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Kentucky Water Resources Research Institute Annual Technical Report FY 2014

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Kentucky Water Resources Research Institute, University of Kentucky

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Introduction

The 2014 Annual Technical Report for Kentucky consolidates reporting requirements for the Section 104(b) base grant award into a single document that includes: 1) a synopsis of each research project that was conducted during the period, 2) citations for related publications, reports, and presentations, 3) a description of information transfer activities, 4) a summary a student support during the reporting period, and 5) notable awards and achievements during the year.

Research Program Introduction

The activities supported by the Section 104(b) program funds and required matching are interwoven into the overall program of the Kentucky Water Resources Research Institute. Additional research, service, and technology transfer activities were funded through a variety of other sponsors. Memoranda of Agreement projects with the Kentucky Division of Water included Nutrient Management Stakeholder Engagement in the Floyds Fork watershed near Louisville and the development of a numerical water quality model for the basin. The Kentucky River Authority supported watershed management services in the Kentucky River basin and a small grant program to fund local grassroots organizations. The National Institute of Environmental Health Services supported research translation activities through the Superfund Public Outreach Program and the development of ground water remediation processes for potential use at contaminated sites. The Department for Hometown Security funded two projects related to security for water infrastructure. The Kentucky Department for Environmental Protection supported 6 students through an Environmental Protection Scholarship Program.

Nine student research enhancement projects were selected by the Institute's Committee on Research and Policy for support through 104(b) 2014 funding. The projects were conducted at the University of Kentucky (4), Northern Kentucky University (1), Western Kentucky University (1), University of Louisville (1), Morehead State University (1), and Murray State University (1). Projects represented a variety of discipline areas including geology (2), biology (2), civil engineering (1), plant and soil sciences (1), chemistry (1), law (1), and forestry (1). The goal of this approach is to support a number of student-based efforts representing a variety of discipline areas at numerous educational institutions throughout the state to develop a broad research capacity related to water resources. Many state agencies are experiencing a significant loss of personnel in their environmental programs through retirements and it is critical that undergraduate and graduate students are well trained and available to fill this void. A total of 14 students were supported through the 104(b) program (7 male, 7 female; 9 undergraduate, 3 masters, and 2 PhD). Reports for the nine 2014 student research enhancement projects follow.

Diverse participation in watershed planning and governance: Building social-ecological resilience in Kentucky Watersheds

Basic Information

Title:	Diverse participation in watershed planning and governance: Building social-ecological resilience in Kentucky Watersheds
Project Number:	2014KY226B
Start Date:	3/1/2014
End Date:	2/28/2015
Funding Source:	104B
Congressional District:	KY 1, 2, 3
Research Category:	Social Sciences
Focus Category:	Law, Institutions, and Policy, Management and Planning, None
Descriptors:	None
Principal Investigators:	Tony Arnold

Publication

1. Arnold, C.A., Jennifer-Grace Ewa, and Alexandra Chase, 2015, Diverse Participation in Watershed Planning and Governance: Building Social-Ecological Resilience in Kentucky Watersheds, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, p. 25-26.

Diverse Participation in Watershed Planning and Governance: Building Social-Ecological Resilience in Kentucky Watersheds

Problem and Research Objectives

The effectiveness and perceived legitimacy of watershed governance depend on the long-term and adaptive participation of multiple, diverse stakeholders. Watersheds are areas of land that drain to a common body of water. Watershed governance includes any decision making processes, whether by government agencies, community groups, or collaborations among many stakeholders, that develop or adopt plans, decisions, or actions to protect, manage, or restore the watershed or particular watershed features. Participation is defined very broadly. It includes attending a meeting about the watershed, sharing one's opinion in any form with watershed decision makers, joining a group that seeks to address watershed issues, participating in cleanup or restoration activities of streams, creeks, wetlands, or parks or even changing one's behaviors that could affect the watershed for the purpose of protecting the watershed. Watersheds are critical scales for developing governance solutions to problems involving relationships between land and water. Watershed governance institutions have grown exponentially in the United States.

This study examined the barriers to, and best methods for, engaging diverse underrepresented groups in governance over time by conducting in-depth interviews of stakeholders from two watersheds: the Beargrass Creek watershed in Jefferson County, and the Green River watershed in south-central and western Kentucky. Interviewees were individuals who live, work, or engage in recreational activities in the watershed, with a focus on farmers and members of traditionally under-represented socio-economic groups. They included both participants and non-participants in watershed governance. The Beargrass Creek and Green River watersheds were selected for two reasons. First, Beargrass Creek is primarily urban, and the Green River is primarily rural. Second, both are watersheds in which a significant amount of planning, management, and other governance activities have already occurred over many years, yet both have watershed planning processes still currently underway. Thus, the choice of watersheds facilitated research of diverse stakeholders with multiple opportunities to participate in watershed governance in the past, present, or future.

Our objectives were to improve the state of knowledge about participation and stake-holder diversity in evolving watershed institutions and to develop applications of this knowledge (i.e. best practices) that will help watershed governance facilitators to improve the breadth, diversity, and quality of participation by stakeholders and therefore the effectiveness and legitimacy of collaborative watershed problem solving.

Methodology

The investigators contacted two-hundred ninety-one (291) government agencies, civic or community-based organizations, environmental groups, business or agricultural groups, and other leading organizations in the two watersheds in order to identify potential interviewees. Over one-hundred twenty (120) potential participants were contacted with the objective of interviewing at least sixty (60). The interviews were conducted either in-person or by telephone using a semi-structured questionnaire of thirty-three (33) questions. The interview questions asked about the participants' experiences with and perceptions of particular processes in watershed planning and governance, including non-participation and barriers to participation. The questions also asked demographic information to reveal to what extent certain groups participate, which participatory methods they prefer, and which obstacles to participation they perceive. The open-ended nature of the majority of interview questions and the qualitative social-science methods used in this study ensured that interviewees could express their actual perceptions and preferences, instead of having to choose among the researchers' pre-determined options and choices that were limited to quantitative measurement.

Potential participants were very enthusiastic about participating in in-depth interviews about their experiences with participating in watershed governance, but the average length of each interview session and unanticipated scheduling conflicts between researcher and participant schedules meant that we were not able to schedule and complete the interviews as quickly as we had hoped. The large volume and variety of qualitative data from asking open-ended interview questions produced high-quality research results reflecting the participants' perspectives in their own words, but the nature, quantity, and diversity of interview answers took longer to synthesize and analyze than we had anticipated.

Principal Findings and Significance

Forty-two (42) interviews were conducted, nineteen from the Beargrass Creek watershed and twenty-three (23) from the Green River watershed. Underrepresented groups made up twenty-one (21) of the responses, with seven (7) underrepresented groups in the Beargrass Creek watershed and fourteen (14) in the Beargrass Creek watershed. Many more members of underrepresented groups were willing to be interviewed in the Green River watershed than in the Beargrass Creek Watershed. Additionally, six (6) of the twenty-three (23) interviewees in the Green River watershed basin are members of more than one underrepresented groups (minority farmers or low-moderate income farmers).

Concerning watershed problems, ninety-three percent (93%) of all interviewees believe watershed problems are important or very important to them personally. However, only sixty-four percent (64%) believe that watershed problems are important or very important to their community. The disparity between personal interest and perceived community interest in watershed problems was greater in the Beargrass Creek watershed than in the Green River watershed.

The study confirmed that citizens of watersheds overwhelmingly want to participate in watershed governance. Over ninety-five percent (95%) of interviewees stated that they want to participate more or that they do participate and are satisfied with their level of participation. Less than five percent (5%) of interviewees expressed no interest in participation. The level of actual participation varied between the watersheds and for underrepresented groups. In the Beargrass Creek watershed, over seventy-one percent (71%) of underrepresented groups participate in watershed governance compared to nearly forty-three percent (43%) in the Green River watershed. Beargrass Creek groups that are not underrepresented participate at nearly eighty-nine percent (89%) while the Green River has a one-hundred percent (100%) participation rate for the same group. Historically, underrepresented groups participate at a lower rate in watershed governance, but the participation rate of underrepresented groups in the Green River watershed is much higher than in the Beargrass Creek watershed. Nearly all participants have participated 7 or more times within the past year.

The methods of participation were very diverse between both watersheds and among all types of groups. The attitudes towards types of actual or desired participation in both watersheds were consistent across four (4) dimensions. Interviewees answered that participation in watershed governance focuses around levels of personal satisfaction, contribution to self-expression, building social capital (cooperation and trust in the community), and the effectiveness of the participation.

Interviewees noted many different positive (e.g., effective, desired) features of participation in watershed governance. Four (4) categories emerged as commonly repeated answers, participation that was hands on (e.g. water quality sampling, river clean-up), activities with immediate effects (e.g. clean-ups and restoration), local community interaction (e.g. community based meetings, collaborative projects), and legal action (e.g. litigation, regulation, enforcement). Legal action was sometimes distinguished as not building community relationships.

Interviewees' responses varied greatly when asked to identify weaknesses in participation. The main categories of negative attitudes towards participation were problems in meetings with government officials, communication with government officials, large-scale planning efforts, and public hearings. Common responses were that government officials are not listening or are non-responsive to communication, that watershed problems are not addressed effectively, that nothing changes, that there exists a lack of follow-up or implementation and a lack of political will. Negative attitudes towards the types of participation were relatively constant between watersheds with one key distinction. Responses from the Beargrass Creek watershed were very critical of local officials, while the Green River watershed interviewees were more critical of federal and state officials with mixed outlooks regarding local officials.

Attitudes towards future participation in watershed governance varied between both watersheds and among all types of groups. In the Beargrass Creek watershed, one-hundred percent (100%) of underrepresented groups want more participation. Beargrass Creek groups that are not underrepresented are split between over fifty-eight percent (58%) wanting more participation in the future and nearly forty-two percent (42%) participating at their desired level. For underrepresented groups in the Green River watershed, nearly forty-three percent (43%) want more participation, nearly another forty-three percent (43%) are participating at their desired level, and a little over fourteen percent (14%) are not interested in participating. Green River groups that are not underrepresented are split between nearly sixty-seven percent (67%) wanting more participation in the future and slightly over thirty-three percent (33%) participating at their desired level. There is less satisfaction with current level of participation in the Beargrass Creek watershed than in Green River watershed. While there is no clear picture about underrepresented groups overall, all underrepresented group interviewees in Beargrass Creek want to participate more.

Interviewees' responses varied when asked to identify barriers to participation, but there were repeated concerns such as time constraints (lack of personal available time to participate), the timing of meetings, the locations of meetings, weaknesses in the information and the communication in regards to the watershed and its problems and issues, the meetings and opportunities to participate and ways to participate. Additionally, interviewees reported a lack of government responsiveness, concerns that participation was ineffective because it did not yield results or implementation, and that a lack of funding for watershed projects was a barrier to participation.

Based on our findings, we recommend that watershed governance systems use diverse methods of participation, instead of trying to select a single "optimal" method, in order to engage a diverse range of participants, including traditionally under-represented groups, such as farmers, low- and moderate-income persons, and racial and ethnic minorities. Our recommendations include scheduling meetings at a variety of times and locations and using meetings to address a variety of important, substantive issues in significant depth, as well as, encouraging government officials be more responsive to citizen input and to implement stakeholder ideas and plans and to follow-up on proposals. Also, legal action can be utilized to support public participation.

Effects of atrazine and metolachlor on willow in riparian zones along the Kentucky River

Basic Information

Title:	Effects of atrazine and metolachlor on willow in riparian zones along the Kentucky River
Project Number:	2014KY227B
Start Date:	3/1/2014
End Date:	2/28/2015
Funding Source:	104B
Congressional District:	KY 4
Research Category:	Biological Sciences
Focus Category:	Ecology, Non Point Pollution, Toxic Substances
Descriptors:	None
Principal Investigators:	Kristine Hopfensperger, David Thompson

Publications

1. Nagel, A., J. Brown, S. Stryffeler, K. Hopfensperger, and D. Thompson, 2014, Quantification of Atrazine and Metolachlor Dispersal Associated with Land Use in Selected Areas of the Kentucky River Watershed, in Proceedings of the Kentucky Academy of Science, Louisville, Kentucky.
2. Nagel, A., J. Brown, S. Stryffeler, K. Hopfensperger, and D. Thompson, 2015, Quantification of Atrazine and Metolachlor Dispersal Associated with Land Use in Selected Areas of the Kentucky River Watershed, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, p. 51.
3. Stryffeler, S., A. Nagel, J. Brown, and K. Hopfensperger, 2015, Exploring the Effects of Herbicides on Willow Seedlings, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, p. 53.

Effects of atrazine and metolachlor on willow in riparian zones along the Kentucky River

Problem and Research Objectives

Clean water is critical for the function of aquatic ecosystems and for the plants and animals that reside within them. While Americans are now more aware of the importance of clean water than they were before policies such as The Clean Water Act were enacted, we continue to add new pollutants with unknown consequences to our streams and rivers. The way humans use the land can influence the type and amount of pollutants that enter our waterways. In the past, scientists have studied how land uses such as clear cutting negatively affected water quality (Likens et al. 1970); and currently scientists are studying how pollutants from activities like mountaintop mining (Lindberg et al. 2011) and fertilization of farm fields (Turner and Rabalais 2003) continue to harm our Nation's water. Along the Kentucky River, agricultural land use has changed after decades of farming tobacco and hay to farming new cash crops such corn and soybeans. Between the 1950s and 1970s, soybean acreage in the state of Kentucky increased over 700% (Herbek and Bitzer 1988). The shift has not just been in the crop being farmed, but also the management methods applied to the land. Herbicide use is common practice when growing corn and soybeans in Kentucky (Johnson et al. 2003). While some research has been completed on the effects of herbicides on fish and amphibians, research is lacking on the effects herbicides may have on non-target, native trees. Resdients who live along the Kentucky River in Henry County brought to our attention the "disappearance of willow trees" from the stream and river banks in the area. In late spring 2013, we collected pilot data in 23 sites and found atrazine present in 21 and metolachlor present in 4 of the sampled sites.. Our objective was to, "investigate herbicide concentration in the lower Kentucky River watershed and its effect on native willow trees using a combined field and laboratory approach."

Our hypotheses were that:

- Higher atrazine and metolachlor concentrations would be found in the soil and water samples with greater upstream agricultural land cover.
- Atrazine and metolachlor would have a negative effect on willow growth and that there would be lower willow coverage in field areas with higher atrazine and metolachlor concentrations.

Methodology

Field Project – Water samples were collected from 12 sites in the Kentucky River watershed in Henry, Owen and Carroll counties at five different times from late April to mid June 2014 (Figure 1). Soil and vegetation tissue samples were collected once from the sample sites. Standard protocol was used to determine atrazine (ATR) and metolachlor (MET) concentrations in water, soil, and vegetation tissue samples via ELISA (Abraxis). Herbicide concentrations were analyzed via Kruskall-Wallis ANOVA, followed by post-hoc comparisons via Dunn's test. We were not able to measure percent of willow coverage at each sample site, because willow were not present at the sample sites. Dominant tree species, including silver maple and sycamore, were sampled for herbicide concentration instead.

Land Cover – Using ARCGIS, land coverage was analyzed 500 meters around and 1000 meters upstream of each sample site for percent of agricultural land use. Land use was compared to herbicide concentrations via mixed linear model analysis.

Greenhouse Project – Thirty willow tree seedlings were divided into nine treatments (with three replicates each) and planted in the Northern Kentucky University greenhouse with standing water and a mixture of different media – sand, gravel, small pebbles, and organic soil (Table 1). The first herbicide application was given on July 1, 2014, and each plant received a total of 10 mL of their designated application 3 times/week thereafter. The last application was given on August 8, 2014. We measured number of leaves, average midrib length, number of branches, chlorophyll fluorescence, and stem length weekly.

Historic Hydrologic Data – We investigated USGS data at three study locations to determine how the Kentucky River has changed in terms of stage, annual average discharge and maximum discharge over time.

Principle Findings and Significance

Field Project **–** The highest atrazine water concentrations during the sampling period were observed in the Drennon Creek watershed (sites 4-6) and Point Park (site 12, the Ohio River; Table 2). The highest metolachlor water concentrations were observed at sites 5 and 12 (Table 2). Additional analyses suggest that these herbicides are accumulating within the soil and tree leaf tissues (Table 3). Note that all sample sites, except 2 and 3, contained atrazine concentrations in water samples at or above 0.1 ppb at some point during the sample period, with the highest single concentration observed in the Drennon Cr. watershed (3.95 ppb, site 4). Previous research found that atrazine at these concentrations can cause adverse effects on non-target species including, but not limited to fish, humans and *Daphnia* (Cox 2001). The concentrations we found were below any guidance levels provided by the U.S. EPA (Table 4).

Land Cover **–** GIS analysis (Table 5) indicated that the amount of land area used for cultivated crops varied among the sample sites, with the highest percentages occurring at sites 4 (36%), 6 (58%), and 12 (37%). Mixed linear model analysis indicated that the percent cultivated crop land use is significantly related to herbicide concentrations in collected water samples ($p<0.0$).

Greenhouse Project – We found no effect of herbicide on willow seedlings compared to our control treatment in our greenhouse study (Table 6). Our study did not find an effect of atrazine or metolachlor on the growth of willow seedlings. It is possible that we did not find an effect of herbicides because the concentration of atrazine and metolachlor applied to the seedlings were too low, or the daily watering from the greenhouse may have diluted the herbicide concentration. However, our atrazine concentration was over the 10 ppb concentration that the US EPA designates as a level of concern to aquatic ecosystems (Table 4).

Historic Hydrologic Data – We investigated USGS hydrologic data at three study locations (Figure 2). Average annual discharge in Eagle Creek has increased since 1916. The increase is likely due to land use conversion to agriculture and impervious surfaces resulting in additional runoff to the creek (Figure 3). We did not find any increasing or decreasing trends in annual average or maximum discharge, or gage height at Lock 2 on the Kentucky River over time. However, the variation in maximum discharge became more stable over time at Lock 14 (Figure 4). This may be the result of human management of river flow, such as the construction of locks and dams.

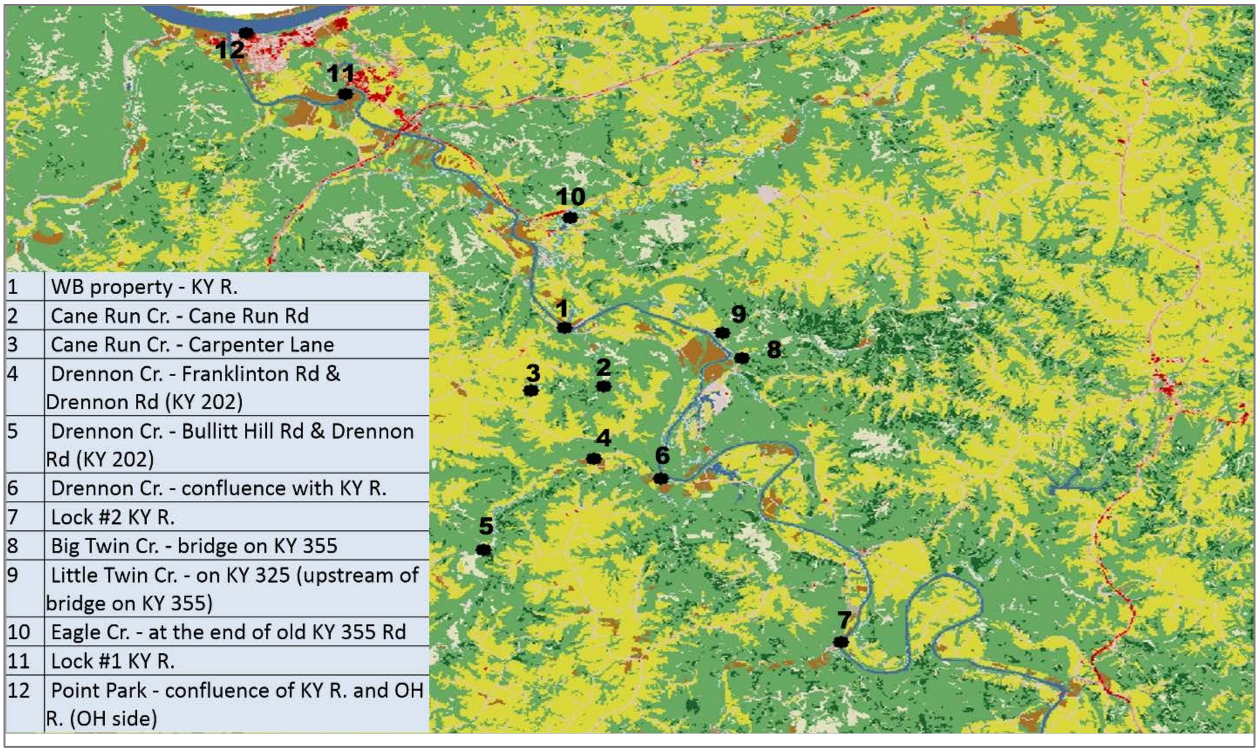


Figure 1. Map of sample sites with land cover (yellow=grass or cropland, green=forest, red=urban).

