. Decrease of total PCB concentrations in pine needles with distance from LCP Superfund site.

tamination by PCBs has resulted in bioaccumulation in organisms producing a variety of health disorders such as dermal toxicity, immunotoxicity, carcinogenicity and adverse effects on reproduction, development, and endocrine function (Van den Berg et al. 1998). Recent studies have shown worldwide contamination by these compounds including remote arctic environments (Simcik et al. 1997; Hafner and Hites 2003; Hermanson et al. 2003; Muir et al. 2003; Stevens et al. 2003; Xu et al. 2003). Most often these areas have been contaminated by atmospheric transport or run-off from highly contaminated areas such as Superfund sites. Evidence shows that people living closer to these highly contaminated sites are more exposed to the chemicals in the air than are people living in other areas (Vorhees et al. 1997; Korrick and Altshul 1998; Osius et al. 1999; White and Aldrich 1999). Therefore, it is important to measure the ambient air levels to determine areas that are at a greater risk to the health of the people living in them.

There are two main ways that air sampling for organochlorine compounds can be conducted. One way is to use large volume air samplers. This method works well for short-term studies, but long-term studies are more difficult and the equipment and analysis can be expensive. The other method, which began in the 1980s, uses plants as biomonitoring indicators (Calberg et al. 1983; Gaggi et al. 1985; Bacci et al. 1986, 1988, 1990; Eriksson et al. 1989; Paterson et al. 1990). In the 1990s onward, pine needles became popular as biomonitoring indicators of lipophillic air pollution (Franich et al. 1993; Kylin et al. 1994, 1996, 1998, 2003; Loganathan et al. 1998; Offenthaler et al. 2003; Xu et al. 2003). Pine needles have been used to understand both local and regional transport of lipophillic air pollution.

In western Kentucky there was no significant difference ($P = 0.053$) in total PCB concentrations between samples collected from residential and industrial locations when all samples were compared. However, when the concentrations in residential samples were compared with those from 'downwind' locations of CCIC, samples I-4 through I-8, there was a statistically significant difference (P-value $= 0.005$). Total PCB concentrations in samples from CCIC 'downwind' locations were significantly lower than those from residential locations. The increased concentration at residential locations over 'downwind' locations of the CCIC was unexpected. A study conducted by Loganathan and coworkers on pine needles collected in 1997 from western Kentucky (Loganathan et al. 1998) found that 'downwind' locations of the CCIC had PCB concentrations in pine needles that ranged from 9.67 to 18.05 ng/g wet wt. The concentrations from 'downwind' CCIC 1997 needles were significantly higher ($P = 0.0002$) than those collected in the present study even without converting the 1997 data from ng/g wet wt. to ng/g dry wt. (usually the moisture in the needles averages 45%). This significant decrease in concentration from 1997 to 2002 at the 'downwind' locations of CCIC suggests that CCIC is no longer a significant source of PCBs.

The concentration of PCBs in samples from the LCP Superfund site in Brunswick, Georgia had concentrations significantly higher (P-value < 0.0001) than those from western Kentucky locations. Because the concentrations in western Kentucky samples are lower than those from the LCP Superfund site, people in western Kentucky have a relatively lower exposure to PCBs than do people living near the LCP Superfund site. Also, the significantly lower concentrations found in pine needles collected in western Kentucky would suggest that western Kentucky does not have a significant source of PCBs such as the LCP Superfund site does.

In western Kentucky in both residential and industrial samples, penta- and lower chlorinated congeners made up over 80% of the concentrations with tetra- and penta- being the dominant homologue groups in residential samples and penta- is the dominant group in industrial samples. At the LCP Superfund site locations the homologue pattern was completely different from that of the western Kentucky locations (Table 2, Figure 3). The LCP Superfund site samples were enriched with octa- and nonachlorobiphenyls. It is known that the LCP Superfund site is contaminated by higher chlorinated PCB congeners. The former chlor-alkali plant utilized Aroclor 1268, an uncommon, more chlorinated technical PCB mixture to lubricate graphite electrodes used in the processing equipment. The enrichment of these highly chlorinated congeners in the pine needles at the LCP Superfund site as opposed to those in western Kentucky suggests that western Kentucky is not severely contaminated by higher chlorinated PCB mixture particularly, Aroclor 1268.

An important observation in this pine needle study is that even higher chlorinated PCB congeners can be transported through atmospheric routes as is evident from the LCP Superfund site pine needle PCB data (Table 2, Figure 4). The congener concentration of the octa- and nona- decreased as the distance from the LCP Superfund site increased (Table 2, Figure 4). This would be expected because the higher chlorinated PCBs are less volatile and would not travel as far from the source as the lower chlorinated congeners. Therefore, it is possible that the people living closer to this site are exposed to PCBs characteristic of this LCP Superfund site from atmospheric routes.

Specific PCB congeners can be used to indicate the potential source of the contamination. The National Institute of Standards and Technology (NIST) reported that PCB-28 and PCB-52 are indicative congeners for Aroclor 1016 and Aroclor 1242 and PCB-101, PCB-138 and PCB-153 are indicative of Aroclors 1254 and 1260 (Rasberry 1986). Presence of PCB-28 and PCB-52 in the pine needle samples from western Kentucky indicates that these samples contained Aroclor 1016 and Aroclor 1242 (Takasuga et al. 2005, 2006). Large quantities of these Aroclors were used in pulp and paper mills and inheat transfer fluids, while smaller quantities were used in electrical transformers and capacitors. It is likely that these congeners arose from industrial discharges in the region as well as from environmental degradation of higher chlorinated PCBs to lower chlorinated forms. Indicative congeners for Aroclor 1254 and Aroclor 1260, such as PCB-101, PCB-153, and PCB-138, also were detected in pine needle samples from western Kentucky. These Aroclors were used in large quantities in electrical transformers, capacitors, pesticides, and hydraulic fluids. These higher chlorinated congeners in the pine needle samples may be due to contribution from old electrical transformers and capacitors used in the region.

Pine needles from the LCP Superfund site and its vicinity contained a high percentage of higher chlorinated PCBs especially, PCB-196, PCB-199 and PCB-206 (Table 2). These congeners are indicative of Aroclor 1268 that was used at the former chlor-alkali plant (Kannan et al. 1997). The presence of congeners PCB-101, PCB-153, and PCB-138 in the LCP Superfund pine needle samples indicate that Aroclor 1254 and Aroclor 1260 were used in the region because these congeners are present in very low amounts in Aroclor 1268. These Aroclors probably arise from transformers and capacitors use. Due to the lower volatility of the highly chlorinated Aroclor 1268 congeners, their relative concentrations are expected to decrease with distance from the LCP Superfund site. Two studies conducted on sediment and biota collected at the LCP Superfund site revealed a characteristic chlorobiphenyl congener pattern with a greater proportion of hepta-CBs through deca-CBs characteristic of the source—Aroclor 1268 (Kannan et al. 1997, 1998). A similar characteristic pattern was evident in this pine needle study suggesting that the distribution of high end PCBs in pine needles can be used to "fingerprint" both the PCB fraction and the location of contamination source. This further confirms that people living near the LCP Superfund site are likely to be exposed atmospherically to the congeners characteristic of this site.

Certain PCB congeners have been found to cause toxic responses similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) by binding to the aryl hydrocarbon (Ah) receptor (Van den Berg et al. 1998). In order to facilitate risk assessment of PCBs, the World Health Organization (WHO) uses toxic equivalency factors (TEFs), as an index of toxicity of these compounds relative to that of 2,3,7,8-TCDD. The TEQs from the residential locations ranged from 0.12–0.17 pg/g dry wt. (Table 3). These TEQs were higher than those from the industrial locations (0.03–0.11 pg/g dry wt.) indicating air at the residential locations appeared to be slightly more toxic. At the LCP Superfund site, TEQs were on the order of 0.24–0.48 pg/g dry wt., which is higher than those recorded for residential and industrial locations in western Kentucky.

PCB concentrations in pine needles from western Kentucky and Georgia were com-

Table 3. Toxic equivalents (TEQs in pg/g dry wt.) of PCB congeners present in pine needles collected from residential, industrial and Superfund Site.

IUPAC No. TEF[a]	77[c] 0.0001	105 0.0001	118 0.0001	170 0.0001	180 0.00001	Total
R-1	0.011	ND[a]	0.04	0.01	0.001	0.16
R-2	0.09	ND	0.03	ND	0.001	0.12
R-3	0.10	0.02	0.03	ND	ND	0.14
R-4	0.10	ND	0.04	ND	ND	0.13
R-5	0.11	0.01	0.05	ND	ND	0.17
I-1	0.06	0.01	0.04	0.01	0.002	0.11
I-2	0.04	0.01	0.03	ND	ND	0.07
I-3	0.06	0.01	0.03	ND	0.001	0.10
I-4	0.02	ND	0.01	ND	ND	0.03
I-5	0.02	0.004	0.01	ND	ND	0.04
I-6	0.02	ND	0.02	ND	ND	0.04
I-7	0.04	0.01	0.02	ND	ND	0.07
I-8	0.03	0.005	0.02	ND	ND	0.05
S-1	0.16	ND	0.06	0.01	0.01	0.24
S-2	0.18	ND	0.06	0.01	0.01	0.25
S-3	0.33	ND	0.10	0.03	0.01	0.48
S-4	0.18	ND	0.05	0.01	ND	0.24

[a] From Vanden Berg et al. 1998; [b] ND, not detected; [c] co-elutes with PCB-154.

pared with PCB concentrations reported in pine needles from other countries (Table 4). Pine needles samples from Tokyo, Japan, had the highest concentration of 73 ng/g PCBs on wet weight (ww) basis followed by Poland (50 ng/g ww), and Germany (47 ng/g dry weight basis (dw). In this study, total highest concentrations of total PCBs were 8.4 ng/g dw, 12 ng/g dw, and 34 ng/g dw in industrial, residential areas of Kentucky and LCP Superfund site at Georgia respectively (Table 4). Global comparison of PCBs using one year old pine needle showed that Central Germany had maximum concentration followed by present study, Croatia, Sweden, Spain, United Kingdom, and Ireland. Very low concentrations of PCBs in pine needles from Spain were attributed to the samples being collected from high mountain rural areas (Grimalt and van Drooge, 2006). The above comparison may not hold up because different plant species accumulate pollutants differently (Ockenden et al. 1998). Furthermore, coniferous trees can have one- and two-year old needles on the same branch. It is likely that needles of different age have different pollutant concentrations, which may confound data interpretation.

Conclusions

Polychlorinated biphenyl measurements made in pine needles collected from selected locations in western Kentucky and a Superfund site located in Brunswick, Georgia revealed several important observations. Pine needles were demonstrated as a fixed site, non-destructive, regenerative, passive monitoring matrix for the evaluation of local and regional distribution of PCBs. Fingerprinting the sources of contamination of PCBs was possible using pine needle PCB data. PCB-28, PCB-52, PCB-101, PCB-153 and PCB-138 in pine needles indicated the use of Aroclors 1016, 1242, 1254 and 1260 in western Kentucky. Pine needles from the LCP Superfund site and its vicinity contained higher chlorinated PCBs especially, PCB-196, PCB-199 and PCB-206 characteristic of Aroclor 1268. Higher concentrations of certain PCB congeners near the source (LCP Superfund site pine needles) and comparatively lower con-

Table 4. Comparison of PCBs concentrations in pine needles from various countries.

Country	Concentration range	Unit	Sampling year	Reference
Central Germany	6.9–47	ng/g dw	1989–1990	Kylin et al. 1994
Sweden	3.8–7.0	ng/g dw	1989–1990	Kylin et al. 1995
Ireland	0.99	ng/g dw	1994	Tremolada et al. 1996
UK	3.2	ng/g dw	1994	Tremolada et al. 1997
USA-Superfund Site	**15–34**	**ng/g dw**	**1997**	**Present Study**
Croatia-1st year needle	ND–7.3	ng/g dw	1998	Romanic and Krauthacker 2006
Croatia-2nd year needle	0.15–12	ng/g dw	1998	Romanic and Krauthacker 2006
Tokyo	3.8–73	ng/g ww	1999	Wyrzykowska et al. 2006
Tokyo	110–420	pg/g ww*	1999	Hanari et al. 2004
USA-Residential	**5.2–12**	**ng/g dw**	**1999**	**Present Study**
USA-Industrial	**1.9–8.4**	**ng/g dw**	**2001–2002**	**Present Study**
Spain	3.0–4.8	ng/g dw	2002	Grimalt and van Drooge 2006
Poland	2.8–50	ng/g ww	2002	Wyrzykowska et al. 2006

* Only dioxin-like PCBs; ND = not detected.

centrations of these congeners far off from the source revealed atmospheric transportability of the compounds dependent on their molecular weight, vapor pressure, etc. This observation is potentially useful in determining what type of compounds people will be exposed to.

Recommendations

Although pine needles are an excellent bioindicator for atmospheric organic pollutants, chlorinated hydrocarbon pollutant data must be used with caution. There are a number of factors that can affect the levels of lipophillic compounds that accumulate in pine needles. For example, the partitioning process between air and vegetation is expected to be dependent on the ambient temperature. According to the Clausius-Claypeyron relationship, a plot of the Ln Kpa vs. 1/T gives a straight line with positive slope. So as the ambient temperature decreases, partitioning to vegetation increases. Further, different species of pine trees may accumulate different levels of lipophillic air pollutants dependent on the lipid content of the species. In addition, leaf surface area, plant architecture (aerodynamic surface roughness), and deposition pathways all influence accumulation of hydrophobic organic pollutants. In this study, with one exception, pine needles from only one species (Loblolly, *Pinus taeda*) were used to avoid species variation. Due to limitation in time and resources, the present study only used pine needle chlorinated hydrocarbon data to qualitatively assess the use of pine needles as a possible bioindicator for atmospheric contamination by PCBs and to describe the environmental distribution and impact of the various congeners. Future pine needle studies conducted by monitoring each particular site over several years will be useful in understanding long-term trends of these pollutants.

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Spatial Distribution of Benthic Macroinvertebrates in a Sidearm Embayment of Kentucky Lake

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ABSTRACT

The macrobenthos of Ledbetter Embayment, Kentucky Lake, were sampled monthly (January 2005 through July 2006) to determine community structure with focus on the physical and chemical factors influencing spatial distribution and density. We collected 38 species, including 27 insects, four mollusks, two crustaceans, and three annelids. Species composition was similar to that observed in other midwestern reservoirs except that some taxa, typically rare in other systems, were very abundant. Mean density was 1158 m^{-2} and density increased with water depth. Macroinvertebrate distribution was patchy. Profundal collector-gatherers were associated with depositional zones created by flow patterns within the embayment driven by the main stem current. Most littoral species showed associations with allochthonous input or substrate heterogeneity provided by incoming streams. The physical structure of Kentucky Lake embayments and commensurate patterns of organic matter deposition, depth, and substrate composition appear to be the primary factors structuring the macrobenthos.

KEY WORDS: macroinvertebrates, benthos, reservoir, embayment, Kentucky Lake

INTRODUCTION

Much of our understanding of benthic community structure has been derived from the study of natural lakes, streams and rivers (Brinkhurst 1974; Wetzel 2001). In the southern United States, however, many of the large, lentic ecosystems are man-made reservoirs. Indeed the surface area of reservoirs in the United States now exceeds that for natural lakes outside the Laurentian Great Lakes (Thornton 1990a). Our understanding of similarities and differences in structure and function between reservoirs and natural lakes is still lacking, and further study is needed to improve our ability to manage and use these resources (Thornton 1990a; White 1990; Wetzel 2001).

Reservoirs differ from natural lakes and rivers in a number of respects including relative drainage basin size (Thornton 1990a), stratification regime and dissolved oxygen dynamics (Cole and Hannan 1990), transport and sedimentary processes (Ford 1990; Thornton 1990b), and water retention time (Wetzel 1990). Variation in such influential characteristics likely affects the structure of benthic communities such that they are expected to differ measurably from natural lacustrine and riverine ecosystems.

Kentucky Lake is the furthest downstream of nearly 50 reservoirs on the Tennessee River system, and was constructed by the Tennessee Valley Authority for power generation, flood control and transportation in 1944. At Kentucky Dam, the Tennessee River is a 8–9th order system, creating what is termed a mainstem impoundment (Thornton 1990b). Mainstem impoundments are characterized by having a deep main channel, a comparatively narrow inundated floodplain, and numerous small sidearm embayments. Kentucky Lake has a length of 296 km, a surface area of 64,750 ha, and a shoreline of 3830 km. It has a rapid turnover time (13–37 days) controlled by Kentucky Dam, which releases water at a mean annual rate of 1800 m^3 sec^{-1} (Yurista et al. 2004). The resulting current in the main channel prevents thermal stratification. The reservoir water level is raised and lowered 1.8

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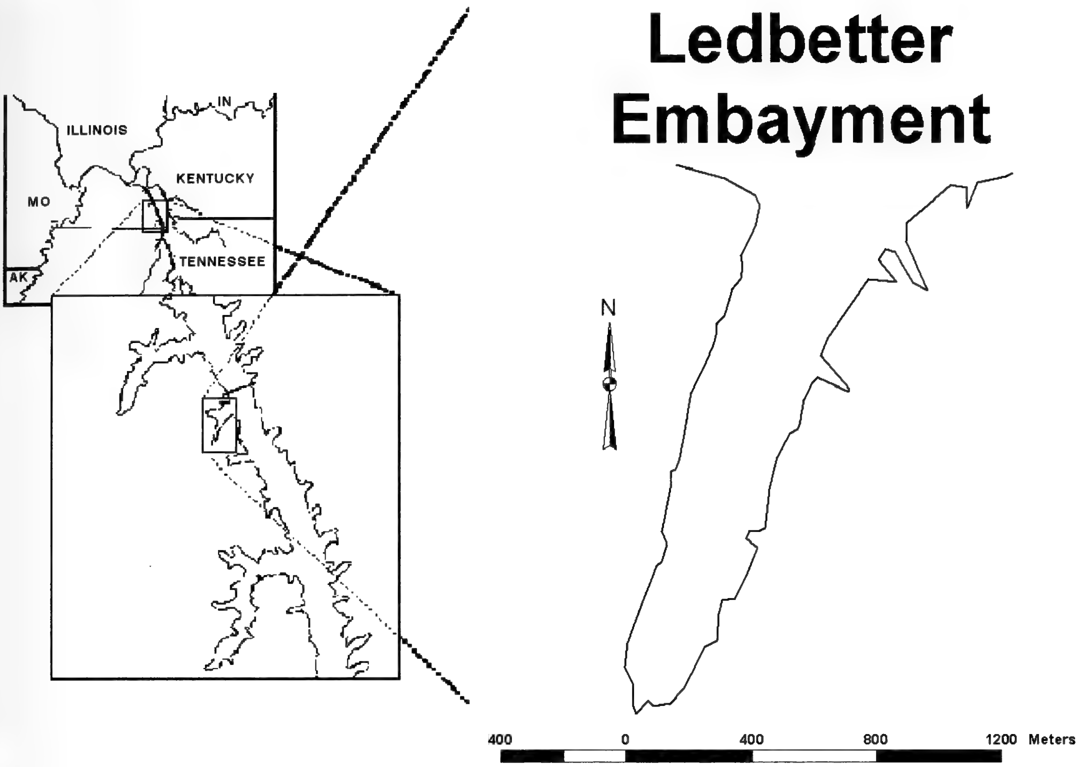

Figure 1. Location of Kentucky Lake in western Kentucky and Tennessee with detail of Ledbetter Embayment shoreline.

m each year between a high summer pool in late spring and a lower winter pool in early fall.

Seasonal drawdown, retention time, and temperature and dissolved oxygen dynamics likely affect the structure of the benthic community in the main body of the reservoir and its embayments (Furey et al. 2006). The goals of the present study were to describe the benthic community of an embayment of Kentucky Lake, examine the patterns of several environmental factors that influence benthic macroinvertebrate structure, and investigate how densities of several dominant taxa change with respect to water depth. Surprisingly little is known about reservoir benthic communities; indeed, there was no discussion of benthos in Thornton et al. (1990).

STUDY SITE

Ledbetter is a 1.2 km long, sidearm embayment located along the western shore of Kentucky Lake at Tennessee River mile 42.5 (68.4 km) (Figure 1). Ledbetter Embayment was created by inflow from Ledbetter Creek, a 3rd order stream, and is typical of bays created by 2nd to 4th order streams entering the Tennessee River. They originally were part of the river's floodplain. Most embayments have submersed bay mouth bars (Figure 2) on the downriver sides that were formed during floods prior to impoundment and that have continued to grow. The bars, in effect, create secondary impoundments with characteristic flow and deposition patterns. For Ledbetter, there is an initial counterclockwise flow pattern followed by smaller counterclockwise gyres. Where the gyres meet, water velocity is reduced resulting in zones of deposition (Figure 2). Water generally exits on the upriver side of the impoundment. Based on mean discharge of Ledbetter Creek, the residence time of water in the embayment is approximately one year (Johnson 1992).

METHODS

Macroinvertebrate sampling consisted of 40 benthic grabs taken each month (October

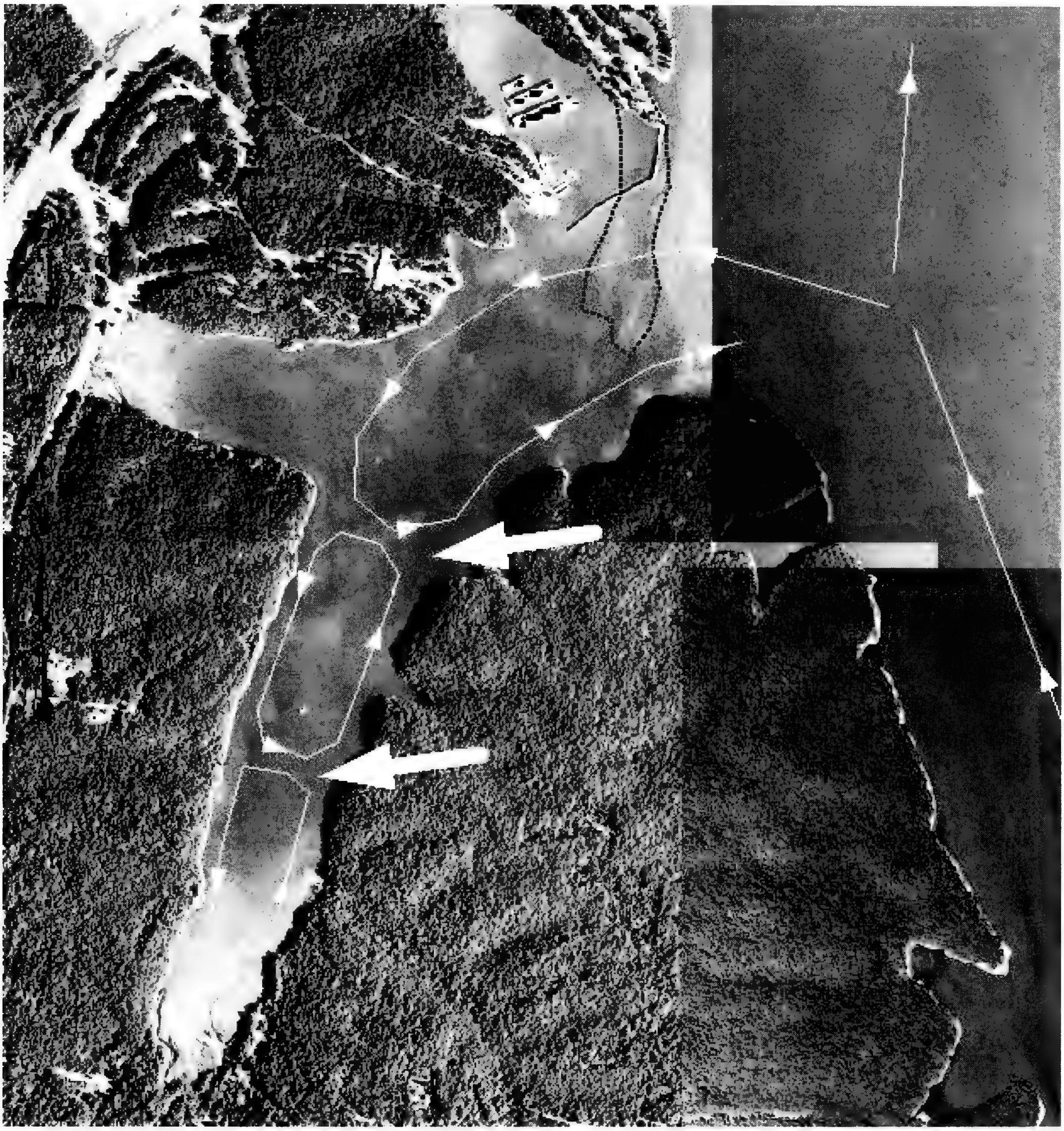

Figure 2. Surface flow patterns of Ledbetter Embayment with direction indicated by small white arrows (Johnson 1992). The submersed bay mouth bar is outlined in black. Zones of low current velocity (potential depositional zones) are indicated by the large white arrows.

2005 through July 2006 with exception of February 2006) at a set of points selected using a stratified, weighted random design. The design was stratified by 1 m depth zones and weighted by the percent area represented by each depth zone. A subset of all points available for each depth zone was taken, and its points were assigned a numerical value (1–300). A random number generator was used to select sampling points. The points were chosen from a 10 × 10 m grid overlay of the embayment with a geographically referenced point in the center of each square on the grid. Benthic samples were taken with a standard PONAR grab sampler (sampling area: 522 cm^2) at each of the selected points. Points were determined with the aid of a Global Positioning System (GPS). Contents of the PONAR were sieved through a 0.5 mm mesh bucket sieve, fixed in 10% buffered formalin containing rose bengal dye, and stored for later processing. Water depth, dissolved oxygen (DO), and temperature at the sediment water interface of each sampling point were measured with a YSI® 600 XL multiprobe sonde.

In the laboratory, formalin was rinsed from each sample before macroinvertebrates were separated from rocks, sand, and course particulate organic matter (CPOM) in white enamel pans. The contents of each pan were searched twice for macroinvertebrates. Samples containing large amounts of debris were split into several pans. Macroinvertebrates were identified, counted, and preserved in 70% ethanol. Coarse particulate organic matter was washed from rocks, sand, and empty mollusk shells using running water and a 0.5 mm sieve, dried for 48 hrs at 40°C, and weighed (±0.01 g).

During January 2006, 100 sediment cores were taken from the embayment with the sampling design described above using a 5.08 cm diameter (2 in) gravity corer. The top 2 cm of sediment from each core was extruded, dried at 40°C for 48 hr, and homogenized. Ash-free dry mass of an 8–20 g subsample was determined by heating it at 550°C for 4 hr then reweighing (±0.01 g). An estimate of the percent combustible organic matter of the sediment samples was obtained by dividing the percent combustible material in half (APHA 2005).

Linear regression was used to analyze the density of several prominent macroinvertebrate taxa against depth using the entire dataset. As some densities were low and many samples contained few or no individuals of some taxa as a result of patchy distribution, a log transformation was used to reduce variance and satisfy normality assumptions (Bartlett 1947; Box and Cox 1964). For each month, temperature and dissolved oxygen concentration were each regressed against depth. Also, the dry mass of CPOM was regressed against depth. All regression calculations were made using SAS® 9.1 (SAS Institute, Inc., Cary, NC). The distributions and densities of taxonomic groups were mapped with ArcView GIS software and compared visually using map overlays of the distribution of sediment organic matter obtained from core content analysis.

RESULTS

Thirty eight macroinvertebrate taxa were collected from the benthos of Ledbetter Embayment (Table 1). The mean density was 1158 m^{-2} with a range of 0–5000 m^{-2}. Distribution was patchy (standard deviation = ±638 macroinvertebrates m^{-2}). Few macroinvertebrates were collected from a submerged roadbed located near the western shore or from areas of the shore with exposed chert gravel and cobble. With few exceptions, the only invertebrates collected from such rocky habitats were tube-dwelling *Chironomus* larvae and *Stenonema* naiads. Regression results suggested a weak increase in macroinvertebrate density with depth ($r^2 = 0.016$, $P = 0.0344$).

The dry mass of CPOM in samples decreased with depth ($r^2 = 0.3269$, $P < 0.0001$). Patches of sandy substrate were present in channels extending from Ledbetter Creek, a spring on the western shore, and a few other small stream inlets. Below a depth of three meters, there was very little CPOM and the substrate was predominantly soft clay. Temperature decreased with depth ($R^2 = 0.16$–0.87, P 0.0116 to < 0.0001), with the exception of the January and May samples when there was no detectable relationship between temperature and depth. Temperature varied less than 3°C among sampling locations each month. Dissolved oxygen concentrations increased with decreasing water depth during all sampling dates ($r^2 = 0.37$–0.78, $P < 0.0001$). Concentrations varied less than 3 mg l^{-1} from October through May. In June and July when DO at water depths ≥4 m dropped to 3–5 mg l^{-1}, shallow areas became supersaturated with oxygen due to high rates of algal photosynthesis.

Map overlays indicated that greater densities of oligochaetes, including both *Limnodrilus* and *Branchiura,* and the fingernail clam, *Pisidium,* were found in association with areas of greater sediment organic matter including both the deepest (5–8 m) area on the northeastern end of the embayment and near a spring outlet on the southwestern edge (Figure 3). *Limnodrilus* and *Pisidium* densities increased with depth ($r^2 = 0.2829$, $P = 0.0054$ and $r^2 = 0.1184$, $P = 0.0296$, respectively) (Table 2). Several species of Chironominae, particularly *Chironomus major* Wülker and

Table 1. Macrobenthic taxa collected from Ledbetter Embayment.

Order	Family	Genus/species
Diptera	Chironomidae	*Coelotanypus tricolor* (Loew)
Diptera	Chironomidae	*Coelotanypus scapularis* (Loew)
Diptera	Chironomidae	*Procladius* sp.
Diptera	Chironomidae	*Ablebesmyia annulata* (Say)
Diptera	Chironomidae	*Microchironomus nigrovittatus* (Malloch)
Diptera	Chironomidae	*Cryptochironomus blarina* Townes
Diptera	Chironomidae	*Tribelos jucundum* (Walker)
Diptera	Chironomidae	*Polypedilum halterale* (Coquillett)
Diptera	Chironomidae	*Cladopelma* sp.
Diptera	Chironomidae	*Chironomus major* Wülker and Butler
Diptera	Chironomidae	*Chironomus crassicaudatus* Malloch
Diptera	Chironomidae	*Chironomus decorus* Johannsen
Diptera	Chironomidae	*Chironomus plumosus* (Linnaeus)
Diptera	Chaoboridae	*Chaoborus punctipennis* (Say)
Diptera	Ceratopogonidae	sp.
Ephemeroptera	Ephemeridae	*Hexagenia bilineata* (Say)
Ephemeroptera	Caenidae	*Caenis* sp.
Ephermeroptera	Heptageniidae	*Stenonema* sp.
Megaloptera	Sialidae	*Sialis velata* Ross
Trichoptera	Hydroptilidae	*Oxyethira* sp.
Trichoptera	Leptoceridae	*Oecetus* sp.
Trichoptera	Polycentropodidae	*Cyrnellus fraternus* (Banks)
Odonata	Coenagrionidae	*Enallagma* sp.
Odonata	Lestidae	*Lestes* sp.
Odonata	Corduliidae	*Macromia* sp.
Odonata	Gomphidae	*Progomphus* sp.
Coleoptera	Elmidae	*Dubiraphia* sp.
Mysidadaecea	Mysidae	*Taphromysis louisianae* (Banner)
Haplotaxida	Tubificidae	*Limnodrilus hoffmeisteri* Claparède
Haplotaxida	Tubificidae	*Limnodrilus udekemianus* Claparède
Haplotaxida	Tubificidae	*Branchiura sowerbyi* Beddard
Rhynchobdellida	Glossiphoniidae	*Placobdella* sp.
Architaenioglossa	Viviparidae	*Campeloma* sp.
Neotaenioglossa	Pleuroceridae	*Pleurocera* sp.
Unionoida	Unionidae	*Quadrula quadrula* (Rafinesque)
Veneroida	Corbiculidae	*Corbicula fluminea* (Müller)
Veneroida	Pisidiidae	*Pisidium compressum* Prime
Veneroida	Dreissenidae	*Dreissena polymorpha* (Pallas)

Butler, were found in greater densities as depth increased ($r^2 = 0.0767$, $P < 0.0001$). Larval densities of Ceratopogonidae, Tanypodinae and *Chaoborus punctipennis* (Say) also increased with depth ($r^2 = 0.1142$, $P < 0.0001$; $r^2 = 0.0235$, $P = 0.0113$; and $r^2 = 0.1605$, $P < 0.0001$, respectively). *Placobdella, Corbicula fluminea* Müller, *Lestes, Enallagma, Oxyethera,* and *Macromia* were found primarily near the mouth of the spring inlet on the southwestern edge of the embayment. Larval densities of *Sialis velata* Ross were not significantly related to depth. Densities of *Caenis* and *Hexagenia bilineata* (Say) naiads, *Placobdella,* and *Gammarus* were all weakly inversely related to depth ($r^2 = 0.1099$, $P < 0.0001$; $r^2 = 0.0177$, $P = 0.0284$; $r^2 = 0.1614$, $P < 0.0001$; and $r^2 = 0.1724$, $P < 0.0001$).