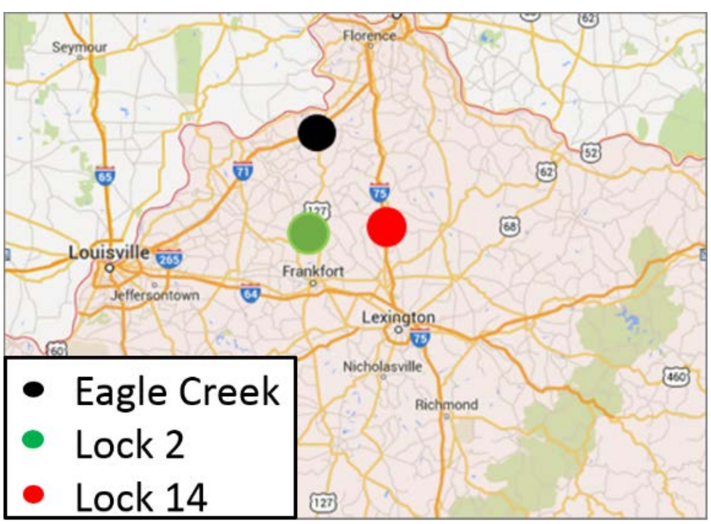
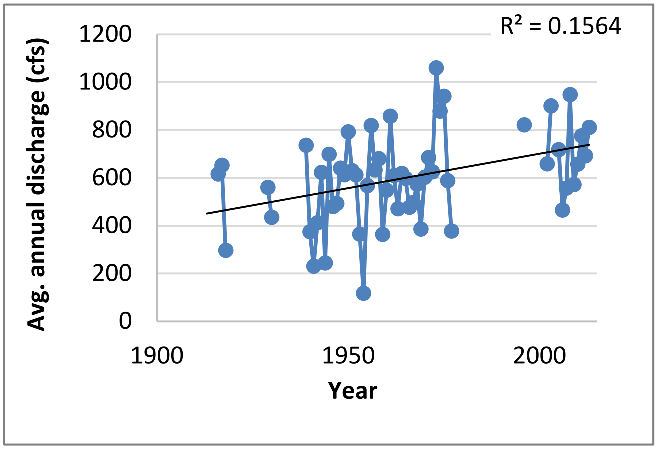


Figure 2. Map of hydrologic data sample sites.



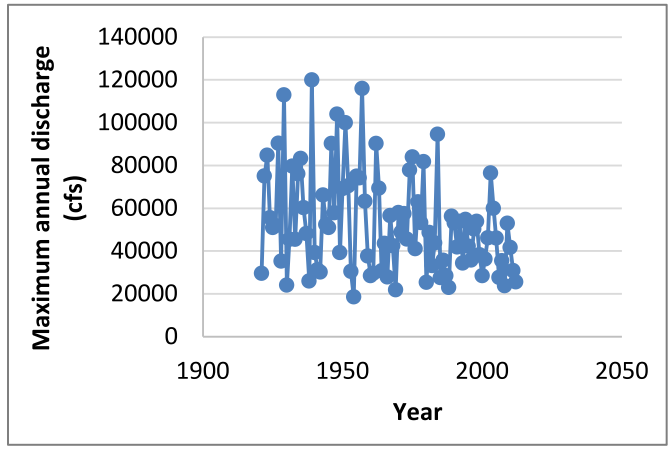


Figure 3. Average yearly discharge at Eagle Creek increased over time.

Figure 4. Maximum yearly discharge at Lock 14.

Table 1. Greenhouse experiment treatments. H=high concentration, L=low concentration, A=Atrazine, M=Metolachlor

Treatment code	Metolachlor (ppb)	Atrazine (ppb)
Control	0	0
LA	0	1.5
LM	0.2	0
HA	0	20
HM	20	0
LMLA	0.2	1.5
LMHA	0.2	20
HMLA	20	1.5
HMHA	20	20

Table 2. Mean ± SEM concentrations during the sampling period. Kruskall-Wallis ANOVA indicated significance for both atrazine and metolachlor ($p<0.05$). Asterisks indicate significant difference from site 3 (lowest herbicide concentrations) via Dunn's test ($p<0.05$). ATR=atrazine and MET=metolachlor concentrations in ppb.

Sites	1	2	3	4	5	6	7	8	9	10	11	12
ATR	0.10 ± 0.04	0.02 ± 0.01	0.01 ± 0.01	1.29 ± 0.69*	1.43 ± 0.61*	0.52 ± 0.17*	0.19 ± 0.07*	0.17 ± 0.08	0.06 ± 0.02	0.08 ± 0.01	0.19 ± 0.06*	1.02 ± 0.29*
MET	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.05 ± 0.05	0.11 ± 0.07*	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.11 ± 0.05*

Table 3. Mean concentrations in the soil and dominant tree leaf tissue (Veg) during the sampling period. ATR=atrazine and MET=metolachlor concentrations in ppb.

Sites	1	2	3	4	5	6	7	8	9	10	11	12
ATR Soil	0.14	0.08	0.09	0.18	0.15	0.09	0.16	0.08	0.07	0.48	0.13	0.29
ATR Veg	4.77	4.25	1.73	3.12	4.04	6	5.49	6.06	3.71	3.3	2.52	6.29
MET Soil	0.45	0.19	0.16	0.17	0.16	0.1	0.06	0.03	0.11	0.06	0.24	0.48
MET Veg	3.77	4.45	5.01	6.16	1.95	3.43	13.08	7.44	5.33	3.44	7.11	3.78

Table 4. Atrazine levels of concern by the US EPA (http://www.epa.gov/oppsrrd1/reregistration/atrazine/atrazine_update.htm).

Drinking water short-term exposure	"Occasional readings of atrazine that are ***below 298 ppb*** in water treated by municipalities do not pose a risk to human health"
Drinking water mid-term exposure	"Our level of concern for drinking water is an intermediate level of exposure where the level is exceeded if, in a 90-day rolling average, the concentration exceeds ***37.5 ppb*** for atrazine and its degradates in raw water."
Drinking water long-term exposure	"A long-term, consistent value above a yearly average of ***3 ppb*** would be of concern"
Aquatic ecosystem	"The EPA is currently estimating the **aquatic ecosystem** level of concern as approximately ***10 ppb*** for atrazine over a 60-day period."

Table 5. Percentage of land area used for cultivated crops at each site.

Sites	1	2	3	4	5	6	7	8	9	10	11	12
Cultivated crops	0	0	4.92	36.0	0	57.8	0	15.9	0	0	0	36.8

Table 6. One-Way ANOVA results from greenhouse study. The response variables measured were not different from the control treatment.

Response variable	F-Statistic	P-Value
Mean Stem Length (cm)	1.236	0.334
Mean # of Branches	0.856	0.569
Mean # of Leaves	0.549	0.805
Mean Midrib Length (cm)	0.424	0.829
Mean Chlorophyll Fluorescence	0.603	0.764

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Turner, RE and NN Rabalais. 2003. Linking landscape and water quality in the Mississippi River basin for 200 years. Bioscience 53:563-572.

Real-time monitoring of phtocatalytic degradation processes

Basic Information

Title:	Real-time monitoring of phtocatalytic degradation processes
Project Number:	2014KY228B
Start Date:	3/1/2014
End Date:	2/28/2015
Funding Source:	104B
Congressional District:	KY 2
Research Category:	Water Quality
Focus Category:	Treatment, Toxic Substances, Wastewater
Descriptors:	None
Principal Investigators:	Matthew Nee

Publications

1. Annarapu, Shashidhar, 2014, Thermal Analysis of Binding of Organic Pollutants to Titanium Dioxide, MS Thesis, Department of Chemistry, Western Kentucky University, Bowling Green, Kentucky, 48 p.
2. Nee, Matthew J., 2015, Thermal Analysis and Raman Studies of the Photocatalytic Degradation of Organic Pollutants, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, p. 5-6.
3. Wallace, Frankie and Matthew J. Nee, 2015, Surface Enhanced Raman Spectroscopy to Study Photocatalytic Degradation of Organic Pollutants, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, p. 57-58.
4. White, Samuel T. and Matthew J. Nee, 2015, Pulsed-Laser Raman and Fluorescence for Environmental and Energy Applications, in Program of the Kentucky EPSCoR 2015 Annual Conference, Kentucky Science and Technology Corporation, Lexington, Kentucky, p. 52.

Real-time Monitoring of Photocatalytic Degradation Processes

Problem and Research Objectives

Recent improvements in the detection of organic compounds in drinking water and water supplies have led to the quantification and relative concern levels of different classes of organic compounds.[1] Some enter water from runoff sources (particularly halocarbons, agricultural materials, and polycyclic aromatic hydrocarbons), while others enter from household or medical sewage streams (notably pharmaceuticals, recently detected in drinking water supplies).[2,3] Among the plans which are promising for the removal of organic compounds is treatment with a photocatalyst (such as titanium(IV) oxide, TiO_2) in the presence of ultraviolet (UV) radiation.[4,5] Raman spectroscopy can be a valuable tool in analyzing the underlying chemical mechanisms involved in this degradation process, with the goals of improving efficiency, efficacy, and safety.[6] The original objectives of the funded project were to fully develop our photocatalytic reactor (Objective 1) and to incorporate a recently funded high-power pulsed laser into our setup to use in a Raman spectrometer (Objective 2). Ultimately, the two objectives will still be merged, though during this annual funding period, some deviation from that path was necessary. During summer 2014, our spectrometer was largely out of the lab for repairs, which interfered with our ability to complete reactor development. Instead, a nanoparticle-based surface enhanced Raman spectroscopy (SERS) technique which shows outstanding potential for the development of quantitative measurement of photocatalytic systems was developed. Subsequently, we successfully developed our pulsed Raman spectrometer to provide preliminary data from that new instrument that will be used to describe trends in how well different compounds adsorb to TiO_2 photocatalysts.

Methodology

The original methodologies remain intact: our new laser has been coupled to a monochromator to detect Raman scattered radiation. New methodologies include the use of SERS for quantitative detection of analytes. We use our modified reactor (Figure 1) to test the usefulness of SERS nanoparticles for the analysis of photocatalytic degradation experiments. Gold nanoparticles have been used for years as substrates to provide million-fold enhancement of Raman signal.[7-9] As a method of quantitative measurement of analyte concentration, however, SERS suffers from inconsistency, both batch-to-batch and spectrum-to-spectrum within a sample. To overcome this difficulty, we have co-adhered carbon-disulfide (CS_2) to our gold nanoparticles. CS_2 has a very strong Raman cross section, while the terminal

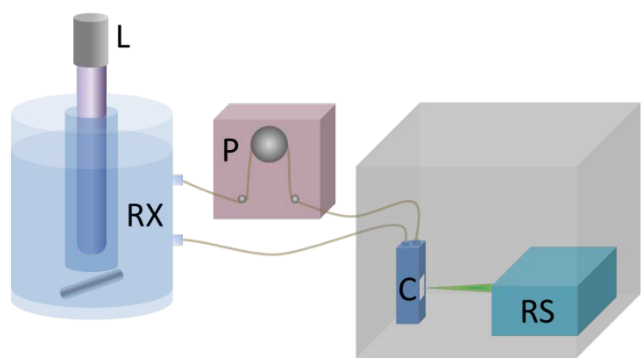

Figure 1. Experimental setup, showing UV lamp source (L) emitting into the center of the reactor (RX), with reaction mixtures pumped (P) to a cuvette (C) in a darkened enclosure for Raman analysis by an appropriate spectrometer (RS).

sulfur groups bind exceptionally well to the gold nanoparticle surface. This powerful combination allows us to use a ratiometric approach, scaling the peak areas of the analyte to the peak area for the single peak in CS_2, even when CS_2 is added in micromolar concentrations.

Thermal analysis techniques have been initiated to study the adsorption of analyte molecules to the photocatalyst, TiO_2.[10] Suspensions of TiO_2 were made in solutions of analytes to be studied. The suspensions were stirred for one hour to ensure equilibration of adsorption of the analyte to the TiO_2 surface and then filtered. The filtrate was slowly heated under carefully controlled thermogravimetric conditions to reveal the temperatures at which adsorbed analyte separates from the photocatalyst. These techniques have been significantly extended to include differential scanning calorimetry and crystallographic analysis. Collectively, these methods provide fundamental insight into the extent to which different compounds are able to interact directly with the photocatalyst surface.

Principal Findings and Significance

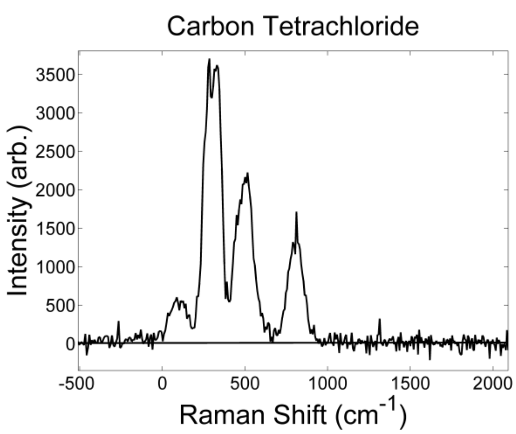

Figure 2. Raman spectrum of carbon tetrachloride collected with our new pulsed-laser instrument. The three peaks from CCl_4 are well resolved. Improvements in both peak width and signal-to-noise ratios are underway.

Figure 2 demonstrates the progress in our pulsed Raman spectrometer by showing a spectrum collected by excitation with our Nd:YAG laser at 532 nm. The laser power is significantly higher than necessary: less than 1% of the maximum power is used to avoid sample damage, which frees the remainder of the laser power to be used for other projects, including a ns fluorescent lifetime instrument, also constructed for use in collaborations with our colleagues at Western Kentucky University. The pulsed Raman spectrometer is nearly ready to be incorporated with our reactor vessel. We had originally hoped to complete this before the end of the funding period, but we feel that the gains accomplished by the introduction of the SERS aspect of the project significantly outshine the slight delay in initial project completion, given that they overcome one of the major limitations of using Raman to study photocatalytic degradation of organic pollutants.

Figure 3 shows a sequence of data to demonstrate the power of our SERS methodology. The SERS nanoparticles were optimized for the identification of rhodamine 6G (R6G). Representative spectra are shown in the left panel, while the right panel shows that there is a direct correlation between analyte concentration in solution and the surface-enhanced Raman signal observed. The most notable feature here is that Raman signal can be detected at nanomolar concentrations of R6G. We are poised to perform quantitative analysis on the destruction of such compounds while adsorbed onto the surface of gold nanoparticles. We have also demonstrated recently that similar spectra can be observed for the pesticide paraquat (not shown here).

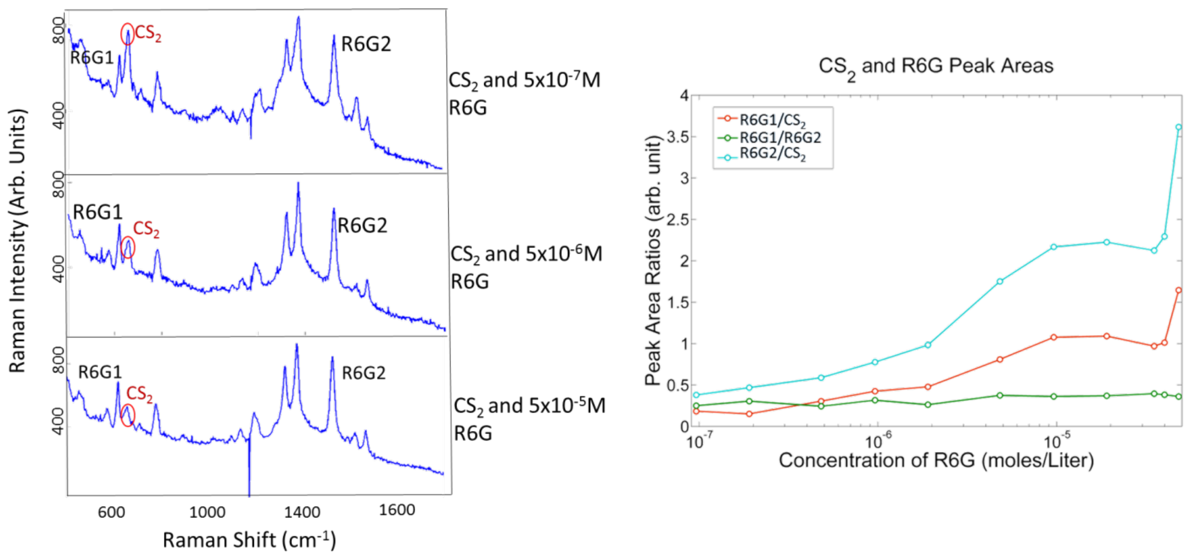

Figure 3. SERS data (left panel) shows that a ratiometric approach can be used with an analyte (the dye R6G) and an internal standard (CS_2) to get quantitative determination of the concentration of the analyte solution (right panel). All spectra use the same amount of CS_2 with differing amounts of R6G.

We are in the process of documenting the adsorption of different compounds to TiO_2. Example data are shown in Figure 4 for the iodinated contrast media studied previously in our group. This will include a comparison of the relative binding strengths of compounds based on structural motifs (particularly the dominant side chains). These data are critical to understanding the extent to which direct oxidation of compounds by electron holes at the TiO_2 surface plays a role in the photocatalytic degradation of organic pollutants in wastewater.

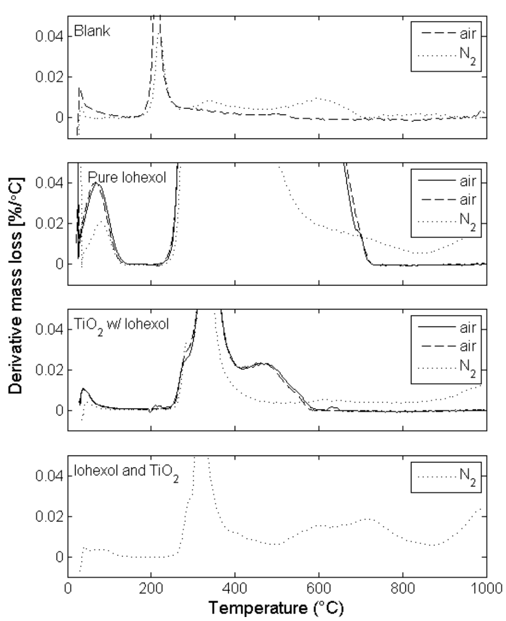

Figure 4. Thermogravimetric analysis curves showing derivative mass loss from iohexol@TiO_2. From top to bottom, results are shown for pure TiO_2, pure iohexol, iohexol adsorbed onto TiO_2, and a simple mixture of iohexol and TiO_2 (no adsorption period in suspension)

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Investigating the influence of Asian carp on primary productivity of Kentucky Lake

Basic Information

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End Date:	2/28/2015
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Descriptors:	None
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Publication

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Investigating the Influence of Asian carp on Primary Productivity of Kentucky Lake

Problem and Research Objectives

The objective of this project was to quantify the influence of invasive silver carp on primary productivity in Kentucky Lake. We conducted diet analysis of invasive bighead (*Hypophthalmichthys nobilis*) and silver carp (*Hypophthalmichthys molitrix*) in Kentucky Lake, but the results only reflect invasive silver carp because of the low sample size of bighead carp (n=3). Long term trends of lake primary production were investigated pre- and post-invasion of silver carp. Long term trends in primary production were analyzed utilizing 23 years of limnological data, collected by Hancock Biological Station (HBS) with the Kentucky Lake Long Term Monitoring Program (KLMP). Investigating long term trends in primary production may help answer the question: Are invasive silver carp impacting phytoplankton dynamics in Kentucky Lake?

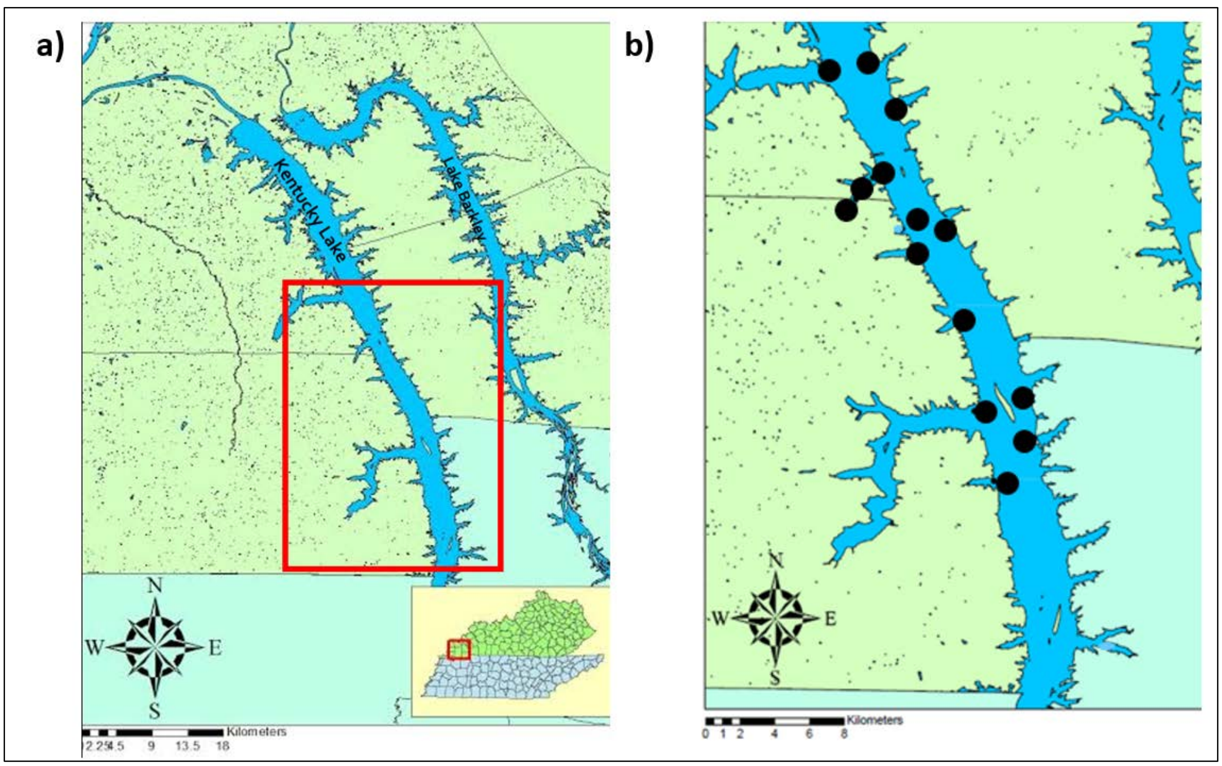

Figure 1: a) The geographic Position of Kentucky Lake and Lake Barkley. Kentucky Lake stretches over 296 km covering a surface area of 64874 hectares from southwest Kentucky to Northwest Tennessee. Hancock Biological Station's (HBS) Kentucky Lake Long Term Monitoring Program (KLMP) samples the lower 30 km of Kentucky Lake, outlined here with a rectangle. b) A magnified view of the fourteen study sites of the KLMP included in this study. Map created by M. Torres.

Methodology

We focused on the diets of invasive silver carp sampled selectively from large reservoir ecosystems in the southern United States (U.S.). To describe invasive silver carp diet, we sampled a combination of embayment, channel and tailwater sites in Kentucky Lake and Lake Barkley of southwestern Kentucky (Table 2). Kentucky Lake is a main-stem reservoir formed in 1944 and is the last of several reservoirs created on the Tennessee River by the Tennessee Valley Authority (TVA). Kentucky Lake is a large, shallow, mesotrophic reservoir of approximately 296 km in length with a surface area of 64874 hectares, making it the largest reservoir of the eastern U.S. We sampled five sites within Kentucky Lake, which encompassed one main channel site and four embayment sites, weekly from May 2014- October 2014. We also opportunistically sampled the tailwaters of Lake Barkley from June-July 2014. Lake Barkley is the last and largest impoundment of the Cumberland River and is geographically parallel with Kentucky Lake (Figure 1a). The two reservoirs are connected with the Barkley Canal located 4.7 km upstream of Kentucky Dam. Invasive silver carp proved difficult to capture so sampling sites were chosen to encompass a range of reservoir habitats and diet information was combined over sampling sites and compared temporally across sampling month.

Fish were collected from Kentucky Lake sites with mono-and-multifilament gill nets (mesh=10.1 cm stretch). Gill nets were typically deployed on a weekly basis in Kentucky Lake sites and checked twice daily, allowing for approximately twelve soak hours between net checks. Gill netting efforts were conducted over a four day period allowing each net to soak for a total of at least 72 hours over the sampling week. Fish were sampled in the tailwaters of Lake Barkley with boat electroshocking, utilizing 5.0 GPP (Generator Powered Pulsator) Electrofishing System from Smith-Root, Inc. We operated at four to six amps at 120 pulses per second at slow speeds behind spillways of Barkley Dam. The hull of the boat acted as the cathode and the anode was constructed as an umbrella array on extended booms submerged partially in the water. Electrofishing occurred on two dates during the sampling period in June and July.

Table 1: Locations and months of entire invasive silver carp catch from September 2013-September 2014 from both Kentucky Lake study sites and tailwaters of Lake Barkley.

Site	Kentucky Lake					Lake Barkley	
	Blood River	**Ledbetter**	**Hancock Bay**	**Anderson Bay**	**Main Channel**	**Tailwaters**	
Sep-13	4	-	-	-	-	-	
Oct-13	-	5	-	-	-	-	
Nov-13	1	-	-	-	-	-	
May-14	8	-	3	-	-	-	
Jun-14	-	14	0	-	-	18	
Jul-14	2	6	-	40	8	-	
Aug-14	-	3	-	8	-	-	
Sep-14	-	-	-	9	5	-	
Total(n)	15	28	3	57	13	18	134

Invasive silver carp captured were processed according to Murray State University Institutional Animal Care and Use Committee (IACUC) protocol number 2014-008. Fish were placed on ice until further processing. Total length (TL) was measured to nearest mm, and weight was measured to nearest kg. The weight resolution was not a concern for our purposes, as our analyses did not compare among size classes, and our sampling methodology selected for similarly sized fish.

The foregut (to the first bend in esophagus) of invasive silver carp was removed by carefully opening the ventral side of the fish from the anus to the base of the operculum (Gelwick and Matthews 2006). Prey items within the foregut were typically less digested and allowed for more accurate prey identification (Suetela and Huusko 2000). Once opened the start of the esophagus was located and pinched off with a binder clip. The esophagus/foregut was followed until the first bend, and an additional binder clip was fixed to contain contents of the study section. The study section of the gut was removed from the fish and contents were extruded into a Whirl-Pak®. Contents within the Whirl-Pak® were then preserved with 70% ethanol and transported to the lab for microscope analysis.

Foregut contents were shaken vigorously inside of the Whirl-Pak® to remove prey contents from gut mucilage (adapted from Sampson et al. 2009). These contents were then transferred to a larger beaker and homogenized via manual stirring. Three, 1 ml aliquots were removed with a pipette from the homogenized gut sample, and placed onto a Gridded Sedgewick Rafter cell (Wildlife Supply Company). Although the field depth of a Sedgewick Rafter cell is more appropriate for zooplankton identification than phytoplankton, we noticed Palmer counting cells (i.e. a method appropriate in nanoplankton identification) became overwhelmed with gut mucilage. Prey contents were identified over twenty five randomly selected views at 400X magnification. Phytoplankton were enumerated by cell count and identified to genus or lowest taxonomic level possible (Wehr and Sheath 2002). Exceptions were made for colonial cyanobacterium (e.g. *Microcystis* sp.*)* because in some instances colonies contained over 1000 cells, for the purposes of our study we measured an approximate area of the colony and assigned a cell diameter of 1 µm (Wehr and Sheath 2002) and extrapolated biovolume from this estimate. Many non-colonial coccoid algae lacked identifiable features, in these cases we were unable to differentiate cyanobacteria from green algae. This required the lumping of these observations under the category of “Little Green Ball” (LGB). Relative biovolume was estimated based on geometric equivalents of phytoplankton taxa (Hillebrand et al. 1999). Originally we aimed to measure dimensions for 20 individuals of each taxa represented, however some taxa had less replication because of low observation. Zooplankton in stomach samples were identified to genus or lowest taxonomic level possible (Smith 2001) and quantified based on relative biovolume with microscope measurements and geometric equivalents.

Temporal differences in carp diet were compared based on relative proportions of prey biovolume across sampling month (May–September 2014) using a one-way ANOVA. Significant comparisons were determined via *post-hoc* Tukey Kramer HSD. We compared diets based on functional groupings. All computations and analyses were performed using R statistical software, version 3.1.2 (R Development Core Team 2014).

We focused on long term limnological data collected by KLMP from the lower 30 km of Kentucky Lake, in southwestern Kentucky (Figure 1). Since its inundation, Kentucky Lake has been home to exotic species including vegetation (*Hydrilla verticillata),* mussels (*Dreissena polymorpha*, *Corbicula* sp.) and zooplankton *(Daphnia Lumholzi*) - (Levine and White *unpublished data*). However, few exotic fish species have been as prevalent or invasive as the Silver and Bighead Carp. Significant population increases of invasive silver carp were reported in 2004 (Paul Rister of KDFWR- *personal communication*). According to data from commercial fish harvest, catch rates of invasive silver carp have continued to increase rapidly, and the harvest of invasive silver carp has increased exponentially (KDFWR *unpublished data*, Figure 2). Utilizing the KLMP we can investigate aspects of primary production before and after the invasion of silver carp.

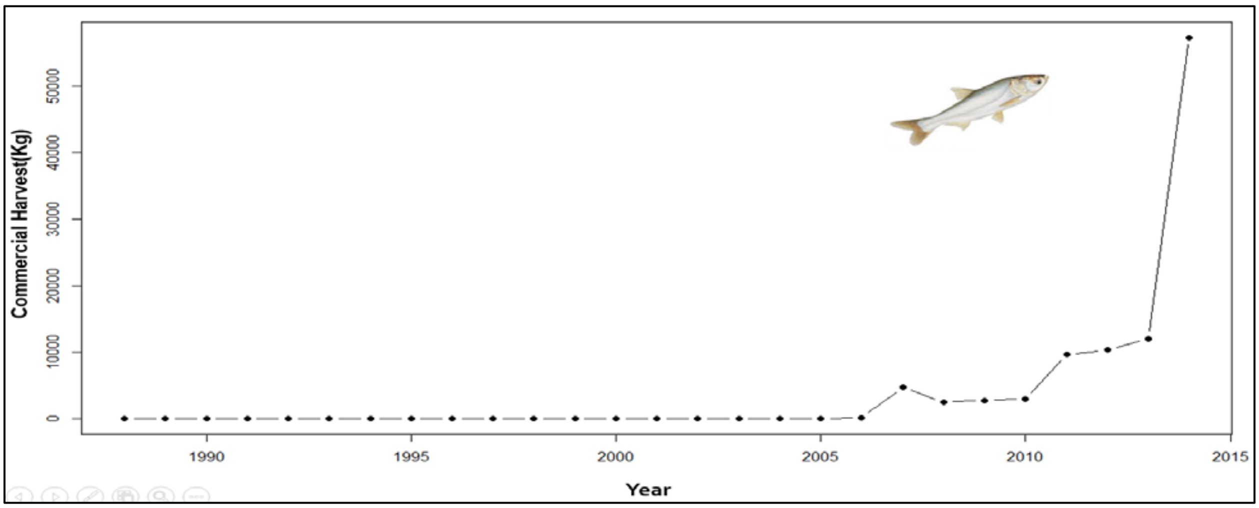

Figure 2: Commercial harvest of invasive silver carp (Kg) from Kentucky Lake from 1988 to 2014. Data accessed through KDFWR 2014.

HBS has conducted sampling cruises on Kentucky Lake since July 1988, with the KLMP. Sampling Cruises occurred every 16 days (32 in winter). On each sampling cruise, the KLMP collected data on over 40 biotic and abiotic limnological parameters, across 16 sites from a range of habitats, of the lower 30 km of Kentucky Lake (White et al 2007). To test our predictions we pared down this data to encompass biologically significant times of year, and a range of reservoir habitats. We were unable to use data from 1988 & 1989 because of differences in sampling methodology, which limited our data set to 23 years of consistent sampling from 1990-2013. We examined a range of limnological variables from the month of May (Table 2. For a more detailed summary see White et al. 2007). May was chosen as a significant time of year because of the potential for biological (e.g. primary production) and chemical (e.g. nutrient availability) maxima (summarized in, Wetzel 2001). In our analysis, we strived to focus on ecologically limiting factors of primary production. We hypothesized that focusing on production patterns during times of ecosystem maxima will be most pertinent in understanding drivers of primary production. An understanding of the system specific limitations to primary production is paramount in determining potential impacts of invasive silver carp. Therefore, the temporal analysis was centered on the timing of the proposed invasion of invasive silver carp into Kentucky Lake. Information on commercial harvest of invasive silver carp were used to define the time periods of “pre-invasion” (1990-2004) and “post-invasion” (2005-2013). We understand the clear limitations in basing our invasion time periods off of agency and commercial harvest reports, however no long term monitoring of fish populations exists for Kentucky Lake.

Table 2: Limnological Measurements utilized in nonmetric multidimensional scaling ordinations from Kentucky Lake Long Term Monitoring Program (KLMP) and Tennessee Valley Authority (TVA).

Measurement	Abbreviation	Units	Data Source
Biological			
Carbon Assimilation	Cassim	µg Carbon/Liter/Hour	KLMP
Chlorophyll-a	Chl-a	µg Chlorophyll-a/Liter	KLMP
Cladoceran Density	Zoopden	Individuals/Liter	KLMP
Physiochemical			
Soluble Reactive Phosphorus	srp	mg Phosphorus/Liter	KLMP
Turbidity	ntu	Ntu	KLMP
Sechhi Depth	secchi	Meters	KLMP
Water Temperature	Temp.	°C	KLMP
Reservoir Discharge	Q	Cubic Feet/Second	TVA
Reservoir Retention	Retention	Number of Days	TVA

Spatial analysis included fourteen of the sixteen sampled sites (two sites were excluded from the analysis because of missing data). The sites chosen encompassed three major reservoir habitats, embayment, embayment mouth, and main channel. These reservoir habitats were hypothesized to express different limnological characteristics. We were interested in investigating these habitat differences, while controlling for site specific dependencies in the temporal analyses. These reservoir habitats were used to develop the framework for our spatial analysis and are hereafter referred to as site type. In addition, a land-use intensity component was added to the spatial analysis framework. Embayment mouth sites on the western side of Kentucky Lake were associated with more intensive land-use (e.g. agriculture and residential development) while sites on the eastern side of Kentucky Lake were associated with low intensity land-use (i.e. Land Between the Lakes National Forest Recreational Area). Understanding habitat specific patterns in primary production may help in determining system specific limnology. Additionally, patterns revealed in the spatial analysis helped with the interpretation of temporal analysis.

Spatial and temporal patterns of primary production were examined using non metric multi-dimensional scaling (NMDS, McCune and Grace 2002). The NMDS ordinated sample units (14 study sites in spatial frame work & 2 periods in temporal framework) in multivariate space were defined by primary production, Chlorophyll-a, and Soluble Reactive Phosphorous. We conducted NMDS using DECODA© (Minchin 2005) following the general procedures including the maximum number of iterations (200), step length (0.2) and the satisfactory stress stopping value (0.0100) recommended by Minchin 1987. The Bray-Curtis Distance was used as a measure of dissimilarity. Starting configurations (100) were randomly generated by the program, stepping down from 3-1 dimensional solutions. Dimensionality was determined by examining scree plots (stress vs. number of dimensions) and interpretability of additional axes. The minimum stress for each chosen ordination and the number of starts needed to arrive at this stress were reported for each ordination.

Vector fitting analysis was performed with sample traits (Table 3) to aid in interpretation of primary production ordination. Sample traits used in the vector fitting analysis were reported to show strength of relationships with environmental variables. Vectors were evaluated by examining the correlation coefficients and *p*-values <0.05 were accepted and plotted. Primary production values were tested for temporal differences based on invasion period, pre-invasion (1990-2004) versus post-invasion (2005-2013). Primary production values were tested for spatial differences based on *a priori* reservoir habitat type (i.e. main channel versus embayment mouth versus embayment). All comparisons were examined using analysis of similarity (ANOSIM, Clarke and Green 1988; Clarke 1993) using DECODA© (Minchin 2005). The Gower metric was used in the ANOSIM analysis (1,000 permutations). Generating an r-value between -1 and 1. Positive values indicated differences among groups and significance was tested at $\alpha<0.05$. Analyses were conducted on May samples from 1990-2013.

Principal Findings and Significance

A total of 134 invasive silver carp were caught for this study, of which 116 were caught from embayment and channel sites of Kentucky Lake and 18 from the Tailwaters of Lake Barkley. An overall catch per unit effort (CPUE) of 0.029 fish/net hour for the duration of the study was calculated based upon a total of 4.062 net hours. CPUE varied by sampling month, ranging from 0 fish/net hour in January & March 2014, to a high of 0.0632 fish/net hour in July 2014. The highest CPUE values were observed during the summer of 2014 (Figure 3).

Table 3: ANOSIM results for ordinations of temporal invasion period and spatial analysis based on site type and location. Invasion period analysis compared pre- (1990-2004) and post-invasion (2005-2013). Spatial analysis compared site types (embayment and main channel) and land-use intensities (east or west shore) from 1990-2013.

	ANOSIM-R	
Invasion Period Ordination	*R*	*P*
Pre-invasion vs. Post-invasion	0.1076	<0.0001 *
Spatial Ordination		
East side vs. West side	0.0186	0.1650
East side vs. main channel	0.0059	0.1290
East side vs. embayment	0.0679	0.0010 *
West side vs. main channel	0.0591	0.0010*
West side vs. embayment	-0.0118	0.06340
Main channel vs. embayment	0.1310	<0.0001*

* Considered significant *p* value <0.05

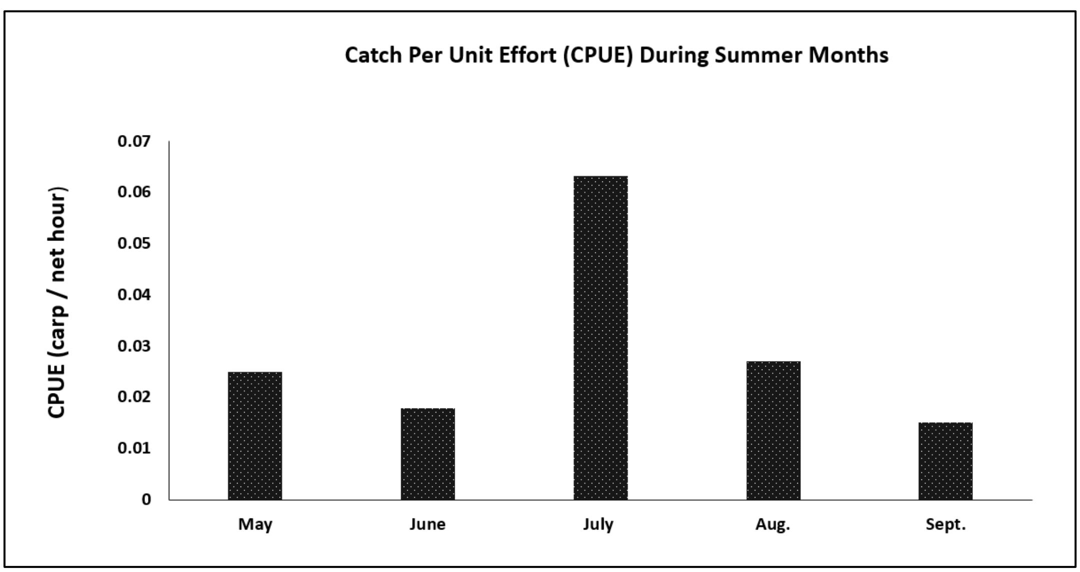

Figure 3: Catch per unit effort (CPUE) measured as number of invasive silver carp per net hour from May-September 2014.

Sixty (60) gut tracts were analyzed for the diet analysis (Appendix 1). Diets included fish captured from May 2014 through August 2014. Fish were collected from Kentucky Lake and the tailwaters of Lake Barkley. The diets were predominantly phytoplankton (≈85%) and secondarily zooplankton (≈15%, Figure 4a). The majority (≈90%) of phytoplankton biovolume in diets was comprised of LGB algae. Diatoms made up approximately 2% of the overall diet, dominant taxa included Fragilariophyceae (*Asterionella* sp.) and Coscinodiscophyceae (*Melosira* sp.). A combination of colonial green algae (Cholophyceae), colonial cyanobacteria (*Microcystis* sp.), dinoflagellates, and desmids made up the remaining proportion of phytoplankton biovolume. Zooplankton in diets were represented by Cladocera, Monogononta, and Maxillopoda (Figure 4a). Cladocera were the least abundant zooplankton prey item; however, they accounted for ≈ 36.2% of the zooplankton biovolume across all diets. Monogononta (*Keratella* sp.) were the most abundant zooplankter in the diets and accounted for ≈ 35.1% of the total zooplankton biovolume across all diets. Maxillopoda (including nauplii) comprised ≈ 28.8% of zooplankton across all diets. When functional groups were compared between sampling month (Figure 4b) the proportion of diatoms of (Bacillariophyceae) were shown to increase significantly ($p<0.05$) in July samples.

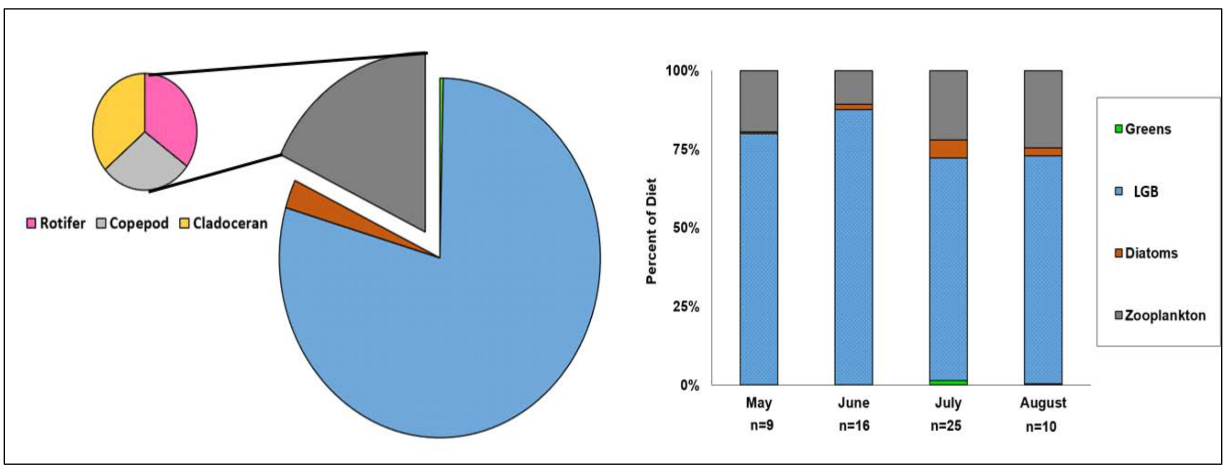

Figure 4: Diet composition of invasive silver carp (n=60) from May to August 2014. a) Overall proportion of diet from all sampling months based on relative biovolume. The exploded portion of the pie diagram explains the relative contributions of zooplankton functional groups to the broader zooplankton category. b) Diets based on sampling month.

The results of the diet analysis of invasive silver carp were consistent with studies completed in other systems (Cremer and Smitherman 1980, Spaturu and Gophen 1985, Starling and Rocha 1990, Williamson and Garvey 2005, Sampson et al. 2009). These studies have investigated invasive silver carp diet in a range of habitats around the world. Studies have reported diets from large rivers in the United States (U.S.) (Mississippi River-Williamson and Garvey 2005, Illinois River- Sampson et al. 2009), reservoirs in Brazil (Starling and Rocha 1990), experimental conditions in lakes of Israel (Spaturu and Gophen 1985) and ponds in the Southern U.S. (Cremer and Smitherman 1980). The relative contribution of phyto-and-zooplankton to invasive silver carp diet varies among these studies. Some of the results of this study differ from other diet studies conducted in the U.S. Sampson et al. (2009) showed that phytoplankton had little contribution to diets, whereas our results indicate it is a predominant aspect of the diet. However, the zooplankton taxa found within the diets of our fish were consistent with Sampson et al (2009). The differences in phytoplankton contribution may be due to study system differences. (i.e. Illinois River vs. Kentucky Lake). Additionally, differences in methodology allowed Sampson et al. (2009) to focus on higher taxonomic resolution of zooplankton, and use that information to quantify diet overlap with native planktivores. The results of our study and other diet studies validate the trophic standing of invasive silver carp as omnivorous planktivores. There is evidence to support that invasive silver carp can affect biota of aquatic ecosystems through a suite of mechanisms (Spataru and Gophen 1985, Burke et al. 1986, Starling and Rocha 1990, Domaizon and Devaux 1999, Xie and Yang 2000, Lu et al 2002, Radke and Kahl 2002, Irons et al. 2007, Guangjie et al. 2011, Sass et al. 2014).