DISCUSSION

The species composition of the Ledbetter Embayment macrobenthos was not greatly different from that of several other midwestern reservoirs, including Lake Texoma (Sublette 1957; Vaughn 1982), Arcadia Lake (Bass 1992), Keystone Reservoir (Ransom and Dorris 1972), Arbuckle Lake (Parrish and Wilhm 1978), and Ham's Lake (Ferraris and Wilhm 1977). Although we report lower species richness in comparison to the studies just mentioned, this is likely due to the sampling method we employed. It excluded smaller organisms (<0.5 mm) and did not include depths less than 0.5 m, whereas other studies often employed multiple collection methods and were driven by qualitative rather than quantitative goals which included taxa such as watermites, and nematodes (Sublette 1957). Also, we did not collect beyond the reach of our sampling boat into the littoral zone to a depth of less than 0.5 m.

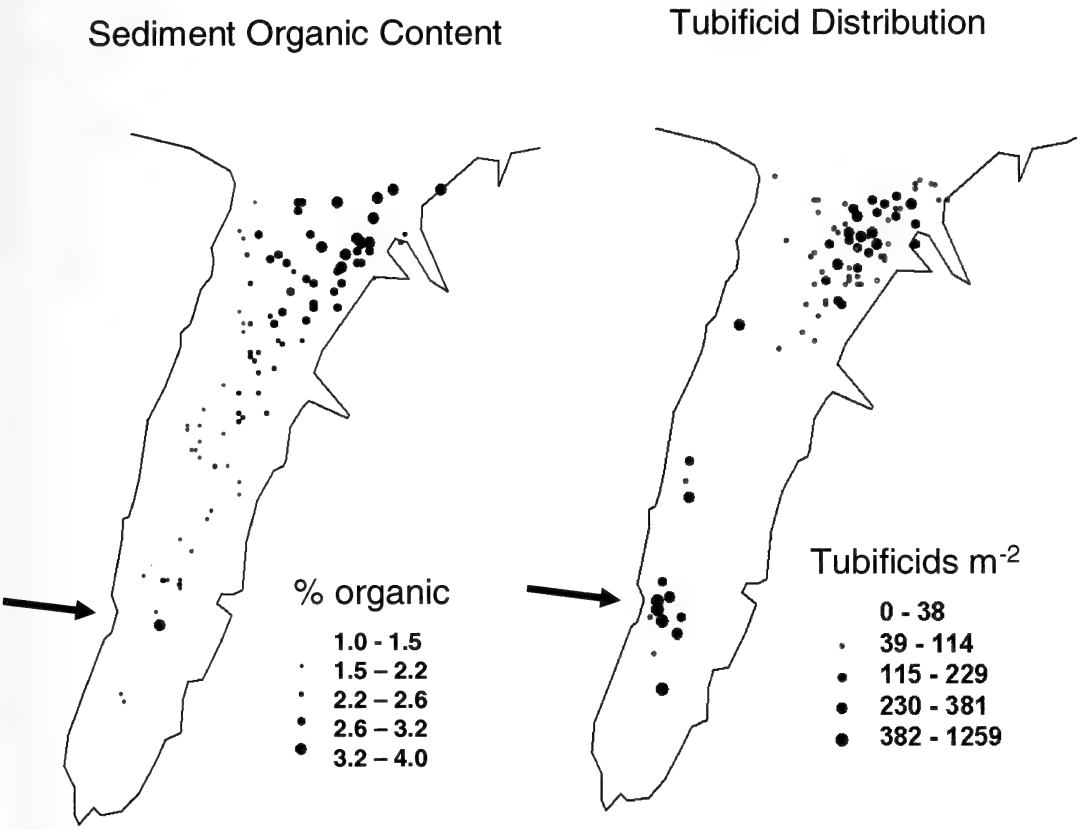

Figure 3. Distribution of sediment organic matter, measured as ½ of % combustible material from subsample (left) and tubificid distribution and density (right). Arrows indicate location of stream inlet.

We recorded several species of interest including the mysid shrimp *Taphromysis louisianae* Banner and the chironomid *Chironomus major* Wülker and Butler. Only a single *T. louisianae* specimen was collected during our study, however, this species is generally found in Gulf Coast regions and has only recently been documented in Ohio marshes (Reeder and Hardin 1992) and the littoral regions of Kentucky and Guntersville Lakes (both Tennessee River impoundments) (Brooks et al. 1998). It appears to be a naturalized species and is a common item in the diet of juvenile largemouth bass (Dreves 1997). *Chironomus major* has been recorded from a few southeastern U.S. reservoirs, but the very conspicuous, blood-red, up to 60 mm long larvae has been considered to be uncommon to rare. It has become the dominant profundal deposit feeder in Kentucky Lake, often reaching densities of 500 larvae m^{-2}. At such densities, *C. major* densities far exceed the only other available figure for *C. major* (Balci et al. 2005), where the maximum density reported in Kentucky Lake was 196 larvae m^{-2}.

The depth trends in macrobenthos density in Ledbetter Embayment were different from trends reported for other reservoirs. Because several other studies on the benthos of reservoirs with seasonal water level fluctuation showed that macroinvertebrate density and biomass tended to be higher immediately below the drawdown zone (Kaster and Jacobi 1978; Furey et al. 2004, 2006), we had expected that macrobenthos density would be greater at 0.5 to 1 m. In Ledbetter Embayment, however, when macroinvertebrate density was plotted against depth, there was a weakly positive yet significant trend despite high variance (Figure 3). The patchy distributions that we observed are not uncommon in the lentic environment, and we suspect that

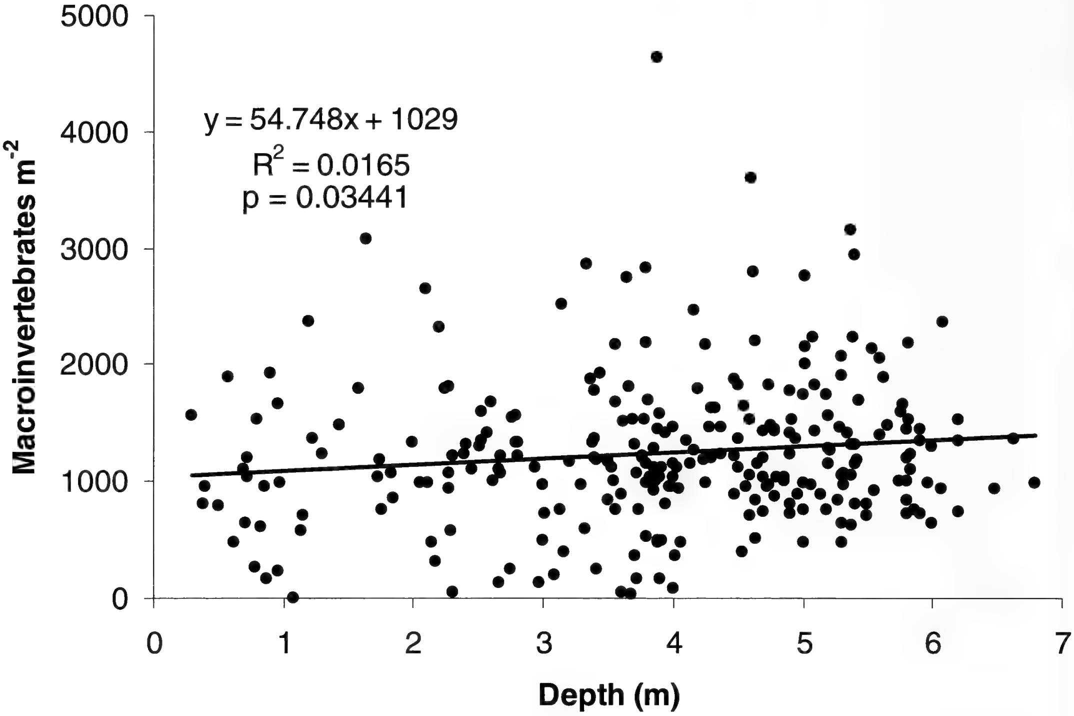

Figure 4. Macroinvertebrate density plotted against depth in Ledbetter Embayment of Kentucky Lake.

a variety of factors contribute to the variable spatial pattern including sediment reworking by benthic organisms, physical variation in the bottom profile (Downing and Rath 1988), and variation in sediment grain size (Sauter and Gude 1996).

Two separate combinations of factors appear to be influencing macroinvertebrate spatial distribution and density in the littoral and profundal zones. Streams entering the embayment are the primary influence in the littoral and sublittoral zones. The erosive force that streams exert and the allochthonous organic matter that they introduce to the system shape the substrate and provide an important food source. The substrate heterogeneity created by erosional forces, exerted by incoming stream inlets, is important for providing suitable habitat for many macroinvertebrates (Brinkhurst 1974). The patches of sand and gravel created by stream flow are particularly important to invertebrates such as *Oxyethira* that do poorly in depositional areas (Wiggins 1996). The influx of allochthonous organic matter is important in shallower regions, especially where it concentrates adjacent to incoming stream channels. The separate and unique assemblage of species inhabiting shallower water suggests that the greater availability of CPOM deposited near shore serves as an important substrate and food source.

Evidence presented here suggests that the pattern of particulate organic matter deposition and depth are important factors influenc-

Table 2. Density regressed with depth for 13 macroinvertebrate taxa in Ledbetter Embayment.

Taxon	Slope	F	*P*	r^2	n
Branchiura	NS	0.689	0.4071	0.0025	271
Caenis	−0.2151	33.23	<0.0001	0.1099	271
Ceratopogonidae	0.457	34.7	<0.0001	0.1142	271
Chaoborus	0.5269	51.455	<0.0001	0.1605	271
Chironominae	0.2127	22.37	<0.0001	0.0767	271
Corbicula	−0.0343	16.16	0.0002	0.2983	40
Gammarus	−0.3651	56.053	<0.0001	0.1724	271
Hexagenia	−0.1644	4.852	0.0284	0.0177	271
Limnodrilus	0.2507	7.834	0.0054	0.2829	271
Pisidium	0.5237	5.104	0.0296	0.1184	40
Placobdella	−0.3617	51.789	<0.0001	0.1614	271
Sialis	NS	3.292	0.0707	0.0121	271
Tanypodinae	0.1561	6.5	0.0113	0.0235	271

ing the profundal macroinvertebrate community structure in Ledbetter Embayment. Areas of greater sediment carbon content, indicative of greater organic matter deposition, support higher densities of profundal deposit feeders (Figures 2, 3). The distribution of oligochaetes was most strongly influenced by organic matter deposition patterns as evidenced by the densely populated patches that coincided with higher sediment organic content revealed using GIS map overlays. In the case of Ledbetter Embayment, and likely in embayments with similar morphology, the direction and velocity of flow propelled by the main-stem current determine where POM deposition occurs. DO levels tend to sag during the summer at water depths of greater than 4 m, and sags may be sufficient to exclude or limit the activities of some taxa. Reduced DO concentrations may have been sufficient to account for the reduced number of tanypod larvae that we observed at depths greater than 5 m despite the positive relationship with depth that linear regression revealed (Table 2).

The distribution and density of the macrobenthos in other 3rd to 4th order stream embayments of Kentucky Lake may be influenced by similar factors. Most of the embayments share morphological characters such as baymouth bars and circulation patterns driven by the mainstem current. This set of conditions appears to be favorable for some taxa that generally are rare in most lakes and reservoirs (e.g., *Chironomus major, Taphromysis*), while reducing populations of species that might be expected to be more common (*Hexagenia*). In order to better understand the structure of benthic communities, it would be of value to determine if other mainstem reservoirs have similarly structured embayments and if the embayments in much more dendritic tributary impoundments (Thornton 1990a) provide the same sets of conditions. More studies like this will not only further our understanding of factors influencing macrobenthos structure in reservoirs, but will serve to clarify the differences between reservoirs and natural lakes thereby improving our ability to manage reservoirs effectively.

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Seasonal Patterns and Abundance of Copepods in Kentucky Lake, USA

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ABSTRACT

The distributions and abundance of free-living copepods in Kentucky Lake were examined for the years 2000, 2002, and 2004. Vertical tow samples were collected from 16 sites every 16 days for April through November and every 32 days for December through March as part of a long-term physicochemical and biological monitoring program. Copepods comprised 14.39% of the zooplankton in tow net samples, but only 6 species were abundant. *Ectocyclops phaleratus, Cryptocyclops bicolor, Mesocyclops tenuis, Eurytemora affinis, Leptodiaptomus siciloides,* and *Acanthocyclops vernalis* accounted for 86.44% of adult copepods. With the exception of *E. affinis* that was found throughout the year, observed seasonal density maxima occurred at differing points during the year for each of the other major species. Most species were present during spring and summer and absent or found in low numbers throughout the remainder of the year. *A. vernalis, M. tenuis,* and *C. bicolor* exhibited density decreases that corresponded with day 160. Day 160 also marked rapid declines of other common zooplankton such as *Bosmina* sp., *Daphnia retrocurva,* and *Diaphanosoma* sp. Similarities in annual distribution patterns among all zooplankton suggest that common factors are exerting influence on abundances and seasonal distributions.

KEY WORDS: Zooplankton, Copepoda, Calanoida, Cyclopoida, Kentucky Lake, seasonal abundance

INTRODUCTION

Copepods are a common component of the zooplankton in most lakes and reservoirs (Wetzel 2001) but often receive less attention than the more abundant and more easily identified Cladocera (Williamson and Reid 2001). Construction of thousands of reservoirs throughout the world over the past 70 years has provided an array of new habitats for aquatic biota (Thornton et al. 1990; Pinto-coelho 1998); however, zooplankton communities have gone largely unstudied in most (Marzolf 1990). In 1988, the Center for Reservoir Research at Murray State University initiated the Kentucky Lake long-term monitoring program (White 1990). A focus of monitoring has been to document long-term trends and spatial variability in water quality, zooplankton, and phytoplankton.

Previous publications on Kentucky Lake have documented seasonal and aerial patterns of zooplankton, primarily Cladocera (Schram and Marzolf 1993), establishment and bioenergetics of *Daphnia lumholtzi* (Yurista et al. 2000, Yurista 2004) and rotifer populations (Albritton and White 2004, 2006). The focus of this study was to examine seasonal distributions of Kentucky Lake copepods based on zooplankton samples archived from the long-term monitoring for the 2000, 2002, and 2004 sampling years.

MATERIALS AND METHODS

Study Site

Kentucky Lake in western Kentucky and Tennessee is the lowermost of nearly 50 reservoirs on the Tennessee River system and was completed in 1944 by the Tennessee Valley Authority (TVA) for electric power, flood control, and navigation. Kentucky Lake is 296 km long and typical of mainstem reservoirs in being narrow (~3.2 km wide) with numerous small side-arm embayments. Water levels are comparatively constant with a summer pool at 109.4 m above msl from April to August and a winter pool of 107.9 m above msl from October through March. Yurista et al. (2004) have documented physical and water chemistry patterns.

Sampling Methods

Monitoring cruises have been conducted every 16 days during the months of March through November and every 32 days during the months of December through February at

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16 sites (Figure 1) since August 1988. Zooplankton have been collected by two methods. One vertical tow using a Wisconsin net (13 cm diameter mouth, 63 μm mesh) was taken at each site from one meter off the lake bottom. Three Schindler trap samples were taken at the depth of the 1% light level (63 μm mesh sieve). All samples were rinsed into 120 mL tissue culture flasks for holding. Samples were returned to the laboratory where they were anesthetized with 2 ml of soda water for 1 hr and then preserved in 10% formalin buffered with $CaCO_3$. Cladocerans were initially identified to species and copepods were identified to order, then samples for each site were composited for archiving.

Counts and Identification

Three years of archived samples were chosen for examination in this study, 2000, 2002, and 2004. Composites for each cruise date were sub-sampled using a sample splitter (Ocean Research Equipment, Inc.) until a sample size of approximately 100 zooplankton was reached. Identification and counts of copepods were done at 100–450×. When necessary, specimens were dissected at 42× and then mounted on glass slides using water miscible CMC-9 (Masters Company, Inc.). Mature calanoids and cyclopoids were identified to species. Harpacticoids were identified to genus except for *Phyllognathopus viguieri*. Keys used for identification of Calanoida, Cyclopoida, and Harpacticoida were Wilson and Yeatman (1959), Pennak (1989), and Williamson and Reid (2001). Copeodites and nauplii were not apportioned.

Analysis

Data from each of the three years were entered into Microsoft Access 2003 for comparison and analysis. Cruise data were obtained from the Kentucky Lake Long-Term Monitoring Program database to determine the total water volume sampled (vertical tows plus Schindler trap samples) on each sampling date along with average water temperatures collected with a YSI 6280 multiparameter probe. Separate years were combined by day-of-year on the same figure in Microsoft Access 2003 to provide visual representations of yearly and temperature related patterns.

RESULTS

In total, fourteen taxa plus copepodites and nauplii were identified, only six of which appeared in any abundance: *Ectocyclops phaleratus, Cryptocyclops bicolor, Mesocyclops tenuis, Eurytemora affinis, Leptodiaptomus siciloides,* and *Acanthocyclops vernalis* (Tables 1, 2). Cyclopoids dominated the total composition of the sub-samples and comprised 71.76% of all copepods identified, while calanoids accounted for 27.00%. Harpacticoids and nauplii comprised 0.71% of the samples only one of which (*Phyllognathopus viguieri*) was identified to species. The remaining 0.53% not reported was due to rounding restrictions. The harpacticoid taxa collected most likely were benthic and are not discussed further beyond the listing in Table 1. Other copepod taxa were present but either were too damaged to identify or could not be identified with certainty.

Total densities ranged from <0.01 l^{-1} in mid-December to 6.77 l^{-1} in mid-June. Peak densities occurred in late spring and early summer (Figure 2a). Cyclopoida densities were highest in early spring, while peak Calanoida densities occurred about 45 days later in early summer (Figure 2b). Cyclopoids dominated the copepod community from January through September, and calanoids dominated from September through December. Peak cyclopoid densities were more than twice those observed for calanoids.

Statistical analyses were run on the resulting distributional data and collected physiochemical data in an attempt to find causal relationships between the two. Our analysis did not reveal any single factor, or combination of factors, that directly affected annual distribution or density. It is quite possible that parameters other than those routinely monitored during our sampling cruises are instrumental in this role.

Late winter–early spring copepod populations were dominated by *Cryptocyclops bicolor,* which was most commonly found between days 80 and 160 and sharply declined before day 180 (Figure 3a). Observed distribution patterns for *C. bicolor* were most similar in 2000 and 2002 and exhibited a roughly bimodal distribution during these years. Distribution during 2004 differed in that it was un-

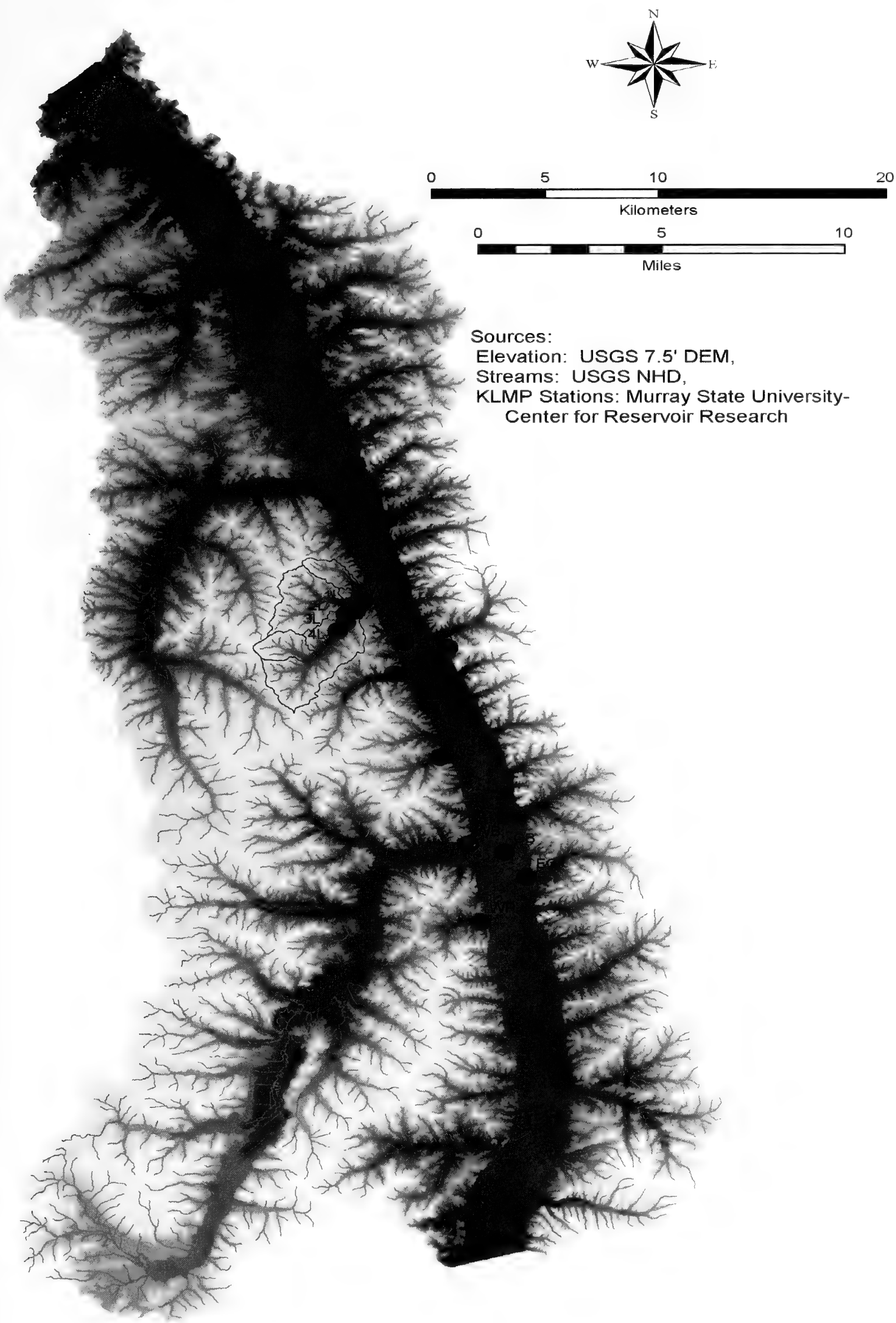

Figure 1. Map of Kentucky Lake showing locations of CRR monitoring stations. Letters refer to site names in the database.

Table 1. Species and average densities of copepods found in CRR Kentucky Lake Long-Term Monitoring Program sub-samples for the years 2000, 2002, and 2004. (0.53% missing due to rounding).

Family	Genus/species	Avg. annual density (liter^{-1})	% of all copepods
Calanoida			
Diaptomidae	*Leptodiaptomus siciloides* (Poppe)	1.09	5.7
Temoridae	*Eurytemora affinis* (Lilljeborg)	0.83	4.3
Copepodites		3.05	15.8
Other[1]		0.24	1.2
Total calanoids		5.21	27.0
Cyclopoida			
Cyclopidae	*Ectocyclops phaleratus* (Koch)	3.31	17.2
	Mesocyclops tenuis (Marsh)	2.43	12.6
	Cryptocyclops bicolor (Sars)	2.28	11.8
	Acanthocyclops vernalis (Fischer)	1.25	6.5
	Cyclops exilis Coker	0.76	4.0
	Mesocyclops edax (Forbes)	0.29	1.5
Copepodides		3.04	15.7
Other[1]		0.47	2.4
Total cyclopoids		13.84	71.8
Harpacticoida			
Tachidiidae	*Microarthridion* sp.	0.06	0.3
Macrobiotidae	*Macrobiotus* sp.	0.02	<0.1
Canthocamptidae	*Canthocampus* sp.	0.02	<0.1
	Moraria sp.	0.02	<0.1
	Maraenobiotus sp.	<0.01	<0.1
Phyllognathopodidae	*Phyllognathopus viguieri* (Maupas)	<0.01	<0.1
Total harpacticoids		0.12	<0.1
Nauplii		0.13	0.7

[1] Includes specimens that could not be identified positively.

Table 2. Peak density and day of year for the six most abundant copepod species found in CRR Kentucky Lake Long-Term Monitoring Program sub-samples for the years 2000, 2002, and 2004.

Species	Peak density (liter^{-1})	Day Of year
Ectocyclops phaleratus	1.25	240
Mesocyclops tenuis	2.34	155
Cryptocyclops bicolor	0.61	111
Acanthocyclops vernalis	0.42	122
Leptodiaptomus siciloides	1.00	189
Eurytemora affinis	0.36	170

imodal, and peak densities were reached around day 100, which corresponded with a trough between peak densities in 2000 and 2002. During all three years examined, there was a late summer increase in densities between days 220 and 260, but densities were low and population increases were not seen again until the following spring.

Following the presence of *C. bicolor, Mesocyclops tenuis* began to appear and was most commonly found between days 90 and 180 (Figure 3b). Observed patterns were different for all three years examined but were most common between 2000 and 2002 when peak densities occurred around day 150. During 2004, the distribution pattern for *M. tenuis* was markedly different, exhibiting lower densities than the other years and being skewed towards early spring. In both 2000 and 2002 there was a sharp decline around day 180. Densities observed for *M. tenuis* in 2002 were the highest for all species in the study and reached 2.34 l^{-1}, nearly twice the density observed in *E. phaleratus,* the next closest in peak density (Table 2).

Acanthocyclops vernalis was present during mid to late spring and summer periods (Figure 3c). Density patterns differed for each of the years examined, with 2000 and 2002 exhibiting the closest patterns. In 2000, two small peaks occurred at days 110 and 150, while the major peak occurred around day 200. For the 2002 sampling year, a roughly bimodal distribution was observed with a longer, more extended peak occurring from days 120–150 and a second peak corresponding with that observed in 2000, around day 200. During 2004 peak densities were found to be approximately half that observed during the other two years and were generally offset by approximately 20 days. Greatest densities were observed during 2002 at 0.42 L^{-1} on day 122.

The most common species through much of the spring and summer was *Ectocyclops phaleratus,* reaching average densities of nearly 0.70 individuals l^{-1} about days 190 and 240 (Figure 3d) with a maximum density of 1.25 l^{-1} (Table 2), in 2004. Peak densities were during the same period of time in 2000 and 2004, while peak densities in 2002 were found approximately 40 days earlier. Although the period during which the 2002 peaks occurred

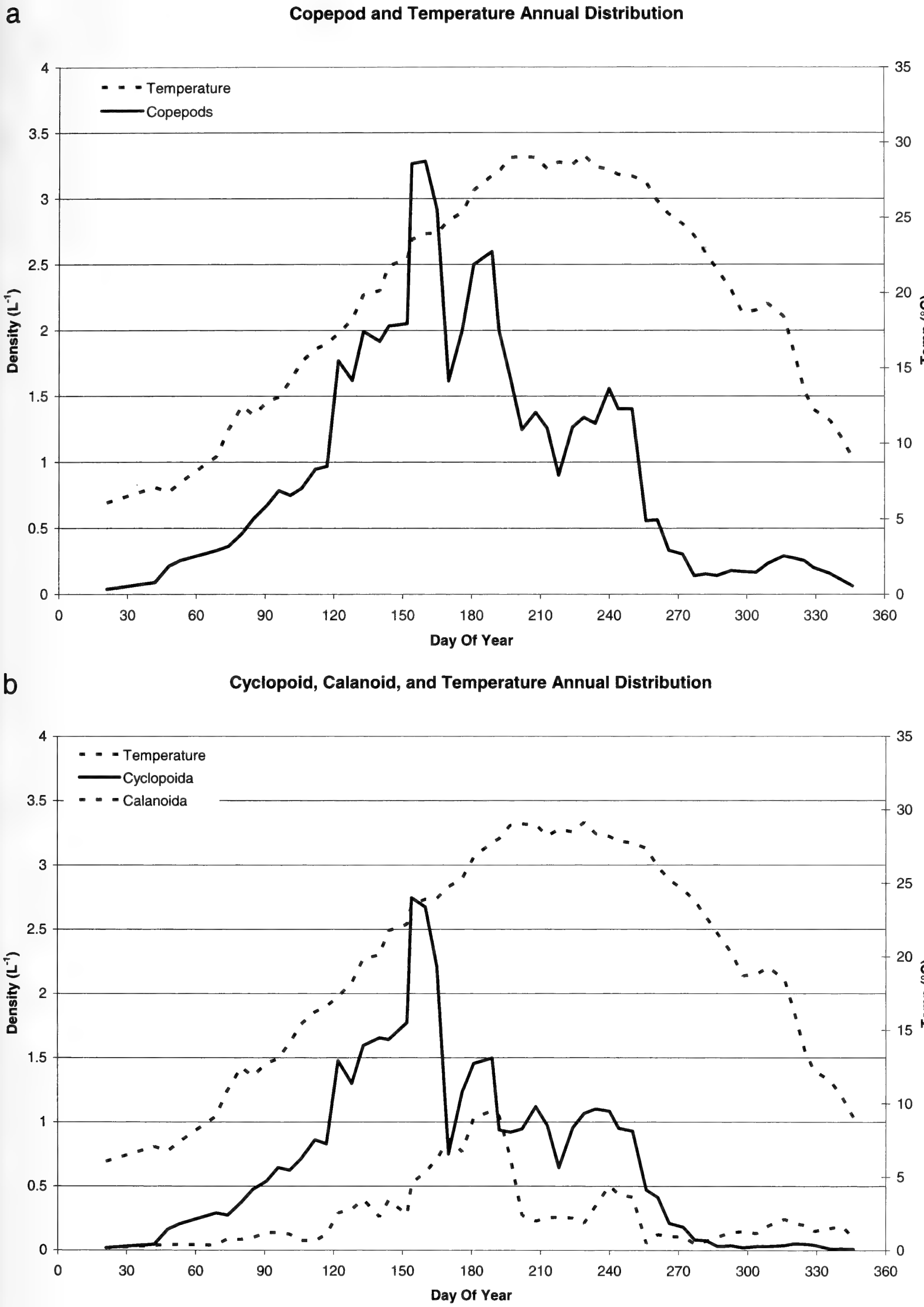

Figure 2. Average densities (3 point moving averages) and temperature distribution by day of year for (a) all copepods and (b) Calanoida and Cyclopoida.

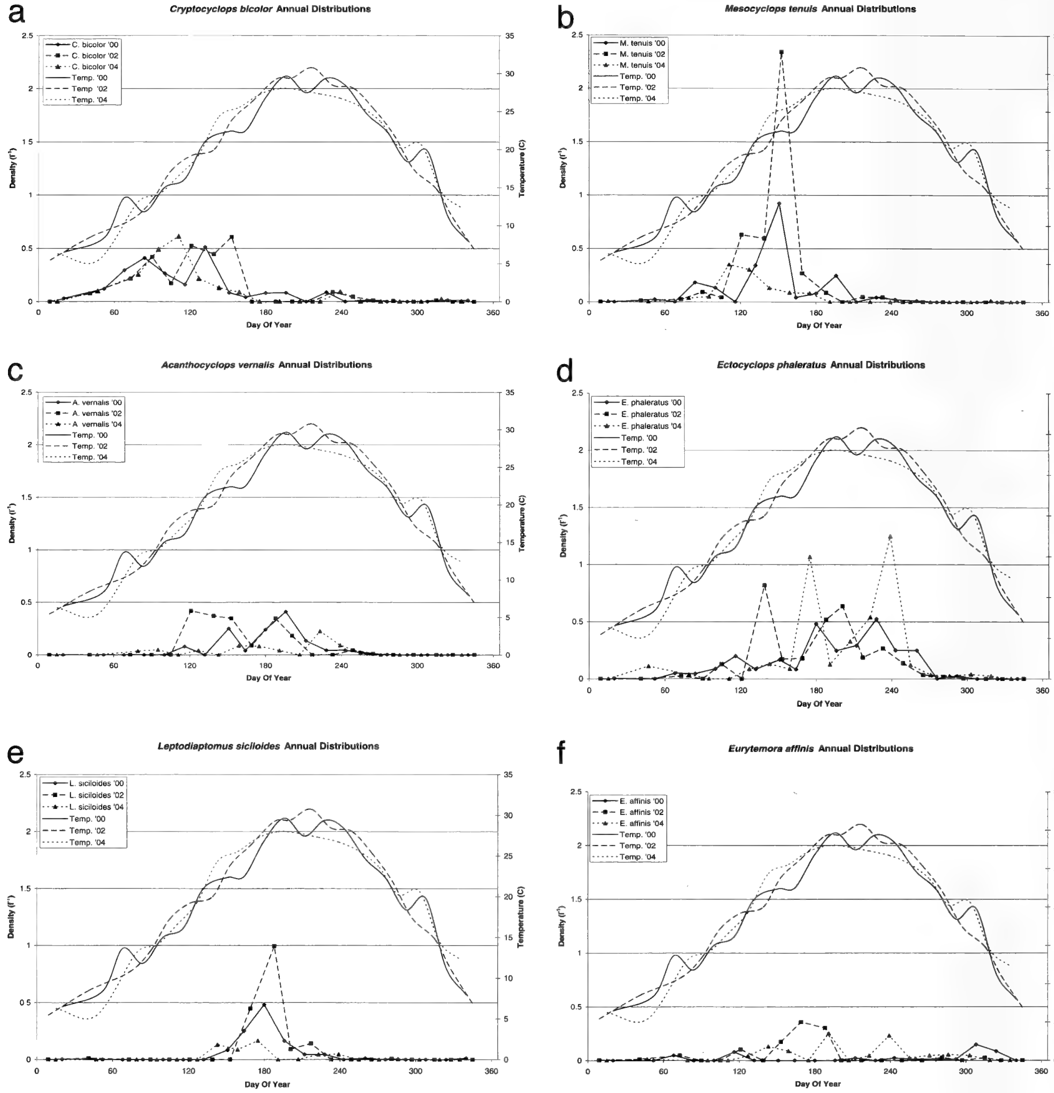

Figure 3. Density and temperature distribution by day of year for (a) *Cryptocyclops bicolor,* (b) *Mesocyclops tenuis,* (c) *Acanthocyclops vernalis,* (d) *Ectocyclops phaleratus,* (e) Calanoida *Leptodiaptomus siciloides,* and (f) Calanoida *Eurytemora affinis.*

was different, a bimodal pattern was still present.

Distinctly concentrated in the middle of the year was the calanoid *Leptodiaptomus siciloides* (Figure 3e). Although differences were seen between the 2000, 2002, and 2004 samples, the same general pattern was observed exhibiting peak densities around day 190. Of the three years sampled, highest densities occurred in 2002 and peaked at 1.00 l^{-1} (Table 2). *L. siciloides* was least common during the 2004 sampling year, reaching densities of approximately 0.20 l^{-1}. With the exception of the period from days 130–250, this copepod was rarely found at any other time of the year, making it the least common of the species identified in this study.

Of the major species present in Kentucky Lake, *E. affinis* was the only one found throughout the year (Figure 3f). Peak densities were at different times in all three years, with the highest density of 0.36 l^{-1} on day 170 in 2002. Peak densities in 2004 exhibited a roughly trimodal distribution with peaks falling around days 140, 200, and 240. Of the three years, densities of *E. affinis* were lowest in 2000, with two small peaks around days 70 and 110 and a larger peak around day 310, the latest date for any of the major species in this study.

DISCUSSION

All copepods found in this study have been reported from other North American lakes and reservoirs (Bowman and Lewis 1989; Taylor et al. 1993; Lee 1999; Reid 1999; Nicholls and Tudorancea 2001). *Eurytemora affinis, Leptodiaptomus siciloides, Ectocyclops phaleratus, Mesocyclops tenuis,* and *Cryptocyclops bicolor* have been reported previously from Kentucky and Tennessee waters (Bunting 1973; Heller and Katz 1982; Novotny and Hoyt 1982; Bowman and Lewis 1989). The total number of species present and the dominance of just a few calanoids and cyclopoids, particularly *Mesocyclops tenuis,* in the Kentucky Lake plankton appear typical for most warmer temperate lakes and reservoirs (Reid 1992; Williamson and Reid 2001). With the exception of *Eurytemora affinis* that is primarily an herbivore, the majority of the species are omnivores feeding on bacteria, phytoplankton, and other zooplankton (Williamson and Reid 2001).

Total copepod densities in this study were comparable to those of Schram and Marzolf (1993) who reported average summer densities of approximately 3.0 l^{-1} in Kentucky Lake based on data from the first three years of the Kentucky Lake Long-Term Monitoring program. The timing of the spring copepod peak appears to have remained constant over the years of the monitoring program independent of water temperatures, lasting about 32 days from late April through May and coinciding with similar peaks in cladocerans and the early development of zooplanktivorous larval fish in Kentucky Lake (Schram and Marzolf 1993). A late spring (days 150–160) change in community composition was observed and is likely due to an increase in larval fish predation and temperature change.

As with cladocerans (Thrope and Covich 2001; Wetzel 2001), several of the copepod taxa reached maximum densities before day 160 and were replaced by other dominants after. For Kentucky Lake, the cyclopoids *Cryptocyclops bicolor* and *Mesocyclops tenuis* were the late spring species (Figures 3a, 3c), while the calanoids *Eurytemora affinis* and *Leptodiaptomus siciloides* peaked in early to midsummer (Figures 3d, 3e). Only the cyclopoids *Ectocyclops phaleratus* and *Acanthocyclops vernalis* were present in similar numbers before and after the day 160 (Figures 3a, 3f). *Eurytemora affinis* (Figure 3d) did not show a density response related to day 160.

At least some cyclopoids and calanoids were present throughout the year, but populations did not greatly increase until water temperatures reached approximately 10°C (Figure 2b), which concurs with summaries in Williamson and Reid (2001). Populations of most species, with the exception of *Ectocyclops phaleratus,* declined before the late summer maximum of 30°C. Similar temperature relationships also have been shown for most Kentucky Lake cladocerans (Schram and Marzolf 1993; Yurista et al. 2000).

Although life histories could not be obtained for all of the major species in this study, they were found for *E. phaleratus, L. siciloides,* and *E. affinis* (Hudson et al. 2003; Lesko et al. 2003). Annual patterns observed for *E. phaleratus* in Kentucky Lake density peaks approximately every 30 days through spring and summer while the reported life cycle of *E. phaleratus* from Minnesota is 12 days. This may be a result of differing environmental factors, the limits of our dataset and sampling frequency, or multiple generations being present concurrently. Annual patterns for *L. siciloides* were observed to rise and decline sharply, peaking around day 180 while their life cycle is reported to last from 26–73 days. These data more closely resemble the reported life history of the species and the single rise in density observed in this species may be the result of these females only being able to produce one clutch of eggs each generation. Finally, *E. affinis* exhibited density peaks approximately every 30 days with the exception of a 60 day peak observed in 2002. Life history accounts report that this species will have a <30 day life cycle at 25°C and corresponds with the density increases observed in our study. The extended peak observed in 2002 may be the result of multiple overlapping generations.

Eurytemora affinis is considered a marine invasive in freshwater systems and generally restricted to temperatures of <17°C (Taylor et al. 1993). Although it was present in Kentucky Lake in low numbers throughout the year, the maximum density was not reached until temperatures reached 17°C and higher. Locally it has been collected from the Ohio River at Louisville, KY (Bowman and Lewis 1989). *Eurytemora affinis* is an herbivore and may take advantage of increasing algal resources at this time of year. Although we have not examined earlier archived samples to determine if this species had been present throughout the entire monitoring period, it is of interest to note that other warm water zooplankton species (e.g., *Daphnia lumholtzi,* Yurista et al. 2000) have invaded Kentucky Lake over the period of monitoring creating a continually changing mosaic of species and species interactions in this human created ecosystem. Continued monitoring of Kentucky Lake zooplankton through the CRR's long-term monitoring program may lead to further insights and a better understanding of copepod ecology and population dynamics.

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Changes in the Freshwater Mussel Community in the Kentucky Portion of Kentucky Lake, Tennessee River, since Impoundment by Kentucky Dam

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ABSTRACT

The unionid mussel fauna of Kentucky Lake has changed significantly over the past century. Prior to the completion of Kentucky Dam in 1944, 42 species had been reported from the mainstem Tennessee River now inundated by Kentucky Lake. After Kentucky Dam was completed, the tailwater fauna experienced minor change in species richness with 38 species being reported between 1978 and 1985. Currently, the Kentucky portion of Kentucky Lake supports 21 species. Four of these were not reported in the historical fauna prior to impoundment: *Anodonta suborbiculata, Plectomerus dombeyanus, Potamilus ohiensis,* and *Toxolasma parvus*; thus only 17 of the original species survived impoundment while four species invaded after Kentucky Dam was constructed. *Amblema plicata* is now the most abundant species. *Plectomerus dombeyanus,* first found in the Tennessee River in 1981, is the second most abundant mussel in the Kentucky portion of Kentucky Lake. *Quadrula quadrula* peaked in relative abundance at 51% in 1989 but subsequently declined to 10% by 2001. Few mussels survive in the deep channel where fine sediment continues to accumulate and anaerobic conditions sometimes occur. The faunal decline is typical following large dam construction, while the continuing change in the mussel fauna may reflect an aging reservoir and invasion of opportunistic species.

KEY WORDS: Unionidae, mussel, Tennessee River, Kentucky Lake, Kentucky Dam, reservoir

INTRODUCTION

Freshwater mussels (Bivalvia: Unionidae) are an important ecological component of many medium to large, flowing rivers, often constituting the predominant benthic biomass (Strayer et al. 1994) and contributing significantly to secondary production (Negus 1966; Vaughn et al. 2004). Mussels filter enormous volumes of water, removing large quantities of suspended material, and depositing large quantities of organic matter utilized by other benthic organisms (Sephton et al. 1980; Spooner and Vaughn 2006; Vaughn and Spooner 2006). Further, mussels serve as sentinels to the health of rivers and lakes. Their long lifespan, averaging 20 years or more and generally sessile existence, make them natural sentinels of water conditions (Maddox et al. 1990; Martel et al. 2003; Ravera et al. 2003; Strayer et al. 2004; Brown et al. 2005). Mussels may be both dense and diverse at a single location, e.g., in the continually flowing, dam tailwater reaches of the Tennessee River, mussels often occur at densities of over 100/m^2, with 20 or more species occurring together (Sickel 1985; Miller et al. 1992; Lewis and Sickel 2004). Mussels now, however, are among the most imperiled animals on the planet, with many species having become extinct in the past century, and many more on the federal endangered species list (Williams et al. 1993).

Mussels constitute an important natural resource in the Tennessee River and Kentucky Lake in western Kentucky. The larger, heavier shelled species were once harvested commercially for the pearl button industry, but this industry disappeared in the 1950s when plastics replaced mother-of-pearl as the material for buttons (Anthony and Downing 2001). Growth of the Japanese cultured pearl industry provided a new market for Tennessee Riv-

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er shells in the 1960s; and once again shells were harvested by the thousands of tons (Isom 1969). Although some harvest continues for the jewelry industry, in western Kentucky the shell industry has declined, and commercial shell harvest in the Kentucky portion of Kentucky Lake is scheduled to cease February 28, 2011 according to the Kentucky Department of Fish and Wildlife Resources regulation 301 KAR 1:085 (KDFWR 2006). Although the direct monetary value of shells may be declining because of market forces in the cultured pearl industry, the full ecological value of mussels remains to be evaluated. Reduced harvest of shells may allow mussels to reach their ecological potential.

There is increasing interest in ecological studies of spatial and temporal heterogeneity in freshwater mussel distributions. Mussels generally are found aggregated within a nonuniform benthic environment (Downing and Downing 1992), and aggregation may influence reproductive success (Downing et al. 1993; Lewis 2001). For resource managers, understanding heterogeneity and temporal changes in mussel populations is essential to regulating activities such as harvest, sand and gravel dredging, or other activities that impact the mussel resources and which could reduce densities of particular species below that needed to sustain reproductive success (Anthony and Downing 2001; Strayer et al. 2004). Periodic surveys, for example at five year intervals, are a way to monitor the mussel resources and determine if existing regulations are sufficient to maintain the mussel communities.

The great diversity of the mussels of North America was recognized in the early 1800s (Rafinesque 1820; Lea 1834), and later it became obvious that the geologically ancient Tennessee and Cumberland River valleys had contributed to the evolution of that diverse fauna (Ortmann 1925). With nearly 300 recognized species in North America, the lower Tennessee River contains about 15%, a number of which are on the federal endangered species list. In the upper Tennessee, Cumberland, and Duck Rivers, about twice that number once occurred (Parmalee and Bogan 1998).

Henry van der Schalie (1939) predicted that Kentucky Dam would significantly alter the habitats of the Tennessee River resulting in the loss of many species. As Kentucky Dam was being constructed, van der Schalie stated, "If, after the impounding of waters, the reaction of the mussels now occupying that region is similar to that of mussels found in ponded areas elsewhere, we can predict safely that the proposed Gilbertsville Dam at Paducah will entirely change the fauna now found in the lower Tennessee. Records that give definitive information as to the ecology and distribution of the naiads of this area prior to the construction of such power dams will consequently be valuable for future studies of the taxonomy and zoogeography of these animals" (van der Schalie 1939). He listed 31 species occurring from Savannah, TN, (TRM 190) to Paducah, KY.

Whenever dams are placed across rivers, sediment accumulates upstream and often covers the natural gravel and sand substrates. Ellis (1936) had stated, "Experimental studies demonstrated that layers of fine silt from one fourth of an inch to one inch thick produced a very high mortality among fresh-water mussels living in gravel or sand beds, and in water which was otherwise favorable." These predictions of the loss of great faunas have been realized in recent years in a number of large reservoirs (Blalock and Sickel 1996; Vaughn and Taylor 1999). No one anticipated the adaptation of many species to the broad floodplains that were to be flooded by Kentucky Dam.

The objective of this paper was to compile data from earlier reports and collections such that the changes in the mussel fauna in the Kentucky portion of Kentucky Lake can be documented. Only two mussel surveys were conducted in the Kentucky portion of the lower Tennessee River prior to impoundment: one by Paul Bartsch in 1907 and one by Ellis in 1930. In each of these surveys only a few sites in Kentucky were investigated. Therefore we relied on other surveys from Tennessee, such as Ortmann (1925) and van der Schalie (1939), to infer what the pre-impoundment mussel fauna may have been. Their surveys extended farther up the Tennessee River indicating a faunal change occurring upstream from Savannah, Tennessee (TRM 190); therefore we used data only from downstream of that point. Because so few samples were made prior to impoundment, we included two extensive surveys of the Kentucky Dam tailwater, one by TVA in 1978 (Gooch et al. 1979) and one by Sickel (1985) to provide a more complete list of species we believe were present in the pre-impoundment fauna and to provide a comparison between the tailwater and impoundment fauna.

STUDY AREA

Kentucky Lake is the largest of the Tennessee Valley Authority (TVA) reservoirs on the Tennessee River and was formed by the construction of Kentucky Lock and Dam completed in 1944 at a point 36 km upstream from the Ohio River at Tennessee River Mile (TRM) 22.4 in Marshall and Livingston counties, Kentucky. Kentucky Lake extends upstream for 296.6 km to Pickwick Landing Lock and Dam (TRM 206.7) in Hardin County, Tennessee. Water storage in the reservoir first began 16 August 1944, with final closure of the dam on 30 August 1944 (Lowery et al. 1990). Minimum pool elevation of 107.9 m above mean sea level (MSL) was reached 7 April 1945, a height of 15.9 m above the original normal river elevation. The scheduled annual surface elevation fluctuation is 1.52 m. Since filling and inundation of the river floodplain and adjacent tributaries, a minimum elevation of 104.6 m occurred once on 11 March 1961 (Lowery et al. 1990). This drop of 3.3 m below minimum pool exposed large areas where mussels may have become established. The length of exposure and effect on any mussels is not known to the authors (Sickel and Burnett 2001).

For approximately half of the length of Kentucky Lake, from Pickwick Landing Dam to about TRM 110 at the mouth of the Duck River in Humphreys County, Tennessee, the reservoir essentially remains within its natural banks, and the sediment in the original channel remains swept clean of silt providing good mussel habitat similar to that of the river before impoundment (Isom 1969). Downstream from this region, sediment accumulates covering the original gravel substrate with varying amounts of fine silt and providing poor habitat for mussels within the main channel. Also, at times of low flow, the deeper areas may become depleted of dissolved oxygen. A TVA study in 1965 (Isom 1969) reported 1.2 m of sediment in the channel 15 km upstream from Kentucky Dam (TRM 31.7) and 0.61 m of sediment at TRM 41, just north of the Eggners Ferry Bridge (TRM 41.75). In reference to the inundated river channel, according to Isom (1969), "The first evidence of a 'flowing water' fauna: bryozoa, sponges, snails and caddisflies, was found above TRM 89." Thus between TRM 110 and 89 a transition from a riverine to a predominantly lacustrine environment occurs. The Kentucky/Tennessee boundary occurs at TRM 49.2 (USACE Navigation Chart No. 8) east of the center of the channel and TRM 62.5 (USACE Navigation Chart No. 10) west of the channel with the boundary extending along the channel line between these points (U.S. Army Corps of Engineers 2007). Therefore, the Kentucky portion of Kentucky Lake is in what we consider to be the lacustrine reach of Kentucky Lake. Within the lacustrine region, outside of the original river channel, broad areas of river floodplain and the lower reaches of tributaries became inundated. These areas now provide most of the habitat for mussels (Sickel and Chandler 1982).

METHODS

To document the mussel fauna of the Tennessee River in the Kentucky portion of Kentucky Lake, data were gathered from published literature, museum records, and numerous unpublished technical reports of mussel surveys conducted by the Tennessee Valley Authority and by Murray State University. All records we could find for the pre-impoundment period were included; however, for the post-impoundment data, only records that added to the overall species list were included. Schuster (1988) compiled a list of mussels occurring in Kentucky from available literature and museum collections, and we depended heavily on this material for historical data and to provide entry points to museum collections now online. His report provided a good source to historical data because he gave museum catalogue numbers for specimens he examined. These numbers were checked and verified online at the Smithsonian National Museum of Natural History, Department of Invertebrate Zoology (http://www.nmnh.si.edu/iz/), the University of Michigan Museum of Zoology (http://www.ummz.umich.edu/

mollusk/databases/index.html), the Ohio State University Museum of Biological Diversity, Mollusks Division (http://www.biosci.ohiostate.edu/~molluscs/OSUM2/), and Carnegie Museum of Natural History (http://collections.carnegiemnh.org/mollusks/specimen/index.cfm). Schuster's (1988) report was summarized by Cicerello et al. (1991) but did not distinguish the mussels reported from Kentucky Lake from those reported elsewhere in the Tennessee River watershed in Kentucky including the Clarks River, so we depended on the original records for location data. Data from mussel surveys conducted at Murray State University were summarized from technical reports. For the Sickel 1989 and the Sickel et al. 1981, 1995, and 2001 surveys, many of the same 94 sites in the Kentucky portion of Kentucky Lake that were surveyed in 1981 were surveyed again in 1989 (56 sites), 1995 (86 sites), and 2001 (89 sites). At each site, 20 1-m^2 quadrats were searched by a diver (only 10 quadrats at each site in 1989). The diver followed an anchored transect line collecting 10 quadrats on each side of the line. The quadrat was a 1-m^2 frame divided by cross bars into quarters to help the diver orient hand sampling under poor visibility conditions. Sampling was accomplished by digging into the sediment and placing mussels in fine mesh bags. All mussels were later identified and counted. Percentage abundance of each mussel species from each of the four surveys was used to examine changes in species composition within Kentucky Lake between 1981 and 2001. We used the taxonomic nomenclature of Turgeon et al. (1998). Earlier reports often used different nomenclature. Synonyms can be found in Parmalee and Bogan (1998).

RESULTS AND DISCUSSION

From 1907 to 2001, 50 species of unionid mussels were reported from the mainstem lower Tennessee River from Savannah, TN, to Paducah, KY, including what is now Kentucky Lake in Kentucky and Tennessee and the Kentucky Dam tailwater but not including the Pickwick Dam tailwater upstream from Savannah where several additional species may occur that probably did not occur in Kentucky (Table 1). All of these species probably occurred in the Tennessee River in Kentucky, but because of the paucity of pre-impoundment data we could not be certain.

Prior to the construction of Kentucky Dam in 1944, only four mussel collections had been made from the region, and van der Schalie (1939) apparently had access to data from only three of these: part of a Bryant Walker/Calvin Goodrich collection now in the University of Michigan Museum of Zoology, Ortmann's 1924 collection in the Carnegie Museum (Ortmann 1925), and the 1930 survey by Ellis in the University of Michigan Museum of Zoology. The first record of the mussels of the lower Tennessee River in western Kentucky was the unpublished collection of Paul Bartsch at the Smithsonian National Museum of Natural History. Bartsch was an assistant curator at the Smithsonian in 1907 (later served as a curator in the Division of Mollusks from 1914–1946) when the U.S. Bureau of Fisheries sponsored the survey of the mussels of the Mississippi, Ohio, and Tennessee Rivers in response to the extensive harvest of shells by the pearl button industry. Smithsonian collection records indicated that on 12 July 1907, the Bartsch expedition began in St. Paul, MN, and followed the Mississippi River to the Ohio River and up the Tennessee River. They reached Paducah, KY, on 19 August 1907 where a collection was made on the Tennessee River at Stiles (probably Stiles Crossing, TRM 7). From there the expedition apparently traveled upstream, perhaps overland, and made several stops for collections as they traveled back downstream. On 27 August, they collected near Savannah, TN, (TRM 190) and then made several stops down the Tennessee River until they collected at Danville Landing (TRM 78) on 30 August 1907. On that same day they collected also at Panther Creek Island (TRM 59) and Birmingham, KY (TRM 31). The last collection of the expedition was made on that day below the "E.P. & S. Bridge" after which Bartsch left to catch the ship, Albatross, for an expedition to the South Pacific. The location marked as "Below E.P.& S.? Bridge" was thought to be near Birmingham, KY, because it was the last collecting station with Birmingham being the previous station, but its precise location remains unknown because no bridge today is called E.P. & S. We found the probable location of the "E.P. & S. Bridge" on an 1895

Table 1. The mussel fauna of the lower Tennessee River before and after impoundment by Kentucky Dam in 1944 at Tennessee River Mile (TRM) 22.4, with X indicating presence, and decimal numbers are percent abundance of each species. Sample locations are indicated by TRM from Savannah, TN to Paducah, KY. Abundance data for Kentucky Lake samples from 1981 through 2001 compare approximately the same 86 to 94 sites where 20 1-m² quadrat samples were collected at each site except in 1989 when only 10 1-m² quadrat samples were collected at 56 sites. Kentucky Dam tailwater samples from 1978 and 1985 are included to provide a more complete estimate of the species probably present in the original river fauna prior to impoundment and for comparison to the present lake fauna.

	Bartsch: Sta. 134–135[1] Paducah, Stiles [Crossing]	Bartsch: Sta. 167 Birmingham and Sta. 168 E. P. & S. Bridge	Bartsch: Sta: 159 Johnsonville to Sta: 164 Panther Creek	Bartsch: Sta: 136 Savannah to Sta: 158 above Johnsonville	Walker/Goodrich Collection: Egner's Ferry	Ortmann: Dixie-Trotters Landing Walker/Goodrich: (*)	Ellis: Fort Henry and Paducah	Ellis: Savannah and Britt's Landing	TVA: Ky Dam Tailwater	Sickel: Ky Dam Tailwater	Sickel and Chandler: Kentucky Lake	Sickel: Kentucky Lake	Sickel, Herod, Blalock: Kentucky Lake	Sickel and Burnett: Kentucky Lake
YEAR	1907	1907	1907	1907	1919?	1924	1930	1930	1978	1985	1981	1989	1995	2001
Species	TRM 0–7	TRM 22–31	TRM 59–100	TRM 102–190	TRM 42	TRM 102	TRM 0–61	TRM 122–190	TRM 5–22	TRM 7–22	TRM 23–62	TRM 23–62	TRM 23–62	TRM 23–62
Actinonaias ligamentina				X		X*				0.02				
Amblema plicata	X	X	X	X	X	X	1.04		7.78	11.92	17.08	20.31	38.74	36.60
Anodonta suborbiculata											0.95	2.39	0.71	0.36
Arcidens confragosus									0.03	0.01	0.73	2.56		0.06
Cyclonaias tuberculata	X	X	X	X	X	X	2.60	5.63	6.71	5.89				
Cyprogenia stegaria				X					0.03					
Dromus dromas				X										
Ellipsaria lineolata	X	X	X	X		X		0.70	4.64	4.91				
Elliptio crassidens	X	X	X	X	X	X	2.08	5.28	1.29	1.38				0.06
Elliptio dilatata	X		X	X		X	0.52	0.35	2.40	1.65				
Fusconaia ebena	X	X	X	X		X	21.35	3.52	40.39	35.36	0.80	1.71	1.97	2.86
Fusconaia flava							2.08		0.03	0.20	4.38	1.71	2.91	2.55
Fusconaia subrotunda		X	X	X		X	0.52		0.21	0.82				
Lampsilis abrupta	X[1]					X	0.52		0.03	0.07				
Lampisilis cardium	X			X		X								
Lampsilis fasciola				X										
Lampsilis ovata	X	X	X	X						0.09				
Lampsilis teres	X			X	X	X	0.52			0.02				
Lasmigona complanata									0.03	0.11				
Leptodea fragilis		X		X		X*				0.78	1.24		0.39	0.61
Lexingtonia dolabelloides				X										
Ligumia recta	X	X		X		X			0.18	0.24				
Megalonaias nervosa	X	X	X	X	X	X	11.46	10.92	3.89	4.11	9.27	8.02	4.65	4.01
Obliquaria reflexa				X		X	6.77	3.52	1.35	3.25	6.93	4.10	6.93	2.74
Obovaria olivaria		X		X		X	1.56	3.87		0.02				
Obovaria retusa	X		X	X		X		0.35		0.04				
Plectomerus dombeyanus											0.15	1.19	18.11	32.46
Plethobasus cooperianus				X		X	1.04	5.63		0.09				

Table 1. Continued.

	Bartsch: Sta. 134–135[1] Paducah, Stiles [Crossing]	Bartsch: Sta. 167 Birmingham and Sta. 168 E. P. & S. Bridge	Bartsch: Sta: 159 Johnsonville to Sta: 164 Panther Creek	Bartsch: Sta: 136 Savannah to Sta: 158 above Johnsonville	Walker/Goodrich Collection: Egner's Ferry	Ortmann: Dixie-Trotters Landing Walker/Goodrich: (*)	Ellis: Fort Henry and Paducah	Ellis: Savannah and Britt's Landing	TVA: Ky Dam Tailwater	Sickel: Ky Dam Tailwater	Sickel and Chandler: Kentucky Lake	Sickel: Kentucky Lake	Sickel, Herod, Blalock: Kentucky Lake	Sickel and Burnett: Kentucky Lake
Plethobasus cyphyus						X		0.35	0.03	0.07				
Pleurobema clava				X										
Pleurobema cordatum	X	X	X	X	X	X	12.50	30.63	10.69	6.96				
Pleurobema plenum						X[2]								
Pleurobema rubrum			X		X	X	5.21	3.52	0.03					
Pleurobema sintoxia								1.76						
Potamilus alatus	X		X	X	X	X			0.60	1.51	0.51	0.68	0.55	1.4
Potamilus ohiensis											0.80			
Ptychobranchus fasciolaris	X		X	X		X				0.04				
Pyganodon grandis										0.11	1.61	0.17	0.63	0.24
Quadrula apiculata										0.20			1.18	2.67
Quadrula cylindrica				X					0.06	0.44				
Quadrula fragosa						X[3]								
Quadrula metanevra	X	X	X	X		X	7.81	10.21	1.89	0.98				
Quadrula nodulata							5.21		0.15	0.49	7.37	6.48	5.83	2.67
Quadrula pustulosa	X	X	X	X	X	X	4.69	10.56	14.31	11.01	0.22		0.39	0.06
Quadrula quadrula	X					X*	9.38		3.05	3.89	47.15	50.68	17.01	10.15
Toxolasma parvus											0.15			0.06
Tritogonia verrucosa	X		X	X		X			0.09	0.58	0.36			
Truncilla donaciformis							1.04	0.35	0.03	1.42	0.15			0.43
Truncilla truncata							2.08	2.46	0.09	1.16				
Utterbackia imbecillis								0.35		0.04	0.15			
Number of species	20	14	17	30	9	29	21	19	27	36	19	12	14	18
Total number collected							192	284	3340	4497	1370	586	1270	1645

Bartsch 1907. Data from the Smithsonian National Museum of Natural History, Department of Invertebrate Zoology.
Walker & Goodrich 1919?. Data from University of Michigan Museum of Zoology with uncertain date, possibly 1919.
Ortmann 1924. Data partly from van der Schalie (1939) and partly from Ortmann (1925) and the collection at the Carnegie Museum.
Ellis 1930. Data from van der Schalie (1939) and the University of Michigan Museum of Zoology.
TVA 1978. Data from Gooch et al. (1979).
Sickel data from Sickel (1985), Sickel and Chandler (1981), Sickel (1989), Sickel et al. (1996), and Sickel and Burnett (2002).

1. In the Paul Bartsch collection at the Smithsonian, *L. abrupta* was reported from Station 135, Tennessee River near Paducah on 19 Aug 1907 but precise location not recorded. Station 134 was indicated as Stiles Paducah Turnpike and collected on the same date. Stiles Crossing is at Tennessee River Mile 7.

2. Ortmann (1925) reported these as *Pleurobema cordatum plenum* and indicated they were rare and found among the more common *Pleurobema cordatum*. Two specimens are at the Carnegie Museum, Catalog No. 61.12006.