Little work has been done to investigate long term ecosystem effects of invasive silver carp. A handful of studies have experimentally shown that invasive silver carp can reduce or change primary production and/or zooplankton communities in short duration enclosure experiments (Spataru and Gophen 1985, Burke et al. 1986, Starling and Rocha 1990, Domaizon and Devaux 1999, Guangjie et al. 2011). An even smaller number of studies have investigated long term effects of invasive silver carp (Xie and Yang 2000, Irons et al. 2007, Sass et al. 2014). Xie and Yang (2000) report that increased stocking of silver and bighead carp induced changes in copepod communities of a shallow eutrophic reservoir. Irons et al. (2007) found that decreases in native fish body condition in the Illinois River USA were related to exponential increases in silver and bighead carp populations. Sass et al. (2014) reported stark changes in zooplankton community structure within the Illinois River after the establishment of invasive silver carp. Findings from long-term analyses illustrate the ecological implications of silver carp invasion, and should motivate researchers to further consider their long-term effects.

A total of 528 samples of primary production were analyzed both temporally and spatially. All nonmetric multidimensional scaling solutions were interpreted within two dimensions at acceptably low stress values (stress < 0.2). Temporal nonmetric multidimensional scaling ordination revealed a high degree of overlap between pre- and post-invasion (Figure 5). Analysis of similarity (ANOSIM) reveal no significant differences between pre and post invasion, and a high degree of overlap (Table 3). However, the post invasion conditions demonstrate greater variability in primary production.

Vector analysis showed that orientation of primary production was correlated ($r > 0.5$) and highly significant ($p < 0.0001$) with Carbon Assimilation (Cassim) Chlorophyll-a (chla) and Soluble Reactive Phosphorus SRP-Table 5. Additionally, vector analysis indicated that primary production was correlated ($r > 0.4$) and highly significant ($p < 0.0001$) with hydrologic factors such as reservoir discharge (Q) and retention (ret), Table 4. Other limnological factors such as water temperature and turbidity (ntu) were highly significant ($p < 0.0001$) but showed lower correlation ($r = 0.32$) with the ordination (Table 4). Additionally, cladoceran density (zoopden) and secchi depth (secchi) were highly significant ($p < 0.0001$), however they expressed little to no correlation ($r = 1.91$ and .266 respectively) with the ordination (Table 4). Portions of the post invasion ordination are correlated with lower values of carbon assimilation based on vector interpretation

(Figure 5). Post-invasion conditions might be associated with lower levels of carbon assimilation than those of pre-invasion. Temporal analysis suggests differences in primary production since the invasion of silver carp. However, this analysis also indicated the importance of other limnological factors such as nutrient availability, hydrology, water temperature and turbidity (Figure 5). It will be important to see how physiochemical variables change over time, in order to better understand the reasons for differences in primary production. Furthermore, this analysis was conducted without differentiating between reservoir habitats. Habitat specific differences in primary production will be important to control in temporal analyses. Additionally, physiochemical processes such as hydrology are expected to influence main channel primary production differently than primary production of embayments.

Table 4: Correlations (r) of all limnological vectors in NMDS ordination for temporal and spatial analysis of Kentucky Lake from 1990-2013.

Limnological Variables	*r*	*P*
Biological		
Carbon Assimilation	0.9214**	<0.0001*
Chlorophyll-a	0.9274**	<0.0001*
Zooplankton Density	0.1906	<0.0001*
Physiochemical		
Phosphorous	0.5977**	<0.0001*
Turbidity	0.3195**	<0.0001*
Secchi	0.2666	<0.0001*
Temp.	0.3194**	<0.0001*
Discharge	0.4790**	<0.0001*
Retention	0.4086**	<0.0001*

* Considered significant *p* value <0.05

**Considered a significant fit and was used as a vector in the final NMDS ordination

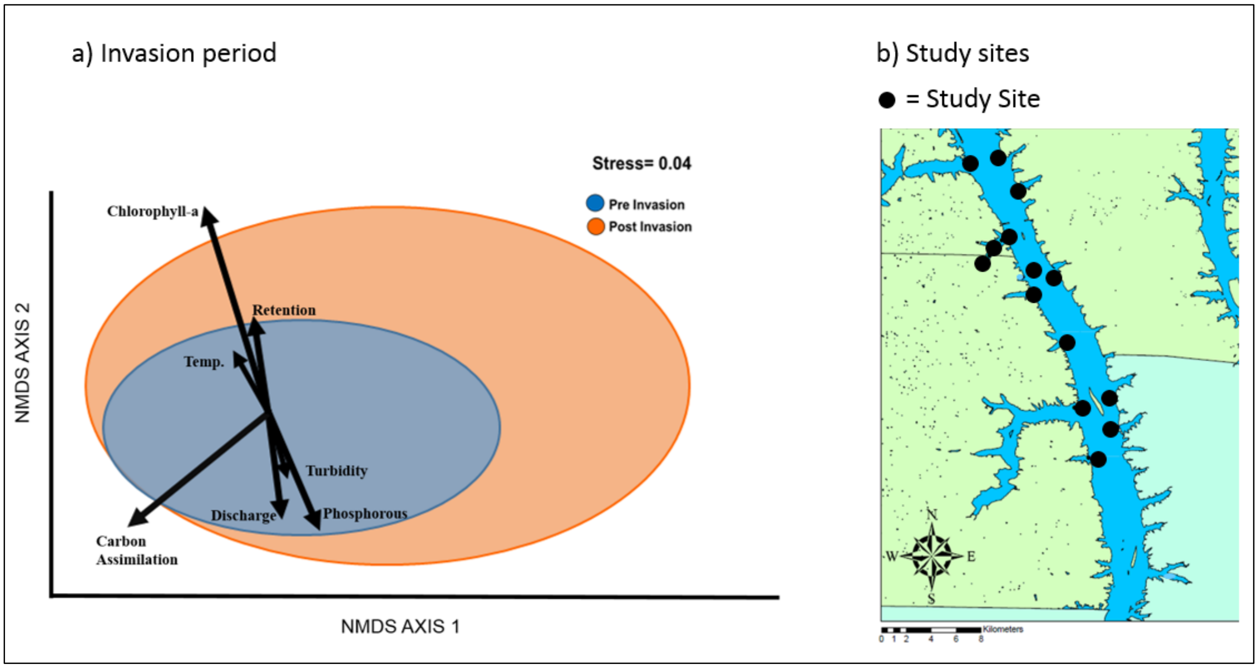

Figure 5: a) Nonmetric multidimensional scaling ordination of Kentucky Lake primary production pre invasion (blue) and post invasion (orange). b) Study sites (n=14) from which limnological variables were equally sampled from 1990-2013. Limnological variables were analyzed from the month of May each year. Significantly correlated limnological variables are shown as vectors. The vector labeled "Chlorophyll-a" is the combination of site specific chlorophyll-a measurements. The vector labeled "Carbon Assimilation" is the combination of site specific carbon assimilation measurements. The vector labeled "Temp." is the combination of site specific water temperature measurements. The vector labeled "Turbidity" is the combination of site specific turbidity measurements. The vector labeled "Phosphorus" is the combination of site specific soluble reactive phosphorus measurements. The vector labeled "discharge" is the measurement of Kentucky Dam discharge from a specific sampling date. The vector labeled "Retention" is the measurement of reservoir hydrologic retention time from a specific sampling date.

Spatial analysis was conducted using NMDS and ANOSIM based on three reservoir habitats and two differing land-use intensities. To investigate spatial patterns, we examined primary production between embayment and main channel habitats. The land-use component of this analysis compared primary production between sites located on the eastern shore (low intensity) and sites of the western shore (high intensity). Ordination based on reservoir habitat and land-use revealed a high degree of overlap between site types based on primary production (Figure 6). The vector fitting was replicated from the temporal analysis (Table 4).

Spatial analysis shows that different habitat types (e.g. main channel or embayment) within Kentucky Lake are very similar in terms of primary production (Figure 7). We suggest that this result is the product of highly variable data.. High intra-site variability presents statistical challenges in detecting biologically relevant differences between site types and across time. Highly variable data may reflect false negatives in regard to temporal and spatial aspects of Kentucky Lake primary productivity.

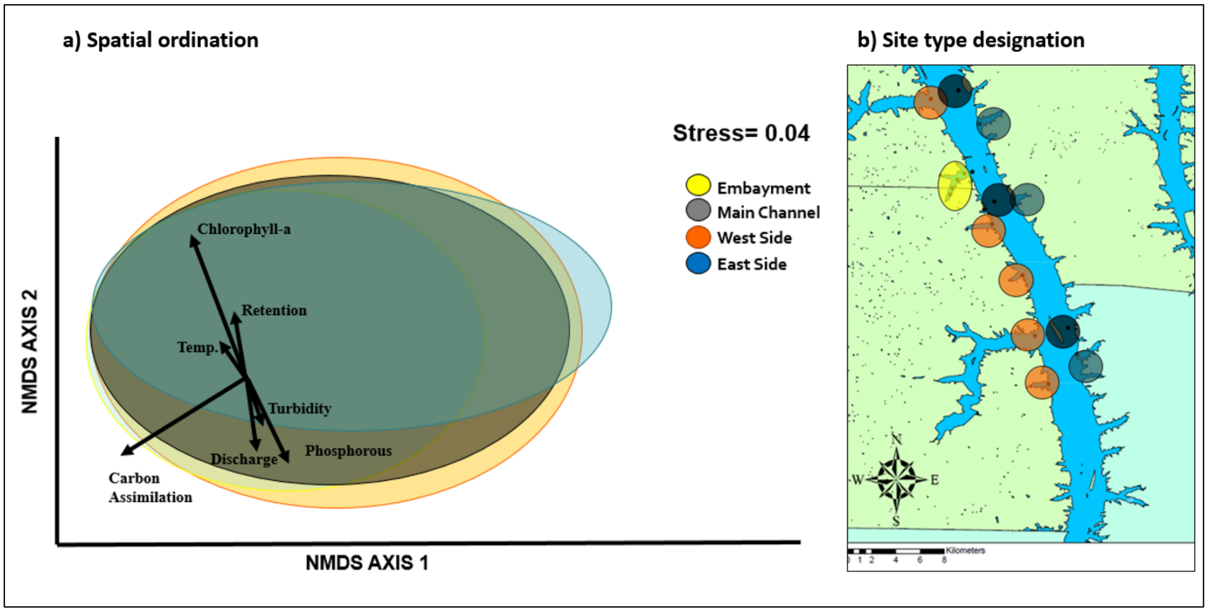

Figure 6: a) Nonmetric multidimensional scaling ordination of Kentucky Lake primary production based on site type, embayment (yellow), main channel (gray), west side embayment mouths (orange), east side embayment mouths (blue). b) Study sites (n=14) from which limnological variables were equally sampled from 1990-2013. Limnological variables were analyzed from the month of May each year. Significantly correlated limnological variables are shown as vectors. The vector labeled "Chlorophyll-a" is the combination of site specific chlorophyll-a measurements. The vector labeled "Carbon Assimilation" is the combination of site specific carbon assimilation measurements. The vector labeled "Temp." is the combination of site specific water temperature measurements. The vector labeled "Turbidity" is the combination of site specific turbidity measurements. The vector labeled "Phosphorus" is the combination of site specific soluble reactive phosphorus measurements. The vector labeled "discharge" is the measurement of Kentucky Dam discharge from a specific sampling date. The vector labeled "Retention" is the measurement of reservoir hydrologic retention time from a specific sampling date.

There were no statistical differences between site types using NMDS and ANOSIM. Additionally, there was no statistical difference in primary production since the invasion of invasive silver carp based on NMDS and ANOSIM. However, additional temporal analyses showed statistically lower values of carbon assimilation and chlorophyll-a since the invasion of invasive silver carp (ANOVA $P<0.001$). The temporal differences in primary production may be the product of changes in reservoir processes (e.g. hydrology) or the intensive feeding of invasive silver carp, however these conclusions need to be investigated further. During the period of post-invasion, Kentucky Lake experienced the second lowest and highest water year on record. Extreme contrasts in environmental conditions (e.g. discharge, water level) influence our ability to detect changes in reservoir responses, even though patterns of primary production are relatively sensitive. Nonetheless, our results show that Kentucky Lake primary production is highly variable spatially and temporally.

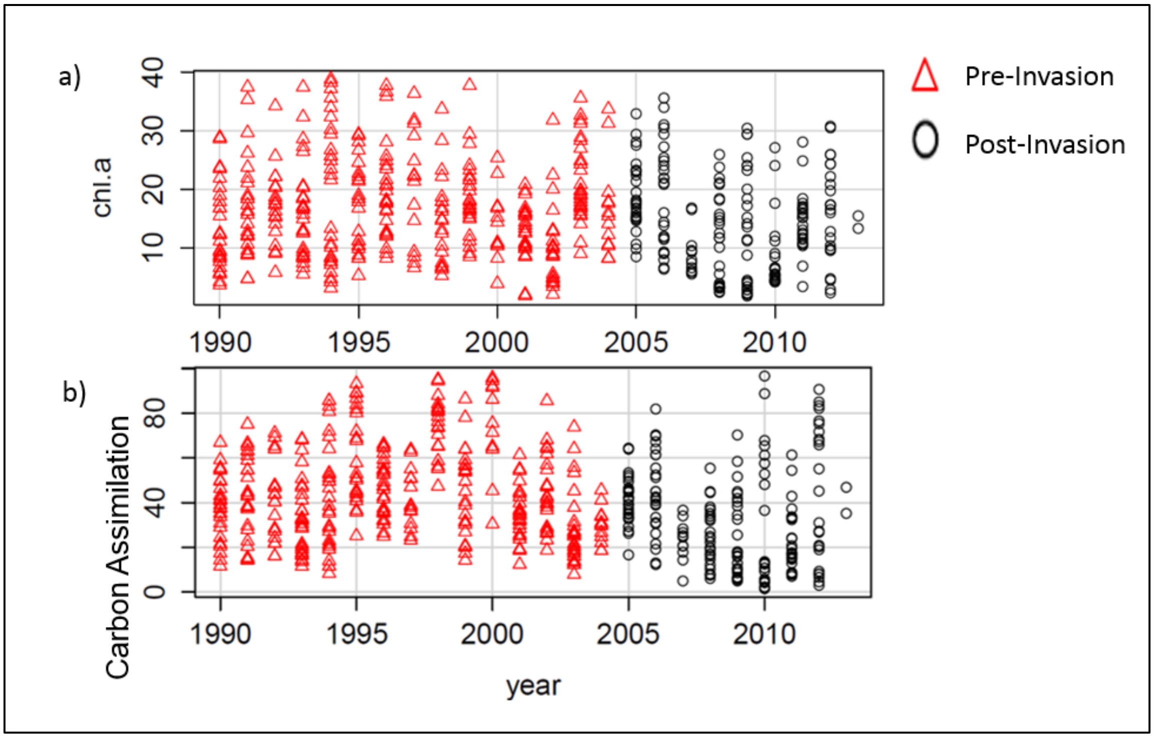

Figure 7: Primary production based on two measurements during the month of May from 1990-2013 from KLMP Cruise Data. a) Primary production measured as chlorophyll-a. b) Primary production measured as carbon assimilation. Both measurements reveal the highly variable nature of primary production in Kentucky Lake. Red Triangles indicate the pre-invasion period (1990-2004) black circles indicate the post-invasion period (2005-2013).

Understanding primary production patterns in Kentucky Lake allows us to assess the potential impacts of invasive silver carp. Given the variation in primary production over time, we can calculate a level of primary production decline that would be statistically detectable. We will apply power analyses to quantify the effect change that could lead to detectable reductions in primary production. Quantifying this effect change allows us to ask important questions about invasive silver carp. Do invasive silver carp pose a threat to ecosystem primary production? It is understood that ecosystem processes such as hydrology and nutrient cycling are principal drivers of primary production in reservoirs (Thorton et al 1990). We can assess the likelihood of changes in these ecosystem processes accounting for detectable changes in system primary production. Initial modeling of primary production in Kentucky Lake suggests that reductions would have to be catastrophic in order to be detectable. We hypothesize that invasive silver carp (at current densities) would not be capable of reducing primary production to statistically significant levels based on the previous patterns over the last 23 years. Additionally, we feel it is unlikely that typical reservoir processes could cause significant levels of reduction in primary production. However, an increase in invasive silver carp densities may reduce primary production to levels that create biologically significant changes, by creating resource limitations for certain organisms. Given our current analyses, we are unable to quantify biologically significant differences in primary production, and the potential ecosystem consequences of these reductions.

An understanding of system specific limnology will be important in discerning between environmentally driven changes in primary production and effects of invasive silver carp. A better understanding of the Kentucky Lake ecosystem and the reservoir response to invasive species is locally applicable to scientific and management interests. Additionally, Kentucky Lake is one of the few reservoirs in the U.S. with robust long-term monitoring which includes environmental and biological data collected before the invasion of silver carp. This dataset allows researchers to investigate the effects of invasive silver carp on other biotic factors (e.g. zooplankton) within Kentucky Lake. Many reservoirs of the Southern U.S. are limnologically similar to Kentucky Lake and have established populations (or are under the threat of invasion) of silver carp. Long-term analyses of Kentucky Lake may prove useful in modeling the effect of invasive silver carp on numerous ecosystems that have been invaded by silver carp across the Southern U.S.

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Appendix 1. Supplemental Diet Information. Relative biovolume of functional prey groups for diets of invasive silver carp(n=60) used in this study. Fish are organized by month.

	May								
Fish ID Number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Relative Biovolume									
Phytoplankton	88.66	50.43	49.69	85.46	99.94	98.77	95.06	92.62	63.69
Chlorophyceae	0.01	0.02	0.03	0.01	0.01	0.01	0.03	0.00	0.01
LGB	92.94	49.47	87.15	85.36	99.83	98.70	94.79	92.57	62.73
Diatom	0.00	0.00	0.00	0.01	0.00	0.00	0.02	0.00	0.00
Bacillariophycea	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fragilariophyceae	0.02	0.09	0.04	0.01	0.00	0.00	0.02	0.01	0.44
Coscinodiscophyceae	0.32	0.84	2.79	0.09	0.11	0.06	0.22	0.00	0.45
Dinophyceae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Desmid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Zooplankton	11.34	49.57	50.31	14.54	0.06	1.23	4.94	7.38	36.31
Monogononta	5.45	49.57	43.82	0.90	0.06	0.05	4.94	0.37	10.95
Maxillopoda	5.90	0.00	6.48	13.64	0.00	1.18	0.00	7.01	25.24
Cladocera	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	June								
Fish ID Number	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
Relative Biovolume									
Phytoplankton	63.76	90.69	87.12	97.18	100.00	78.52	78.52	98.86	98.30
Chlorophyceae	0.49	1.43	0.23	0.00	0.00	0.02	0.01	0.00	0.01
LGB	73.94	82.18	86.19	97.09	99.56	99.39	99.39	98.82	98.01
Diatom	2.47	5.88	0.71	0.09	0.02	0.41	0.34	0.11	0.25
Bacillariophycea	0.02	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fragilariophyceae	0.49	1.26	0.18	0.04	0.00	0.34	0.28	0.09	0.14
Coscinodiscophyceae	1.96	4.61	0.53	0.04	0.02	0.07	0.06	0.02	0.11
Dinophyceae	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Desmid	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Zooplankton	36.24	9.31	12.88	2.82	0.00	21.48	21.48	1.14	1.70
Monogononta	17.11	4.18	12.88	2.82	0.00	21.48	21.48	0.00	0.27
Maxillopoda	19.13	5.10	0.00	0.00	0.00	0.00	0.00	1.14	1.43
Cladocera	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Appendix 1 Cont.

	June						
Fish ID Number	**19**	**20**	**21**	**22**	**23**	**24**	**25**
Relative Biovolume							
Phytoplankton	97.50	65.31	86.65	90.81	99.96	99.96	93.91
Chlorophyceae	0.00	0.29	0.03	0.25	0.00	0.00	0.00
LGB	97.46	62.63	84.93	76.13	99.68	99.59	93.65
Diatom	0.06	2.16	1.61	10.75	0.02	0.07	0.25
Bacillariophycea	0.00	0.04	0.00	0.05	0.00	0.00	0.00
Fragilariophyceae	0.01	1.37	1.25	6.93	0.02	0.05	0.20
Coscinodiscophyceae	0.04	0.74	0.36	3.76	0.00	0.02	0.05
Dinophyceae	0.00	0.19	0.08	0.00	0.00	0.00	0.00
Desmid	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Zooplankton	2.50	34.69	13.35	9.19	0.04	0.04	6.09
Monogononta	0.00	26.46	0.84	9.17	0.04	0.04	6.09
Maxillopoda	2.50	8.20	12.51	0.00	0.00	0.00	0.00
Cladocera	0.00	0.00	0.00	0.00	0.00	0.00	0.00

	July									
Fish ID Number	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**
Relative Biovolume										
Phytoplankton	72.09	82.93	93.58	91.53	82.10	62.64	100.00	98.93	98.13	79.84
Chlorophyceae	0.09	0.25	0.09	1.67	1.07	0.23	10.66	0.13	0.70	0.04
LGB	69.92	79.69	92.13	22.76	63.65	46.74	66.53	96.21	92.18	20.93
Diatom	0.03	0.04	0.01	0.51	0.22	0.24	0.21	0.03	0.05	0.42
Bacillariophycea	0.00	0.01	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00
Fragilariophyceae	1.44	1.84	0.79	24.27	7.54	8.05	20.94	1.24	2.95	0.37
Coscinodiscophyceae	0.60	1.04	0.53	24.06	9.80	7.35	0.04	1.32	2.10	0.35
Dinophyceae	0.04	0.10	0.04	0.00	0.00	0.24	0.00	0.00	0.00	0.34
Desmid	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Zooplankton	27.91	17.07	6.42	8.47	17.90	37.36	0.00	1.07	1.87	20.16
Monogononta	0.76	16.00	1.12	8.47	8.05	14.73	0.00	1.07	1.87	20.16
Maxillopoda	27.15	1.06	5.30	0.00	9.85	22.63	0.00	0.00	0.00	0.00
Cladocera	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	97.60

Appendix 1 cont.