3. Ortmann (1925) reports all of these from Dixie as *Quadrula quadrula fragosa* and indicates this form to be common in the Cumberland River but rare in the Tennessee River. He distinguishes it from *Q. quadrula* that occurs elsewhere in the Tennessee River. Four specimens are at the Carnegie Museum, Catalog No. 61.11982.

Atlas as being the railroad bridge, Elizabethtown-Paducah-St. Louis RR, which crossed the Tennessee River at approximately the present location of Kentucky Dam, TRM 22.4. Apparently the shell collection was deposited at the Smithsonian and no record was ever published. Smithsonian staff recently has placed a portion of the collection records online.

The Bartsch collection includes 33 species of mussels collected from Savannah, TN, to Paducah, KY, which includes most of the region of the river now under Kentucky Lake and the tailwater downstream from Kentucky Dam. Four species he collected that were not characteristic of the Kentucky portion of the Tennessee River were *Dromus dromas* (I. Lea, 1834), *Lampsilis fasciola* Rafinesque, 1820, *Lexingtonia dolabelloides* (I. Lea, 1840), and *Pleurobema clava* (Lamarck, 1819). These species were characteristic of the Muscle Shoals of northern Alabama with *D. dromas* and *L. dolabelloides* belonging to the Cumberlandian fauna (Ortmann 1925) typically found in the middle and upper Tennessee, Cumberland, and Duck Rivers (van der Schalie 1939). The other twenty-nine species he collected were reported by others for the lower Tennessee River (Table 1). These species are the typical Interior Basin (or Ohioan) fauna of big rivers (Ortmann 1925).

The next report of lower Tennessee River mussels was that of Ortmann (1925). His only collection within what is now Kentucky Lake was made in 1924 at Dixie/Trotters Landing, which is in the vicinity of New Johnsonville, Tennessee at TRM 102. He reported 26 species, and much of that collection is at the Carnegie Museum. Three species in the collection were not recorded by Bartsch (*Plethobasus cyphyus* (Rafinesque, 1820), *Pleurobema plenum* (I. Lea, 1840) and *Quadrula fragosa* (Conrad, 1835)). All the species he collected at Dixie except *P. plenum* and *Q. fragosa* have been reported from the Kentucky portion of the Tennessee River post-impoundment, but most have been reported only from the Kentucky Dam tailwater and not from the reservoir. Ortmann was one of the authorities on mussels in the early twentieth century, so if he determined these two species to be *P. plenum* and *Q. fragosa,* they probably were; however, neither has been reported before or since from the lower Tennessee River. The specimens are at the Carnegie Museum, and could be examined: *P. plenum* (Cat. No. 61.12006) and *Q. fragosa* (Cat. No. 61.11982).

An unreported collection mentioned briefly by van der Schalie (1939) was a collection from Trotters Landing by Goodrich. We found a reference to this collection as the Bryant Walker and Calvin Goodrich collection online at the University of Michigan Museum of Zoology, but the date was uncertain—possibly 1919. This collection contained 23 species, adding three to the Ortmann list: *Actinonaias ligamentina, Leptodea fragilis* and *Quadrula quadrula.* We included these together in Table 1 because they came from the same location, TRM 102, in Tennessee. While searching the UMMZ database, we found nine entries in the Goodrich and Walker collection from Egners Ferry in Kentucky. There were no additional species that had not been reported by Bartsch, but we included it in Table 1 because it is the only pre-impoundment collection from that area. The date is uncertain because it was not included in the database, but because part of the Goodrich and Walker collection from Trotters Landing was dated 1919, we used this date. This needs to be verified if possible.

The final pre-impoundment collection was that of Ellis in 1930. He reported 26 species from the lower Tennessee River with 21 from Kentucky. His Kentucky sites were at Fort Henry, Stewart Co., TN/Calloway Co., KY, (TRM 61) and Paducah. These collections included six species that had not been reported by Bartsch or Ortmann (*Fusconaia flava* (Rafinesque, 1820), *Pleurobema sintoxia* (Rafinesque, 1820), *Quadrula nodulata* (Rafinesque, 1820), *Truncilla donaciformis* (I. Lea, 1829), *Truncilla truncata* Rafinesque, 1820, and *Utterbackia imbecillis* (Say, 1829)).

The Kentucky Dam tailwater mussel data of TVA in 1978 (Gooch et al. 1979) and Sickel (1985) (Table 1) represent the post-impoundment tailwater fauna that was similar to the pre-impoundment fauna with the addition of four species that had not been reported previously for the Kentucky section of the river: *Arcidens confragosus* (Say, 1829), *Lasmigona complanata* (Barnes, 1823), *Pyganodon grandis* (Say, 1829), and *Quadrula apiculata* (Say, 1829). This tailwater fauna closely resembles the pre-impoundment fauna. After impoundment, dramatic changes began to occur upstream from the dam, especially within the Kentucky portion of Kentucky Lake.

There is a large body of post-impoundment information on the mussels of Kentucky Lake starting with the investigation by Bates (1962) in which he pointed out that a number of species were adapting to the ''mud shallows'' as the deep main channel habitat was accumulating silt. Additional studies were conducted by Williams (1969) and Isom (1969), but they focused primarily on the main channel and did not record accurate sample locations. Bates (1962) reported the first occurrence of *Potamilus ohiensis* (Rafinesque, 1820), *Toxolasma parvus* (Barnes, 1823), and *Anodonta suborbiculata* Say, 1831. Isom (1969) reported two additional species from the riverine region between TRM 136–195.5: one valve of *Pleurobema oviforme* (Conrad, 1834) and *Cumberlandia monodonta* (Say, 1829). *Pleurobema oviforme* is considered a Cumberlandian species (Ortmann 1925) and probably did not occur in the Kentucky region. Although a weathered dead shell of *C. monodonta* was reported in 1991 at TRM 15.8 (Sickel 1991), we did not add it to the Kentucky Lake list because it had never been reported alive in the Kentucky region of the river.

Yokely (1972) conducted an extensive study of Kentucky Lake in Tennessee from Pickwick Landing Lock and Dam at TRM 206.7 downstream to mile (TRM) 93. Hubbs (2002) updated information on Kentucky Lake mussels within Tennessee. Bates (1975) conducted a reservoir wide survey of channel and overbanks, but relatively few samples were collected in Kentucky and they were collected with brail or PONAR grab, so they may not be representative of species composition. In his report few mussels were collected from the Kentucky section, and no additional species were reported.

The first systematic survey designed to establish accurately located sites for future monitoring in the Kentucky portion of Kentucky Lake was that of Sickel and Chandler (1982). In 1981 they collected at 94 locations within Kentucky from TRM 23–62. In this survey, divers utilized quadrat sampling to estimate mussel density, and sample sites were selected to represent four habitat types: old river levee, old river floodplain (referred to as overbank by Bates (1962)), main lake shoreline, and embayment. The surveys in 1989 (Sickel 1989), 1995 (Sickel et al. 1995), and 2001 (Sickel and Burnett 2001) resampled most of the same sites. Although those surveys were conducted by different divers who sometimes sampled 10 m^2 rather than 20 m^2 at some of the locations, the sampling was consistent enough that we believe they provide comparable data useful for documenting a progressive change in the mussel fauna.

By 1981 (Sickel and Chandler 1982) dramatic changes already had taken place in the mussel fauna in the Kentucky portion of Kentucky Lake compared with the pre-impoundment fauna. A number of pond or lake forms had appeared that were not found in the pre-impoundment, free flowing river, *Anodonta suborbiculata, Potamilus ohiensis,* and *Toxolasma parvus.* Also, a species new to the Tennessee River appeared, *Plectomerus dombeyanus* (Valenciennes, 1827). Two live individuals of *P. dombeyanus* were found at one of the sites sampled by Garry Pharris on 10 August 1981 while diving with Carol Chandler and accompanied by James Sickel, John Bates and Sally Dennis (Pharris et al. 1984). In 1989 only seven *P. dombeyanus* were found, and all occurred at the same site as in 1981. By 1995 their number had increased dramatically as the population was expanding up and down the lake from the original point of introduction. This same sample site had the highest mussel density in 1995 (113 mussels in 20 m^2, 33 *A. plicata,* and 54 *P. dombeyanus,* the largest number of that species found at any site in 1995). Again in 2001 this sample site had the greatest number of mussels (133 individuals in 20 m^2, 33 *A. plicata,* and 75 *P. dombeyanus*). The *P. dombeyanus* population continued to expand, and by 2001 it had become the second most abundant species within the sample area in Kentucky, making up 32% of the mussel community. Also in 2001, it was found for the first time below Kentucky Dam in the tailwater (Sickel and Burnett 2001).

Plectomerus dombeyanus is a Gulf coastal drainage species which had never been reported in the Tennessee River prior to 1981. However, its migration northward from Gulf tributaries had been noted as early as 1895 when Samuel N. Rhoads found it in Reelfoot Lake in west Tennessee, and Henry A. Pilsbry stated, "The species has not before been reported from so far north, east of the Mississippi, so far as I know" (Pilsbry and Rhoads 1896). Rhoads found two young specimens, identified as *Unio trapezoides* Lea (Academy of Natural Sciences of Philadelphia Cat. No. 69216 (http://data.acnatsci.org/biodiversity_databases/snails.php)), in Reelfoot Lake at Samburg, Obion County, Tennessee. This location is but 110 km from the Tennessee River, yet it took nearly a century for the species to appear in Kentucky Lake even though it obviously is well adapted to that habitat.

Anodonta suborbiculata was first reported in Kentucky Lake in Tennessee by Bates (1962), and its first appearance in Kentucky was reported by Morgan Sisk who collected three specimens on 14 August 1971. The Sisk specimens were identified by David Stansbery and reside in the Ohio State University Museum of Biological Diversity (Cat. No. 26528). Within Kentucky Lake, *A. suborbiculata* is never abundant and generally restricted to the back of bays in soft mud.

Potamilus ohiensis is a thin shelled species found predominantly in ponds and lakes in fine sediments or rivers in slow current (Parmalee and Bogan 1998). First reported in Kentucky Lake in Tennessee by Bates (1962) (as *Leptodea laevissima* (I. Lea, 1830)), we found it only in 1981 (Sickel and Chandler 1981); however, the shallow water in the back of bays where this species may occur were not sampled in later systematic surveys. It is commonly found along the shoreline as dead shells, probably brought up by muskrats or dying as the water recedes during late summer and fall drawdown. The lake form can be confused with small individuals of the lake form of *Leptodea fragilis* or very young *Potamilus alatus.*

Toxolasma parvus, first reported from Kentucky Lake by Bates (1962) (as *Carunculina parva* (Barnes, 1823)), is a small mussel generally found in bays and often overlooked because of its size. It can become abundant under conditions where it is protected from fish predation. We found only two specimens in 1981 and one in 2001. In a separate study (Berg et al. 1995) in which *Quadrula quadrula* were being held in mussel cages designed to protect them from muskrats, *T. parvus* became very abundant along with *Utterbackia imbecillis* after a period of two years. In the sediment outside the cages, none of either species were found. The cage mesh permitted small fish to enter but excluded larger fish, and small sunfish were seen in the cages. Both *T. parvus* and *U. imbecillis* utilize bluegill and green sunfish as hosts for their glochidia (Watters 1994). We believe they were brought in as glochidia on the gills of small sunfish. Larger sunfish and other predators that might have eaten the small mussels were excluded from the cages by the small mesh size. *Utterbackia imbecillis* was reported in the surveys only in 1981. It is found in mud substrate in the shallow water of bays.

Quadrula quadrula is widely distributed in Kentucky Lake. In 1981 it was the most abundant species (47%) and was one of the most sought after commercial species. By 2001, it had declined to 10% as both *P. dombeyanus* and *Quadrula apiculata* began to be found in the same habitat. *Quadrula quadrula* and *Q. apiculata* appear to intergrade and may experience more intense competition. *Quadrula apiculata* was not reported until 1995, although it was present but had been confused with *Q. quadrula.* Wendell Haag pointed out the distinction between the two morphs while working at the Hancock Biological Station with David Berg on mantle biopsies, and later they were shown to be distinct populations (Berg et al. 1995, 1998). Although overall the percent of *Q. apiculata* was only 3% in 2001, relative to *Q. quadrula* it is increasing as *Q. quadrula* declined in abundance from 51% in 1989 to 10% in 2001. Some of the decline of *Q. quadrula* may be attributed to commercial harvest in the 1990s, but Lewis (2001) showed that *Q. quadrula* was experiencing low fertilization success compared with *Amblema plicata* and *Megalonaias nervosa.*

Amblema plicata has become the most abundant species in the Kentucky portion of Kentucky Lake, increasing from 17% relative abundance in 1981 to 37% in 2001. It is found in all habitats sampled. In the shallow waters and sandy-mud substrate in the back of some shallow bays, juvenile *A. plicata* often can be seen moving toward deeper water in the fall as the drawdown is occurring. This species grows rapidly, has a thick pearly shell, and is a valuable commercial species. *Megalonaias nervosa* is found with *A. plicata,* but its abundance declined from 9% to 4% from 1981 to 2001.

The most abundant species in the Kentucky Dam tailwater, *Fusconaia ebena,* was found only rarely (0.8%) in the Kentucky portion of the reservoir in 1981; however, it increased to an abundance of 3% in 2001. It is mostly restricted to the levees along the river channel where there is still some current when discharge is occurring at the dam. The levee habitat contained the most species, probably because it is a habitat where sediment is not accumulating. Although the levee substrates generally consist of firm clay and the original river bank soils, in some areas there is gravel mixed in with the clay. This is not like the original gravel bottom of the channel but may provide a habitat most similar to the flowing water habitat where most of these mussels evolved.

Most mussel species with low abundance in the reservoir in this study are generally found on the levees. These include *Arcidens confragosus, Elliptio crassidens* (found only once), *Fusconaia flava, Quadrula nodulata, Quadrula pustulosa, Tritogonia verrucosa,* and *Truncilla donaciformis.* The other three species found in the reservoir, *Leptodea fragilis, Potamilus alatus,* and *Pyganodon grandis,* generally occur near shore or in bays and at low abundance. This gives a total of 21 species that have been found in the Kentucky portion of Kentucky Lake post-impoundment, with four of these new to the fauna. The other 25 species that once occurred in the Tennessee River in western Kentucky and Tennessee (Table 1) apparently were not able to adapt to the conditions found in the lower, lacustrine region of the reservoir or their fish hosts do not occupy these areas. Many of these species still occur in the tailwater of Kentucky Dam and also the Pickwick Dam tailwater at the upper end of the reservoir, but they do not persist in the lacustrine environment.

Why some species thrive in the reservoir environment and others do not is not understood at this time. Numerous factors probably determine mussel success or failure. The most obvious might be the occurrence and behavior of the host fish. The most common mussel in the Kentucky Dam tailwater is *Fusconaia ebena* yet it is rare in the reservoir. Its primary host is the skipjack herring (Watters 1994) which is very abundant below dams in the spring when *F. ebena* is gravid. The skipjack is primarily a plankton feeder in schools near the surface. In the deep water of the reservoir it would not be on the bottom where the mussels are located. The most abundant mussel in the reservoir is *Amblema plicata* which can utilize many hosts, e.g., black and white crappie, largemouth bass, sauger, sunfish and yellow perch (Watters 1994) all of which are abundant along the bottom of the reservoir. Once transformed, juvenile mussels drop from their host fish, and they encounter many dangers as they settle into the sediment and begin feeding. Swift current moving large gravel can crush the juveniles while fine silt in slack water may cover the juveniles and prevent access to food and oxygen (Isely 1911). At that stage there are many predators such as turbellarians that feed on the tiny mussels, and as the mussels grow, larger predators such as mollusk eating fish, drum and shell crackers, become important. In shallow water muskrats and otters may prey on larger mussels. Obviously, changing the environment from a shallow flowing river to a deep lacustrine reservoir favors some mussels while being detrimental to a larger number of species. While mussel densities in the reservoir seldom exceed 5/m^2 compared with 100/m^2 sometimes seen in the Kentucky Dam tailwater, overall the mussel community appears to be thriving although at a lower species richness in the reservoir compared with the tailwater (Sickel and Burnett 2002).

CONCLUSIONS

The unionid mussel fauna in the Kentucky portion of Kentucky Lake has undergone major changes over the past century and continues to change in species composition and abundance today. Of the original 42 species that had been reported in the Kentucky Lake reach of the Tennessee River before impoundment, only 17 species now survive in the Kentucky portion of the reservoir. Four additional species that were not reported prior to impoundment have invaded and survive bringing the present total to 21 species. One of these, *Plectomerus dombeyanus,* which first appeared in 1981, has become the second most abundant species. The most abundant species (47% relative abundance) in 1981 was *Quadrula quadrula,* but it declined to 10% of the community in 2001 while *Amblema plicata,* which made up 17% of the community in 1981, increased to 37% and is the most abundant mussel. As the reservoir ages, we predict continued changes in the mussel community as opportunistic species invade and as habitats change.

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Seasonal Dynamics of the Bacterial Community of a Mudflat at the Mouth of a Major Kentucky Lake Reservoir Tributary

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ABSTRACT

Sediment samples were taken from the mudflat of Ledbetter embayment at the mouth of the stream, at summer pool when it was submerged and at winter pool when it was exposed. DNA was extracted and molecular techniques were applied to avoid problems associated with bacterial culture. Amplified Ribosomal DNA Restriction Analysis (ARDRA) found 96 different fingerprints in 350 clones containing amplified sediment 16S rDNA. There were 54 different patterns in the 180 clones examined from summer samples and 55 in the 170 clones examined from winter samples. Only 13 ARDRA patterns were found in both winter and summer. These were among the most abundant organisms found and were found frequently in both summer and winter. Sequence analysis of clones with different ARDRA patterns showed that the α and β Proteobacteria were the most abundant bacteria in these samples and were abundant in both winter and summer. The α Proteobacteria, however, were the most abundant group in winter and the β Proteobacteria were the most abundant group in the summer. Data suggest that there was seasonal turnover in the bacterial community and that the organisms that can thrive under, or at least tolerate, the differences in water level, temperature, nutrient, and oxygen availability are the most abundant species in the community throughout the year.

KEY WORDS: microbial diversity, bacterial community, littoral zone, microbial ecology, sediment microbiology, Kentucky Lake

INTRODUCTION

Very little is known about the microbial communities of the littoral zones of major reservoirs (Jones 1980). Kentucky Lake is the final of the nine mainstem reservoirs on the Tennessee River and the largest reservoir in the southeastern United States (White et al. 2007) impounded for the purposes of power generation, flood control, and transportation. The water level of Kentucky Lake is dropped during late summer to "winter pool" and raised in spring to "summer pool." Natural lake littoral zones conversely are exposed in the summer and flooded in the fall, winter, and spring. The littoral zones of tributary embayments of Kentucky Lake total approximately 43,000 ha. (White et al. 2007).

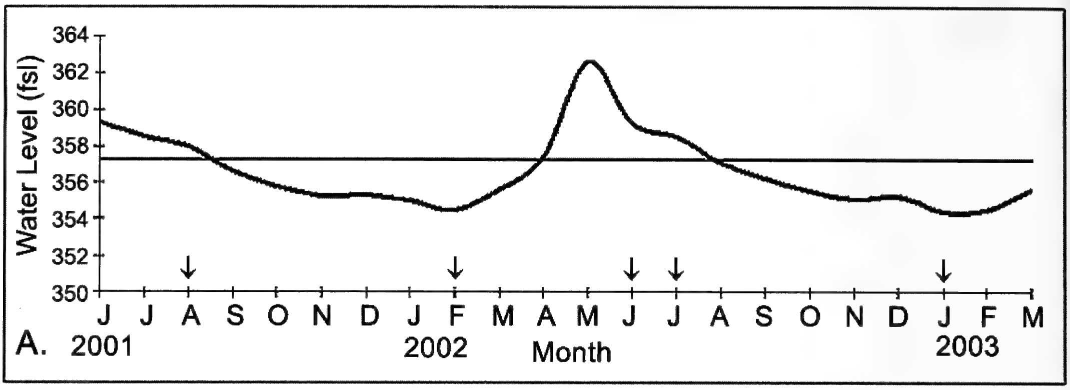
Molecular techniques have been applied to the study of microbial communities in sediments and soils in recent years (Amann et al. 1995; Gruber and Bryant 1997; Wise et al. 1997; Pace 1997; Rappe′ and Giovannoni 2003). The techniques, however, have not yet been applied to examine the microbial communities of littoral zones of a major reservoir. Much of the littoral zone of Kentucky Lake embayments is steep clay/gravel bank, but at the mouths of the tributaries there often is a mudflat where the streams have deposited sediments. Because temperature changes through the year, the mudflat is exposed in the winter and submerged in the summer, and there is seasonal input of allochthonous matter via the stream, thus it seems likely that the microbial community of the mudflat sediment would be dynamic.

The purpose of this investigation was to characterize and compare the bacterial community of the mudflat of a major reservoir tributary while it was exposed and while it was submerged. Molecular techniques were applied to this study to avoid the problems inherent in culturing microorganisms (Rappe′ and Giovannoni 2003).

MATERIALS AND METHODS

Sampling the Study Site

The mudflat at the mouth of the Ledbetter Creek where the stream runs into an embayment of Kentucky Lake reservoir was chosen for this study. The mudflat is approximately 200 meters × 300 meters in size. Sediment samples were collected from within 3 meters



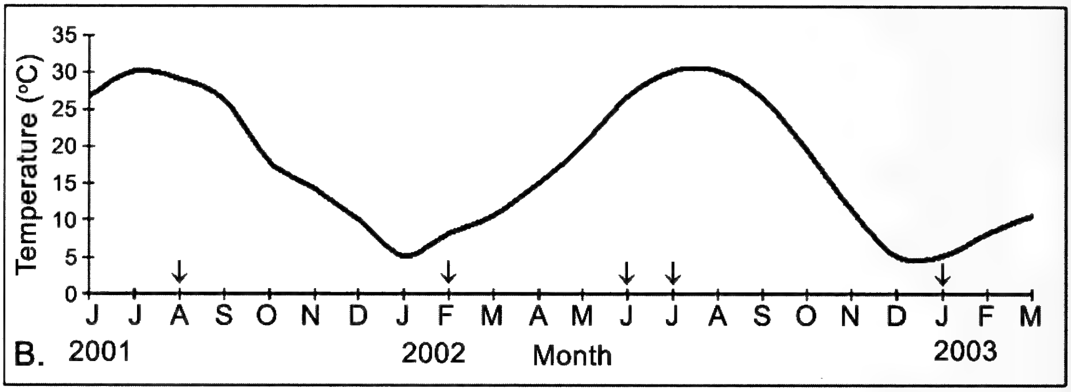


Figure 1. A, Water level of Kentucky Lake from June 2001 to March 2003. Line indicates the level above which the mudflat sample sites of Ledbetter embayment were submerged (masl: meters above sea level). B, Water temperatures of Kentucky Lake Ledbetter embayment measured at site 4L of the Murray State University monitoring program. Arrows on A and B indicate when the samples were taken.

of 5 permanently installed piezometers that transect the study site. The piezometers were installed for the monitoring program of the Murray State University Center for Reservoir Research (CRR) (Fryar et al. 2007; White et al. 2007). Samples were taken during the summer months of August, 2001, June, 2002, and July, 2002 when Kentucky Lake was at summer pool and the mudflat was submerged (Figure 1). The average embayment water temperatures on these dates were 29.01°C, 26.73°C, and 30.26°C respectively (CRR monitoring program database, unpubl.). The sites also were sampled during the winter months of February 2002 and January 2003 when Kentucky Lake levels were at their lowest (Figure 1) and the mudflat exposed. The average water temperatures on those dates were 8.13°C and 5.18°C respectively (CRR monitoring program database, unpublished). Samples were collected by first removing and discarding the top 2 cm of sediment and then filling a 60-ml centrifuge tube with sediment from 2 cm to 8 cm deep. Samples were stored on ice during transport to the lab where they were frozen at −20°C.

DNA Extraction, Amplification, and Cloning

DNA was extracted using a MO BIO UltraClean Soil DNA Kit with the BIO101 FastPrep Machine set at a speed of 5.5 for 30 sec (Martin-Larent et al. 2001). Approximately 200 ng of DNA was used as template in PCR reactions to amplify rDNA representing the bacterial community. The reactions included 5 units of Tfl DNA Polymerase and 1X Tfl Polymerase buffer (Epicentre), 25 pMol of the Bacteria specific primers 68F (5′ TNA NAC ATG CAA GTC GAK CG 3′) and 1392R (5′ ACG GGC GGT GTG TRC 3′)

(Wise et al. 1997), 5 mM dNTPs, MasterAmp PCR Enhancer with Betaine®, and 3 mM MgCl. Reactions were then run on a PTC-200 Thermal Cycler (MJ Research). The reaction conditions were 94°C for 1.5 min followed by 35 cycles of 94°C for 30 sec, 51°C for 45 sec plus 1 sec per cycle, and 70°C 1 min plus 5 sec per cycle. There was a final extension step at 74°C for 7.5 min (Wise et al. 1997). The amplified products of each reaction were checked on 0.8% agarose gels to ensure that the amplification yielded a unique fragment of approximately 1300 bp. PCR products were purified using a Prep-A-Gene DNA purification kit (BioRad) and inserted into the pGEM®-T Easy vector (Promega). The ligated plasmid was then transformed into *E. coli* DH5α, and transformed cells with inserts were selected and screened on LB agar with 50 μg/ml ampicillin, 40μg/ml X-gal, and 120μg/ml IPTG.

Amplified Ribosomal DNA Restriction Analysis (ARDRA) of 16S rDNA Clones

Plasmid DNA was purified from the transformants with the Wizard® Plus SV Miniprep DNA Purification System (Promega) and used as template to reamplify the inserts. The reaction mixture included 100 ng of template, 4 units Tfl DNA polymerase and 1X buffer, 25 pMol of M13F and M13R primers (Messing et al. 1981), 5 mM dNTP, MasterAmp PCR Enhancer with Betaine®, and 3 mM MgCl. The amplification protocol was 94°C for 2 min followed by 35 cycles of 94°C for 40 sec, 54°C for 1 min, and 72°C for 1 min. There was a final extension step at 72°C for 3 min. PCR products were then checked on a 0.8% agarose gel to insure they were the right size. The amplified fragments were purified using a Prep-A-Gene DNA purification kit (BioRad). To perform ARDRA analysis (Arturo, et al. 1995), 100 ng of amplified DNA was cut with Alu I and run on 10cm 4–20% PAGEr™ Gold Precast TBE Gels (Bio Whittaker) at 20V/cm per gel for 1 hr. The gels were stained in 1X TBE buffer with ethidium bromide for 2 min, and washed with water for 1 min. The gels were photographed with a BioRad® Gel Doc 1000 and fragment sizes determined using Quantity One Software (BioRad®).

DNA Sequencing and Sequence Analysis

The 16S rDNA inserts were sequenced using a LiCOR Global Edition IR² NEN™ DNA Sequencer 4200L. The reactions were performed using Thermosequenase™ (USB Kit 78500) according to the kit instructions. Sequence data were obtained from the scanner using e-Seq DNA Sequencing and Analysis software Version 3.0 (LiCOR) and edited using the software EditSeq™ and SeqMan™II sequence analysis software Version 4.05 (DNASTAR Inc.). Sequences were then examined using the program CHIMERA_CHECK version 2.7 (Michigan State University RDP-II) to eliminate any sequences that resulted from chimeric errors. Online search of NCBIs database (http://www.ncbi.nlm.nih.gov/BLAST/) and Michigan State Universities RDP-II database (Maidak et al. 2001; http://rdp.cme.msu.edu/html/) was then carried out on the sequences to determine closest matches. Sequence alignments were created using the algorithm in Clustal W (Gap Penalty of 15, Gap Length penalty of 6.66, and Gap separation penalty range of 8) in the program BioEdit Version 7.0.1.

RESULTS

ARDRA

ARDRA analysis was carried out on a total of 350 clones. There were 96 different ARDRA patterns, and because each ARDRA fingerprint indicates a different organism, 96 different organisms were identified. Specifically, there were 54 different organisms found in submerged sediment samples (Table 1) and 55 different organisms from clones from exposed sediment samples (Table 2). Of the 96 ARDRA patterns only 13 were found both when the sediment was submerged and exposed. There were two organisms that were very common, found 37 and 20 times. Both were found in submerged and exposed sediment samples. All other patterns repeated much less frequently than these or were found only once.

Blast Analyses of Sequenced Clones

Blast analyses of the sequences of the 96 different organisms as indicated by ARDRA were performed to identify the major taxa to which these organisms belong (Table 1 and Table 2). Pairwise alignments of all but seven

Table 1. Sequenced summer clones RDPII and NCBI blast similarity matches.

Clone	RDPII Best Matched Organism (S_ab)	NCBI Best Matched Organism (bp and %)	Group	Frequency
1B30	—	589/598 (98%) *Acidobacteria*	—	4
2B20	0.766 *Nostoc* group	1239/1299 (95%) *Cylindrospermum*	CyanoBacteria	3
3.2.5	0.722 *Burkholderia*	1233/1317 (93%), β Proteobacterium	β Proteobacterium	4
3.2.6	0.811 *Acidimicrobium*	1076/1161 (92%) *Actinobacterium*	Actinobacteria	2
3.2.8	0.203 *Microbispora bispora*	—	—	4
3B12	0.925 *H.palleronii*	1326/1342 (98%) *Hydrogenophaga*	β Proteobacterium	2
3B15	0.841 *Methylobacterium*	1250/1300 (96%) *Methylobacterium*	α Proteobacterium	1
3B20	0.81 *P. aminophilus*	1198/1247 (96%) *Paracoccus*	α Proteobacterium	1
4.2.2	0.58 *E. asburiae* subgroup	860/898 (95%) Uncultured *Actinobacteria*	—	2
4.2.3	0.801 *Oxalobacter* group	1233/1343 (91%) *Ralstonia*	β Proteobacterium	3
4.2.4	0.602 *Enterobacter asburiae*	1252/1358 (92%) γ Proteobacteria	γ Proteobacterium	4
4.2.8	0.772 *Oxalobacter* group	1278/1357 (94%) β *Proteobacterium*	β Proteobacterium	5
4.2.9	0.853 *Nitrosomonas* group	1235/1341 (92%) β proteobacterium	β Proteobacterium	2
4B18	0.86 *J. lividum*	1292/1337 (96%) *J. agaricidamnosum*	β Proteobacteirium	3
4B19	0.835 *Rhodobacter*	1233/1273 (96%) *Rhodobacter*	α Proteobacterium	2
4B20	0.9 *Actinomadura*	1013/1036 (97%) *Actinomycete* species	Actinobacteria	1
5.2.4	0.71 *Desulfuromonas* group	1246/1318 (94%) γ proteobacterium	γ Proteobacterium	2
5.2.6	0.787 *Acidovoraz*	1275/1346 (94%) β proteobacterium	β Proteobacterium	1
5.2.7	0.816 *Blastomonas*	1239/1306 (94%) α proteobacterium	α Proteobacterium	22
5.2.8	0.843 *C. lituseburense*	1265/1317 (96%) *C. lituseburense*	Clostridia	3
5.2.10	0.573 *Frankia* group	292/308 (94%) Chloroflexi bacterium	—	4
5.2.9	0.621 *Pseudomonas putida*	828/911 (90%) *Nitrosococcus* halophilus	—	5
5.2.11	0.823 *Nitrospira multiformis*	948/986 (96%) β proteobacterium	β Proteobacterium	4
5.2.12	0.856 *Leptothrix discophora*	1274/1316 (96%) β proteobacterium	β Proteobacterium	4
5.2.13	0.855 *B. elkaniil*	1252/1304 (96%) *Bradyrhizobium*	α Proteobacterium	4
5.2.14	0.753 *Oxalobacter* group	1285/1355 (94%) β proteobacterium	β Proteobacterium	4
5.2.16	0.693 *Enterobacter asburiae*	1261/1361 (92%) γ proteobacterium	γ Proteobacterium	6
5B16	0.540 *A. hydrophila*	1239/1292 (95%) Uncultured bacterium	γ Proteobacterium	4
1.2.9	0.856 *Acidiphilium acidophilum*	1362/1417 (96%) *Acidiphilium rubrum*	α Proteobacterium	2
1.2.13	0.847 *Ahrensia kielensis*	1287/1320 (97%) *Agrobacterium kieliense*	α Proteobacterium	3
1.2.14	0.885 *Bacillus subtilis*	1380/1414 (97%) *Bacillus subtilis*	Bacilli	4
4.2.15	0.809 *Alcaligenes*	1281/1367 (93%) *Alcaligenes*	β Proteobacterium	1
3.3.5	0.737 unidentified pseudomonas	1335/1371 (97%) *Cellvibrio* sp	γ Proteobacterium	1
3.3.7	0.849 *Cytophaga*	1328/1365 (97%) *Cytophaga*	Sphingobacteria	2
4.2.23	0.856 *Legionella*	1352/1391 (97%) *Legionella*	γ Proteobacterium	1
4.2.24	0.894 *Methylobacter*	1332/1371 (97%) *Methylobacter*	γ Proteobacterium	3
N611	0.603 *P. vitellinum*	1292/1369 (94%) γ proteobacterium	γ Proteobacterium	7
N617	0.728 *Nitrospina* subdivision	1066/1142 (93%) *Holophaga* sp	δ Proteobacterium	5
N820	0.739 *D. zoogloeoides*	849/912 (93%), 151/177 (85%) β proteobacterium	β Proteobacterium	5
4.3.8	0.895 *Rhodopseudomonas*	1264/1305 (96%) *Rhodopseudomonas*	α Proteobacterium	1
4.3.12	0.874 *Nostoc*	1287/1322 (97%) *Nostoc*	Cyanobacteria	1
1.4.2	0.86 *Nitrospina* subdivision	1290/1375 (93%) *Planctomycetales* bacterium	Planctomycetacia	3
4.3.14	0.896 *Caulobacter*	1251/1278 (97%) *Caulobacter*	α Proteobacterium	1
4.3.15	0.904 *Rhizobium* sp	1286/1317 (97%) *Rhizobium* genosp	α Proteobacterium	1
2B25	0.918 *R. lindaniclasticus*	1362/1394 (97%) *R. lindaniclasticus*	γ Proteobacterium	3
1.3.10	0.901 *Rickettsia moreli*	1321/1348 (97%) *Rickettsia* sp	α Proteobacterium	2
4.2.16	1 *Streptomyces*	1310/1351 (96%) *Streptomyces*	Actinobacteria	1
5.3.6	0.672 *S. thermosulfidooxidans*	1249/1276 (97%) *Sulfobacillus* sp	Bacilli	4
4.4.9	0.814 *Gallionella ferruginea*	1239/1294 (95%) *Gallionella ferruginea*	β Proteobacterium	12
5.3.7	0.737 *Desulfobulbus*	1341/1378 (97%) *Desulfobulbus*	δ Proteobacterium	2
5.3.8	0.850 *Hyphomicrobium*	1262/1296 (97%) *Hyphomicrobium*	α Proteobacterium	1
4.3.18	0.918 *Desulfobacter*	1359/1377 (98%) *Desulfobacter*	δ Proteobacterium	2
5.3.10	0.813 *Microbacterium*	1303/1340 (97%) *Microbacterium*	Actinobacterium	3
1B33	0.791 *Rhodopila*	1114/1164 (95%) *Rhodopila*	α Proteobacterium	1

Table 2. Sequenced winter clones RDPII and NCBI blast similarity matches.

Clone	RDPII Best Matched Organism (S_ab)	NCBI Best Matched Organism (bp and %)	Group	Frequency
1.4.1	0.856 *M. phosphovorus*	1055/1081 (97%) *Nocardioides jensenii*	Actinobacteria	2
1.4.2	0.86 *Nitrospina* subdivision	1290/1375 (93%) *Planctomycetales* bacterium	Planctomycetacia	4
1.4.6	0.719 *Cytophaga fermentans*	1237/1322 (93%) *Cytophaga* sp	Shingobacteria	4
1.4.8	0.849 α proteobacterium	1224/1302 (94%) α proteobacterium	α Proteobacterium	6
2.4.5	0.820 *Phenylobacterium immobile*	1230/1273 (96%) *Caulobacter* sp	α Proteobacterium	8
2.4.7	0.807 *Desulfobulbus propionicus*	1298/1370 (94%) *A. dehalogenans*	δ Proteobacterium	7
3.2.5	0.722 *Burkholderia*	1233/1317 (93%), β Proteobacterium	β Proteobacterium	3
3.4.2	0.689 *Acetobacter xylinum*	827/873 (94%) α proteobacterium	α Proteobacterium	2
5.2.11	0.823 *Nitrosospira multiformis*	948/986 (96%) β proteobacterium	β Proteobacterium	4
4.2.3	0.801 *Oxalobacter* group	1233/1343 (91%) *Ralstonia*	β Proteobacterium	4
4.4.1	0.792 *Prosthecobacter* group	1263/1360 (92%) *Verrucomicrobia* bacterium	Verrucomicrobia	3
4.4.2	0.745 *Aquabacterium*	1227/1287 (95%) *Aquabacterium*	β Proteobacterium	2
4.4.3	0.650 *Pseudomonas putida*	1191/1282 (92%) γ proteobacterium	γ Proteobacterium	2
4.4.7	0.550 *Nitrospina* subdivision	458/513 (89%) *Acidobacteria*	—	3
4.4.9	0.814 *Gallionella ferruginea*	1239/1294 (95%) *Gallionella ferruginea*	β Proteobacterium	8
4.4.10	—	—	—	1
4.4.11	0.757 Rhizobium group	1065/1138 (93%) *Afipia* genosp	α Proteobacterium	3
4.4.12	0.602 *Chloroflexus*	1239/1300 (95%) *Chloroflexus*	Chloroflexi	2
4.4.13	—	—	—	1
4B18	0.860 *J. lividum*	1292/1337 (96%) *J. agaricidamnosum*	β Proteobacterium	5
5.2.7	0.816 *Blastomonas*	1239/1306 (94%) α proteobacterium	α Proteobacterium	15
3.5.1	0.962 *Alicyclobacillus*	1352/1363 (99%) *Alicyclobacillus*	Bacilli	3
2.5.11	0.966 *Alteromonas macleodii*	1378/1385 (99%) γ proteobacterium	γ Proteobacterium	1
5.5.2	0.974 *Azoarcus*	1375/1380 (99%) *A. fungiphilus*	β Proteobacterium	2
1.2.14	0.885 *Bacillus subtilis*	1380/1414 (97%) *Bacillus subtilis*	Bacilli	5
3.5.11	0.914 *C. braakii*	1374/1400 (98%) *Citrobacter*	γ Proteobacterium	1
1.5.3	0.972 *D. kamchatkensis*	1351/1365 (98%) *D. kamchatkensis*	γ proteobacterium	2
4.2.4	0.602 *Enterobacter asburiae*	1252/1358 (92%) γ Proteobacteria	γ Proteobacteria	2
1.5.4	0.906 *Enterococcus*	1337/1360 (98%) *Enterococcus*	Bacilli	1
5.5.6	0.836 *Escherichia*	1324/1360 (97%) *E.coli*	γ Proteobacterium	2
3.5.3	0.846 *Flexibacter*	1321/1346 (98%) *Flexibacter*	Flavobacteria	1
5.5.9	0.888 *Maricaulis*	1281/1310 (97%) *Maricaulis*	α Proteobacterium	2
1.5.17	0.884 *Mycobacterium*	1301/1327 (98%) *Mycobacterium*	Actinobacteria	2
4.5.12	0.755 *Brevundimonas*	1081/1122 (96%) *Brevundimonas*	α Proteobacterium	1
N611	0.603 *P. vitellinum*	1292/1369 (94%) γ proteobacterium	γ Proteobacterium	4
N617	0.728 *Nitrospina* subdivision	1066/1142 (93%) Holophaga sp	δ proteobacterium	4
N820	0.739 *D. zoogloeoides*	849/912 (93%), 151/177 (85%) β proteobacterium	β Proteobacterium	3
4.5.3	0.861 *Mesorhizobium*	1268/1301 (97%) *Mesorhizobium*	α Proteobacterium	1
5.5.1	0.893 *O. maris*	1357/1382 (98%) *O. maris*	γ Proteobacterium	2
3.5.6	0.961 *Phaeospirillum molischianum*	1335/1349 (98%) *Rhodospirillum molischianum*	α Proteobacterium	1
3.5.8	0.775 *Methylomicrobium*	1304/1350 (96%) *Methylomicrobium*	γ Proteobacterium	3
2.5.22	0.907 *Rhodobium marinum*	1272/1292 (98%) *Rhizobiaceae* bacterium	α Proteobacterium	1
2.5.7	0.848 *Nitrobacter*	1275/1306 (97%) *Nitrobacter*	α Proteobacterium	2
1.5.20	0.888 *R. sodomense*	1321/1353 (97%) *R. sodomense*	α Proteobacterium	3
4.5.2	0.758 *Nitrospira*	999/1076 (92%) *Nitrospira*	Nitrospira	4
5.3.6	0.672 *S. thermosulfidooxidans*	1249/1276 (97%) *Sulfobacillus* sp	Bacilli	4
2.5.31	0.931 *Vibrio*	1366/1370 (99%) *Vibrio*	γ Proteobacterium	2
1.5.22	0.813 *Azospirillum lipoferum*	1292/1317 (98%) *Azospirillum*	α Proteobacterium	1
2.5.12	0.797 *Pelobacter*	1301/1346 (96%) *Pelobacter*	δ Proteobacterium	3
2.5.2	0.680 *Synechococcus*	1235/1303 (94%) *Synechococcus*	Cyanobacteria	1
1.5.6	0.751 *Proteus*	1193/1239 (96%) *Proteus*	γ Proteobacterium	3
2.5.16	0.763 *Sphingomonas*	1276/1304 (97%) *Sphingomonas*	α Proteobacterium	2
3.5.22	0.690 *Sulfitobacter*	1111/1153 (96%) *Sulfitobacter*	α Proteobacterium	3
3.5.16	0.880 *Agromyces*	1345/1376 (97%) *Agromyces*	Actinobacteria	1
5.5.12	0.823 *Sinorhizobium*	1271/1305 (97%) *Sinorhizobium*	α Proteobacterium	2

of the clones exceeded 90% identity with sequences in the NCBI database. The data identified representatives of the major divisions of the Domain Eubacteria. The majority of sequenced clones, 68, fell within the phylum Proteobacteria. Clones also represented members of the groups Actinobacteria, Chloroflexi, Firmicutes, Nitrospira, Cyanobacteria, Planctomycetes, and Flavobacteria. The ARDRA analysis found one pattern 37 times in the 350 clones examined, 22 times in the 180 summer isolates and 15 times in the 170 winter clones. That sequence had a 94% identity to an α Proteobacterium. A search of the RDPII database showed this sequence to have a similarity score (S_ab) of 0.816 with a member of the genus *Blastomonas.* Similarity scores were calculated as the number of unique oligomers shared between the unknown sequence and a given RDP sequence divided by the lowest number of unique oligomers in either of the two sequences. The second most common ARDRA pattern was found 20 times. It was found 12 times in summer samples and 8 times in winter samples. This sequence showed 95% identity with *Gallionella ferruginea,* a β Proteobacterium that is a chemolithotroph known to oxidize Fe^{2+} which is abundant in this environment (Madigan et al. 2002). Of the seven that were not identified to group by the Blast search, three were placed into a group by constructing a phylogenetic tree of the sequences. The phylogenetic tree was constructed using the software TOPALi Version 0.22 that uses the Jukes Cantor model of nucleotide substitution to estimate pairwise distances and the tree was then estimated using the Neighbor-Joining method. The software conducts 1000 Bootstrap iterations to produce phylogenetic trees. The other four sequences could not be placed in any group using either the sequence database comparisons or phylogenetic tree analysis and may have come from groups of organisms that have yet to be described. Chimera analysis showed that they were not artifacts of the amplification process.

Analysis of all isolated summer clones revealed that 29%, 42 clones, belonged to the subdivision α Proteobacterium, 35%, 50 clones, to the subdivision β Proteobacterium, 17%, 25, to subdivision γ Proteobacterium, 6%, 9 clones, to the subdivision δ Proteobacterium, 6%, 8 clones, to the Class Bacilli, 3%, 2 clones each, to Classes Planctomycetacia and Clostridia, and 1% each to the subdivisions Cyanobacteria, and Actinobacteria and the Class Sphingobacteria (Figure 2). Analysis of all isolated winter clones revealed that 35%, 53 clones, belonged to the subdivision α Proteobacterium, 23%, 35 clones, to the subdivision β Proteobacterium, 12%, 19, to subdivision γ Proteobacterium, 10%, 16 clones, to the subdivision δ Proteobacterium, 8%, 13 clones, to the Class Bacilli, 3%, 4 clones, to Class Planctomycetacia, 3%, 5 clones, to Class Sphingobacteria, 2%, 3 clones each, to subdivision Actinobacteria and the Phylum Verrucomicrobia, and 1% each to the Phylum Cyanobacteria and the Class Chloroflexi (Figure 2).

DISCUSSION

The data presented here show that the near surface microbial community of the sediment of the mudflat at the mouth of Ledbetter Creek where the stream enters an embayment of Kentucky Lake reservoir is dynamic. Of 350 isolates examined, there were 96 organisms identified, and only 13 were found in both winter and summer samples. Further, there was a change in the most abundant group of organisms between the α Proteobacteria in the winter and the β Proteobacteria in the summer. Both groups, however, were relatively abundant in both summer and winter samples. The α and β Proteobacteria include the purple nonsulfur bacteria, a diverse group of anoxygenic phototrophs that get their carbon from organic compounds (Nealson 1997; Madigan et al. 2002); nitrifying bacteria, chemolithotrophs that can oxidize nitrogen compounds using O_2 to fix CO_2 and are abundant in soils where ammonia is present; sulfur, iron, and hydrogen oxidizing bacteria (Nealson 1997; Madigan et al. 2002); methanotrophs and methylotrophs, organisms that oxidize one carbon compounds; and nitrogen fixing bacteria (Nealson 1997; Madigan et al. 2002). Also included in these subdivisions are the enteric organisms, many of which are facultative anaerobes (Nealson 1997; Madigan et al. 2002); *Spirilla,* a diverse group of sheathed filamentous organisms common to freshwater environments rich in organic mater (Madigan et al. 2002); pseudomonads; and sulfur and

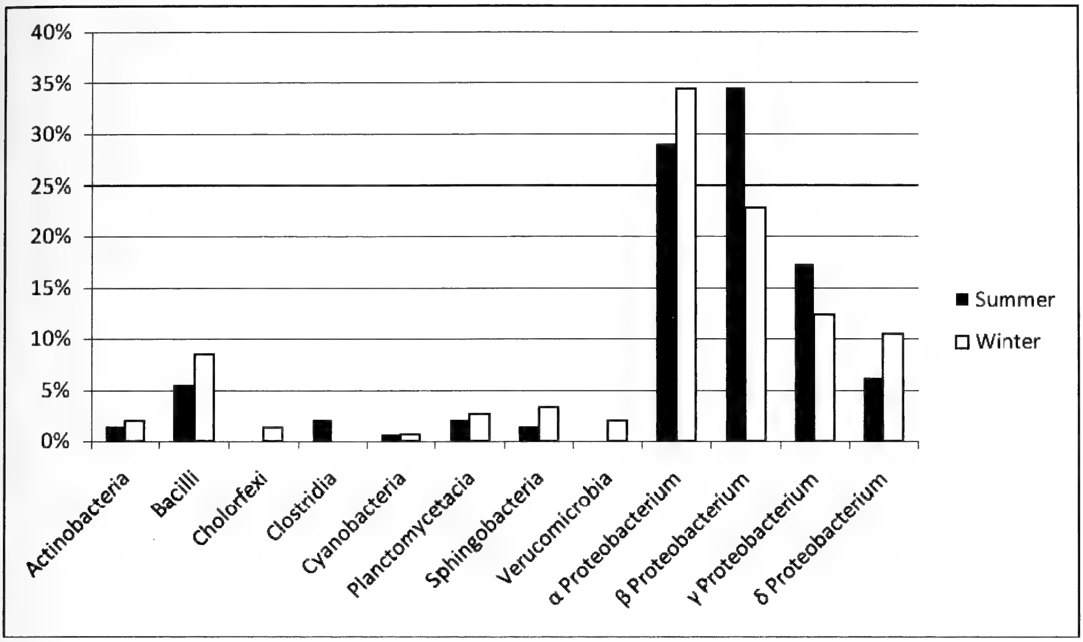


Figure 2. Histogram of the major taxa found in submerged samples (summer) and exposed samples (winter). Percent of total includes the total number of clones that identified a particular major taxon.

sulfate reducing bacteria. Other subdivisions of the Proteobacteria were also abundant and many of the organisms described above include species that have been placed in those subdivisions.

The turnover of the microbial community composition no doubt resulted from a variety of factors including but perhaps not limited to temperature, nutrient availability, oxygen availability, and the selection pressure of chemical pollutants including pesticides and herbicides. During the summer season the warm water holds less dissolved oxygen than when at colder temperatures and the increased microbial activity at higher temperatures creates anoxic conditions. The Ledbetter watershed is 55% second growth oak-hickory forest. Approximately 45% of the watershed is under cultivation for part of the year. The seasonal deposition of allochthonous organic matter from forests and the sporadic input of pesticides and nutrient fertilizer most likely affect the microbial community composition by enriching some species while selecting against others. Whether or not the manipulation of the reservoir level has a direct effect on the microbial community composition is unclear. It may simply contribute to the distribution of sediment across the mudflat, therefore having an indirect effect.

While the bacterial community in our study was dynamic, it also was surprisingly lacking in diversity in that there were only 96 different bacteria found and two species made up 16% of the 350 clones examined. The *Blastomonas*-like α Proteobacterium made up 12% of the bacterial community in the summer and 6% in the winter. The *Gallionella*-like β Proteobacterium made up 8% and 5% of the bacterial community in summer and winter, respectively. Further study of these organisms and their selective advantage to life in this environment will require cultivation in the laboratory, however their abundance in both summer and winter, and the relative abundance of the other species that were found under both conditions, indicate that the organisms that can thrive in or at least tolerate the changing conditions in the mudflat created at the mouth of this stream are the dominant organisms in the community.

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Polygonum cuspidatum (Polygonaceae) Genetic Diversity in a Small Region of Eastern Kentucky

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ABSTRACT

Polygonum cuspidatum Sieb. & Zucc. is an invasive species that has spread across Britain, Europe, Canada, and the United States. Prior to this research, no analysis of genetic diversity within, or among, populations had been conducted in the United States, although population genetic diversity had been examined in Britain. An analysis of genetic diversity was carried out using Polymerase Chain Reaction (PCR)-based DNA fingerprinting on plant populations along two creeks in Rowan County, Kentucky. One creek was sampled at one-meter intervals and the other at intervals of one to nine km. The results of this analysis indicate that the plants along both of these creeks are genetically dissimilar and not clonal. The data did not support the hypothesis that *P. cuspidatum* is reproducing and dispersing by asexual means.

KEY WORDS: DNA fingerprinting; *Fallopia japonica*; genetic diversity; Japanese knotweed; *Polygonum cuspidatum*; RAPD analysis

INTRODUCTION

Japanese knotweed (*Polygonum cuspidatum* Sieb. & Zucc., synonyms *Fallopia japonica* (Houtt.) R. Decr., *Reynoutria japonica* Houtt.) is an invasive plant species introduced into northeastern North America in the late 1800s. Since that time, it has spread across the United States and into Canada. It is widely distributed in the northeastern United States, and its distribution decreases as one moves south and west of Kentucky (USDA, NRCS 2006). In the United States, *P. cuspidatum* is listed as a noxious weed in 35 states, including Kentucky. *P. cuspidatum* prefers a riparian habitat along streams and ditches but can grow almost anywhere (USDA, NRCS 2006). Because *P. cuspidatum* has a rapid growth rate and produces an extensive rhizome system, it rapidly outcompetes native species, forming dense stands along stream banks thus making it a threat to native plant populations. Furthermore, it is an economic burden to those entities that maintain roadsides, ditches, and streams.

A plant's mode of reproduction significantly impacts that plant's mechanism for dispersal. In the case of an invasive plant such as *P. cuspidatum,* understanding the mechanism of dispersal can lead to more informed and effective management practices. *P. cuspidatum* exhibits both asexual and sexual modes of reproduction. *P. cuspidatum* has a tremendous capacity for vegetative propagation from rhizomes and aerial stem fragments. The rhizome has been observed to grow horizontally for 15–20 m (Locandro 1973; Conolly 1977), and a fragment of rhizome as little as 0.7 g in size can produce a new plant (Brock and Wade 1992). *P. cuspidatum* also can regenerate from aerial stem tissue. DeWaal (2001) found that node-containing aerial stem sections 40 mm-long produced new shoots and adventitious roots when placed on moist soil.

In terms of sexual reproduction, *P. cuspidatum* is dioecious with the fruits being achenes. It also has been described by Beerling et al. (1994) as being gynodioecious. Bailey (1994) reported that the *P. cuspidatum* populations in Britain contained only male-sterile and hermaphroditic plants. No purebred seeds have been found in Britain (Beerling et al. 1994). *P. cuspidatum* can hybridize with the closely related species *F. sachalinensis* and *F. baldschuanica* (Beerling et al. 1994). The implication of these studies is that reproduction and dispersal are the results of vegetative propagation, at least in Britain.

In contrast, *P. cuspidatum* produces large quantities of viable seeds in its native range in Japan (Maruta 1983). Survival of first-year

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seedlings is heavily dependent on their ability to attain a ''critical dry matter production'' due to environmental stresses. Furthermore, patch establishment in Japan was found to be the result of seed dispersal as opposed to vegetative propagation, while patch development/expansion was found to be a combination of these two modes of reproduction (Zhou et al. 2003).

In the United States, large quantities of seeds can be produced by *P. cuspidatum,* but there are conflicting reports regarding the viability of these seeds and survivability of the seedlings. Locandro (1973) reported that in the presence of fertile males, seeds were produced that germinated, but that these seedlings did not survive the first year. In the absence of fertile males, seeds were produced, but the achenes were empty. Seiger (1993) reported that seed germination rates were low (from 10–63%, depending on their period of dormancy). Seedling survival was low in the laboratory, and none was observed in the wild. It was the conclusion of Locandro (1973) and Seiger (1993) that sexual reproduction played a very minor role in the reproduction and dispersal of *P. cuspidatum* in the United States.

In contrast, Forman and Kesseli (2003) reported high mean germination rates of 65–79% for *P. cuspidatum.* These germination rates were dependent upon treatment with some seed sets showing 100% viability. Survival of these seedlings ranged from 0–100% with a mean of 65%. Similarly, Bram and McNair (2004) reported a high degree of seed viability as measured by seed germination. They found that seed germinability was dependent upon maturation of the seeds. Less than 10% of seeds produced early in the season germinated, while germination was greater than 90% for seeds formed later in the season. Forman and Kesseli (2003) and Bram and McNair (2004) concluded that sexual reproduction may play a very important part in the reproduction and dispersal of this species.

Observations of the lack of male-fertile plants in Britain imply that vegetative propagation is the primary means for reproduction and dispersal, at least in some locations. If this hypothesis is correct, then all plants within a population should be genetically identical to each other. In Britain, this hypothesis has been tested and confirmed using molecular techniques. DNA extracted from plants across Britain was subjected to Random Amplified Polymorphic DNA (RAPD) analysis. Hollingsworth et al. (1998) and Hollingsworth and Bailey (2000) showed that the vast majority of *P. cuspidatum* populations in Britain were genetically identical and thus clonal. The populations that were not genetically identical to the rest were found to be hybrids between *P. cuspidatum* and *F. sachalinensis* (Hollingsworth et al. 1998). A study from the Czech Republic by Bímová et al. (2004) came to a similar conclusion based on isozyme analysis; vegetative propagation is the primary means of reproduction, but sexual reproduction can occur.

Considering the contradictory reports of seed germinability and seedling survival for *P. cuspidatum* plants in the United States, the role for asexual compared with sexual reproduction is not clear. Furthermore, no genetic analysis of *P. cuspidatum* populations in the United States has been performed.

In order to determine the degree of genetic diversity of *P. cuspidatum* populations in the United States, *P. cuspidatum* populations were sampled in a localized region of eastern Kentucky. RAPD analysis was utilized to genetically characterize plants along two streams.

MATERIALS AND METHODS

Plant Material

Plant samples for this study were collected along two streams, Evans Branch and Triplett Creek, which flow through Morehead, Kentucky. Evans Branch is a tributary of Triplett Creek (Figure 1). Sampling for Evans Branch was carried out where the stream runs through a parking area before emptying into Triplett Creek (Figure 1). This patch of *P. cuspidatum* formed one continuous stand, approximately 20 m long. Two samples were collected at 1-m intervals along a linear transect parallel to the creek. Sampling for Triplett Creek was carried out at intervals of 1 to 9 km and included areas inside and outside of the Morehead city limits but within Rowan County (Figure 1). The patches of *P. cuspidatum* along Triplett Creek were discontinuous. Two leaf samples were collected at each site, but only one was used for the genetic analysis.

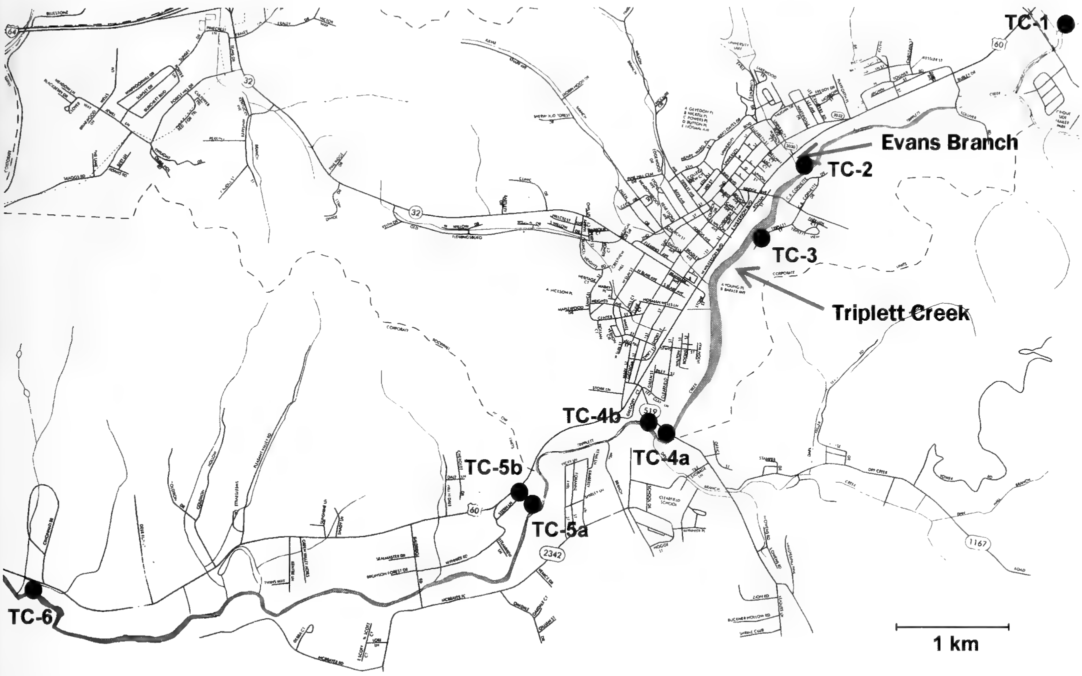

Figure 1. Map of Evans Branch with respect to Triplett Creek and the sampling sites along Triplett Creek. Samples were collected both upstream and downstream of Morehead, Kentucky, USA.

DNA Extraction

DNA extraction was carried out using the Plant DNAzol reagent (Invitrogen, Carlsbad, California, USA). When samples were processed immediately, DNA extraction was carried out according to instructions by the manufacturer. Some plant samples were not processed until the plant material had become dry. In this case, the manufacturer's instructions were altered so that a higher volume of Plant DNAzol reagent (3- or 4-fold more) was used per gram of tissue to compensate for water loss. DNA was quantified by spectrophotometric absorbance at 260 nm.

RAPD Analysis

RAPD amplification reactions were performed in a total volume of 20 μL with the final concentrations of 1.25 units Thermo-Start DNA polymerase, 3.0 mM $MgCl_2$, 0.2 mM dNTPs, 10 ng plant DNA, and 3 or 30 μM primer. PCR reagents were supplied in Thermo-Start PCR Master Mix (ABgene, Surrey, United Kingdom) supplemented with additional $MgCl_2$.

More than thirty 10-mer oligonucleotide primers were tested including those from the Operon A and F sets. The following primers were chosen for their ability to produce an appropriate number of reproducible bands: OPA-6, OPA-16, OPF-16, RAPD-A01, and RAPD-A09. Sequences of the primers are given in Table 1.

Amplification was carried out essentially as reported by Congiu et al. (2000) using an Applied Biosystems (Foster City, California, USA) GeneAmp 7200 thermal cycler set to the following parameters: (1) 3 min of enzyme activation at 94°C; (2) 45 cycles of: 40 s at 94°C, 70 s at 48°C, 120 s at 72°C; (3) 7 min at 72°C. Each reaction was carried out in triplicate.

PCR products and molecular weight standards were separated on 1.6% agarose gels and stained with ethidium bromide. Images of

Table 1. Sequences of the RAPD primers used for PCR.

Primer	Sequence 5′-3′
OPA-06	GGT CCC TGA C
OPA-16	AGC CAG CGA A
OPF-16	GGA GTA CTG G
RAPD-A01	CAG GCC CTT C
RAPD-A09	GGG TAA CGC C

the gels were captured using the Kodak (Rochester, New York, USA) EDAS 290 1D system and were analyzed using Phoretix 1 D software (Nonlinear Dynamics, Newcastle-Upon Tyne, UK). Bands were scored only if they were present in two of the three replicates. The resulting RAPD markers for each plant were pooled and used to generate dendograms with Phoretix 1 D software by the unweighted pair group method with arithmetic averaging (UPGMA).

Controls

Several plant samples were collected for use as outgroups. The LR sample was taken from the Lower Lick Fork, a tributary of the Licking River into which Triplett Creek flows. The LR sample was taken approximately 40 km from the Triplett Creek samples. The NW sample was taken from a site along North Wilson Street in Morehead that was geographically isolated from both Evans Branch and Triplett Creek.

To gauge the amount of genetic similarity that could be detected, two leaves from the same plant (P1L1 and P1L2) were collected and analyzed as if they were independent plants. The plants were collected from a site geographically separated from both Evans Branch and Triplett Creek. Leaves from two separate plants were analyzed in this way, and leaves from the same plant were always grouped together in preliminary analyses. Only one plant was included in the dendrograms presented.

RESULTS

Evans Branch

Two samples from Evans Branch failed to yield useable DNA (sample #5 and #6) and were not included in the analysis. Sample #4 was not adequately amplified by two primers, so it also was excluded from analysis. RAPD analysis along Evans Branch using five primers yielded 61 RAPD markers, of which 95% were polymorphic. Markers ranged in size from 120 bp to 1500 bp. A sample gel is shown in Figure 2. The number of RAPD markers across all primers ranged from 10–15.