	July									
Fish ID Number	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**
Relative Biovolume										
Phytoplankton	96.04	76.72	99.83	100.00	88.20	40.19	99.47	84.05	86.31	84.25
Chlorophyceae	0.20	0.57	0.01	0.01	0.02	0.13	1.05	0.47	1.14	0.01
LGB	92.19	68.97	98.78	99.83	80.86	21.17	63.04	50.31	42.06	83.06
Diatom	0.03	0.10	0.01	0.00	0.07	0.63	0.03	0.27	0.41	0.01
Bacillariophycea	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00
Fragilariophyceae	1.44	4.37	0.12	0.03	0.74	5.68	0.90	8.51	21.84	0.25
Coscinodiscophyceae	1.25	2.73	0.08	0.04	0.33	3.36	2.02	8.84	12.90	0.21
Dinophyceae	0.00	0.00	0.00	0.00	0.00	0.23	1.02	2.08	3.15	0.00
Desmid	0.01	0.02	0.02	0.00	0.00	0.06	0.00	0.00	0.13	0.00
Zooplankton	3.96	23.28	0.17	0.00	11.80	59.81	0.53	15.95	13.69	15.75
Monogononta	3.96	5.24	0.17	0.00	7.95	47.51	0.53	15.95	13.72	15.75
Maxillopoda	0.00	18.04	0.00	0.00	3.84	12.33	0.00	0.00	0.00	0.00
Cladocera	0.00	0.00	73.80	0.00	82.29	58.82	28.78	0.00	0.00	0.00

	July				
Fish ID Number	**46**	**47**	**48**	**49**	**50**
Relative Biovolume					
Phytoplankton	82.79	98.36	90.55	99.52	77.65
Chlorophyceae	0.09	0.08	0.23	0.01	0.83
LGB	82.17	91.49	78.32	99.30	59.85
Diatom	0.01	0.02	0.10	0.00	0.09
Bacillariophycea	0.00	0.00	0.04	0.00	0.00
Fragilariophyceae	0.08	0.46	1.59	0.03	5.06
Coscinodiscophyceae	0.45	0.40	1.84	0.09	1.91
Dinophyceae	0.00	0.13	0.59	0.08	0.00
Desmid	0.00	0.01	0.00	0.00	0.47
Zooplankton	17.21	1.64	9.45	0.48	22.35
Monogononta	0.55	0.80	9.45	0.48	4.97
Maxillopoda	16.66	0.83	0.00	0.00	17.48
Cladocera	0.00	44.61	59.53	0.00	0.00

Appendix 1 cont.

	August									
Fish ID Number	**51**	**52**	**53**	**54**	**55**	**56**	**57**	**58**	**59**	**60**
Relative Biovolume										
Phytoplankton	99.62	99.33	98.33	61.57	93.95	55.77	71.97	89.74	73.22	44.74
Chlorophyceae	0.02	0.01	0.01	2.60	0.01	0.21	0.14	0.20	0.05	0.06
LGB	97.74	99.15	98.09	0.00	93.85	48.18	19.97	66.98	68.77	39.80
Diatom	0.34	0.16	0.08	10.38	0.04	2.62	1.19	3.92	1.19	1.36
Bacillariophycea	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.02	0.00	0.00
Fragilariophyceae	0.10	0.13	0.04	3.11	0.02	1.27	0.58	2.26	0.42	0.46
Coscinodiscophyceae	0.24	0.03	0.04	8.70	0.01	1.43	0.63	1.81	0.79	0.91
Dinophyceae	0.54	0.00	0.13	33.73	0.05	4.65	20.51	18.04	1.36	1.03
Desmid	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.07	0.00
Zooplankton	0.38	0.67	1.67	38.43	6.05	44.23	28.03	10.26	26.78	55.26
Monogononta	0.38	0.21	0.00	38.43	0.00	2.12	2.02	1.84	2.77	5.51
Maxillopoda	0.00	0.46	1.67	0.00	6.05	42.12	26.01	8.42	24.02	49.75
Cladocera	0.00	0.00	0.00	0.00	0.00	0.00	31.43	0.00	30.56	0.00

Development of passive sampling techniques for the characterization of subsurface contamination

Basic Information

Title:	Development of passive sampling techniques for the characterization of subsurface contamination
Project Number:	2014KY230B
Start Date:	3/1/2014
End Date:	2/28/2015
Funding Source:	104B
Congressional District:	KY 6
Research Category:	Ground-water Flow and Transport
Focus Category:	Geochemical Processes, Water Quality, Methods
Descriptors:	None
Principal Investigators:	Kelly G Pennell

Publication

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Development of Passive Sampling Techniques for the Characterization of Subsurface Contamination

Problem and Research Objectives:

Vapor intrusion of volatile organic compounds (VOCs) which originate from hazardous waste sites has gained more widespread attention in recent years because of adverse human health effects. Indoor air quality is often overlooked in terms of chemical exposures; and, vapor intrusion of VOCs from contaminated groundwater emanating from hazardous waste sites into buildings is one of the pathways that has been shown to greatly impact indoor air quality. Transport of VOC vapors through soil; and the effects of various factors such as wind pressure gradients, soil permeability, and soil porosity make this process complicated for analysis and modeling. Laboratory analytical methods capable of measuring VOC concentrations present in indoor environments exist; however, to date there is a lack of measurement methodologies that are directly relevant to the vapor intrusion pathway.

Sorbent-based mass flux measurement devices have been used to evaluate hazardous waste contamination in groundwater and soil vapor with some success. Using groundwater flux devices, such as the Passive Flux Meter (PFM) (Hatfield et al., 2004; Annabel et al., 2005; Cho et al., 2007) is a superior technique (as compared to chemical concentrations) for assessing risks associated with groundwater contamination. However regulatory reliance on concentration-based clean-up standards limits the wide-spread use of PFMs for groundwater. The emergence of the more recent concern, vapor intrusion, provides a new opportunity to consider the advantages of flux measurements. As part of this research, we evaluated vapor flux measurements using direct vapor measurement techniques, as well as passive sampling techniques that incorporate permeable sorbent media onto which vapor-phase VOC contaminant mass is passively sorbed. The research was aimed at evaluating mass flux rates in soil columns so that flux measurement techniques could be developed. The major objectives of the research were to: 1) develop analytical capabilities to conduct vapor phase sampling, and; 2) to design, build and test a soil column for vapor flux analysis.

Methodology

Preliminary experimental work focused on sorbing contaminant vapors (tetrachloroethene, PCE) onto sorbent media. Following vapor-phase sorption, the contaminant was desorbed from the sorbent media using solvent extraction. Two different solvents (methylene chloride and ethyl acetate) were compared for extraction and desorption efficiency. Ultimately, methylene chloride performed best. Following chemical extraction, the solvent was injected into a gas chromatograph (GC) for analysis. Aside from sorbent-based sampling, we also evaluated direct injection of vapor samples into the GC.

To investigate diffusive flux rates of VOCs through soils, we designed and built a soil column. Figure 1 shows the general layout of the soil column. Column 1 serves as the chemical source. It can be connected to Column 2, which is packed with uniform sand. We conducted experiments with the soil column to evaluate vapor phase flux. Samples were collected from the ports at specified times and analyzed using the GC methods discussed above. Table 1 provides soil property information.

Table 1. Summary of Soil Column Properties

Soil characteristics (ASTMD422 Test &Constant Head Method Test)	
Size mm	D30: 0.28 , D50: 0.35
Density of soil (kg/m^3)	1.57 E3
Specific Gravity	2.65
Porosity	41.42 %
Permeability (m/s)	6.234E-4
Intrinsic permeability (m^2)	6.385E-11
Properties of PCE	
D air-PCE (M^2/S)	7.65E-06
D Water-PCE (M^2/S)	1.09E-09
Henry's Law Constant	0.754
Effective Diffusion (M^2/S) (water content function)	0 - 100 % water content 2.36136E-06 - 4.44295E-10

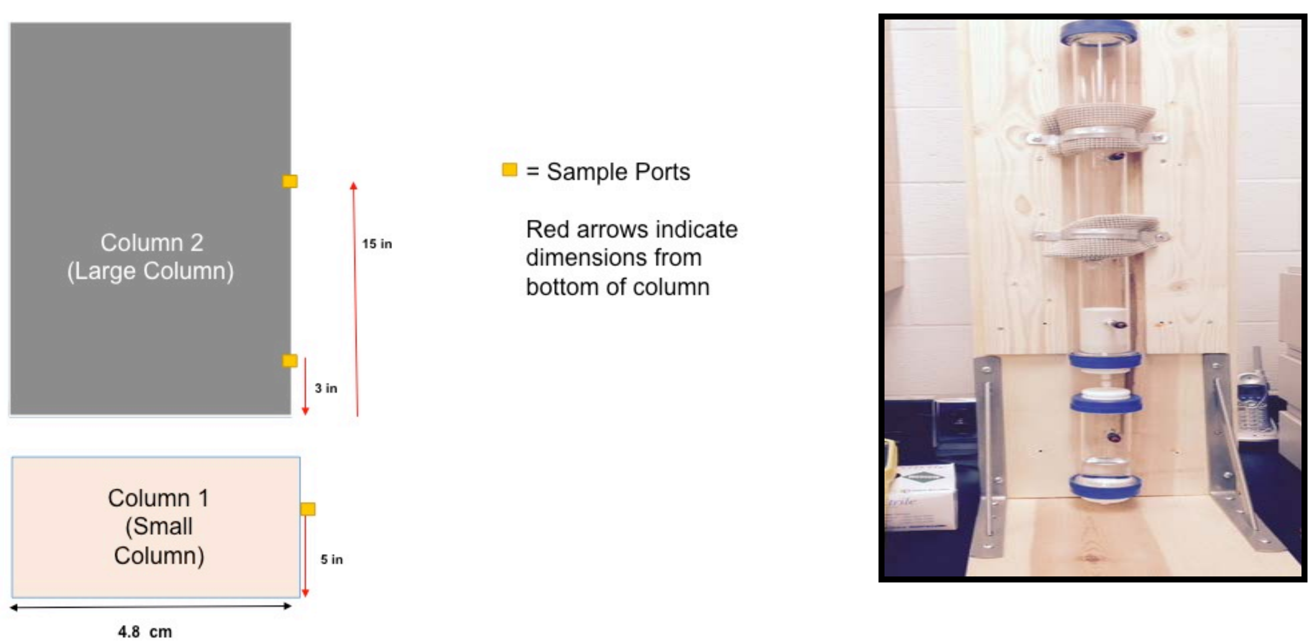


Figure 1. Soil Column Schematic (Left) and Photo (Right)

A chemical reservoir is located at the bottom of the column. The reservoir containing a water and methanol mixture was spiked with tetrachloroethylene (PCE). PCE was used as model VOC. Throughout the experiments, column seals were inspected using a MiniRAE 3000

Portable Handheld VOC Gas Monitor. The column was equipped with three sampling ports: (1) headspace above reservoir; (2) within soil strata; and (3) headspace above soil. Chemical transport was monitored by collecting vapor samples using quick-stop luer check valve syringes and analyzing the vapors using a GC/FID.

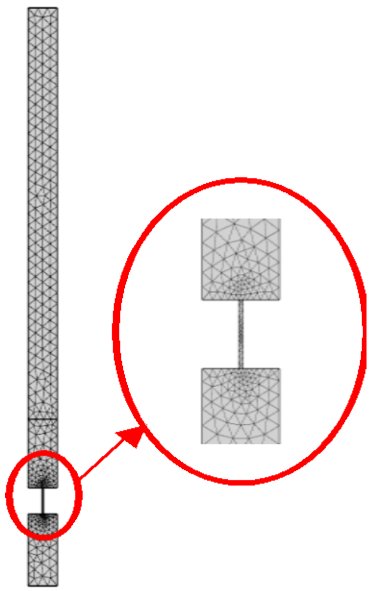


Figure 2. Meshed Model Domain of Soil Column

The column was modeled using COMSOL Multiphysics 4.4.0.248 using the Transport of Diluted Species module with a time dependent study. Figure 2 shows the meshed model domain. All solid boundaries within the domain do not allow chemical flux except at the interfaces between volumes that allow diffusion.

Principal Findings and Significance

Extensive sampling of soil vapors, indoor air and groundwater is often required to determine whether or not vapor intrusion poses a risk at a given site. Mass flux, rather than soil gas concentration, is well known to be a critical variable when characterizing vapor intrusion exposures; however, there are currently no robust methods for measuring flux at vapor intrusion sites. As part of this research, we evaluated vapor flux measurement techniques. We conducted laboratory soil column experiments, and compared the results with a computational model that uses a commercially available software package, COMSOL Multiphysics. The results provide the length of time needed for steady-state conditions and also estimates of mass flux. The time to reach steady state and the mass flux vary depending on the specific scenario being tested. Example results are included as Figure 3. Generally speaking, the time to reach steady state was on the order of days to weeks. Future experiments are investigating the effect of soil moisture and cyclic pressure variations to simulate wind-induced pressures.

By considering the laboratory data with the modeling results, we can investigate critical fate and transport processes that govern vapor intrusion (diffusion coefficients, tortuosity, permeability, etc.). The findings of this research provide insight about new methods for characterizing vapor intrusion as well as a better conceptual understanding of the vapor intrusion process to aid in remediation efforts.

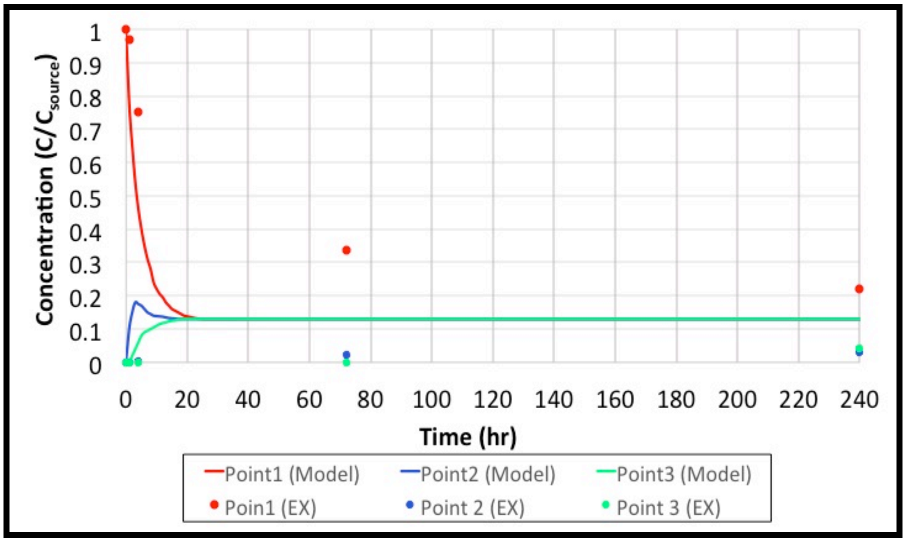
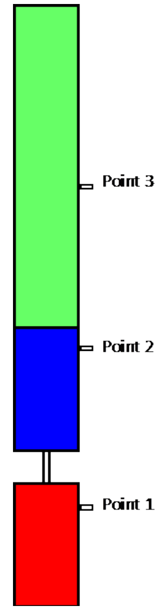


Figure 3. Model and Experimental Results for Column Study



Does logging and surface mining increase the vulnerability of stream-associated salamanders to chytrid fungus infection?

Basic Information

Title:	Does logging and surface mining increase the vulnerability of stream-associated salamanders to chytrid fungus infection?
Project Number:	2014KY231B
Start Date:	3/1/2014
End Date:	2/28/2015
Funding Source:	104B
Congressional District:	KY 5
Research Category:	Biological Sciences
Focus Category:	Ecology, Water Quality, None
Descriptors:	None
Principal Investigators:	John J Cox

Publications

1. Spaulding, Sarah, 2015, An Investigation Into the Occurrence of Bactrachochytrium dendrobatis Infection in Plethodontid Salamander Communities in Robinson Forest, MS Thesis, Department of Forestry, University of Kentucky, Lexington, Kentucky, 33 p.
2. Hamilton, S.M., J.J. Cox, A.N. Drayer, J.M. Richards, and J.J. Treanor, 2015, Assessment of Chytrid Fungus (Bactrochochytrium dendrobatidis) Occurrence and Prevalence in Plethodontid Salamanders Across a Forest Disturbance Gradient in Southeastern Kentucky, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, p. 47.

Does Logging and Surface Mining Increase the Vulnerability of Stream-Associated Salamanders to Chytrid Fungus Infection?

Problem and Research Objectives

Significant amphibian declines have been documented on a global level and at a highly accelerated rate over the past three decades (Stuart *et al*., 2004; McCallum, 2007). Global population assessments indicate that amphibians are declining more rapidly than other vertebrates, and current amphibian extinction rates may be more than 200 times greater than background extinction rates (McCallum, 2007). Amphibians are known to host a wide range of microparasites (i.e. viruses, bacteria and fungi) and macroparasites (e.g. nematodes, mites and copepods) that can cause disease and mortality (Blaustein *et al*., 2012). The chytrid fungus (*Batrachochytrium dendrobatidis;* Bd) has been known to cause numerous population-level declines and species extinctions (Berger *et al.* 1998, Blaustein *et al*., 2012). Susceptibility to infection and the manifestation of chytridiomycosis can vary greatly among different species, and by habitat (Moss *et al*., 2008; Blaustein *et al*., 2012).

The greatest concentration and diversity of North American salamanders are located in the Appalachian region, where they comprise a significant amount of trophic biomass and are the dominant vertebrate predators (Davic & Welsh, 2004). The forest ecosystems of this region have been severely degraded due to the pollution, sedimentation and habitat degradation caused by various resource extraction practices (e.g. surface mining, timber harvest). Environmental degradation may increase susceptibility to pathogen infection and mortality in amphibians by compromising the immune systems of local amphibians and negatively impacting their physiology (Blaustein *et al*., 2012). Recently, Maigret *et al.* (2014) and Muncy *et al.* (2014) demonstrated that the abundances of several species of salamander were negatively affected by timber harvest and surface mining, respectively, in Robinson Forest, Kentucky; however, questions remain about whether and how Bd might be impacting salamanders in these areas affected by anthropogenic resource extraction.

In spring 2013, we conducted research to determine whether Bd was present in one of the more isolated and mature mixed-mesophytic forest tracts of southeastern Kentucky, and compared infection intensity and prevalence to nearby sections of this forest impacted by timber harvest and surface mining, two regionally common extractive activities. We hypothesized that salamanders inhabiting streams impacted by surface mining and timber harvest would have a higher prevalence of Bd than those inhabiting streams in mature forest.

Methodology

Sampling for the pathogenic fungus *Batrachochytrium dendrobatidis* (Bd) was conducted in order to: 1) determine the presence of chytrid infection in stream-associated plethodontid salamanders of southeastern Kentucky, and 2) evaluate differences in infection intensity between salamanders residing in intact forest streams, timber-harvested streams and surface-mined streams. Samples were collected from ephemeral streams within University of Kentucky's Robinson Forest, located in southeastern Kentucky within Breathitt, Knott and Perry counties. The forest contains the Clemons Fork and Coles Fork watersheds, both of which are considered reference streams by the Environmental Protection Agency. Between 2008-2009, six headwater streams were logged within Clemons Fork using a two-aged deferment harvest, in accordance with one of three separate streamside management zone (SMZ) treatments (Witt *et al*., 2013; Maigret *et al.,* 2014). Samples from the control and harvest treatments were obtained from randomly selected ephemeral streams within the Clemons Fork watershed.

Nine ephemerals were randomly selected for sampling within the control treatment, which consists of the aforementioned 90 year-old second-growth mesophytic forest. We randomly chose eight streams within one randomly selected streamside management zone configuration to use as our harvested treatment sites; this treatment consisted of streams harvested with a mandatory retention of channel bank trees, limited equipment tracking, and improved ephemeral stream crossings (Witt et al. 2013).

In addition to collecting DNA samples from control and harvested streams within the Clemons Fork watershed, we also collected samples from ephemeral streams located within the Laurel Fork watershed, a 2200-acre section of Robinson Forest located approximately 5km southwest of the main block surface mined for coal in the 1990s. Five ephemerals in the Laurel Fork watershed were randomly selected for sampling within the mined treatment. Sampling areas for the control, harvested and mined sites were approximately equivalent in size. Sampling sessions were conducted in March, April and May of 2013, when air temperatures were relatively cool and average relative humidity high.

We focused on collecting DNA samples from salamanders within the family Plethodontidae; however, samples were obtained from other amphibians (*i.e.* frogs, newts) when encountered. Starting at 20m upstream from where the ephemeral joined an intermittent or perennial stream, and continuing upstream until physical access to the stream was impossible, each ephemeral was sampled once by turning over logs and rocks within or near the water. Leaf litter searches were also conducted within 3m of the stream bank.

Each captured amphibian was restrained by placement in a clear plastic Ziplock bag before being identified and examined visually for gross abnormalities characteristic of Bd infection (*e.g.* lesions or sloughing skin), and then released following DNA sampling. DNA samples were obtained by using a sterile, individually wrapped cotton-tipped swab to rub the limbs, in between the digits, and the ventral surface of the salamander 20 times in accordance with Hyatt *et al.* (2007). Swabs were placed into individual tubes filled with ethanol, and were then labeled and stored at 4˚C until they were shipped to colleagues in an external lab for molecular analysis. Quantitative polymerase chain reaction (qPCR) was used to determine the presence and quantity of *Batrachochytrium dendrobatidis* (Bd) DNA in the samples. Chi-square analysis was used to test differences in Bd infection among treatment groups.

Principal Findings and Significance

During 14 sampling sessions occurring between March and May of 2013, we collected DNA samples from 306 individual salamanders within 8 species from the family Plethodontidae; additional amphibians (i.e. frogs, newts) were opportunistically sampled when encountered (Table 1). Approximately 2.1% of the salamanders and 50% of the frogs sampled from intact streams, 2.3% of the salamanders and 80% of the frogs sampled from harvested streams, and none of the salamanders and 100% of the frogs sampled from mined streams tested positive for Bd. No significant differences in occurrence of Bd or infection intensity were detected between the treatment sites ($\chi^2 = 0.59$; p-value = 0.75), or between individuals of a species from different treatment sites.