To determine the degree of genetic similarity that might be expected from clonal plants, two leaf samples from the same plant were included in the analysis as if they were taken from independent plants. These plants were collected from a location that was geographically separated from Evans Branch and Triplett Creek. The dendogram produced for plants along Evans Branch (Figure 3A) revealed two genetically distinct groups; one at each end of the sampling area. As expected, the control samples (that were known to be genetically identical) grouped with each other and were separate from the rest of the plants along Evans Branch (Figure 3A). Similar clustering was observed when the data were analyzed using a neighbor-joining algorithm (data not shown). Samples #2 and #3 were the only samples that were genetically indistinguishable from each other (and thus presumed to be clonal). All of the other plants sampled along Evans Branch (at 1-m intervals) were more diverse from each other than the known clonal samples, indicating that they were not genetically identical. Relative plant positions

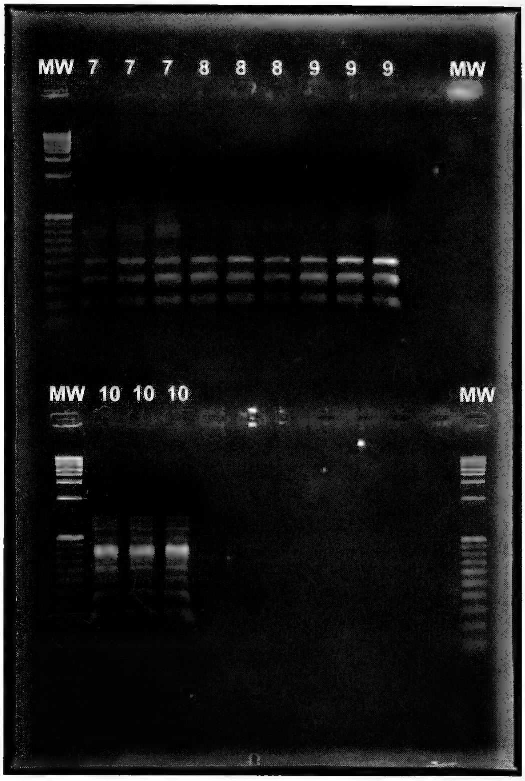

Figure 2. Electrophoretic gel illustrating the genetic diversity of the plant samples.

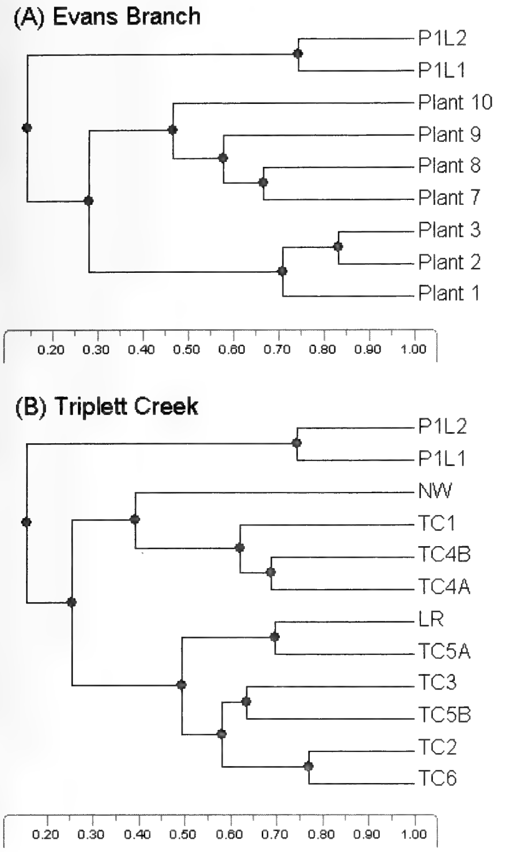

Figure 3. UPGMA dendogram, based on RAPD data, for individuals of *P. cuspidatum* collected (A) along Evans Branch and (B) along Triplett Creek.

at the study site appeared to influence clustering.

Triplett Creek

Samples were collected at intervals along Triplett Creek and at two sites distant from the creek. One site (NW) was from a ditch that was about 0.8 km away from the creek. The other was from the Lower Lick Fork, a tributary of the Licking River (LR) into which Triplett Creek flows but about 40 km away. RAPD analysis of these samples using five primers yielded 80 markers, 97% of which were polymorphic. Markers ranged in size from 70 bp to 2300 bp. The number of RAPD markers across all primers ranged from 13–21. The dendogram produced for the plants along Triplett Creek indicated that only two plants were clonal (TC2 and TC6; Figure 3B). Sample TC2 was approximately 9 km upstream of TC6. None of the plants from neighboring sites grouped together with the exception of TC4a and TC4b. One supposed outgroup, LR, grouped more closely with Triplett Creek samples than did Triplett Creek samples group with each other. NW also grouped with the Triplett Creek samples, although not as closely. The control samples (known to be clonal) grouped with each other and were separate from the rest of the plants along Triplett Creek (Figure 3B), as expected. Similar clustering was observed when the data were analyzed using a neighbor-joining algorithm (data not shown). Relative plant positions did not appear to influence clustering.

DISCUSSION

Although *P. cuspidatum* observed and sampled along Evans Branch form a single, large population, only two genetically identical samples were observed (located 1 m apart). The same lack of genetic similarity was observed for the plant populations along Triplett Creek. It is apparent that multiple genotypes of *P. cuspidatum* exist in the United States and even along approximately 12 km of Triplett Creek.

These results stand in sharp contrast with the results from populations sampled throughout Britain (Hollingsworth and Bailey 2000) where all samples were found to be genetically identical. Hollingsworth and Bailey (2000) attributed this clonal relationship to the observation that plants in Britain are male-sterile (Beerling et al., 1994). Forman and Kesseli (2003) and Bram and McNair (2004) provide evidence that *P. cuspidatum* plants in the United States are fertile producing viable seeds that can over-winter. In Japan, Zhou et al. (2003) have shown that seed dispersal is a mechanism by which *P. cuspidatum* can spread.

In the United States, *P. cuspidatum* is found primarily in locations that have been disturbed by humans; either humans have purposefully planted *P. cuspidatum* or humans have accidentally transferred the plant in the process of transferring soil from one place to another. The original source(s) of *P. cuspidatum* in Rowan County is unknown. Along the stretch of Evan's Branch that was sampled, soil was brought in from at least one remote site when a parking lot was built. The origin of the plants along Triplett Creek is not known; although all of the areas sampled had been disturbed by humans. The plant populations along Triplett Creek could have formed from rhizome fragments or seeds in transported soil, seeds carried by air or water, or stem fragments carried by water. If the dispersal giving rise to these populations were by fragmentation or extension of the rhizome system (thus, asexual), then it would be expected that plants positioned next to each other would be genetically identical (clonal). Only two of the plants along Triplett Creek were found to be clonal, and there was no clustering of plants based on their relative location. This excludes reproduction and dispersal by an asexual mechanism. The data are consistent with the hypothesis that diverse genotypes are the result of diverse genetic backgrounds as the result of introduction from multiple sources, suggesting genetic diversity extends beyond Rowan County, Kentucky. It is also possible that these plants are reproducing sexually, but given the great distance between the two clonal samples along Triplett Creek, the evidence does not support this hypothesis.

The data are different along Evans Branch. Plants 2 and 3 were found to be clonal. There was a clustering of the genotypes based on the plants' relative locations; plants on opposite ends of the transect grouped separately. The two clonal samples were only 1 m apart and may represent asexual reproduction by either fragmentation or rhizome extension. The genotypic clustering within the population may indicate that neighboring plants are exchanging pollen and are in-breeding. Thus, along Evan's Branch, there is some evidence for sexual reproduction as a dispersal mechanism.

The populations of *P. cuspidatum* studied were found to be genetically diverse, even within one county of Kentucky. Thus, multiple genotypes exist in the United States. It is interesting to speculate that this genetic diversity might be beneficial for population expansion given the wide distribution of *P. cuspidatum* in the United States (USDA, NRCS 2006). Little evidence of dispersal by asexual reproduction was observed along either creek studied; however, there was some evidence for sexual reproduction among neighboring plants within a large stand of knotweed plants along Evans Branch.

ACKNOWLEDGMENTS

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Formation of Apex Natural Arch, Apex, Kentucky

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ABSTRACT

Apex Natural Arch located in northeastern Christian County, Kentucky, is the result of surface water infiltration along parallel joints, basal erosion along a weaker section of the outcrop extending to existing joints, removal of basal support, and collapse of sandstone slabs. The arch is formally named here for the village of Apex, Kentucky.

KEY WORDS: natural arch, western Kentucky arch, Apex Arch

INTRODUCTION

Natural bridges and arches are some of the most unusual and beautiful landforms in the Commonwealth. Probably the most well-known features of this type are located in central and eastern Kentucky and have been described by McFarlan (1943). Corgan and Parks (1977) discussed numerous bridges within the state, few of which are located in western Kentucky. Kind and Shelby (1985) and Kind et al. (1991) have since published articles on natural bridges and arches in Livingston and Caldwell counties of western Kentucky.

The natural 'bridge' in Christian County, Kentucky was originally referenced by Collins (1882):

> ''Situated in the northern extremity of the county, near ''Harrison's tanyard,'' about twenty miles from Hopkinsville, is a Natural Bridge, somewhat similar, but on a reduced scale, to the celebrated rock bridge in Virginia, which was considered by Mr. Jefferson the greatest natural curiosity in the world. The bridge in question crosses a deep ravine, is thirty feet in height, with a span of sixty feet, and a magnificent arch. The surface is perfectly level, and the general width about five feet. The scenery in the vicinity of the bridge is remarkably romantic, and presents great attractions to the lovers of the picturesque in nature.''

According to Fairbridge (1969), the term ''arch'' is used when the feature does not span a stream channel. Therefore, the feature at Apex is more correctly termed a natural arch. We propose that the 'bridge' be formally named Apex Natural Arch, as it is in close proximity to Apex, Kentucky, and has been heretofore formally unnamed.

LOCATION

Apex Natural Arch (Figure 1) is located approximately 0.6 km east of Apex, Kentucky and about 0.45 km southeast of Atkinson Cemetery (Figure 2). Apex and the Atkinson cemetery are located on the Haleys Mill 1:24000 United States Geological Survey topographic map (Mullins 2005). Apex consists of only a few buildings along Kentucky State Highway 189. Access to the arch can be gained by an unimproved private gravel road that passes beyond Atkinson Cemetery. An obvious footpath leads to the arch, which is and has been a popular scenic attraction. As a result of its accessibility and total disregard for the rarity of this landform by some visitors, the arch and adjacent rock outcrops have been irreparably damaged by graffiti. Only a more distant view allows appreciation of this interesting natural feature.

Universal Transverse Mercator (UTM) coordinates at the northwest corner of the arch are Zone 16N, 470161.5mE, 4105803.1mN as determined by a sub-meter accurate GPS re-

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Figure 1. Apex Natural Arch looking north.

ceiver. Elevation of the span is approximately 187 m above mean sea level. Orientation of the span is N73°W, as evidenced by joint planes on either side of the span and the back of the window behind the arch.

ARCH DIMENSIONS

The arch is a long, slender feature which is nearly uniform across the span (Figure 3). The window behind the arch is also elongate, narrow, and quite uniform, being controlled by

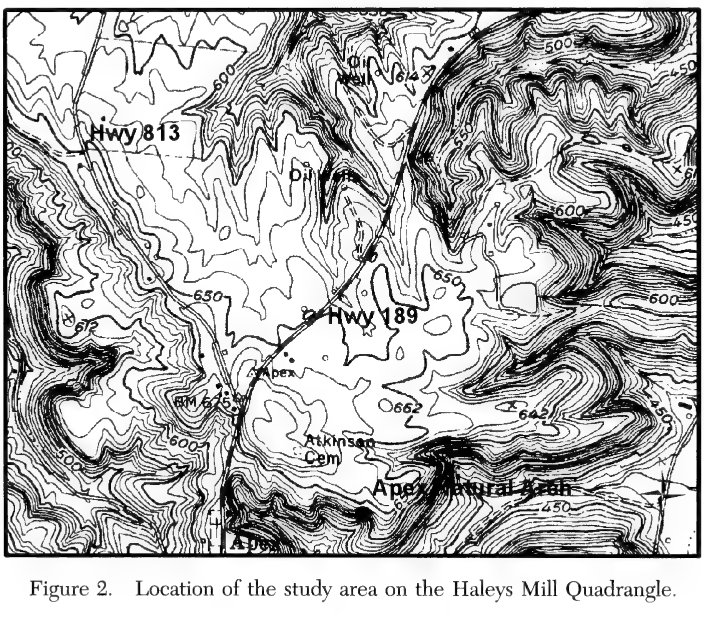

Figure 2. Location of the study area on the Haleys Mill Quadrangle.

Figure 3. Arch dimensions: span length is 23 m; window length is 44 m; span width varies from 1–2 m; window width from 4–6 m; and span height is 10 m.

the northwest trending joint set mentioned above.

The span of Apex Natural Arch is 23 m with the length of the window being 44 m. Width of the window varies from 4–6 m. The span is quite narrow and thin ranging from 1–2 m in width and thickness. Height of the span along its length is about 10 m (Figure 3).

GEOLOGY

The regional physiographic setting for Apex Natural Arch is the Shawnee section of the Interior Low Plateaus (Fenneman 1938). The uplands, which are nearly flat to gently rolling, are deeply dissected by streams with flat valley floors. The slopes between are steep, as the uplands are capped with units resistant to erosion. Elevations range from 124 to 270 m.

The arch formed in the upper portion of the lower Pennsylvanian Caseyville Formation (Figure 4) that is dominated by sandstone. The sandstone is light to medium gray and weathers to light yellowish-brown. It is coarse- to fine-grained and locally conglomeritic in the lower portion of the unit. The sandstone is sometimes interbedded with light to dark gray shale and siltstone but not within the outcrop in which the arch is developed. The sandstone is a cliff-forming unit that locally may be up to 15 m thick. Limited amounts of honeycomb weathering (tafoni) (Figure 5) are found on the southwestern faces of the outcrop in the immediate area of the arch. The Caseyville unconformably overlies the Mississippian Chesterian Kincaid Limestone in the study area (Trace 1977).

Faults of the Pennyrile Fault System (PFS)

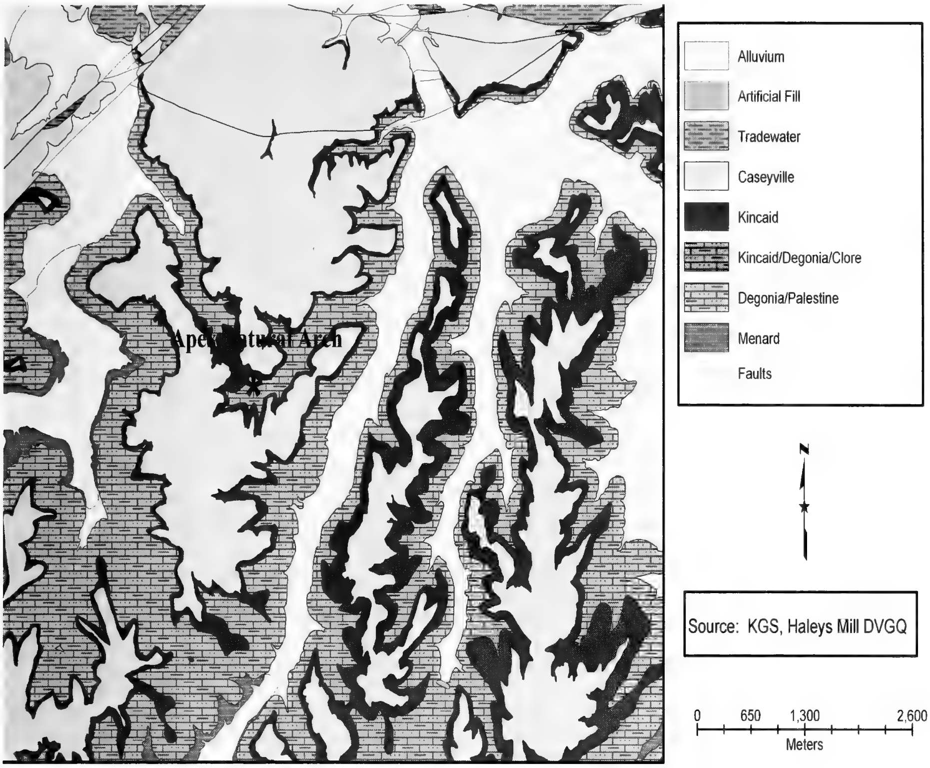

Figure 4. General geology of the study area.

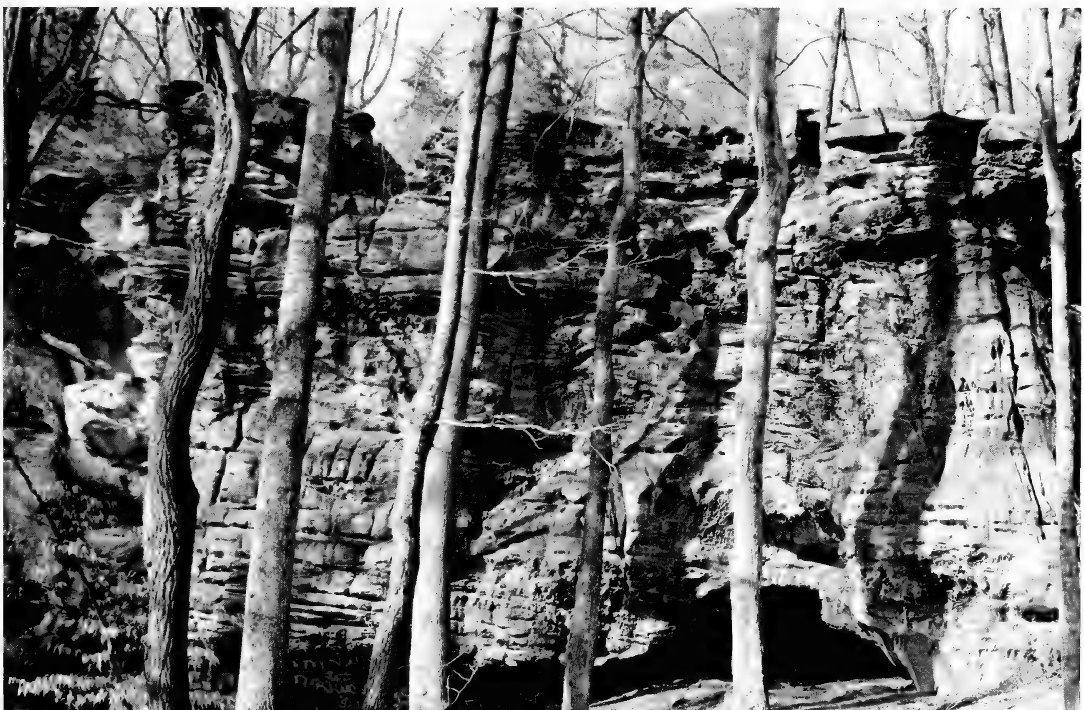

Figure 5. Honeycomb weathering and basal erosion at Apex Natural Arch.

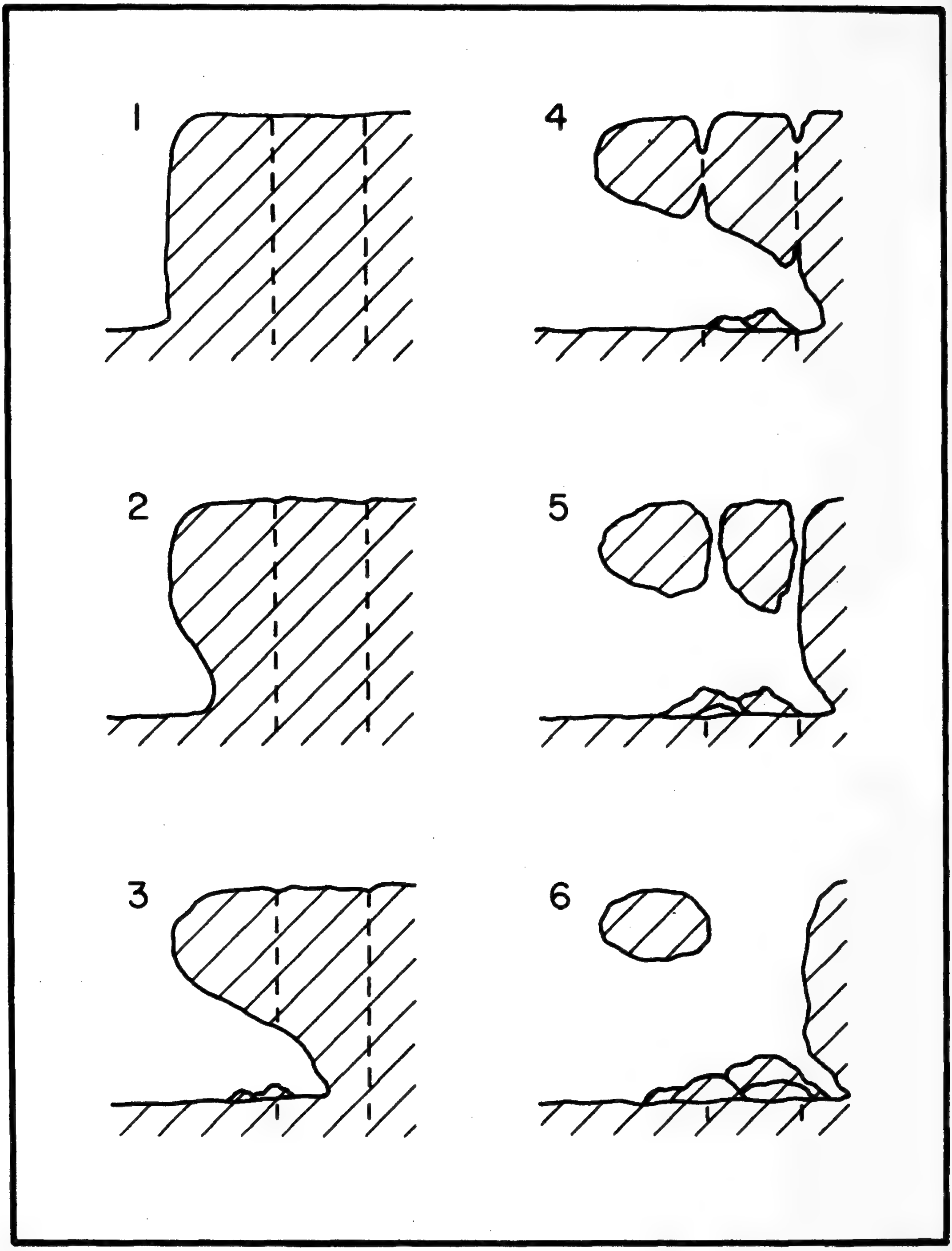

Figure 6. Proposed sequence of arch formation.

are found approximately 1.6 km north and 3.2 km to the northwest of the arch. There are also faults from the southern section of the PFS located about 8 km south of Apex. The known faults are vertical to high-angle trending almost due east; some also trend east-northeast suggesting extension and associated twisting (Trace 1977). Joint orientations measured along the front of the arch trend N73°W. The joint bearings along the inside of the window and the back of the arch also trend N73°W (almost east-west). Some transverse joints on the top of the span were measured at N5°W and others at N2°E. The fractures are related to the tectonism associated with the post-Devonian, pre-Quaternary flexure that occurred along the southern edge of the Rough Creek Graben that generated the PFS (Trace 1977).

PROPOSED SEQUENCE OF ARCH FORMATION

Apex Natural Arch appears to have formed in much the same way as Mantle Rock located in Livingston County, Kentucky (Kind and Shelby 1985). Physical and chemical weathering processes have been active along the sandstone cliff and in associated joints (Figure 6). Infiltration of surface waters along the parallel joints also likely aided in formation. The weathering processes first removed material from the base of the cliff (Steps 1 and 2, Figure 6). Undercutting at the base of the cliff eventually met a fracture, which paralleled the cliff face (Step 3, Figure 6). As weathering progressed beneath the cliff, another fracture was encountered (Step 4, Figure 6). Continued weathering beneath the cliff and along the joint planes resulted in removal of support and progressive collapse of sandstone slabs (Step 5, Figure 6). Material finally collapsed to form the window area as a result of continued weathering and mass wasting (Step 6, Figure 6).

SUMMARY

Apex Natural Arch is a very delicate structure compared with others that have been documented by the authors (Kind and Shelby 1985), (Kind et al. 1991). Rather than being quite massive, the span is very thin and narrow. The mode of formation is also quite common among natural bridges and arches, in general being related primarily to physical and chemical weathering processes along parallel joints with some assistance from surface runoff and mass wasting. Conditions in the Shawnee section of the Interior Low Plateaus, uplands capped with massive, horizontal, jointed sandstones, certainly support the presence of other bridges/arches that are yet to be documented.

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Abstracts of Some Papers Presented at the 2006 Annual Meeting of the Kentucky Academy of Science

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AGRICULTURAL SCIENCES

Organic Control of Japanese Beetles (*Popillia Japonica*) in Grapes. ANGIE WHITEHOUSE*, SANJUN GU and KIRK W. POMPER, Community Research Service, Kentucky State University, Frankfort, KY 40601.

The Japanese beetle (JB) is one of the major insect pests of grape vines in Kentucky. Feeding by adult JBs on grape leaves can result in a loss of leaf area which reduces both yield and fruit quality. In an effort to control JB feeding using organically approved substances, Surround®, Neemix 4.5® (at the label rate or at the doubled label rate) and water (control) were applied to 'Chambourcin', 'Chancellor', 'Chardonnay', and 'Chardonel' grapevines in caged choice studies in late June to early August, 2006. Additionally, Surround and water (control) were applied to 'Chambourcin', 'Norton' and 'Chancellor' grapevines in the KSU research vineyard. Surround and Neemix 4.5 significantly reduced the incidence and severity of JB damage to grape leaves in the caged choice study, compared to control vines. Surround reduced the incidence of damage at a greater rate than either Neemix 4.5 treatment; however, there was no difference in incidence and severity of JB damage between the two Neemix 4.5 treatments. In the field choice study, the number of JBs present on the grapes was counted daily from June 21st to June 30th (after the application of Surround on June 20th). The number of JBs feeding on grapes treated with Surround was significantly lower than those treated with water, indicating a feeding deterrent effect of Surround on JBs.

Using Simple Sequence Repeat Markers to Distinguish Scion and Rootstock Tissues on Grafted Pawpaw Trees. JEREMIAH D. LOWE*, KEIDRE LONG and KIRK W. POMPER, Land Grant Program, Kentucky State University, Atwood Research Facility, Frankfort, KY 40601-2355.

Pawpaw [*Asimina triloba* (L.) Dunal] is a tree fruit native to much of the eastern United States that has production potential in Kentucky. Pawpaw cultivars are routinely propagated by chip budding cultivar scion wood onto seedling rootstocks. When chip budded trees are planted in orchards, adventitious shoots often form on rootstock trunks and the scion shoot and adventitious rootstock shoots can be confused. It has also been suggested that several pawpaw cultivars, such as Mitchell, have been switched with other genotypes during propagation in nurseries. The objectives of this study were to determine whether microsatellite markers unique to the pawpaw cultivar Mitchell could be identified and then used to distinguish adventitious rootstock shoots from the Mitchell scion. Young leaves were collected from 30 pawpaw cultivars, including Mitchell, and from three trunk shoots (A, B, and C) from a Mitchell tree where rootstock and scion shoots were indistinguishable. Genomic DNA was extracted from leaves using a DNAmite Kit. SSR-PCR was carried out using primers B103 and B118 developed by Genetic Information Services (Chatsworth, CA). Amplified products were separated via electrophoresis on 2.5% agarose gels and stained with ethidium bromide and visualized with ultraviolet light. Both primers produced scorable markers for the cultivar Mitchell and these markers indicated that branches A and B were identical to the 'Mitchell' control and that branch C was not, and was therefore a rootstock shoot. Microsatellite markers provide a powerful tool for distinguishing scion and rootstock tissues on grafted pawpaw trees.

Is Flame Cultivation a Viable Method for Organic Weed Control in Pawpaw Orchards? KIRK W. POMPER* and SHERI B. CRABTREE, Land Grant Program, Kentucky State University, Atwood Research Facility, Frankfort, KY 40601-2355.

Pawpaw [*Asimina triloba* (L.) Dunal] is a tree fruit that has potential as a new niche crop for small farmers in the eastern United States. Flame cultivation uses a torch-directed flame to kill weeds by causing the plant cells to rupture and offers an organic alternative to herbicide application for the control of grass and perennial weeds. The objectives of this study were to determine if flame cultivation would control grass/weed coverage around pawpaw trees, and if flaming would damage tree trunks. A completely randomized experimental design was implemented using 12 five-year-old seedling trees treated with either 1) control (weed eating), 2) flaming with avoidance of the trunk, or 3) flaming without avoiding flame contact with the trunk. There were four replicate trees in each treatment. On July 25 and August 2 and 18, 2006, a three foot area around treatment trees was either subjected to the flaming treatments or weed eating (to a height of 2 inches). On August 25, and September 8 and 15, 2006, regrowth in plots was rated from 1 to 10, with 1 having no grass/weed coverage and 10 having total grass/weed coverage. By August 25, all flame plots had significantly less grass/weed coverage (about 2.25 rating) than control plots (7.75). On September 15, flame treatment plots had increased grass/weed coverage (about 4.75), but less coverage than control plots (9.5). By September 15, trees in either flaming treatment did not display noticeable trunk damage or wilting. Trunk damage will be evaluated again in 2007.

A Comparison of Agarose, Metaphor Agarose, and Polyacrylamide Gel Electrophoresis Systems in Resolving Pawpaw Simple Sequence Repeat Markers. LAUREN A.

COLLINS*, JEREMIAH D. LOWE and KIRK W. POMPER, Land Grant Program, Kentucky State University, Atwood Research Facility, Frankfort, KY 40601-2355.

Pawpaw [*Asimina triloba* (L.) Dunal] is a tree fruit native to much of the eastern United States. Since 1994, Kentucky State University (KSU) has served as the USDA National Clonal Germplasm Repository, or gene bank, for pawpaw; therefore, the assessment of genetic diversity with molecular markers in pawpaw is an important research priority for the KSU program. Using the polymerase chain reaction (PCR) with simple sequence repeat (SSR) primers and pawpaw template DNA, products between 150 to 500 bp are usually amplified and visualized via electorphoresis. The objective of this study was to determine if SSR-PCR products separated on agarose, metaphor agarose, and polyacrylamide gel electrophoresis systems display unique scoring patterns that result in different genetic separation upon analysis for nine pawpaw cultivars. DNA was extracted from young leaves collected from the pawpaw cultivars: Cales Creek, Davis, Middletown, NC-1, Overleese, Rebeccas Gold, Taytwo, Wilson, and Zimmerman using a DNAmite kit. SSRs primers B129 and C104 were developed by Genetic Information Services (Chatsworth, CA) and were used to amplify the pawpaw template DNA. PCR products were separated by electrophoresis on 3% agarose, 3% metaphor agarose, and 5% polyacrylamide gels. Following electrophoresis, gels were stained with ethidium bromide and photographed. Gel analysis was carried out using Kodak 1D software. Dendrograms were produced from the marker scoring data using NTSYSpc v2.11T. Each electrophoresis system produced a unique separation of the pawpaw cultivars, although some groupings were similar using the different systems.

Can Grape Black Rot Disease be Controlled with Application of the Particle Film Product Surround? SANJUN GU*, ANGIE WHITEHOUSE and KIRK W. POMPER, Community Research Service, Kentucky State University, Frankfort, KY 40601.