While no significant differences were found in the occurrence and infection intensity of Bd from individuals inhabiting the different extractive resource treatments, this study is the first to document Bd infection in salamanders of this highly biodiverse forested region of Kentucky. Although Bd was not detected in salamanders captured in the surface mined watershed, the possibility of infected salamanders being present cannot be ruled out due to difficulty in finding amphibians to sample (sample size n = 29), and the fact that samples collected from cohabitating ranid frogs within these mined streams tested positive for Bd. We recommend widespread sampling of Kentucky amphibians in order to determine the occurrence of Bd infection in our highly biodiverse and amphibian-rich state so as to better understand and perhaps manage for these fungal diseases that have been implicated in the global loss and decline of many amphibian species.

Table 1. Bd infections organized by species and ephemeral treatment for amphibians captured between 13 March - 1 May 2013 from Clemons Fork and Laurel Fork watersheds, Robinson Forest, Kentucky. The number of individuals testing positive for Bd, per species for each treatment, can be found within parentheses, followed by the percentage testing positive, per species for each treatment. Salamander data are presented first, data for the two frog species encountered are given last.

	Treatment			
Species	Control	Harvest	Mined	*Total*
D. fuscus	55(1) 1.8%	20(1) 5%	4(0)	*79(2) 2.5%*
D. monticola	66(1) 1.5%	38(1) 2.6%	-	*104(2) 1.9%*
D. spp	6(0)	-	-	*6(0) 0%*
E. cirrigera	35(1) 2.8%	8(0)	-	*43(1) 2.3%*
E. longicauda	-	-	7(0)	*7(0) 0%*
G. porphyriticus	10(0)	9(0)	-	*19(0) 0%*
P. glutinosus	-	3(0)	1(0)	*4(0) 0%*
P. richmondi	16(0)	10(0)	16(0)	*42(0) 0%*
P. ruber	2(1) 50%	-	-	*2(1) 50%*
N. viridescens	-	-	1(0)	*1(0) 0%*
Total (salamanders)	190(4) 2.1%	88(2) 2.2%	29(0) 0%	*307(6) 1.7%*
L. clamitans	10(5) 50%	5(4) 80%	1(1) 100%	*16(10) 62.5%*
L. palustris	-	-	1(1) 100%	*1(1) 100%*
Total (frogs)	10(5) 50%	5(4) 80%	2(2) 100%	*17(11) 64.7%*
Total (all captures)	200(9) 4.5%	93(6) 6.5%	31(2) 6.5%	*324(17) 5.2%*

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Streambank stability and riparian habitat relationships and mapping in the Triplett Creek watershed

Basic Information

Title:	Streambank stability and riparian habitat relationships and mapping in the Triplett Creek watershed
Project Number:	2014KY232B
Start Date:	3/1/2014
End Date:	2/28/2015
Funding Source:	104B
Congressional District:	KY 5
Research Category:	Climate and Hydrologic Processes
Focus Category:	Geomorphological Processes, Management and Planning, Sediments
Descriptors:	None
Principal Investigators:	April Haight

Publication

1. Meade, Nicole and Toney Phillips, 2015, Streambank Stability and Riparian Habitat Relationships and Mapping Tools in the Triplett Creek Watershed, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, p. 21.

Streambank Stability and Riparian Habitat Relationships and Mapping in the Triplett Creek Watershed

Problem and Research Objectives

The Triplett Creek watershed is located in the Knobs region of Eastern Kentucky, about 60 miles east of Lexington, within the Daniel Boone National Forest. The steep terrain and lack of privately owned land forces much of the development to be concentrated along Triplett Creek and its tributaries. Triplett Creek is about 23 miles long, drains 180 square miles (approximately 2/3 of Rowan County), and flows into the Licking River. Triplett Creek is a meandering, high gradient stream that is impaired; with high levels of bacteria, nutrients, and sediments. Bank erosion is the main source of sediment (65% from streambanks) within the waterway (Emrich, Garnear, Haight, and Reid, 2013). The objectives of this study were to further our understanding of the causes of the streambank erosion.

Our efforts focused on developing an understanding of the causes of streambank erosion from a watershed implementation perspective. Specifically, the objectives of the study were to 1) know where to focus funding for streambank stability projects, 2) better understand the relationship between impervious surfaces and streambank erosion, and 3) investigate the relationship between the quality of riparian habitat and streambank erosion. The methodology developed for this study included documentation of the amount of impervious surfaces, the quality of riparian habitat, and the severity of streambank erosion.

Methodology

The methods used in this study focused on increasing our understanding of landuse practices and streambank erosion; and identifying priority areas for streambank stability projects. Thirteen sites along the main stem of Triplett Creek were selected for study. These sites were located where wadeable tributaries entered Triplett Creek. The sites represented a wide variety of subwatershed sizes and of landuse practices (pervious vs. impervious). Data collection was conducted in the field and using a geographic information system (GIS).

Field Methods: The severity of erosion and quality of riparian habitat were recorded for 600 foot sections on each tributary, and upstream and downstream on the main stem of Triplett Creek. The severity of streambank erosion was measured using a Modified Bank Erosion Hazard Index (mBEHI) developed by the Southwest Michigan Planning Commission (Meersman, 2008). This method is a modified version of Rosgen’s Bank Erosion Hazard Index (2001). The mBEHI uses four simple metrics to assign a rating to streambank erosion. The metrics include root depth, root density, surface protection, and bank angle. Habitat data were recorded on the Habitat Assessment worksheet for high gradient streams used by Kentucky Watershed Watch (2011). This measurement tool evaluates instream habitat as well as the quality of the riparian zone, using 20 parameters. Generally speaking, the parameters measure the size and quality of the riparian zone, bank erosion, and instream habitat.

GIS Methods: GIS allowed us to spatially analyze our data. Watershed data layers and topographic base maps were used to create the specific subwatersheds of the tributaries selected for this study. The calculated sizes of the subwatersheds were joined with impervious surface layers to calculate the percent of impervious surface for each individual subwatershed (Figure 1). A stream data layer was used to encompass a 120-foot riparian habitat zone along each of our tributaries and then joined with the same percent imperviousness layer. This allowed us to calculate a percent imperviousness for 120-foot-width corridors along each tributary. GIS also allowed for spatial data to be converted directly into data tables for statistical analysis.

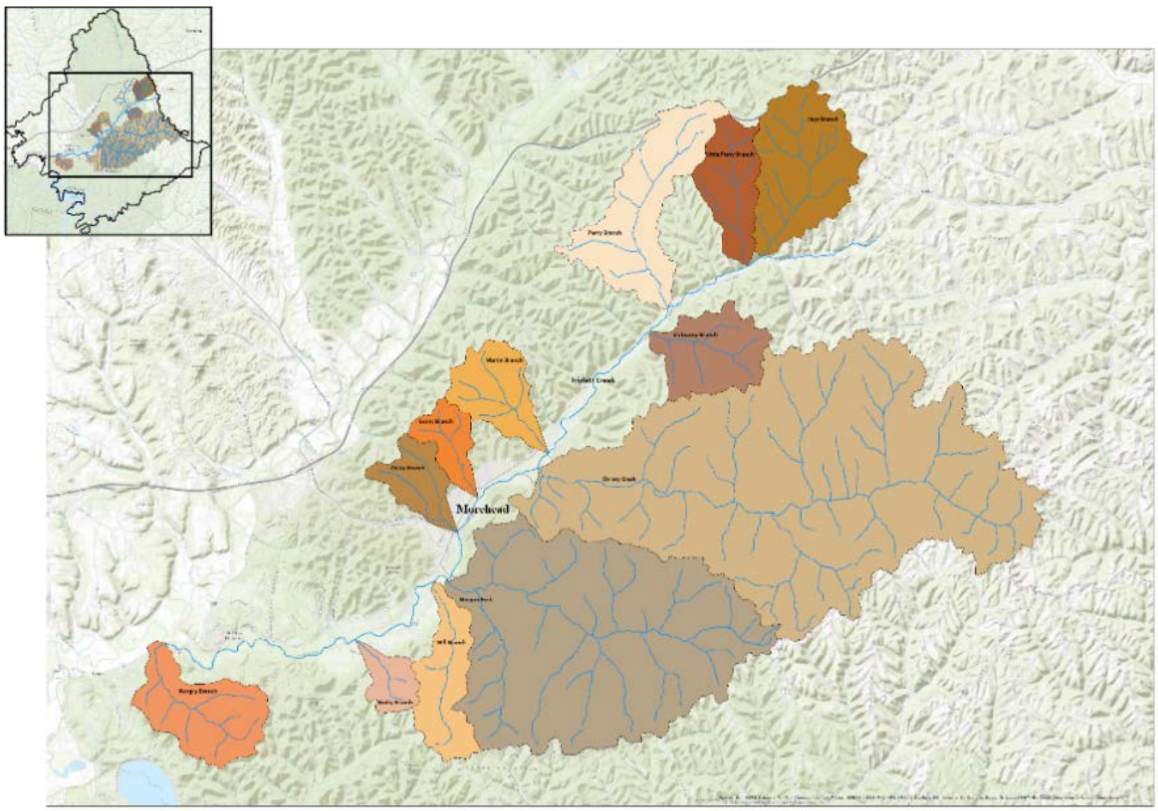

Figure 1. Impervious surface layer.

Principal Findings and Significance

The field and GIS data were evaluated using both nominal and statistical analysis. At each of the 13 tributary intersections, we calculated the overall mBEHI and Habitat Assessment values for 600-foot upstream and downstream sections on the main stem of Triplett Creek, and the last 600 feet at the mouth of each tributary. Overall average values were calculated for each 600 foot section. The average scores and the individual parameters for each site were subsequently evaluated using statistical analysis to investigate 1) correlations between the field method parameters and streambank erosion, 2) correlations between GIS data and streambank erosion, and 3) differences between tributaries and the main stem of Triplett Creek.

Nominal analysis: The mBEHI and Habitat Assessment were analyzed nominally. The mBEHI calculations resulted in 196 of the 234 streambank sections receiving a high or extreme category rating. Then the 200 foot streambank sections were averaged into 600 foot sections that were also found to be in the high to extreme categories. Habitat Assessment scores for individual 300 foot sections ranged from 42 (Poor) to 92 (Fair). When averaged into 600 foot sections the range narrowed to 52 to 89. These scores were still poor to fair. By using GIS tools, we were able to visualize mBEHI and Habitat Assessment data. We did not find that the erosion values were worse upstream (where the gradient and consequently the velocity are higher). In addition, the mBEHI values were not greater at downstream locations (where the total flow is greater).

Statistical Analysis: The data were evaluated further using statistical analysis. The results include:

- The mBEHI upstream and downstream of each tributary on the main stem of Triplett Creek sections were not statistically different.
- The mBEHI and Habitat Assessment do not correlate on Triplett Creek main stem. Our initial thought was that the Habitat Assessment at any location on Triplett Creek would correlate to the mBEHI at that same location. This proved to be incorrect. This correlation produced an r-squared value of 0.1999 with a P value of 0.248.
- There was no correlation between bank height and mBEHI. The Triplett Creek watershed contains many tall, steep, conspicuous, barren streambanks (some over 20 ft), but statistical analysis shows that their mBEHI ratings were no worse than the lower streambanks (some of which are only 1 ft high).
- mBEHI values for the tributaries were statistically higher than those of the main stem.
- The size of a subwatershed did not correlate with the mBEHI value found at the tributary’s mouth.
- No correlation was found between the mBEHI of a stream and the type of immediate landuse at that location (commercial, parking lot, residential, forest, etc.)
- The percentage of impervious surfaces within a 120-foot width riparian area along a stream did not correlate with the mBEHI at that location.
- Multiple Regression Analysis yielded an important finding. The percentage of impervious surface of each entire tributary’s watershed correlated quite well with the average mBEHI value at the tributary’s mouth. The P value was 0.0049. There is significant evidence that impervious percent of the entire watershed is a significant predictor of bank erosion at the mouth.

The results of this study have several potential implications for watershed managers. First, streambank erosion stability efforts should be focused at the mouths of the wadeable tributaries, not on the main channel. Secondly, these efforts should focus on reducing the amount of impervious surfaces anywhere within a tributary, not just those in close proximity to the streambanks. Lastly, in order to address ongoing and future streambank erosion, impervious surfaces within the watershed should be mitigated through the use of low-impact designs, green infrastructures, and more traditional techniques that retain runoff during precipitation events.

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Phosphorus leaching from compost bedded pack dairy barn waste

Basic Information

Title:	Phosphorus leaching from compost bedded pack dairy barn waste
Project Number:	2014KY234B
Start Date:	3/1/2014
End Date:	2/28/2015
Funding Source:	104B
Congressional District:	KY 4 & 6
Research Category:	Water Quality
Focus Category:	Agriculture, Nutrients, Water Quality
Descriptors:	None
Principal Investigators:	Mark Steven Coyne, Joseph Taraba

Publications

1. Leite, Mauricio Cunha Almeida, Leslie Hammond, Ann Freytag, Mark Coyne, and Joseph Taraba, 2015, Phosphorus Transport form Land-Applied Compost Bedded Pack Dairy Barn Waste, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, p. 61-62.
2. Hammond, Leslie, Mark Coyne, and Joseph Taraba, 2015, Phosphorus Dynamics of Compost Beded Pack Dairy Barn Waste, 2015, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, p. 63-64.

Phosphorus Leaching From Compost Bedded Pack Dairy Barn Waste

Problem and Research Objectives

The central problem we addressed was the extent to which compost bedded pack barn dairy (CBP) waste changes soil test phosphorus (STP) values and, consequently, the mobility of phosphorus in land-applied wastes. With the completion of preliminary work, an aerobic mineralization study, and a field study, we addressed the following central research objectives of the proposal:

1) Demonstrated changes in soil test phosphorus as a consequence of mineralization dynamics of composted bedded material amended soils.

2) Provided phosphorus release characteristics for aerobically incubated material mixed with a common Kentucky soil, Faywood silt loam that has high and low soil test phosphorus values.

3) Monitored the distribution of various phosphorus fractions in field-applied waste as a function of depth.

Methodology

We addressed the first and second objectives by performing aerobic mineralization studies in conjunction with data generated from related studies performed at the same time. The Crider silt loam with high and low STP levels that was originally proposed for use in the investigation was determined to be influenced by prior management; the resulting high STP levels could be attributed to the addition of organic P materials to the soil. Consequently these soils would not provide a valid comparison. For this reason, a Faywood soil was substituted for the aerobic mineralization study. The Faywood soil is a series with native phosphorus mineralogy that exhibits high and low STP as an effect of mineralogy. To each soil, CBP or fresh manure was added on a total P basis at 0, 25, 50, or 100 mg kg^{-1} P. This is roughly equivalent to 0, 56, 112, and 224 kg ha^{-1} P. At thirty day increments for 120 days, STP, orthophosphate, dissolved organic P, ammonium, and nitrate readings were made.

To better understand the CBP material in a field setting, we monitored the distribution of various phosphorus fractions in field-applied CBP as a function of depth. The field experiment outlined in the proposal was not completed as originally described. The research was conducted at a field site at Spindletop Research Farm in Lexington, KY. CBP was applied at various rates and the distribution and form of P were measured by taking subsequent samples at various depths. The analyses described in the P-fractionation methodology were applied to the field study to assess the phosphorus dynamics and transport in bluegrass soils exhibiting high STP that were amended with compost bedded pack dairy barn material.

Principal Findings and Significance

Aerobic Mineralization

As a result of our work, we devised an innovative phosphorus fractionation strategy that allows researchers to measure the phosphorus dynamics of land-applied organic waste sources. The fractions are hypothesized to correspond with nutrient availability or lack thereof. Additionally, the fractions reveal the forms of P that would likely leach or be bio-available, and the forms that would be fixed or recalcitrant to leaching. The fractionation procedure involved UV-assistance to decompose organic P forms into PO_4-P. The samples were subjected to a colorimetric PO_4-P assay before and after UV light exposure. The difference in readings indicated the dissolved organic-P fraction. The procedure was calibrated by the use of National American Proficiency Test Program soils, a designated collection of soils set aside by the Soil Science Society of America.

From the aerobic mineralization study, we determined that any P-source, no matter the form, increased the bioavailable P and the STP of both high and low STP soils at the time of addition (Figures 1 and 2). As incubation advanced, bioavailable P decreased from the initial readings for all amended soils (Figures 3-6), while the bioavailable P in the control remained generally constant. The decrease in bioavailable P is most likely due to adsorption or precipitation of secondary P-containing minerals. In general, CBP yielded more bioavailable P than fresh manure with time in both high and low STP soils. However, soils amended with fresh manure tended to yield greater amounts of organic P, which suggests that the organic P pool of fresh manure is more labile than that of CBP waste.

The overall contributions of organic P in both soils were negligible relative to the amount of inorganic water soluble P. However the ratio of dissolved organic plant available P to dissolved inorganic P increased in general as the incubation progressed, which suggests that some of the inorganic P becomes less available due to sorption or precipitation, and/or P cycling is occurring such that much of the P is incorporated into microbial biomass and is therefore conserved.

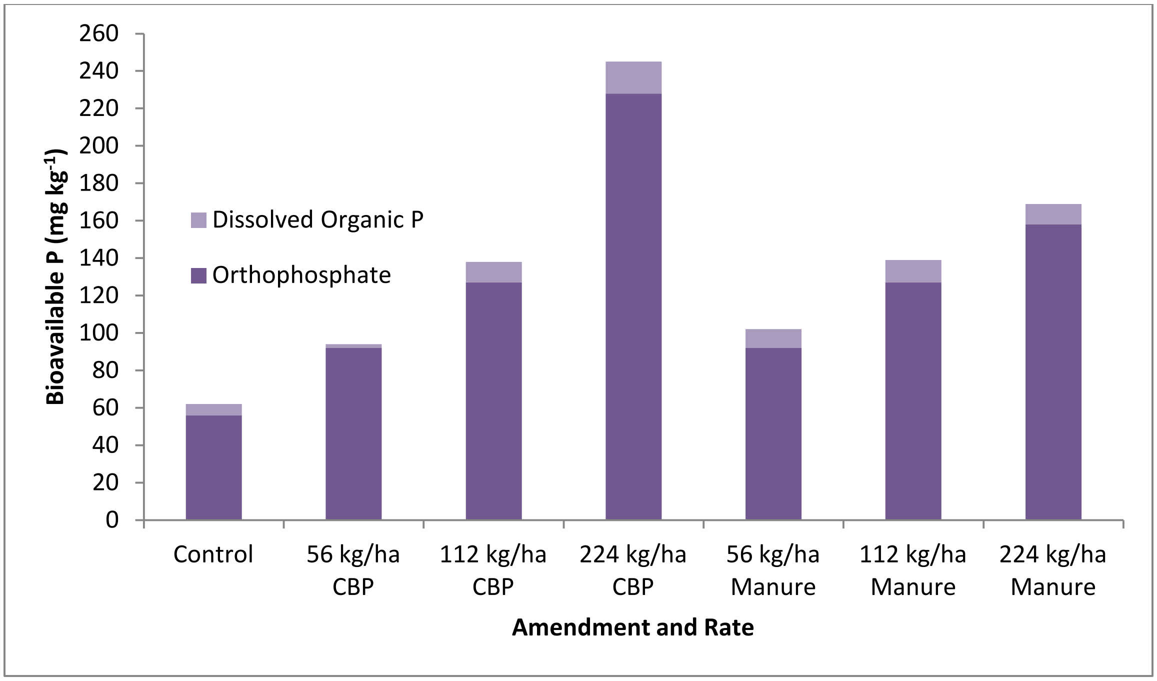

Figure 1: Bioavailable P in the high STP soil as a function of amendment and rate at the beginning of incubation.

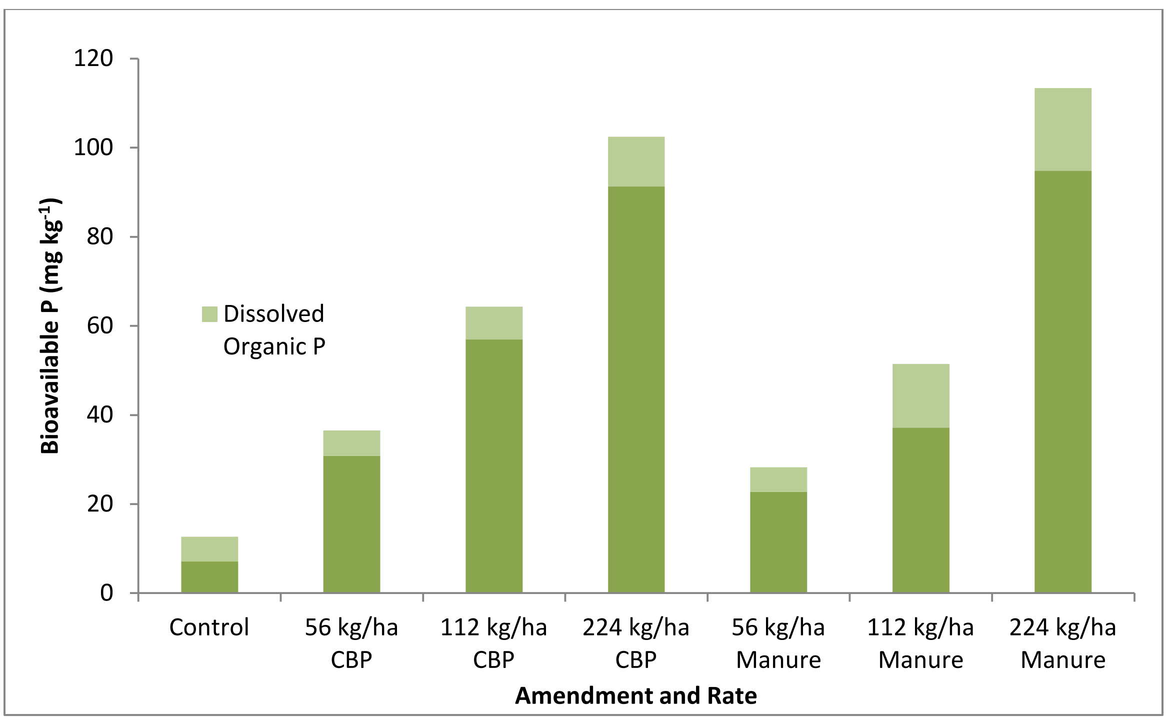

Figure 2: Bioavailable P in low STP soil at the start of the incubation as a function of amendment and rate.