Black rot, *Guignardia bidwellii,* is the most prevalent and important fungal disease of grapes in Kentucky. Fruit production from grape vines is severely limited if this disease is not controlled during the growing season. In an effort to identify sustainable control measures, the effectiveness of the organic compound Surround® was compared to Manzate® and water (control) applications in controlling the incidence of black rot on two-year old 'Norton', 'Chambourcin' and 'Chancellor' grape vines in the KSU research vineyard. An experiment was initiated with a split-plot design in completely randomized blocks, with three blocks. Within each block, there were three cultivars (main plot) with nine vines each, and treatments were applied to three consecutive vines (split plot) randomly. Treatments were applied every 7 to 10 days from late May to the end of June, 2006. Natural foliage incidence of black rot was recorded before (May 19th) and after (July 18th) treatments. 'Norton' showed a significantly lower disease incidence than 'Chancellor' or 'Chambourcin' late in the season, indicating a difference in cultivar susceptibility to black rot. Although there was a trend for reduced black rot incidence on vines treated with Surround and Manzate, these treatments were not significantly different from the control. Low natural black rot inoculum levels in the new vineyard likely contributed to the low disease incidence rate in both treatments and the control. Manual inoculation of black rot conidia on leaves and fruit clusters will be attempted next year to challenge all treatment and control vines.

Rootstock Leaf Retention Aids Bud Break in Chip-Budded Pawpaw (*Asimina triloba).* SHERI B. CRABTREE* and KIRK W. POMPER, Community Research Service, Land Grant Program, Kentucky State University, Frankfort, KY 40601.

The pawpaw (*Asimina triloba)* is a tree native to the eastern U.S., producing large, edible fruit. Pawpaw cultivars are propagated by chip-budding the desired variety onto seedling rootstock. It has been suggested that when chip-budding pawpaw, it is beneficial to leave 6–8 leaves on the rootstock seedling. The photosynthetic activity of these leaves provides energy for the scion bud, until the bud has broken and initiated leaves, at which time the rootstock's leaves would be removed. To test this hypothesis, a 2x2x2 factorial experiment was implemented, with 2 scions (Sunflower and Susquehanna), 2 seedling rootstocks (Sunflower and K8–2), and 2 leaf treatments (removing leaves vs. leaving 6–8 leaves). Trees were chip-budded in late June 2006. Leaf number was counted weekly. The remaining rootstock leaves were removed after 6 weeks. Retaining leaves on the rootstock seedling increased scion bud break, with 75% of buds breaking on rootstocks with leaves remaining, and 53% bud-take on rootstocks with all leaves removed, before leaf removal. Removing the remaining rootstock leaves after 6 weeks had a positive effect, with an additional 13% of buds on rootstocks that had previously retained their leaves breaking by 4 weeks after leaf removal, vs. only 2% more buds breaking on rootstocks that had leaves removed from the beginning. Removing the rootstock's leaves upon budding had a positive effect on scion leaf number, with scions budded onto rootstocks whose leaves had been removed having an average of 9 leaves, and scions budded onto rootstocks with leaves remaining having 5 leaves.

Heavy Metal Concentrations in Vegetables Grown in Soil Incorporated with Sewage Sludge. GEORGE F. ANTONIOUS*, Kentucky State University, Land Grant Program, Department of Plant and Soil Science, Frankfort, KY 40601 and JOHN C. SNYDER, Department of Horticulture, University of Kentucky, Lexington, KY 40546.

Sewage sludge from municipal wastewater treatment plants can be used in agriculture to circulate nitrogen, phosphorus, potassium, organic matter and micronutrients from food consumers to food producers for sustainable agriculture. Three consecutive years of field experiments were carried out to investigate the effect of different soil management practices on the concentration and accumulation of seven heavy metals in potato tubers, bell pepper fruits, and broccoli heads. The study was conducted on a 10% land slope at Kentucky State University Research Farm. Eighteen plots of 22 × 3.7 m each were separated using metal borders to prevent cross contamination between treatments. The soil in six plots was mixed with sewage sludge, six plots were mixed with yard waste compost, and six plots were used for comparison purposes. During a 3-year study, plots were planted with potato (year 1), pepper (year 2), and broccoli (year 3). The objectives of this investigation were to 1) determine the concentration of seven heavy metals (Cd, Cr, Ni, Pb, Zn, Cu, and Mo) in sewage sludge and yard waste compost; and 2) monitor heavy metal concentrations in edible portions of plants at harvest. Concentrations of heavy metals in sewage sludge were below the USEPA limits. Analysis of potato tubers grown in sludge-amended soil showed that Cr, Ni, and Pb were not significantly different from control plants. Concentrations of Cd, Zn, Cu, and Mo were significantly greater in tubers grown in sludge compared to control tubers. In broccoli, only Zn, Cu, and Mo concentrations were higher in sludge-grown crops, and in pepper, only Cu and Mo were higher than control crops.

Lethal Effects of Hot Pepper Extracts on the Cabbage Looper, *Trichoplusia ni,* Hübner. JANET E. MEYER* and GEORGE F. ANTONIOUS, Land Grant Program, Department of Plant and Soil Science, Kentucky State University, Frankfort, KY 40601.

Investigation of natural pesticides has become increasingly important due to the fact that synthetic pesticides may cause ecological harm and are increasingly removed from the market due to legal restrictions. Consumer and grower interest in safe alternatives to synthetic pesticides has increased rapidly over the past few years and is expected to continue. Hot pepper extracts have traditionally been used by various cultures for pest control. Previous research has indicated the effectiveness of compounds found in pepper fruits against various agricultural pests including the spiny bollworm, *Earias insulana*; onion maggot, *Delia antique*; and spider mite, *Tetranychus urticae.* Cabbage looper, *Trichoplusia ni,* is one insect of economic importance which attacks various cole crops, consuming up to three times its body weight in leaf material daily. This study investigates the mortality of laboratory-reared third instar cabbage looper larvae. The larvae were sprayed with water-based pepper fruit extract, which was prepared from 23 different accessions of hot pepper fruits grown at the Kentucky State University research farm in summer 2005. Mortality was recorded at 6 and 24 hrs after treatment. At 24 hours, average mortality ranged from 22–93% as compared to control. Accessions PI-593566 (*C. annuum*), PI-281340 (*C. baccatum* var. *pendulum*), PI-586675 (*C. frutescens*), PI-439381 (*C. baccatum*), and PI-438614 (*C. chinense*) showed 93, 85, 82, 78, and 75% mortality, respectively.

Mobility of Trifluralin and Napropamide Under Field Conditions. MICHAEL O. SOMUAH*, ZACHARY RAY, TEJINDER S. KOCHHAR and GEORGE ANTONIOUS, Department of Plant and Soil Science, Land Grant Program, Kentucky State University, Frankfort, KY 40601.

Agrochemicals use has increased dramatically over the years. Farmers' anticipation to increase yield and compete with global agriculture market has led to a high dependency on agrochemicals. The objective of this study was to trace the mobility of two herbicides (trifluralin and napropamide) that are commonly used in crop protection, in runoff and infiltration water from soil treated with three management practices. A field study was conducted on a 10% land slope at Kentucky State University Research Farm. Eighteen plots of 22 × 3.7 m each were separated using metal borders and the soil in six plots was mixed with sewage sludge, six plots were mixed with yard waste compost, and six unamended plots were used for comparison. During a subsequent 3-year study, plots were planted with potato (year 1), pepper (year 2), and broccoli (year 3). Once the soil was sprayed with trifluralin and napropamide, runoff and infiltration water were collected following natural rainfalls and the herbicide residues were quantified. Addition of sewage sludge increased soil retention of trifluralin and napropamide, lowering their concentration in runoff, and reducing their transport into streams and rivers.

Yield and Quality of Hot Pepper Grown at Kentucky State University. JAMI A. ROGERS*, JANET E. MEYER and GEORGE F. ANTONIOUS, Land Grant Program, Department of Plant and Soil Science, Kentucky State University, Frankfort, KY 40601.

With the growing popularity of "hot foods" in the U.S., hot peppers offer a wide variety of marketing outlets. Various outlets for hot peppers include farmer's markets, contracts with retail stores or mass production of capsaicinoids for industrial purposes. The objective of this study was to select candidate hot pepper accessions for potential mass production of capsaicinoids. Twenty-four *Capsicum* accessions selected from the USDA *Capsicum* germplasm collection were grown at the Kentucky State University Research Farm. Six accessions from *Capsicum chinense,* six accessions from *Capsicum frutescens,* six accessions from *Capsicum annuum,* and seven accessions from *Capsicum baccatum* were selected. Mature fruits from each accession were harvested, weighed, and the numbers of fruits from each plant were recorded. Measurements of the length and width of individual fruits were taken. Accessions PI-438649 (*C. annuum*), PI-438643 (*C. chinense*), and PI-435916 (*C. chinense*) produced the greatest yield, while PI-632921 (*C. annuum*) produced the lowest yield compared to other accessions tested. Plants from PI-238061 (*C. baccatum*) produced the highest number of fruits. Overall, PI-*438649 (C. annuum*) had the greatest yield (1,528 g/plant) and the greatest fruit quality among other accessions tested.

Mass Spectrometric Analysis of Decanoic Acid Methyl Esters in Hot Pepper Accessions, HU YOON-HYEON* and GEORGE F. ANTONIOUS, Land Grant Program, Department of Plant and Soil Science, Kentucky State University, Frankfort, KY 40601.

Long chain saturated fatty acids and their esters are common components of plant lipids. Crude extracts of hot pepper accessions of *Capsicum chinense, C. frutescens, C. baccatum,* and *C. annum* were prepared using methanol and analyzed for pentadecanoic acid methyl esters, hexadecanoic acid methyl esters, and octadecanoic acid methyl esters. Mass spectrometry in total ion mode of hot pepper fruit extracts indicated that the molecular ions at m/z 256, 270, and 298.5, which correspond to pentadecanoic acid methyl ester, hexadecanoic acid methyl ester, and octadecanoic acid methyl ester, respectively, have two common ion fragments at m/z 74 and 87 that can be used for monitoring decanoic acids in hot pepper fruit extracts. Bioassays of pure standards of decanoic acid methyl esters using cabbage looper, *Trichopulsia ni,* larvae have shown that pentadecanoic acid methyl ester was the most effective (74% mortality) compared to hexadecanoic and octadecanoic acid methyl esters. Concentrations of decanoic acid methyl estes varied among hot pepper accessions. Accession PI-632921 (*C. annuum*), PI-257051 (*C. frutescens*), and PI-239703 (*C. frutescens*) contained the highest concentration of pentadecanoic acid methyl ester (827.5 μg/g), hexadecanoic acid methyl ester (969.7 μg/g), and octadecanoic acid methyl ester (1546.6 μg/g), respectively. This research provides background information on the level of pentadecanoic acid methyl ester in pepper fruits and may provide an opportunity for use in crop protection as alternative to synthetic pesticides.

Quality and Yield of Eggplant and Bell Pepper Grown with Sewage Sludge. ZACHARY M. RAY*, MICHAEL O. SOMUAH and GEORGE F. ANTONIOUS, Kentucky State University, Land Grant Program, Department of Plant and Soil Science, Frankfort, KY 40601.

Nutrients in sewage sludge are used to replace a supplement commercial fertilizer, while sewage sludge organic matter can improve crop yield and quality. Sewage sludge as a soil amendment provides not only a means for sewage sludge disposal, but can also improve fertility and physical properties of soil. The objective of this study was to compare yield and quality of bell pepper and eggplant from three soil management practices. The three soil management practices were: 1) municipal sewage sludge mixed with native soil at 15 t/acre, 2) municipal sewage sludge mixed with yard waste compost at 15 t/acre, and 3) rototilled bare soil used for comparison purposes. Field studies were conducted on a Lowell silty loam soil at Kentucky State University Research Farm, Franklin County, KY. Six replicates of each soil treatment were established in 18 plots of 22 × 3.7 m each. Seedlings of bell pepper and eggplant were planted in rows at 12 inches apart. Mature bell pepper and eggplant were harvested from each plot, weighed, and graded according to USDA standards for grades of fresh sweet bell pepper and eggplant. Total harvest weight and number of Fancy, US #1, and US #2 were obtained from each soil treatment. Our results have indicated that application of sewage sludge mixed with yard waste compost provided the greatest total yield (85,323 lbs/acre) compared to sewage sludge only (54,702 lbs/acre) or no mulch treatment (65,185 lbs/acre). In addition, the greatest eggplant yield of US #1 and U.S. #2 was obtained from sewage sludge mixed with yard waste compost.

Transition of Agriculture in the Appalachian Counties of Kentucky. ARCHANA LAKKARAJU* and ELMER GRAY, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101.

Regions of Kentucky have developed different agricultural systems resulting from interactions of natural geological conditions, economic developments and human cultures. The Appalachian area comprises 49 counties in eastern Kentucky and constitutes the state's most diversified agricultural region. The Tobacco Settlement Program began in 2000 when the Kentucky General Assembly passed HB611 calling for the state to invest half of the settlement money to reduce dependence on tobacco income and to diversify into other products. Objectives of the present treatise were (1) to conduct a time analysis of changes in productivity of agricultural enterprises for pre- and post-2000 periods and (2) to compare changes for the Appalachian Counties with those for the State. Census, USDA, NASS and Kentucky Department of Agriculture data were used to make comparisons between the Appalachian Counties (AC) and State (KY) for various agricultural indexes. Since 1980, AC have contributed about 28% of KY population of people and have included about 34% of KY farms which averaged about 86% of the size of KY farms. Number and size of farms which averaged for AC and KY followed similar trends. Total market value of agricultural products reached a peak in 2004; however, AC contribution decreased recently. In general, for both AC and KY, the market value of crops increased prior to 2000 but decreased subsequently reflecting the reduced production of tobacco. For the market value of livestock, the continued decline in dairy production was offset by broiler production resulting in continued increase in the value for both AC and KY. Comparisons of cash receipts for 2005, showed that the livestock contributed 68% and crops 32%, reflecting increase in horses and broilers and decrease in tobacco and soybeans. Although the economic impact is minor at present, increase in commodities such as goats, rabbits, bison, vegetables, fruits, nuts, and ornamental flowers indicated that Tobacco Settlement Funds are impacting Kentucky Agriculture.

Weed Management in an Established Orchard of *Vaccinium corymbosum* 'Bluejay' (highbush blueberry) in South Central Kentucky. KYLE DANIEL* and MARTIN STONE, Western Kentucky University, Department of Agriculture, Bowling Green, KY 42101.

In response to the changing economy of Kentucky tobacco, producers are seeking an economically viable alternative that can be produced on a similarly small acreage. Blueberries are an emerging crop that satisfies the needs of these producers and are popular with consumers for their flavor and health benefits. The effects of four weed management schemes were investigated on berry yield components and new growth in an established orchard of 'Bluecrop' blueberries in Metcalfe County, Kentucky. The experimental design was a randomized complete block with four replications. Plots consisted of six established plants but data was collected on the innermost four. Treatments were weed-free strips 0.609 m or 1.828 m wide within the row, mowing, and an untreated control. Weed-free strips were maintained as necessary with directed sprays of labeled rates of glyphosate, a phloem-mobile, non-selective herbicide. Highly significant differences in new growth were noted during both years from the herbicide-treated plots compared with the non-treated plots. In 2005, highly significant differences were noted within total berry weight and berry weight per plant from the herbicide-treated plots compared with the non-treated plots. In 2006, highly significant differences were noted within total berry numbers, number of clusters per plant, and mean berries per cluster from the herbicide-treated plots compared with the non-treated plots. Eliminating weed competition, most likely for water, increased new growth in the short term and improved yield components in subsequent years.

Asexual Propagation of Four Cultivars of *Vaccinium corymbosum.* KYLE DANIEL* and MARTIN STONE, Western Kentucky University, Department of Agriculture, Bowling Green, KY 42101.

In response to the changing economy of Kentucky tobacco, producers are seeking an economically viable alternative that can be produced on a similarly small acreage. Blueberries are an emerging crop that satisfies the needs of these producers and are popular with consumers for their flavor and health benefits. In addition to selling the berries, local producers are experiencing much success selling blueberry plants to homeowners and other producers. However, the protocol for propagating specific cultivars under local environmental conditions is unknown. Rooting percentages for producers has been extremely variable. A two year study conducted at Western Kentucky University investigated asexual propagation of four commercially significant cultivars of *Vaccinium corymbosum,* highbush blueberry, 'Jersey', 'Elliot', 'Bluecrop', and 'Bluejay'. The study was a randomized complete block design with four replications. Cuttings were taken from a producer's field in Metcalfe County and planted in a bed of pure peat under a mist system at the Western Kentucky University Agricultural Research and Education Center. Propagation techniques were designed to closely mimic the systems used by producers. The effect of cutting phenology, rooting hormone, and cutting location along the stem was investigated as they affected rooting percentage, and dry matter mass of leaves, shoots, and roots. Hormones had no effect on rooting or growth of cuttings. The greatest rooting percentages and dry mass gain was found in descending order, 'Jersey', 'Elliot', 'Bluecrop', and 'Bluejay'. There was a correlation between location of the cutting and time of the year the cutting was acquired. Basal cuttings performed well early in the season, while apical cuttings performed well later in the season. To ensure consistent success, producers of blueberry plants via asexual propagation under the growing conditions of south central Kentucky must understand the interaction of time of year and location of cutting along the stem for each cultivar.

Body Condition Score and Blood Glucose Relationships of Grazing Horses. ASHLEY MONFORT* and CHARLES ANDERSON, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101.

Recent studies have reported that blood glucose levels and founder in horses consuming forage/concentrate mixed diets are positively correlated. Other studies have reported body condition score and the incidence of founder are also positively correlated. Few studies have monitored these parameters for horses consuming all forage diets, even though the incidence of grass founder is quite high. In the present study, a group of aged quarter horses were allowed six months to adapt to an all forage diet consisting of free choice access to a mixed grass pasture. Four thin, four moderate and four obese horses were grouped with two mares and two geldings per group. Blood glucose samples were collected at four- hour intervals, during a twelve hour grazing period. Statistical analysis indicated no significant blood glucose variation due to time of sampling, sex, or body condition scores. The mean blood glucose level for horses consuming this pasture diet was 77 mg/dl. These results indicate that there is no significant short term variation in blood glucose levels of horses consuming only grass pasture diets and blood glucose levels alone are not correlated to body condition score. The results also indicate that the mean normal blood glucose level of horses consuming grass pasture diets is similar to that of horses consuming forage/concentrate mixed diets. These results imply that blood glucose and body condition are not correlated and suggest blood glucose alone may not be a valid indicator of the metabolic disorder founder.

Landscape of the Past: the Felts Log Cabin at Western Kentucky University. ELIZABETH ALEWINE* and MARTIN STONE, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101.

The Felts House was constructed between 1800 and 1820 in Logan County, KY by Archibald Felts. The house, which features a dogtrot floor plan, V-notched logs, and other historical architectural features, was donated to the University in 1980. The landscape design for the Felts House is intended to be an interpretative representation of farming homesteads in south-central Kentucky during the early 1800s. A small section will be devoted to period farm crops and an orchard. A kitchen garden, complete with heirloom vegetables, herbs, medicinal plants, and dye plants will occupy an area close to the cabin. Other areas close to the cabin will be devoted to outdoor demonstrations, such as a replica of a bee hive and outdoor living. The remaining area will be designed to recreate the native woodland vegetation of Kentucky. Suggestions will be included with the design to address issues of soil erosion and the unique needs to maintain such gardens. Additional information that will be provided with the design includes a materials list and historical references.

Impact of Cry 3Bb Bt Transformed Corn Kernels on Life History Attributes of Several Stored Grain Beetle Pests. ADRIENNE FLEMING*, KAREN L. FRILEY and JOHN D. SEDLACEK, Community Research Service, Kentucky State University, Frankfort, KY 40601.

Laboratory experiments were conducted to examine the impact of *Bacillus thuringiensis* (Bt) corn hybrids containing Cry 3Bb protein genes on mortality and progeny production of maize weevil, *Sitophilus zeamais*; red flour beetle, *Tribolium castaneum;* and sawtoothed grain beetle, *Oryzaephilus surinamenis,* three common pests of stored grain. Approximately 8.5 g of Yieldgard® Corn Borer, Yieldgard® Rootworm, Yieldgard® Plus, or conventional corn (Bt) used as a control were placed in ventilated vials. Nine maize weevils, 10 red flour beetles or 10 sawtoothed grain beetles were added to each vial. The vials were placed in an environmental growth chamber set to 27 $\pm$ 1°C, $\geq$ 60% RH and complete darkness. Three replicates, each containing five vials of each treatment were used. Mortality was quantified after one week and adults were removed. Progeny checks began two weeks after adults were removed and were done daily for six weeks. Percent mortality was low with all insects in all treatments. Maize weevil progeny emergence was the same in all treatments. However, progeny emergence was lowest in the conventional corn used as a control for both red flour beetle and sawtoothed grain beetle. Results indicate that these three Bt corn hybrids have no negative impact on maize weevil, red four beetle or sawtoothed grain beetle life history attributes.

Impact of Benallure® Beneficial Insect Attractant on Populations of Predatory Insects in Sweet Corn. CARMEN HAYNES*, KAREN L. FRILEY, STEVE L. HILLMAN and JOHN D. SEDLACEK, Community Research Service, Kentucky State University, Frankfort, KY 40601.

Sweet corn is among the major vegetables grown in Kentucky during summer months. Unfortunately, several insect pest species cause damage to sweet corn ears. These pests have been controlled primarily with chemical spray programs. However, excessive spraying may negatively impact non-target organisms, contaminate ground water, and farmers, consumers, and homeowners may be exposed to unacceptable levels of insecticides. An alternative method of pest control involves beneficial insect introduction or biological control. Sometimes attractants are used to lure predaceous insects to prey upon pest insects. A commercially available attractant called Benallure® is claimed to be attractive to lady beetles, green lacewings, and syrphid flies. Benallure is composed of two corn plant volatiles which have been shown to be attractive to these insects in laboratory studies. However, no field studies have yet been performed examining efficacy of Benallure in the field. Thus, the objective of this study was to evaluate the effect of Benallure as a predaceous insect attractant in organically grown sweet corn. Yellow sticky traps were used to quantify beneficial insects in Benallure and control plots. Pink or 12-spotted lady beetle, *Coleomegilla maculata,* was the most abundant predator caught. Asian multicolored lady beetles, *Harmonia axyridis,* and green lacewings, *Crysoperla carnea,* were captured but were not abundant. Few syrphid flies were caught. Populations of lady beetles and lace wings in control vs. Benallure treated plots did not differ. Thus, this rather expensive beneficial insect attractant could not be recommended for use in late planted central Kentucky sweet corn.

Ground Beetle Populations in Organic, Conventional, Genetically Engineered Sweet Corn: Preliminary Results. KAREN L. FRILEY* and JOHN D. SEDLACEK, Community Research Service, Kentucky State University, Frankfort, KY 40601.

Ground beetles (Coleoptera: Carabidae) are important predators of many agricultural insect pests. Economically important insect pests that they may prey upon in sweet corn include corn earworm, *Helicoverpa zea* (Boddie); European corn borer, *Ostrinia nubilalis* (Hübner); southwestern corn borer, *Diatreae grandiosella* Dyar; fall armyworm, *Spodoptera frugiperda* (J.E. Smith); and Japanese beetle, *Popillia japonica* Newman. In this study, sweet corn was grown using three different cropping methods: conventional, genetically engineered, and organic. Concerns regarding reduction in biodiversity and non-target impacts in genetically engineered crops have been expressed. Thus, the objective of this research is to identify and enumerate ground beetle species in the three cropping methods of sweet corn. Pitfall traps were used to capture ground dwelling insects. Four pitfall traps were placed equidistant from edges and from each other within the middle row of corn in the first two of the three subplots in each plot. Pitfall traps were made from 473.2 cc plastic cups. Two cups were placed in each hole with the edge flush with the soil surface. A rain cover, supported by 3 wooden blocks, was made from a 22.8 cm diameter plastic plate. A 118 cc 1:1 ratio of ethylene glycol and distilled H_2O was placed in each trap to preserve insects until collected. Pitfall traps were serviced at weekly intervals throughout the growing season. Initial analyses for one sampling period revealed greater numbers of carabids in Bt plots than organic plots. The most abundant ground beetle was *Harpalus pennsylvanicus* followed by *Poecilus chalcites.*

Perceptions of Modern Agricultural Production Practices and Taste Preferences Among Patrons of a Farmers' Market Concerning Sweet Corn Grown Organically, Conventionally or Genetically Engineered. JOHN D. SEDLACEK*, SUSAN B. TEMPLETON and KAREN L. FRILEY, Community Research Service, Kentucky State University, Frankfort, KY 40601.

As part of a larger study comparing the ecological/environmental impact of organic, conventional, and biotechnology enhanced production of sweet corn, we examined consumer knowledge and taste preferences concerning these production practices. Consumer concerns were quantified using a 10 question survey administered at the local Frankfort Farmers' Market. The questionnaire quantified attitudes and perceptions concerning sweet corn grown in each of the three cropping types. The survey culminated with taste paneling of corn harvested from each of the three cropping methods. An informal acceptance and sensory evaluation using informed/blind testing procedures was employed. Color, juiciness, sweetness, crispness, overall appearance, overall flavor, and overall texture were evaluated. The majority of respondents were female, Caucasian, non farmers ranging in age from 45–64. Before completing the taste perception component of our survey, over 50% of the respondents believed that the organically grown sweet corn tastes better. Surprisingly, respondents preferred the genetically engineered sweet corn over that which was conventionally and organically grown for color, juiciness, sweetness, and crispness characteristics. Overall, taste test respondents preferred the genetically engineered sweet corn to the organically grown sweet corn by at least a 3:1 ratio for appearance, flavor, and texture. Differences in fertilizer amendments between the genetically engineered and conventional plots vs. the organic plots may be responsible for these differences. Another possible explanation could be weed pressure in the organic plots that was much more severe than in the conventional and genetically engineered plots.

CELLULAR AND MOLECULAR BIOLOGY

Synthetic Genetic Array Slow Growth Suggests a New Role for Protein Kinases Pkh1 and Pkh2 in mRNA Degradation. MARC ETTENSOHN*[1], MICHAEL CONSTANZO[2], CHARLES BOONE[2], ROBERT C. DICKSON[1] [1]Department of Molecular and Cellular Biochemistry, University of Kentucky, Lexington, KY 40506, and [2]Department of Medical Genetics and Microbiology, University of Toronto, Ontario M5S 1A8, Canada.

In the baker's yeast, *Saccharomyces cerevisiae,* sphingolipid long chain bases, such as phytosphingosine, regulate the protein kinases Pkh1 and Pkh2, which control a wide variety of cellular processes including aging, growth, and translation. To identify new roles for Pkh1/2 we used a procedure called synthetic genetic arrays (SGA). In SGA two strains, each containing a deletion of a nonessential gene, are crossed to create a double mutant, which is analyzed to determine if it is viable or grows slower or faster than either single mutant. For these experiments we used a strain with a temperature-sensitive *pkh1* gene and a deletion of *pkh2.* This strain was crossed in pair wise combinations with over 4,000 yeast strains each deleted for one non-essential gene. The SGA data suggested that Pkh1/2 were playing a role in decapping and breakdown of messenger RNAs (mRNAs) because the growth of a *pkh1/2* and *kem1* mutant strain was reduced compared to the single mutants. Kem1 is involved in mRNA breakdown and cells lacking Kem1 are sensitive to the antifungal drug myriocin, which inhibits synthesis of long chain bases, and therefore, also inhibits Pkh1/2 activity. We then determined if strains deleted for individual genes involved in mRNA decapping and breakdown are sensitive to myriocin. We found several deletion mutants were sensitive to myriocin including *ccr4Δ, rtf1Δ, leo1Δ, cdc73Δ, ctr9Δ* and *nab6Δ.* Future studies will determine if Pkh1/2 directly plays a role in mRNA decapping and breakdown, as suggested by our initial data.

CHEMISTRY

Trace Level Analysis of Polybrominated Diphenyl Ethers in Fish Tissue Extracts Using a Gas Chromatograph-Electron Capture Detector. ANNETTE FOWLER* and BOMMANNA G. LOGANATHAN, Department of Chemistry and Center for Reservoir Research, Murray State University, Murray, KY 42071.

Polybrominated diphenyl ethers (PBDEs) are well known global environmental pollutants. These compounds are widely used in industrial and domestic applications. Limited research is available on the levels of these chemical pollutants in fish tissues, and human exposure via consumption of contaminated fish. In this study, PBDE standards were prepared following appropriate handling procedures, and run on a gas chromatograph (GC) equipped with an electron capture detector (ECD) to study the response of ECD to PBDEs. Response factors were determined, and then 15 fish tissue extract samples from bottom feeding Costal Georgia fish were analyzed using GC-ECD. Analytes were identified and quantitated using Excel. The PBDE concentration data was tabulated and compared to concentrations of PBDEs in fish from several rivers and lakes in the United States to establish baseline concentrations from this region for future research. Results revealed that PBDEs 47, 99, 100 and 28/33 were frequently detected in fish samples. Inter-species and intra-species differences were noticed. Accumulation pattern of the PBDEs in fish tissues shown the following order 47>99>100>28/33>66.

ECOLOGY AND ENVIRONMENTAL SCIENCE

Fluctuating Asymmetry as a Measure of Stress from Habitat Disturbance. TIFFANY L. HEDRICK*, COURTNEY A. THOMASON, PATRICK HOWELL and TERRY L. DERTING, Department of Biological Sciences, Murray State University, Murray, KY 42071.

As human activities continue to impact wildlife populations, it is increasingly important to understand the relationships between habitat disturbance and stress. Prior research indicated that white-footed mice (*Peromyscus leucopus*) in disturbed patches of habitat experience less moderate-term stress than mice from undisturbed patches. Our objective was to determine whether long-term stress is also reduced with disturbance. One tool for examining prolonged stress in free-living animals is fluctuating asymmetry (FA). We tested the null hypothesis that FA does not differ between mice from disturbed and undisturbed habitats and between seasons. Adult male mice were trapped from disturbed and undisturbed habitats during the summer and winter of 2003. Disturbed patches were adjacent to either residential or agricultural areas. Undisturbed patches were located in the Land Between the Lakes National Recreation Area. Using digital photographs, we measured eight bilateral characters of the dental, cranial, and post-cranial skeleton. *Peromyscus leucopus* exhibited significantly lower levels of FA in disturbed habitats and during the summer season. *Peromyscus leucopus* from the winter-undisturbed group exhibited significantly more FA than mice from any other group. Our results indicated that at least some forms of human disturbance are associated with reduced long-term stress in our model species. These results were consistent with previous reports indicating reduced moderate-term stress in mice from disturbed habitat patches. We attribute this reduction in stress primarily to the availability of high quality food resources, such as human refuse and soybean and grain crops, adjacent to disturbed habitat patches.

Temporal Change as a New Landscape Metric in a New Kentucky Land Cover Map. DEMETRIO P. ZOURARAKIS, Kentucky Division of Geographic Information, Frankfort, KY 40601.

Temporal land cover change, such as the increase in impervious surfaces due to urbanization and the inter-conversion between forested, agricultural and mined lands, is critical to landscape analysis work. The NASA-funded Kentucky Landscape Snapshot Project (KLS) provided an update to Kentucky's 1992 National Land Cover Dataset (NLCD92; http://seamless.usgs.gov; http://kygeonet.ky.gov), creating the NLCD01, a baseline for land cover change detection derived from classification and regression tree methodologies. This study was elicited by new dataset distributed by the US Geologic Survey, an image-to-image change mask for Kentucky, ca. 2000s vs. ca. 1990s at an Anderson Level I (AL-I). An algorithm is presented that enables the dissection of land cover change. In a first step, spatial regions of change made up of contiguous pixels, and measuring less than 1 ha are eliminated, the remaining regions are categorized in ''from-to'' and ''to-from'' change. Thus, 5.7% of the ''from-to'' regions contained 42.7% of the change, while 3.3% of the ''to-from'' regions contained 37.8% of the change (total change of 418,645 ha. in regions <1ha). In subsequent stages, the area size frequency displacement for individual AL-I classes are plotted. The AL-1 classes showing largest discrepancy in area size frequency between the ''from-to'' and ''to-from'' groupings were: forest, urban, grass-shrub and wetland. The temporal element is thus incorporated to the direction of class migration as a new type of landscape metric.