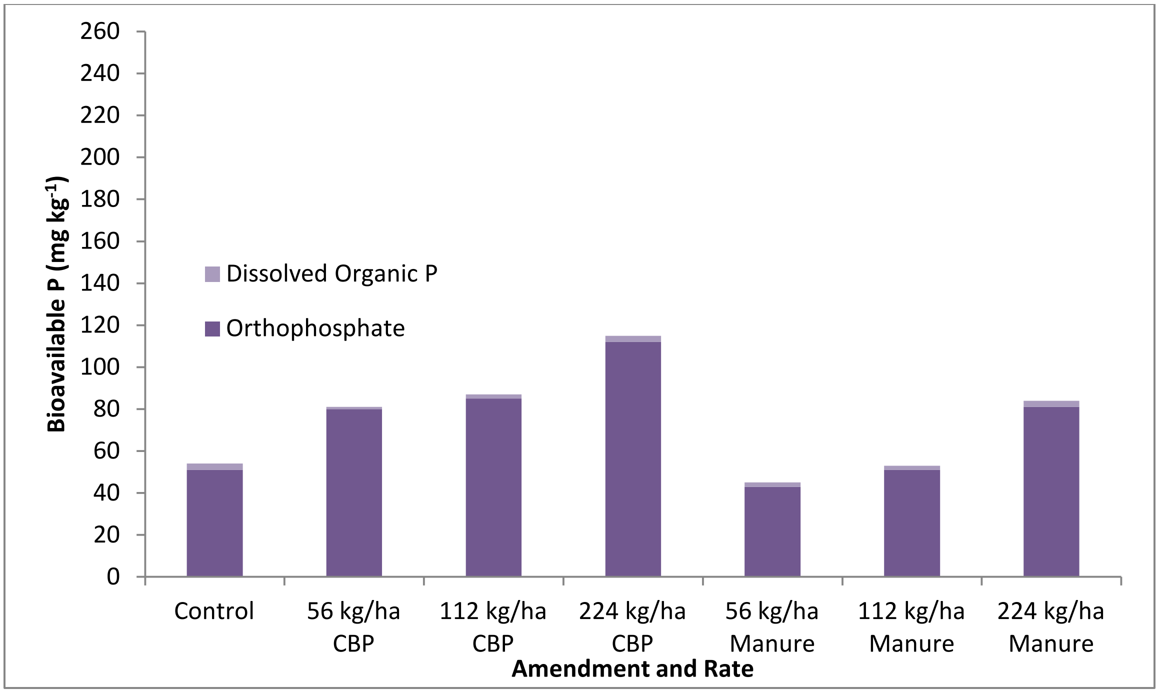

Figure 3: Bioavailable P in the high STP soil as a function of amendment and rate at day 60 of the incubation.

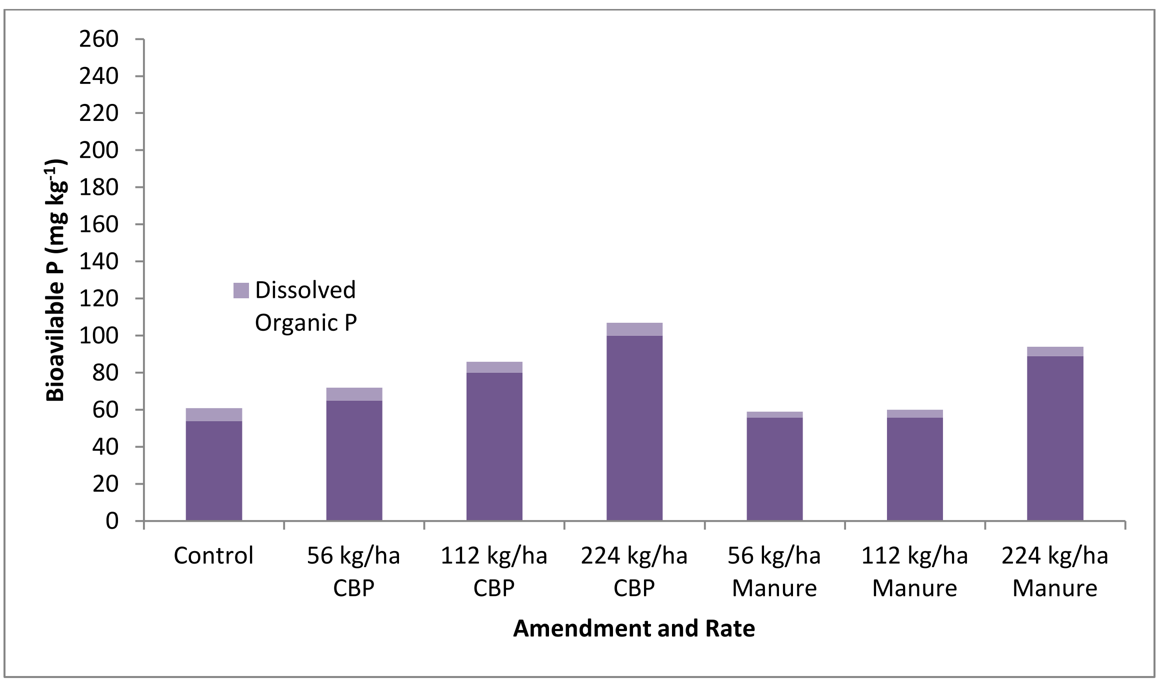

Figure 4: Bioavailable P in the high STP soil as a function of amendment and rate at end of the incubation.

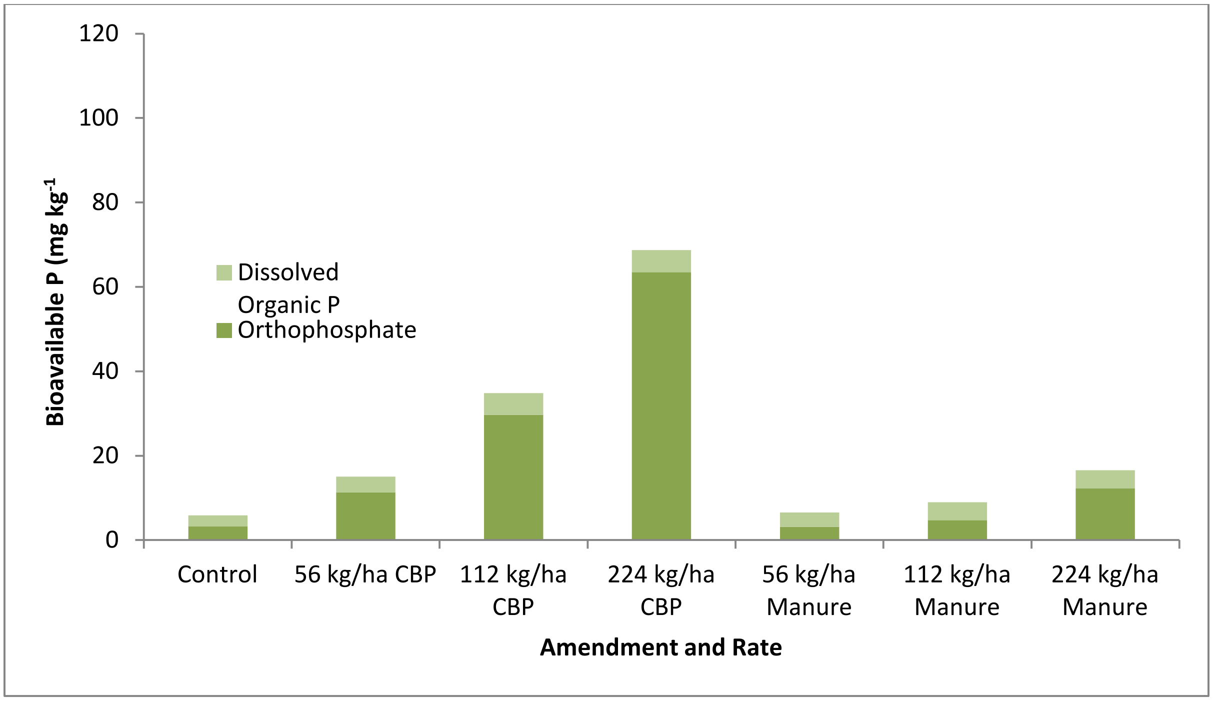

Figure 5: Bioavailable P in the low STP soil as a function of amendment and rate at day 60 of the incubation.

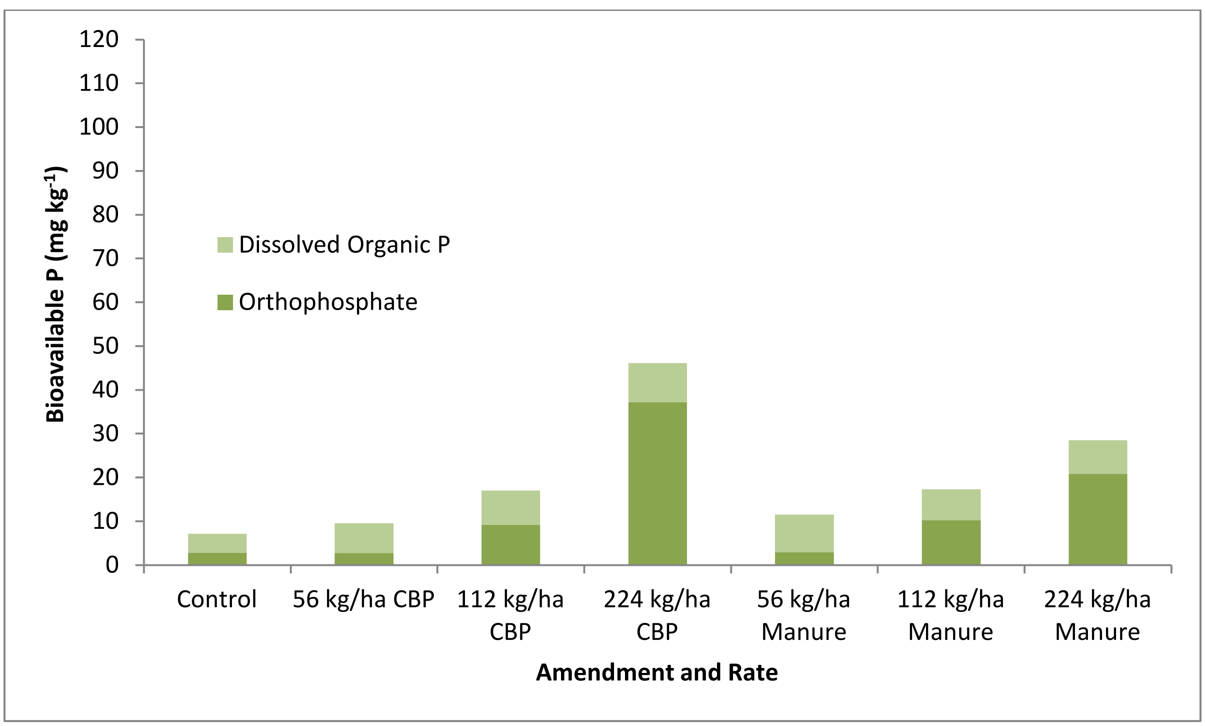

Figure 6: Bioavailable P in the low STP soil as a function of amendment and rate at the end of the incubation.

On the whole, the low STP soil buffered changes in STP at low application rates, regardless of amendment. In addition, the addition of CBP generally resulted in greater changes to STP than that of manure, regardless of soil type. For both soil types, the change in STP exhibited by both amendments was proportionally comparable at low application rates. The difference in the change in STP between amendments was more significant at higher rates of application; CBP changed STP to a greater extent than manure added at the same rate.

In general, STP increased at the time of addition for both soil types which was followed by a rapid decrease after 30 days incubation (Tables 1 and 2). The result was a net increase in STP for all treatments (except for the control) with time. The initial spike in STP for all treatments could be due to the flush of water soluble P in the system as a result of the rewetting effect. The trend for bioavailable P was the same in both soils in that there was an immediate increase followed by a statistically significant decrease for nearly all treatments ($p<0.01$). The two soil types behaved similarly in that each source of variability had a significant effect on orthophosphate yields in each soil (Table 3) The most notable differences among rates in terms of soluble P and Mehlich III P are at the beginning of the incubation, which are to be expected.

Table 1: Soil Test Phosphorus levels in the high STP soil as a function of time and amendment.

Time	Control	56 kg ha^{-1}		112 kg ha^{-1}		224 kg ha$^{- 1}$
	(mg kg^{-1})	CBP (mg kg^{-1})	Manure (mg kg^{-1})	CBP (mg kg^{-1})	Manure (mg kg^{-1})	CBP (mg kg^{-1})	Manure (mg kg^{-1})
0 Days	189	208.5	201	291.5	210	273	237
30 Days	184	200	197.5	216	204	263.5	216
60 Days	171.5	196.5	195	208.5	196.5	236.5	229.5
90 Days	188.5	212.5	199	223	219.5	257.5	242.5
120 Days	190.5	208.5	205	218.5	222.5	249	245.5

Table 2: Soil Test Phosphorus levels in the low STP soil as a function of time and amendment.

Time	Control	56 kg ha^{-1}		112 kg ha^{-1}		224 kg ha^{-1}	
	(mg kg^{-1})	CBP (mg kg^{-1})	Manure (mg kg^{-1})	CBP (mg kg^{-1})	Manure (mg kg^{-1})	CBP (mg kg^{-1})	Manure (mg kg^{-1})
0 Days	18	48	20.5	65.5	26.5	115.5	41
30 Days	15.5	22	24	33	30.5	61.5	44.5
60 Days	16	21	24	39.5	29.5	72	42
90 Days	16	19	23.5	29.5	29.5	53.5	43
120 Days	16	21	23	32	28	76.5	45.5

Table 3: Source Interactions for orthophosphate yield in high and low STP soil.

Source	P value
Time	<0.001
Rate	<0.001
Amendment	<0.001
Time*Rate	<0.001
Time*Amendment	<0.001
Rate*Amendment	<0.001
Time*Rate*Amendment	<0.001

We do not fully understand the mechanism behind the generally greater increase of STP or bioavailable P as a result of CBP amendment, but hypothesize that the amorphous nature of the CBP material prevents P from adsorbing as easily to colloid surfaces, allowing it to be more easily extracted.

The dynamics of P in both high and low STP soils suggests that there may be regulatory implications due to the variability in STP measurements occurring during the 120 day incubation period. Soils may exhibit different STP values depending on the number of days following incorporation. However, this experiment was performed on soils without vegetation, so the change in STP may be less in a field setting with potential plant uptake of P. Moreover, the threat to water quality may be reduced in a field setting where plant-available P may be retained.

Litterbag Mineralization

A litterbag study was performed to evaluate the decomposition of land applied CBP waste as a function of particle size. Material from the upper 30 cm of a CBP barn was collected and incubated in mesh bags for a 180-day period. There was no statistically significant difference in mass loss as a function of particle size (Fig. 7). In general, CBP waste decomposed faster than a commercial product - Louisville Green - even though the Louisville Green had a smaller particle size. The slope of all decomposition model lines was greater than that of Louisville Green ($p<0.0001$) except for the largest particle size. The mass loss was best fit by a linear rather than first order decomposition model.

The relative uniformity in mass loss exhibited across all particle sizes of CBP was most likely due to the starting material being essentially the same. Lack of uniform breakdown might be exhibited by different particle sizes removed from deeper depths in the CBP barns because the different depths had different nutrient characteristics.

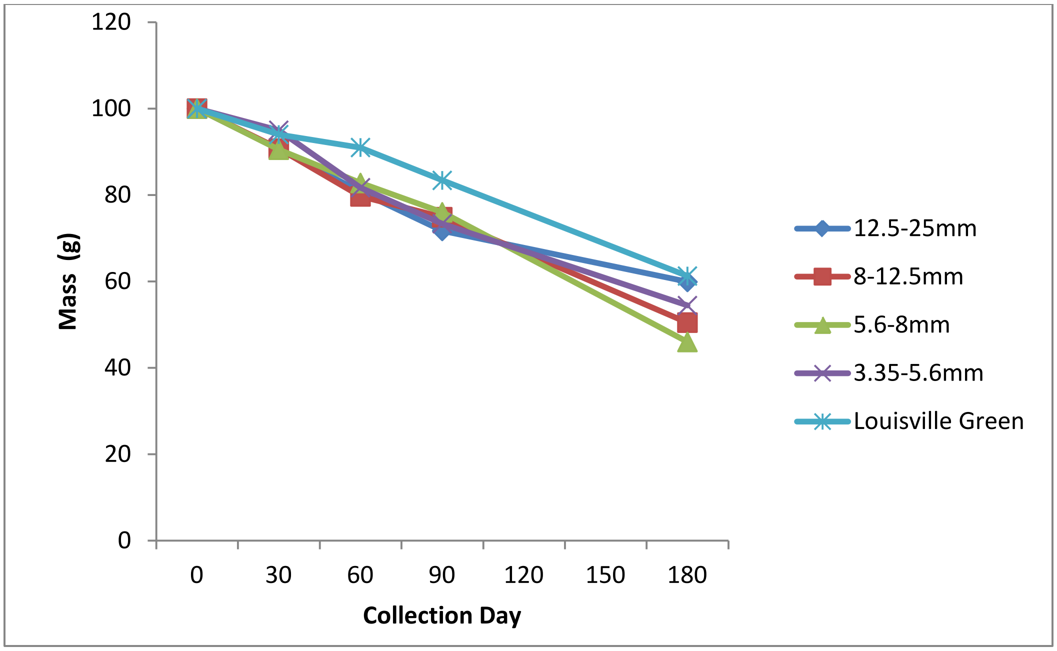

Figure 7. Mass of CBP and Louisville Green remaining as a function of particle size.

Mineralizable P (7-d anaerobic incubation) as a function of total P decreased in all CBP samples for the first 60 days (Fig. 8). Thereafter, the percentage of mineralizable P increased more or less uniformly with the CBP waste, which suggested some environmental or biological weathering of the material to increase the relative pool of labile P.

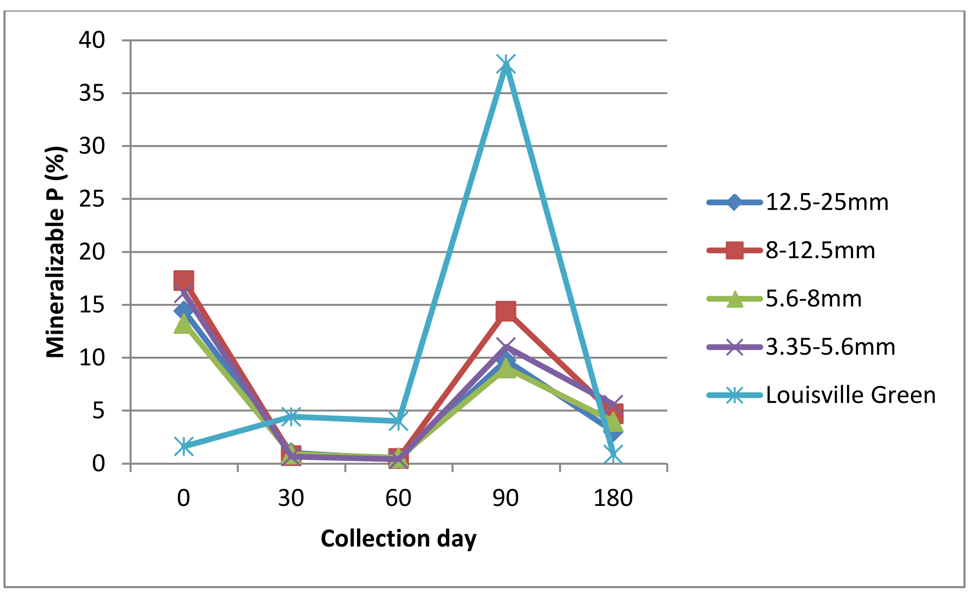

Figure 8. Percent mineralizable P as a function of particle size, amendment, and time.

Land Application and Transport

At the time of application, the average water soluble P in the Maury silt loam soil to which the CBP waste was applied was 37 mg kg^{-1} (range 25 to 50 mg kg^{-1}) at 0-15 cm and 11 mg kg^{-1} (range 7 to 17 mg kg^{-1}) at 15-30 cm. Average labile organic P was 9 mg kg^{-1} (range 7 – 12 mg kg^{-1}) at 0-15 cm and 3 mg kg^{-1} (range 2 to 6 mg kg^{-1}) at 15-30 cm depth. Labile organic P represented about 20% of total water soluble P at either depth. There was a significant effect of depth on initial water soluble and labile organic P (Table 4).

Table 4. Concentrations (mg kg^{-1}) of P_i and P_o as a function of different plots and depths in a Maury silt loam soil prior to CBP application. Values followed by a different letter within the same analysis and depth are significantly different ($P < 0.05$).

Plots	Pi		P UV (i + o)		Po	
	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm
A	34.5 d	12.2 b	43.7 c	15.8 c	9.16 a	3.5 a
B	35.9 d	12.6 b	45.6 c	15.8 c	9.7 a	3.2 a
C	24.9 f	9.3 c	32.1 f	14.9 c	7.2 a	5.6 a
D	30.1 e	7.0 d	37.3 e	9.0 d	7.1 a	2.0 a
E	30.2 e	6.8 d	38.3 e	10.5 d	8.1 a	3.6 a
F	49.1 a	16.9 a	60.2 a	21.7 a	11.1 a	4.8 a
G	45.2 b	9.7 c	56.5 b	13.3 c	11.2 a	3.6 a
H	35.0 d	10.7 c	43.5 c	13.7 c	8.5 a	3.0 a
I	30.2 e	11.0 c	40.6 d	14.2 c	10.4 a	3.2 a
J	41.8 c	13.5 b	53.6 b	17.6 b	11.7 a	4.1 a
K	36.4 d	9.7 c	44.9 c	12.2 d	8.5 a	2.4 a
L	50.0 a	16.3 a	60.2 a	19.3 b	10.2 a	2.9 a
CV(%)	7.82		5.87		37.42	

Table 5 shows the net change in water soluble P by depth and treatment. After 30 days, the net change in water soluble P from 0-1 cm depth relative to an unamended control core from the same plot ranged from 23 to 103 mg kg^{-1}. Labile organic P was an inconsequential fraction of the P after compost addition.