GEOLOGY

Preliminary Results of a Nutrient Source Study in Wilgreen Lake, Madison County, Kentucky. WALTER S. BOROWSKI* and ERIN JOLLY, Department of Earth Sciences, Eastern Kentucky University, Richmond, KY 40475.

Wilgreen Lake (Madison County, Kentucky) is an eutrophic lake formed by damming Taylor Fork, part of the Silver Creek watershed. Two principal tributaries drain urban areas of the city of Richmond, agricultural land typified by cattle grazing, and a high-density residential area using septic systems. The lake is ''nutrient impaired,'' so it is likely that anthropogenic nutrient loading is affecting water quality. Our study aims to first characterize the physical characteristics and water quality of the lake (2006), and then determine the specific proportion of nutrient inputs (2007) to the lake with the aim of remediating any possible degradation of water quality. We anticipate using nitrogen isotopes and microbial DNA templates to identify specific nutrient sources. Research started in May 2006 with work occurring throughout the 2006 field season with the intent of establishing a baseline for key lake parameters. We used an YSI probe to measure temperature, conductivity, oxygen concentration, and pH and assayed for total ammonia nitrogen using the sodium hypochlorite, colorimetric method. The lake was already strongly stratified in May with disoxic and anoxic water below about 4 meters. Stratification strengthened in the summer with the disoxic-oxic boundary moving upward to about 3 meters, and showing a sharper gradient between oxic and disoxic waters. Ammonium concentrations are typically zero in the oxic zone, and increase in concentration with increasing water depth in anoxic waters to about 6 ppm. We anticipate that phosphate and nitrate concentrations will mirror ammonium concentration profiles in character.

A Preliminary Study of Sulfide Mineral Formation in Methane-Rich, Marine Sediments Associated With Anaerobic Methane Oxidation, Cascadia Continental Margin, Offshore Oregon. DAVID DEIGERT* and WALTER S. BOROWSKI, Department of Earth Sciences, Eastern Kentucky University, Richmond, KY 40475.

Within gas hydrate settings, sulfide mineralization in marine sediments is likely controlled by two microbially-mediated, sulfate-depleting processes: anaerobic methane oxidation (AMO) and sulfate reduction. If large amounts of methane are delivered to the sulfate-methane interface (SMI), predominantly by diffusion, larger amounts of solid sulfide sulfur should occur there as dissolved sulfide combines with iron, forming an authigenic precipitate. We measure the amount of diagenetic sulfide sulfur at three locations in the Hydrate Ridge vicinity by extracting the bulk sedimentary sulfide-phase minerals (S°, FeS, and FeS_2) through chromium reduction, precipitating sulfide sulfur as silver sulfide, and gravimetrically determining concentration. Two of three sites show authigenic sulfide sulfur levels of approximately 0.27 and 0.7 weight percent (wt %) sulfur, occurring immediately above the SMI. Lower concentrations of 0.12 and 0.41 wt % sulfur, respectively, occur below the SMI. The remaining site has no discernable pattern to the vertical distribution of sulfide sulfur concentration, but shows peak amounts of 0.52 wt % sulfur above the SMI. At the first two sites, we infer peak amounts of sulfide sulfur are precipitated due to the production of sulfide sulfur via AMO. We can test this interpretation by determining the sulfur isotopic composition (□^{34}S) of the bulk sulfide minerals. Sulfide sulfur forming at the SMI should also be enriched in heavy sulfur relative to sulfide minerals forming in the sediments above. If these sulfur isotopic enrichments are unique to methane-rich settings associated with gas hydrates, then these diagenetic fingerprints can be recognized in the rock record.

Nonscientific Attitudes and Biases towards Evolutionary Theory in Introductory College Science Courses for Education Majors. EDWARD L. CRISP, Geology Department, West Virginia University at Parkersburg, Parkersburg, WV 26104.

Teaching of biologic evolution in the public school system in the U.S. is currently under serious attack from the intelligent design movement, a movement that is taking advantage of the scientific illiteracy of the general public to emplace a faith-based, nonscientific alternative to evolutionary theory into the public school science classroom. This is really a social, cultural, and political attack rather than one that has any scientific merit. It is a ''stealth'' attempt to undermine real scientific inquiry and emplace the old-fashioned creationistic view into the public school science classroom. This movement is also becoming ever more evident in college and university introductory science classrooms. During the summer and fall 2005 and spring 2006 semesters at West Virginia University at Parkersburg, a science attitudes survey was administered to 206 students in physical science, earth science, physical geology, astronomy, and some sections of introductory biology. Over 50% of these students are education majors. This ongoing process for several semesters will eventually allow a determination of any changes in attitudes by administering both a pre-course and post-course survey. Current results of the pre-course survey indicate that 59% of the 206 students think that ''creation science'' should be taught in the public schools alongside evolutionary theory. This decreases to 46% for the 150 students that have completed the post-course survey. About 57% of pre-course survey students think that the Bible is an accurate and adequate explanation for the origin and development of Earth, life, and humans; as compared to 48% for post-course survey students. Today, with the dangers to science education inherent in the intelligent design movement, explaining evolutionary theory and stressing its strong basis in sound scientific inquiry to college students, some that are future public school teachers, is extremely important.

Evidence of Lower Mississippian Glaciation in the Sunbury Shale of East-Central Kentucky. ROBERT T. LIERMAN*, Department of Earth Science, Eastern Kentucky University, Richmond, KY 40475 and CHARLES E. MASON, Department of Physical Sciences, Morehead State University, Morehead, KY 40351.

This is a preliminary report on the recent discovery of a large, granite boulder found imbedded within the Sunbury Shale in Rowan County, Kentucky. The boulder measures 1.7 by 1.3 m and is at least 0.75 m thick. Compositionally it was originally biotite granite, with quartz, K-feldspar (microcline) and biotite mica as the main mineral constituents. Thin-section examination of samples taken from the boulder shows it to have been subjected to low-grade (greenshist) metamorphic alteration. This is evidenced by the presence of highly strained quartz, along with bent or kinked biotite that has been altered to chlorite. The boulder is imbedded within the Sunbury Shale (an organic-rich, black shale) as evidenced by the presence of upturned shale layers or a ''mud drape'' along the edges of the boulder. The boulder itself is unweathered, though it appears quite worn, with beveled or flatted surfaces and rounded corners and edges. Factoring in its size and density (2.70 g/cm3), we estimate the boulder to weight at least 3 1/2 tons. Taking into account the size, weight, overall shape and exotic lithology of this boulder, we suggest that it represents an ice-rafted ''dropstone'' that was transported to and then released from a melting iceberg at this site. There is strong evidence that during late Devonian and early Mississippian times, portions of Gondwana (specifically north-central South America) were covered by a major episode of glacial ice. Given the fact that Kentucky was located at approximately 30 deg. south latitude at this time, we suggest that this boulder was rafted by an iceberg derived from the Gondwana ice sheet some 30 deg. to the south. This suggests that ocean currents were moving counterclockwise (northwards) along the west coast of Gondwana and that it was these currents that brought icebergs into the area just west of the Acadian Mountains and eventually into Kentucky.

An Undergraduate's Research Experience at the NASA Haughton-Mars Project, Devon Island, Nunavut, Canada. WESLEY C. SMITH* and CHARLES E. MASON, Department Physical Sciences, Morehead State University, Morehead, KY 40351.

This summer, Charles E. Mason and I traveled to the NASA Haughton-Mars Research Camp located on Devon Island in the new territory of Nunavut in Canada. The purpose of this project was to collect rock specimens of clasts from impact breccia and possible ejecta blocks. We are currently extracting the conodonts from these samples with the intention of using their Color Alteration Index to determine the temperatures these samples experienced upon impact. Temperatures obtained will be compared between both the samples and other techniques of temperature determination. I was able to contribute to this research project thanks to Charles Mason and support from Morehead State University, the Kentucky Space Grant Consortium, and the NASA Haughton-Mars Project. While at the Haughton Crater I was able to work with scientists from around the world. Working with such a wide variety of scientists, whose backgrounds, ideas, and cultures differed greatly, allowed me to get real world experience. This kind of work setting makes you consider all opinions of everyone involved and allowed me to see how a group of people who speak different languages, are experts in different fields, and have different views can all come together to work toward a common goal. While at the Haughton Crater I not only was able work on our project, but also was able to learn and help others with their projects. An undergraduate research experience allows a student to experience things that can't be experienced in the classroom and forces a student to apply everything that has been taught. Before being a part of this project I did not realize the importance of everything you learn and how all the concepts are interconnected. In other words this experience enabled me to see the need for what I have learned so far, and what I need to learn in the future to function as a scientist in my profession. The most important point I learned of all those I experienced was collaboration.

Taphonomy of Late Ordovician Cyclocystoids from the Millersburg Member, Lexington Limestone, Central Kentucky. NEIL E. RUSSELL* and FRANK R. ETTENSOHN, Department of Earth & Environmental Sciences, University of Kentucky, Lexington, KY 40506.

Eight specimens from the very rare echinoderm class Cyclocystoidea were recovered on the base of a bed from a former outcrop along I-64 near Winchester, Kentucky. Although no longer extant, the outcrop exposed nodular limestones and shales and a few, coarse-grained, through-going limestone beds in the Millersburg Member from uppermost, Edenian parts of the Lexington Limestone. Based on marginal ossicle count (40–60), disc percent of test (83%–86%), test diameter (16–50 mm), the circle-to-ovoid skeletal morphology, and presence in Edenian rocks, the specimens most likely represent the species *Polytryphocycloides depressus*. Occurrence of this species in central Kentucky may possibly expand its known geographic range. Cyclocystoids are typically encrusters, found on the tops of firm- or hardground beds. In this occurrence, however, the specimens occur on the base of one of the through-going beds. Notably, the cyclocystoids are part of a basal, lag-like concentration with bryozoans, brachiopods, and gastropods in rocks that exhibit subtle planar cross-bedding and graded bedding, features that are indicative of shallow, open-marine storm deposits in the Lexington and Millersburg. The cyclocystoids display taphonomic evidence of transportation and deposition, including contorted and fractured marginal rings, misaligned and missing ossicles, thecae draped over other fossil shells, and possible upside-down orientation. Such a transported fossil assemblage containing cyclocystoids is a heretofore unreported phenomenon and implies that the organisms were gregarious, as well as the nature of the community and environment in which they lived. Previously unknown aspects of hydrodynamic stability, thecal strength, and the nature of attachment are also suggested by the occurrence.

Characterizing Potential Environmental Controls on Stromatolite Morphology Using of Stable-Isotope Analysis and Micro-Stratigraphy. NATHAN W. LANDRUM, MARC F. ETTENSOHN and FRANK R. ETTENSOHN, Department of Earth & Environmental Sciences, University of Kentucky, Lexington, KY 40506.

Characterizing extremophiles, organisms which thrive in extreme environmental conditions, is becoming increasingly important as science tries to grapple with the origin of life and the possibility of extraterrestrial life. Bahamian inland lakes are commonly characterized by hyper- or hyposaline ''extreme'' environmental conditions perfect for such organisms. One such lake, Storrs Lake on the Bahamian island of San Salvador, harbors easily accessible bacterial, archaean, and eukaryotic extremophile assemblages in the form of thrombolites and stromatolites. Storrs Lake is hypersaline alkaline lake which varies drastically in both pH and salinity. The stromatolites in Storrs Lake are carbonate structures precipitated in finely laminated layers to well-cemented thrombolitic clots with inner voids. δO^{18} and δC^{13} isotope analyses have been used as indicators of paleoclimate conditions such as temperature and salinity. We examined the δO^{18} and δC^{13} stable-isotope ratios from a single, Storrs Lake stromatolite head to see if there was some correlation between isotope ratios and morphology, which might suggest environmental control on stromatolite morphology. During the project, a single stromatolite from Storrs Lake was sectioned and stratigraphically characterized as patterns of laminated and thrombolitic layers. Samples were taken accordingly for isotope analyses. δC^{13} values showed a 7.5% variation, both above and below the standard for precipitated carbonates. δO^{18} values remained above the carbonate standard, as would be expected for the extreme environment found in Storrs Lake. Clots layers and individual clots showed lower levels of both heavy isotopes in comparison to laminar layers. There also appeared to be alternating periods with higher and lower levels of heavy isotopes relative to the age and depth of the samples. More visible within the stromatolite are a set of four recognizable 'sequences' which follow a repeating pattern of sediment, laminar layer, then clots. These patterns could be indicative of a long term record of heavy storm activity and might be more plausible as environmental indicators than the microstraticraphy, which, due to graphical noise seems to be more reflective of micro-environmental changes within individual stromatolites.

HEALTH SCIENCES

Is There a Ballistic Gelatin That Mimics the Soft Tissue of a Human Forearm Subjected to a BB Gun Shot? DREW DUERSEN*, DAVID PORTA and BILL TIETJEN, Department of Biology, Bellarmine University, Louisville, KY 40205.

The misuse of BB guns causes a significant number of injuries each year. One of the authors, a healthy, 37 year old man, was subjected to such misuse and suffered a penetrating wound to the right forearm. The gun was a CO_2 powered Daisy-brand Powerline Model 1200. The accident occurred in a laboratory setting and all parameters were recorded. From radiographs it was noted that the BB penetrated 63 mm. Using this precise data, the opportunity was taken to compare the human BB penetration to a laboratory-based series of experiments utilizing ballistic gelatins. A standard gelatin powder (250 Bloom Type A Ordinance Gelatin) purchased from Kind & Knox Gelatine, Inc. (Sioux City, Iowa) was used. Five published methods for the preparation of 10% gelatin were used. The recipes called for relatively minor differences in temperatures and standing times. A total of 25 shots from 126 cm were recorded for each recipe. The mean penetration distance for each recipe was: 1) 62.24 mm; 2) 43.25 mm; 3) 56.13 mm; 4) 64.22 mm, and 5) 40.98 mm. Statistical analyses showed significant differences between each recipe except 1 and 4. The mean values for these particular recipes were closest to the actual penetration of the human subject. Based on this, it seems the preparation method for ballistic gelatin effects the penetration distance of BB gunshots. Although the human data only consists of a population of 1, it appears to be the only such data in the medical literature.

Mandibular Impact Study: Can a Chin Impact Cause a Basilar Skull Fracture? STEPHEN FOWLER* and DAVID PORTA, Department of Biology, Bellarmine University, Louisville, KY 40205.

Basilar skull fractures have significant rates of morbidity and mortality. They are usually caused by impact forces transmitted through facial and/or vertebral bones. Chin impacts, in particular, have been indicated to be a major mechanism for this injury due to the fact that the mandible articulates with the base of the skull at the temporomandibular joint (TMJ). In order to investigate this, 7 embalmed human cadaver craniums were procured. A 1.5 m moment arm was constructed with square tube steel and a wood base with fulcrum. The impacting end consisted of a steel plate (6x6 cm and 1.25 cm thick) connected by a force transducer (Omega DLC101–5k) to the arm. The superior aspect of each cranial vault was removed in order to place each specimen chin up on a flat surface. The surface consisted of clear lexan so that the basilar skull could be viewed by way of an angled mirror placed below. The impact was captured on high speed video at 2000 frames/sec (Photron Fastcam Ultima 1024). The desired impact angle was a line, marked by laser, from symphysis menti to the TMJ. This angle was chosen under the theory that it would maximize mandibular stability, thus transmitting a maximum force to the base of the skull. The mean impact force reading was 2.85 kN (range 2.00 kN to 4.12 kN). Post-test analysis included thorough dissection. Documented injuries consisted mostly of mandibular fractures to the ramus, body, and condylar neck, but one specimen had a basilar skull fracture.

Physical Activity Levels and BMI Status of Preschool Children and Their Caregivers. SUSAN B. TEMPLETON* and MARTHA A. MARLETTE, Human Nutrition Research, Kentucky State University, Frankfort, KY 40601.

Overweight and obesity are increasingly prevalent in America, and the rates are growing fastest among children. One contributing factor often cited is a lack of physical activity. This study was designed to capture data regarding physical activity levels of preschool-aged children and their primary caregivers. Children aged 3–5 were recruited from public preschool programs and at various community events. The physical activity of each child and his/her primary caregiver (if available) was recorded with an Actical accelerometer over seven days. The height and weight of each participant were measured and Body Mass Index (BMI) was computed. Weight status was classified according to CDC guidelines. Five female and eight male children, mean age 3.9 years, participated; ten female caregivers, mean age 32.4 years, also took part. Eleven children (85%) were classified as ''Normal weight''; one was classified as ''At risk of overweight'' and one as ''Overweight.'' Conversely, most caregivers were classified as either ''Overweight'' (17%) or ''Obese'' (58%). Sedentary activities accounted for 67% of time spent by ''Normal weight'' children, compared to 81% of time spent by ''At risk/Overweight'' children. The mean percent of time spent in sedentary activities (68%) was the same for children of ''Normal weight'' and ''Overweight/Obese'' caregivers. However, the percent of time spent at the sedentary level by the caregiver and by the child were positively correlated, suggesting that it is the activity level of the caregiver, not caregiver's weight status, that influences the activity level of the child.

Body Fat Analysis: Comparison of Two Years Measurements during the Kentucky State Fair. MARTHA A. MARLETTE* and SUSAN B. TEMPLETON, Human Nutrition Research, Kentucky State University, Frankfort, KY 40601.

Kentucky is sixth highest in the nation for the number of people that are overweight and obese. At the request from Kentucky Cabinet of Health and Family Services, body fat analysis as by bioelectrical impedance analysis (BIA) for adults and body mass index (BMI) was conducted during eleven days of the Kentucky State Fair. BIA was conducted for 1286 adults in 2005 and 1101 adults in 2006. BMI was calculated for 167 children (under age 21) in 2005 and 147 in 2006. After analysis, each person was consulted on how to improve or maintain his or her body weight and insure adequate nutrient intake and adequate exercise. In 2005, 19% adult males were classified as overweight as compared to 18.3% in 2006. There was little change in the percent of adult males classified as obese, 22.1% in 2005 and 22.5% in 2006. However, the percent for adult females classified as overweight was lower in 2006, from 33.4% in 2005 down to 29.2% in 2006. There was no change in the percent of children whose BMI were classified as ''At Risk for Overweight'' 11.2%. However, for the percent with BMI classified as overweight was lower in 2006, from 22.3% in 2005 down to 6.9% in 2006.

PHYSIOLOGY AND BIOCHEMISTRY

Nicotine Stimulation of the Nitrosylation of Tyrosine Residues in Rat Striatum *in vivo.* RACHEL A. VICKERS* and BOBBY R. BALDRIDGE, Department of Biology, Asbury College, Wilmore, KY 40390.

The leading form of drug abuse is tobacco dependence and many labs are searching to find suitable pharmacotherapies for this problem. Dopamine is involved in reward and motivation, which is why we look to dopamine to find the pathway to addiction. Earlier studies have shown that nicotine causes dopamine transporters (DAT) to approach the synaptic terminal in the medial prefrontal cortex. However, in the striatum nicotine does not bring DAT to the terminal. This study was conducted in order to determine what DAT is doing intracellularly by looking at the novel nitrosylation pathway. Rats were injected with varying dosages of nicotine or saline and the striatum was obtained. The proteins were purified and the immunoblot procedure was performed. The data are not yet fully analyzed.

The effects of diet and social stress on humoral and cell-mediated immunity in *Peromyscus leucopus.* COURTNEY A. THOMASON*, TIFFANY L. HEDRICK and TERRY L. DERTING, Biological Sciences, Murray State University, Murray, KY 42071.

Increased anthropogenic disturbance of natural habitats can create greater physical, nutritional, and social stress than wild animals would normally experience. Human activities also lead to increasing habitat fragmentation. As habitats become more fragmented, population densities of wild animals can change dramatically. Diets can also change, reflecting the disturbed habitats animals occupy. Previous research on *Peromyscus leucopus* suggests that white-footed mice in disturbed areas have ready access to high quality diets, which can contribute to a stronger immune system. We studied individual and combined effects of poor diet quality, in the form of dietary protein, and density on immunocompetence in wild-captured adult white-footed mice. We hypothesized that social stress, in the form of high density, takes a larger toll on the immune system than diet stress, in the form of low protein. Our objective was to determine which stressor, or which combination of protein and density variables, has a greater effect on the immune system in order to better understand the effects of anthropogenic disturbance on white-footed mouse populations. Low dietary protein had a significant negative effect on the cell-mediated immune response in *P. leucopus,* as compared to their counterparts fed a high protein diet. White-footed mice housed in pairs mounted a significantly weaker humoral immune response than those housed individually. *P. leucopus,* a known vector of Lyme disease, are common in and around residential and agricultural areas. Reduced immunocompetence resulting from change in diet quality and/or population density may affect their contribution to transmission of disease to human populations nearby.

Two Germline Variants of the *TGFBR1* Gene in Men with Prostate Cancer. ADAM PFENDT*, AMANDA SCHALK, MICHELLE DUENNES, EMILY DONAWORTH, CHRIS HARTMANN, TONY LIOI, BRIAN WHEELER, MICHAEL MARKEY, JAMES DEDDENS, TAIPING CHEN, LARRY DOUGLASS, BRUCE COLLIGAN, JULIA CARTER and JAMES SCHAEPER, Wood Hudson Cancer Research Laboratory, Newport, KY 41071.

One of six men will be diagnosed with prostate cancer. In the USA, there will be an estimated 234,460 new cases of prostate cancer diagnosed in 2006 and 27,350 men will die from this disease. The objective of this study was to determine if two variants (Int7G24A and TGFBR1*6A) in the transforming growth factor beta receptor 1 gene (*TGFBR1*) are associated with prostate cancer susceptibility and poor clinical outcome. Polymorphisms of the *TGFBR1* gene have been linked to advanced breast, bladder, cervical, kidney, and ovarian cancers. To determine if these two variants are related to an increased incidence of prostate cancer, tissue from patients with prostate cancer as well as non-tumor controls were obtained from archived formalin-fixed, paraffin-embedded surgical specimens. DNA was extracted from the samples and amplified by PCR with one of the primers being fluorescently labeled for TGFBR1*6A studies. After amplification, the samples were injected into an ABI 310 Genetic Analyzer capillary electrophoresis system. An internal size standard was added allowing mobility to be normalized across the sieving matrix, thereby eliminating run-to-run variability and generating accurate sizing across this matrix. Differences in electrophoretic mobility of the fragments correlated with differences in the molecular length of the fragments. The GeneScan Analysis software calculated the unknown DNA fragment size by generating a calibration curve calculated from migration times of different sized fragments thereby permitting identification of TGFBR1*6A. To confirm the presence of the Int7G24A allele, the PCR amplified intron 7 fragment was digested with BsrI. After 18 hr incubation at 65°C, the mixture was loaded onto an acrylamide gel to resolve the BsrI digestion bands and obtain the allelic status. Patients were categorized as having either wild type *TGFBR1* or having either the intronic variant (Int7G24A), the deletion (TGFBR1*6A), or both variants. The frequency of

TGFBR1 variants did not differ in men without cancer and men with prostate cancer. The frequency of *TGFBR1* variants also did not differ in men who died with or from prostate cancer, who survived less than 10 years, or who developed bone or other metastases. We conclude that the Int7G24A and TGFBR1*6A variants in the *TGFBR1* gene are not associated with prostate cancer susceptibility or clinical outcome.

PSYCHOLOGY

The Impact of Major Depressive Disorder Symptoms on Broad and Narrowband AD/HD Rating Scales. COURTNEY L. BROWN* and SEAN P. REILLEY, Department of Psychology, Morehead State University, Morehead, KY 40351.

Rating scales are commonly used in the diagnostic process for determining Attention Deficit/Hyperactivity Disorder (AD/HD). At present, little data are available to clinicians for using these measures to discriminate between attention problems due to AD/HD and those which are secondary to psychiatric disorders. This is especially problematic when the clinical picture is clouded by a history of periodic childhood attention problems. Our prior work has shown that broad symptoms of dysphoria are sufficient to yield highly probable scores on some AD/HD rating scales. The present study examined the extent to which particular broadband (i.e., Brown ADD Scales and non-DSM-IV AD/HD scales from the Conners Adult AD/HD Rating Scales) and narrowband (i.e., Adult AD/HD Self Report Scale and DSM-IV AD/HD scales from the Conners Adult AD/HD Rating Scales) were influenced by Major Depressive Disorder (MDD) symptoms in the absence of AD/HD. Two hundred thirteen college adults without positive histories of AD/HD completed several AD/HD rating scales and the MDD subscale of the Psychiatric Disorders Screening Questionnaire. Significant moderate correlations ($r > 0.30$) were hypothesized and found between the MDD scale and AD/HD symptoms from both broad and narrow band AD/HD rating scales. Partial support was found for the hypothesis that broadband rating scales would yield significantly higher correlations with the MDD measure relative to narrowband AD/HD instruments. Selection of specific Adult AD/HD rating scales is discussed for enhancing accurate detection of AD/HD. Research supported by a Morehead State University Undergraduate Research Fellowship and a grant from KY Statewide EPSCoR.

The Impact of Test Anxiety on Reports of Inattention and Hyperactive-Impulsive Behaviors on the Adult AD/HD Self Report Scale. RICHARD CATES* and SEAN P. REILLEY, Department of Psychology, Morehead State University, Morehead, KY 40351.

Attention Deficit/Hyperactivity Disorder (AD/HD) is a lifespan disorder that frequently goes undetected in adults. Assessment of AD/HD includes self report attention rating scales which rarely have comparative clinical data from psychiatric groups. Such groups frequently have secondary attention problems due to their psychiatric conditions. Prior work has demonstrated significant rates of misclassification of AD/HD on attention rating scales when significant dysphoria is present. In a recent study, chronic high arousal yielded significant false classifications of AD/HD on the well used Adult AD/HD Self Report Scale (ASRS). The present study examined the relationship between a related anxiety construct, test anxiety, and ASRS scores. Some clinicians have argued that test anxiety is part of a more chronic, generalized anxiety condition that becomes pronounced during evaluation situations. Based on prior work demonstrating significant relations between reports of chronic, high arousal and frequent attention problems, we predicted high levels of test anxiety would be significantly related to reports of attention problems on the ASRS. Further, it was predicted that test anxiety would yield a stronger relationship with inattention symptoms in contrast to hyperactive-impulsive symptoms. Two hundred college adults without positive histories for AD/HD completed the ASRS and an assessment of test anxiety using the Test Anxiety Inventory. High levels of test anxiety were significantly correlated ($r > 0.30$) with both inattention and hyperactive-impulsive scores on the ASRS as predicted. However, the magnitude of the relationship was not significantly greater for inattention symptoms. Research supported by a grant from KY Statewide EPSCoR.

The Impact of Specific Symptoms of Anxiety Disorders on Adult AD/HD Self-Report Scale Scores. BERNARD VOSS* and SEAN P. REILLEY, Department of Psychology, Morehead State University, Morehead, KY 40351.

Attention Deficit/Hyperactivity Disorder (AD/HD) is a chronic neurobiological disorder involving difficulties in activity regulation, behavioral inhibition, and inattention. Research studies have shown that anxiety symptoms negatively impact attention, many anxiety disorders share overlapping symptoms with AD/HD, and ADHD is frequently comorbid with a variety of Anxiety Disorders. An overlooked area in adult AD/HD assessment is the degree to which AD/HD rating scales are susceptible to symptoms of DSM-IV Anxiety disorders. Prior work has shown that chronic, high levels of anxiety yield clinically elevated scores classifiable in the AD/HD range on several popular attention rating scales. In the present study, it was hypothesized that symptoms of several DSM-IV Anxiety disorders with a broad worry component (e.g., General Anxiety Disorder) would yield significant relations with scores on the ASRS in the absence of a positive history of AD/HD. Two hundred college adults without positive histories of AD/HD completed the ASRS and the Anxiety Disorders subscales (Agoraphobia, Social Anxiety Disorder, Panic Disorder, PTSD, and OCD) of the Psychiatric Disorders Screening Questionnaire (PDSQ). Partially consistent with predictions, the strongest correlations emerging ($r > 0.30$) between the ASRS and scores on the PDSQ Anxiety Disorder subscales were for those with a broad worry component, including Generalized Anxiety and Social Anxiety Disorders. The applications of these findings to clinical assessment for AD/HD are discussed. Research supported by a Morehead State University Undergraduate Research Fellowship and a grant from KY Statewide EPSCoR.

SCIENCE EDUCATION

Useful Parameters for Assessing Classroom Student Learning Outcomes: A Case Study. JOHN G. SHIBER, Division of Health Sciences & Related Technologies, Kentucky Community & Technical College System-Big Sandy District, Prestonsburg, KY 41653.

In a continuing study of how best to assess classroom learning outcomes in community college biology courses, 593 student performance records from six biology courses, pre-/entry & post-/exit test scores, and end-of-semester survey responses were amassed and reviewed. The survey asked students what percentage of material covered over the semester they felt they had learned and what importance they place on class attendance, graded assignments and the teacher-prepared instructional objectives (TPOs) for their respective courses. They were also asked to comment on the instructional approach and offer suggestions for improvement. The survey responses, together with the instructor's record of the percentage points each student earned for attendance, individual involvement, extra credit work & 'attitude' (labs only) and their final averages, were tabulated then arranged according to the final letter grade earned. The results indicate that using many parameters support better understanding of classroom learning outcomes and are most useful when considered in conjunction with the students' perceptions of their learning and the instructional methods employed to facilitate it. Furthermore, because nearly all students (98%) said that good attendance in class is important to learning and that attendance was found to correlate well with the grades students earned in these classes, it is suggested that community colleges consider creating a fair, uniform attendance policy that would reinforce such a consensus. Requiring regular attendance may help them develop better self-discipline, which many students presently lack. The study also concludes that pre-/post- testing is an important parameter in measuring learning outcomes that ought to be established as a routine assessment tool across the disciplines in two-year colleges.

ZOOLOGY

Kentucky Butterfly Net: An Online Database to Facilitate Research and Education Programs about the Lepidoptera of Kentucky. JEFFREY M. MARCUS, Department of Biology, Western Kentucky University, Bowling Green, KY 42101.

There are approximately 2500 species of Lepidoptera (butterflies and moths) known from the state of Kentucky. Largely through the efforts of Dr. Charles V. Covell, formerly of the University of Louisville, collection information for virtually all of the documented observations of Lepidoptera in Kentucky has been organized into a Microsoft Access database. The database contains almost 50,000 individual records, and covers the years 1872-present. Until recently, this database was not accessible by the public. The database is now available on the web as the "Kentucky Butterfly Net" at http://www.kybutterfly.net. The web implementation includes several new features. First is a password-protected data entry form by which members of the public can report new observations of the Lepidoptera. Once observations are entered into the form, they are evaluated by Kentucky Butterfly Net database administrators with extensive knowledge of the state fauna who can ask the observer for more information, accept the record into the main database, or reject the record. Once entered into the main database, records can be searched by species, locality, or date through open-access query functions. A search by species not only results in a list of all of the records for that species, it also dynamically generates a range map that updates itself as additional records are added to the database. To my knowledge, this is the first on-line implementation of a dynamic range map generator, and it could easily be adapted to dynamically produce range maps for other taxonomic groups in Kentucky.

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