Table 5. Effect of compost bedded pack dairy waste amendment on net change in water soluble P by depth (mean $\pm$ sd, n=3)

Application Rate (Mg/ha)	0-1 cm depth (mg kg^{-1})	4-5 cm depth (mg kg^{-1})
1	23.3 $\pm$ 16.3	29.4 $\pm$ 13.5
2	25.9 $\pm$ 16.2	15.1 $\pm$ 13.6
4	40.5 $\pm$ 9.1	19.6 $\pm$ 13.8
8	103.3 $\pm$ 67.6	8.2 $\pm$ 11.1

The initial results of this study suggest that increasing the rate of the compost material results in an immediate increase in water soluble P in the first cm of soil that is proportional to the rate applied. However, the increase in water soluble P at deeper depths was not consistent and reaffirms the relative immobility of soluble P in the soil environment immediately after application.

Conclusions

From these studies, we learned that CBP material often yields more bioavailable P in soils than fresh manure if added on a uniform total P basis. Any addition of dairy waste may increase STP with more pronounced changes occurring at higher rates of application, as the soil is less able to buffer this change, regardless of initial STP. As literature suggested, greater changes in STP occurred in soils with higher native STP, and these soils exhibited higher bioavailable P content. For this reason, soils with higher STP should be amended with CBP material on a P basis to mitigate the risk of overloading soils with P.

We hypothesize that greater amounts of bioavailable P and Mehlich III P resulting from soils amended with CBP are due to the possible amorphous nature of CBP, which may hinder adsorption to soil colloid surfaces. If a producer is utilizing a high STP soil, we recommend that CBP additions should be incorporated to prevent P runoff risk.

Use of time-lapse electrical resistivity to image solute transport in a karst conduit

Basic Information

Title:	Use of time-lapse electrical resistivity to image solute transport in a karst conduit
Project Number:	2014KY238B
Start Date:	3/1/2014
End Date:	2/28/2015
Funding Source:	104B
Congressional District:	KY 6th
Research Category:	Ground-water Flow and Transport
Focus Category:	Groundwater, Methods, Solute Transport
Descriptors:	None
Principal Investigators:	Audrey Hucks Sawyer

Publication

1. Atcher, Clay, Audrey H. Sawyer, Junfeng Zhu, and James Currens, 2015, A Test of Combining Time-Lapse Electrical Resistivity Imaging and Salt Injection for Locating Karst Conduits, 2015, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, p. 59.

Use of Time-Lapse Electrical Resistivity to Image Solute Transport in a Karst Conduit

Problem and Research Objectives

Karst conduits are difficult to image using traditional geophysical methods. Yet it is critical to understand the location of karst conduits and their ability to convey solutes and contaminants in order to better manage water resources in karst aquifers. Improved prediction of a conduit's location can be used to site new groundwater wells and understand a conduit's size and depth. One promising method for imaging karst conduits is to combine a time-lapse resistivity survey with a salt tracer injection. The appearance of salt solution in the conduit will decrease bulk electrical resistivity in the conduit, and differences over time in the two-dimensional resistivity field from time-lapse electrical resistivity surveys may be useful for resolving the conduit location. Time-lapse electrical resistivity methods have been used to successfully quantify mass transfer in fractured aquifers [Singha et al., 2007] and surface water-groundwater interaction in streams [Ward et al., 2010].

The objective of this study was to assess whether time-lapse electrical resistivity methods hold promise for detecting karst conduits. This research could potentially yield a new method for detecting conduits in karst and would provide new information about a conduit feature that conveys water to Royal Spring, the municipal water source for Georgetown, Kentucky.

The novelty of this study lies in the combined use of a salt tracer injection and remote geophysics (time-lapse electrical resistivity imaging, or ERI) to detect changes in electrical resistivity within a known conduit. We validated results of ERI using in-situ conductivity measurements from a well that intersects the conduit. The salt injection was performed by the Hydrogeology Class at the University of Kentucky on April 9, 2014. Salt was injected into a karst window in the Kentucky Horse Park (Lexington, Kentucky) upgradient from a monitoring well and the electrical resistivity transect (Figure 1).

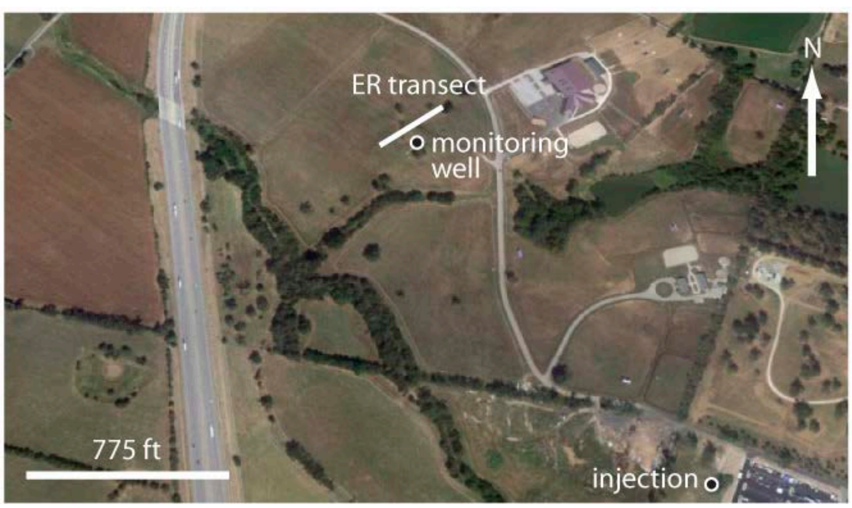

Figure 1: Map of injection site, monitoring well, and electrical resistivity transect at the Kentucky Horse Park in Lexington, KY.

Based on data from the monitoring well, electrical resistivity in the conduit decreased from its initial value by approximately 20% during passage of the peak tracer concentration. This projected supported the inversion and analysis of the ERI data to image tracer movement in the conduit.

Methodology

Two-thousand pounds of salt dissolved in 900 gallons of water were injected into a karst window at the Kentucky Horse Park (Lexington, KY) on April 9. The injection duration was approximately 45 minutes. Electrical resistivity in the conduit was monitored approximately 1000 m downgradient using two methods: in-situ well monitoring and remote electrical resistivity imaging. The well was equipped with a conductivity sensor deployed at the conduit depth of ~18.5 m below ground surface. Conductivity (the inverse of electrical resistivity) was logged every ten minutes. The electrical resistivity data were collected with a transect of 42 electrodes spaced 10 feet apart and located approximately 50 feet from the monitoring well. Background data collection consisted of collecting three sets of pre-injection measurements. Data collection continued for approximately five hours after injection ended to monitor flushing of the tracer from the conduit. Data were collected at approximately 20-minute intervals.

Electrical resistivity data were inverted using the research code R2 (v2.6, Generalized 2-D Inversion of Resistivity Data, available at (http://www.es.lancs.ac.uk/people/amb/Freeware/freeware.htm). We implemented the difference inversion strategy of LaBrecque and Yang [2001] that seeks the change in model parameters from the initial model by inverting on the change in observed electrical data. This inversion scheme minimizes systematic errors in inversion models and reduces artifacts in the inversion images. The observations from the monitoring well were used to validate ERI results. All analyses were performed by Mr. Atcher and PI Sawyer on a desktop computer.

Principal Findings and Significance

Time-lapse ERI in combination with salt tracer injection is a valuable tool for characterizing solute transport in karst aquifers. In this case study, salt tracer breakthrough was imaged at a known conduit depth and confirmed with in-situ conductivity data from a nearby monitoring well. The breakthrough behavior inferred from the conductivity sensor in the well and time-lapse ERI differed markedly, though the approximate timing of solute arrival was similar. In-situ observations from the monitoring well yielded detailed information about the tail of the breakthrough curve that was below detection in ERI inversions. Meanwhile, time-lapse ERI provided new information about the conduit path in the vicinity of the monitoring well. Pixel breakthrough behavior in ERI inversions was bimodal unlike breakthrough behavior at the observation well, likely due to out-of-plane detection of solute movement along the three-dimensional conduit. Pixel breakthrough curves from ERI inversions are only an apparent representation of solute transport and cannot replace in-situ observations.

Time-lapse ERI is ideally suited for locating and mapping approximate conduit locations, in part due to its affordability. However, time-lapse ERI also suffers from spatial and temporal smoothing and relatively large detection limits, so quantitative interpretation of solute transport and conduit size ultimately requires installation of monitoring wells and in-situ observations. Where monitoring wells exist, time-lapse ERI is a highly complementary approach that can provide additional spatial and temporal information on solute transport and conduit morphology.

Information Transfer Program Introduction

Information transfer activities are an important part of the overall program of the Kentucky Water Resources Research Institute. There are two main components, an annual symposium and the institute web sites. The institute also participates in and supports numerous other technology transfer activities throughout the year.

Kentucky information transfer project

Basic Information

Title:	Kentucky information transfer project
Project Number:	2014KY235B
Start Date:	3/1/2014
End Date:	2/28/2015
Funding Source:	104B
Congressional District:	Ky 6
Research Category:	Not Applicable
Focus Category:	None, None, None
Descriptors:	None
Principal Investigators:	Lindell Ormsbee

Publication

1. Proceedings Kentucky Water Resources Annual Symposium, 2015, Kentucky Water Resources Research Institute, Lexington, Kentucky, 86 p.

Kentucky Information Transfer Project (2014KY235B)

Problems and Objectives

The Water Resources Research Act requires that Institutes or Centers shall:

1) plan, conduct, or otherwise arrange for competent applied and peer reviewed research that fosters -

- (A) improvements in water supply reliability
- (B) the exploration of new ideas that -
  - (i) address water problems; or
  - (ii) expand understanding of water and water-related phenomena;
- (C) the entry of new research scientists, engineers, and technicians into water resources fields; and
- (D) the dissemination of research results to water managers and the public.

2) cooperate closely with other colleges and universities in the State that have demonstrated capabilities for research, information dissemination, and graduate training in order to develop a statewide program designed to resolve State and regional water and water related land problems.

Each institute shall also cooperate closely with other institutes and other organizations in the region to increase the effectiveness of the institutes and for the purpose of promoting regional coordination.

Kentucky information transfer activities are conducted in support of these objectives.

Methodology

Information transfer activities are an important part of the overall program of the Kentucky Water Resources Research Institute. There are 2 main components, an annual symposium and the Institute's web sites. The Institute also participates in and supports other technology and information transfer activities throughout the year.

The Associate Director develops the program for the Annual Water Resources Symposium. Presentations in both platform and poster format allow researchers and practitioners to share progress on planned, ongoing, and completed water-related activities throughout the Commonwealth each year. Recipients of the 104(b) student research enhancement grants are required to present the results of their projects at the symposium.

The Information Specialist Senior assists with posting program announcements and the proceedings volume for the symposium on the web site. She develops and maintains content for several web sites including the main Institute page at: www.uky.edu/waterresources/. Links for additional sites describing projects and activities (for example, volunteer sampling results and watershed pages for the Kentucky River basin) are provided on the main web site. Research translation to make results accessible for a variety of audiences is a major goal for all of the technology transfer activities of the unit.

The Institute cooperates closely with other groups and agencies in planning additional technology transfer activities in the Commonwealth. These efforts included support for seminars/lectures, support for other web sites, and open houses during Earth Science Week and Engineering Day. Institute staff members serve a variety of support roles on technical committees and advisory panels for agencies and volunteer organizations to help disseminate relevant information about ongoing activities and research results.

Principal Accomplishments and Activities

Kentucky Water Awareness Month is an educational program of the University of Kentucky Cooperative Extension Service, Environmental and Natural Resources Issues (ENRI) Task Force (the Associate Director of KWRRI serves on this group). The program promotes overall water awareness for citizens of Kentucky During May each year. Materials are developed by a committee at the state level and distributed to all of the 120 county extension offices in the state. Individual county agents are encoruraged to tailor the program to fit their county's specific needs and to use the materials to enhance their program efforts. The materials remain available throughout the year for use by classroom teachers, 4-H volunteers, and others interested in water issues through the ENRI internet site: www.ca.uky.edu/enri/ The Task Force is also working to encourage Project WET training for extension agents across the Commonwealth. A separate educational program for local elected officials is also under development to inform them of potential water resource issues in local communities.

Water Week, September 15-20, 2014, was a week-long series of events designed to inform faculty, staff, and students on the University of Kentucky campus of the importance of water in the environment. This was the pilot year and plans are to build this into an annual fall event. The project was a collaborative between the College of Agriculture, Food, and the Environment, the College of Arts and Sciences, the College of Engineering, the Kentucky Geological Survey, the Tracy Farmer Institute for Sustainability and the Environment, and the Kentucky Water Resources Research Institute. Featured events for 2014 included a scavenger hunt and photo contest, a film screening and panel discussion ("Last Call at the Oasis"), a water career panel (informal discussion with science, industry and government experts), a lecture ("Rivers and Flooding in the 21st Century," Nicholas Pinter, Department of Geology, Southern Illinois University), a second film screening ("Watermark"), and a student field trip to Robinson Forest to explore aquatic habitats. These events were organized by a multidisciplinary group of researchers working toward the advancement of water-related research and education at the University of Kentucky.

An open house was held on Wednesday evening 10/15/14 in association with Earth Science Week. This event was co-sponsored with the Kentucky Geological Survey. KWRRI staffed a water exhibit for the elementary, middle school, high school students, and their parents who attended the event (approximately 200 people).

Engineers Day, or E-Day, is a celebration of everything engineering has to offer. From building bridges to discovering new medications to writing the software that powers our cell phones, engineers and computer scientists do the things that make the 21st-century world work. The 2015 E-Day celebration at the University of Kentucky in Lexington was on Saturday, Feb. 28, from 9 a.m. to 1 p.m. E-Day comes at the end of Engineers Week, an annual event sponsored by a coalition of more than 100 professional societies, major corporations and government agencies dedicated to promoting math and science literacy and ensuring a diverse and well-educated future engineering workforce. KWRRI staffed an Enviroscape exhibit demonstrating sources on nonpoint source pollution for participants at the event.

Cyberseminars provided through the Consortium for the Advancemnt of Hydrolgoic Sciences, Inc. were made available of the University of Kentucky Campus for interested faculty, staff, students, and local professionals. The initial University of Kentukcy membership in CUAHSI was underwritten by the KWRRI.

The Kentucky Water Resources Annual symposium was held on March 9, 2015. Although the date of the symposium fell outside of FY2014, most of the planning and preparation for the event occurred during the fiscal year. An opening plenary session featured 3 oral presentations. This was followed by a session including 18 poster presentations. Two concurrent sessions provided time slots for 22 oral presentations to round out the program. The noon luncheon provided an opportunity for presentation of annual awards acknowledging outstanding contributions in the areas of Water Research, Water Practice, and Water Quality. Approximately 120 people attended the meeting. Abstracts for all of the presentations were distributed to participants on the day of the meeting: Proceedings of the Kentucky Water Resources Annual symposium, 2015, Kentucky Water Resources Research Institute, Lexingtonm KY, 86 p. The full proceedings document is also available online through a link on the institute web site: www.uky.edu/waterresources/ The document includes contact information for all of the authors and presenters. Symposium attendees also receive a list of attendees providing basic contact information for each individual who pre--registered for the symposium. Attendees include researchers, personnel from local, state, and federal agencies, undergraduate and graduate students, participants from volunteer groups and NGOs, and members of the general public. Conference registration fees are kept low through partial subsidy of symposium expenses (using 104(b) technology transfer and matching funds) to ensure accessibility to individuals from all potential audiences. All of the 104(b) student research enhancement projects funded through the Institute are required to present their results at the symposium.

Maintenance of the institute web site provides open access for those interested in the activities of the Institute. The site also provides additional links to related sites and information maintained by others. Creation and maintenance of the web site are ongoing throughout the year. Links on the site provide direct access to the Kentucky Center of Excellence for Watershed Management, the University of Kentucky Superfund Research Center, the Kentucky Research Consortium for Energy and Environment, the Kentucky River Watershed Watch, the Tracy Farmer Institute for Sustainability and the Environment, the Environmental Research and Training Laboratory, and the Kentucky Geological Survey.

As a part of the University of Kentucky Superfund Research Program, the Kentucky Water Resources Research Institute planned and presented 6 seminars for employees in the Kentucky Department for Environmental Protection.

2014 Superfund Seminar Series

Lindell Ormsbee, PhD and Kelly Pennell, PhD, University of Kentucky, Wednesday February 4, 2015, **National highlights of the Superfund Program**

Bradley Newsome, Ph.D., University of Kentucky, Thursday, November 20, 2014 at 12:00pm, ***Taking a holistic approach to risk reduction: biomedical intervention, pollutant remediation, and research translation***

Wendy Heiger-Bernays, Ph.D., Boston University, Wednesday, October 29, 2014 at 12:00pm, ***Derivation of TCE Toxicity Values and Implications for Risk Management***

Kevin J. Pearson, Ph.D., Wednesday, April 2, 2014 at 12:00pm, ***Developmental Programming: Effects of Diet, Exercise, and Polychlorinated Biphenyl Exposure during Pregnancy on Long-term Health in Offspring***

Lindell Ormsbee, Ph.D., Wednesday, March 5, 2014 at 12:00pm, ***Best Strategies for Solving Environmental Problems***

Kelly G. Pennell, Ph.D., Wednesday, February 5, 2014 at 12:00pm, ***Characterizing Vapor Intrusion Exposure Risks***

All of these brown-bag luncheon seminars were held at the Kentucky Department for Environmental Protection Training Room, 300 Fair Oaks Drive, Room 301D, Frankfort, KY, *Co-Sponsored by the UK-Superfund Research Program, the Kentucky Water Resources Research Institute, and the Kentucky Division of Waste Management (Kentucky Department for Environmental Protection).*

USGS Summer Intern Program

None.

Student Support					
Category	Section 104 Base Grant	Section 104 NCGP Award	NIWR-USGS Internship	Supplemental Awards	Total
Undergraduate	9	0	0	0	9
Masters	3	0	0	0	3
Ph.D.	2	0	0	0	2
Post-Doc.	0	0	0	0	0
Total	14	0	0	0	14

Notable Awards and Achievements

2014KY226B - Jennifer-Grace Ewa's work on this project led to the award of a prestigious post-doctoral fellowship from the University of Denver in open space and unequal distribution and access to open space, through the Interdisciplinary Research Incubator for the Study of (In)Equality (IRISE). Alexandra Chase's work on the project led to an American Law Institute Environmental Law Scholarship to attend the 2015 American Law Institute educational program on environmental law in Washington, DC

2014KY228B - Undergraduate student Sam White was selected as an Ogden College Scholar at Western Kentucky University. He was also selected as a recipient of the George V. and Sadie Skiles Page Award for Excellence in Scholarship. Undergraduate student Frankie Wallace received awards for outstanding session presentations at the 2014 Kentucky Academy of Sciences and the 2015 Western Kentucky University Student Research Conference. Wallace also received a Western Kentucky University FUSE (Faculty-Undergraduate Student Engagement) Grant to continue his research ($4,500 for travel, supplies, and other support from 1/1/2015 to 12/31/2015).

2014KY229B - Benjamin B. Tumolo, Master of Watershed Science candidate, received three institutional awards from Murray State University resulting from his participation in the research project: 1) Jenkins Scholarship for Reservoir Research (February 2015, $1,000), 2) Jones College of Science, Engineering, and Technology Graduate Conference Travel Grant to present the results of the research (March 2015, $1,200), and 3) Dr. Morgan Emory Sisk Sr. Memorial Scholarship (April 2015, $3,500).

2014KY230B - Undergraduate student researcher (Evan Willett) was named the University of Kentucky Department of Civil Engineering Outstanding Junior in 2015. The results of this research project helped to inform the Principal Investigator's proposal for her recently awarded NSF CAREER Grant entitled "Vapor Intrusion, Environmental Health, and Knowledge Brokers: A Three Dimensional Perspective" (Dr. Kelly Pennell - grant dates 2015-2020).

2014KY232B - Undergraduate students Nicole Meade and Nelson Phillips received an award for their verbal presentation at the Morehead State University Student Scholarship Celebration.

Publications from Prior Years

1. 2012KY208B ("Determining groundwater flow velocities and discharge rate at the Kentucky Horse Park Royal Spring conduit monitoring station") - Conference Proceedings - Currens, J.C., C.J. Taylor, S. Webb, J. Zhu, S. Workman, C. Agouridis, J. Fox, and A. Husic, 2015, Initial Findings from the Karst Water Instrumentation System Station, Royal Spring Groundwater Basin, Kentucky Horse Park 2010-2014, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, p. 9-10.
2. 2012KY204B ("Field-scale water and bromide transport as affected by land use and rainfall characteristics") - Conference Proceedings - Yang, Y. and Ole Wendroth, 2015, Space-Time Behavior of Soil Water Status Measured Across Two Land Use Systems, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, p. 29.
3. 2010KY137B ("A coupled hydrologic and biogeochemical modeling approach to understand if in-stream depositional zones are a carbon source or sink to the atmosphere") - Conference Proceedings - Husic, A., J. Fox, C. Agouridis, J. Currens, S. Workman, W. Ford, C. Taylor, 2015, Sediment Organic Carbon Fate and Transport Mechanisms in a Fluvial Karst System in the Bluegrass Region, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, p. 35-36.
4. 2010KY137B ("A coupled hydrologic and biogeochemical modeling approach to understand if in-stream depositional zones are a carbon source or sink to the atmosphere") - Conference Proceedings - Al Aamery, Nabil and Jimmy Fox, 2015, Climate Change Impact on the Transport of Water, Sediment, and Sediment Carbon in the Inner Bluegrass, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, p. 73-74.
5. 2009KY129B ("Study of turbulent processes driving sedimentation in the rivers of Kentucky") - Conference Proceedings - Ghasemi, Amirreza, Jimmy Fox, and Jeremy Puckett, 2015, Macroturbulence in Uniform and Nonuniform Hydraulically Rough Open Channel Flow, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, p. 75-76.