

Kentucky Water Resources Research Institute

Annual Technical Report

FY 2002

Introduction

The FY 2002 Annual Technical Report for Kentucky consolidates reporting requirements of the Section 104(b) base grant award in a single technical report that includes: 1) a synopsis of each research project supported during the period, 2) a list of related reports, 3) a description of information transfer activities, 4) a summary of student support during the reporting period, and 5) notable achievements and awards during the year.

Research Program

The activities supported by Section 104(b) and the required matching are interwoven into the Kentucky Water Resources Research Institute's total program. Other elements of the Institute's program during FY 2002 included: 1) the Environmental Systems Graduate Certificate, 2) the Environmental Protection Scholarship Program, and 3) Research, Service, and Technology Transfer Activities funded by other sources.

Memorandum of Agreement projects with the Kentucky Division of Water included: 1) TMDL development for pH, nutrients, and pathogens in several Kentucky streams, 2) Evaluation of the impacts of gravel dredging on Buck Creek, 3) Planning and management of the Kentucky Semi-Annual Nonpoint Source Conference (9/23/02) and the EPA Quad Regions NPS Annual Conference (9/24-25/03), and 4) Diagnostic watershed model for pathogen speciation and mitigation.

Several additional projects were funded by the Natural Resources and Environmental Protection Cabinet (NREPC), the Kentucky Cabinet for Health Services (CHS), the Kentucky Department of Military Affairs (DMA), the Kentucky River Authority (KRA), the National Institute of Environmental Health (NIEH), and east Kentucky PRIDE (Personal Responsibility in a Desirable Environment): 1) Environmental Protection Scholarship (NREPC), 2) Technical support for the Maxey Flats Nuclear Disposal Site (CHS), 3) Technical support for the Paducah Gaseous Diffusion Plant (Federal Facilities Agreement and Agreement in Principle - CHS), 4) Technical support for environmental construction (DMA), 5) Technical support for solid waste management (DMA), 6) Evaluation of possible models for use in developing an operational model for the Kentucky River (KRA), 7) Watershed management services and water resources planning (KRA), 8) Superfund public outreach program for Kentucky (NIEH), and 9) Kentucky PRIDE water quality assessment.

Evaluating site remediation success using a sensitive biochemical indicator in fish

Basic Information

Title:	Evaluating site remediation success using a sensitive biochemical indicator in fish
Project Number:	2002KY1B
Start Date:	3/1/2002
End Date:	2/28/2003
Funding Source:	104B
Congressional District:	Sixth Kentucky
Research Category:	Biological Sciences
Focus Category:	Toxic Substances, Water Quality, Ecology
Descriptors:	PCBs, biomonitoring, toxicology
Principal Investigators:	ADRIA ELSKUS

Publication

1. Brammel, B.F., J.S. McLain, J. T. Oris, and A.A. Elskus, Submitted, Use of Rainbow Trout as a Biomonitoring Tool for Evaluating the Effectiveness of a PCB Remediation Project, Environmental Toxicology and Chemistry.
2. Brammel, B. F., D. J. Price, X. Arzuaga, W. J. Birge, and A. A. Elskus, 2003, CYP1A Expression in Longear Sunfish as a Biomarker of PCB Exposure, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, Kentucky, p. 1.
3. B. F. Brammel, J. S. McLain, J. T. Oris, and A. A. Elskus, 2003, Use of rainbow trout as a biomonitor for evaluating the effectiveness of a PCB remediation project, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, Kentucky, p. 3.
4. Arzuaga, X. And A. A. Elskus, 2003, Resistance to Polychlorinated Biphenyl (PCB) and Polyaromatic Hydrocarbon (PAH) Mediated Induction of CYP1A in a PCB Resistant Population of Fundulus Heteroclitus, 2003, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, Kentucky, p. 7.
5. Price, D. J. and W. J. Birge, 2003, The Stoneroller Minnow as an In Situ Monitor of PCB Contamination in Freshwater Systems, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, University of Kentucky, Lexington,

Kentucky, p. 5.

Problem and Research Objectives

Polychlorinated biphenyls (PCBs) are ubiquitous aquatic pollutants with significant toxic effects in both humans and fish, including altered reproduction, immunosuppression, carcinogenesis, and neurotoxicity. Significant levels of environmental PCBs in Kentucky have led to the posting of fish advisories in several waterways (Kentucky Division of Water). The focus of the present study was the Town Branch-Mud River (TB/MR) system in Kentucky, a PCB-contaminated site currently under remediation. This project addressed several needs identified by the *Water Science and Technology Board* [3], including the need to understand the impact of contaminants on higher organisms, to monitor the time course of recovery following contamination, and to evaluate the effectiveness of management efforts to improve water quality. Water quality in Kentucky is evaluated based on contaminant concentrations in water, sediment or biota, and/or on biological indices of species diversity. Contaminant concentrations alone provide no information on organism response, and diversity indices do not distinguish between response to contaminants, habitat disturbance, or natural stressors. For example, there is no information on whether exposure to PCBs in the TB/MR system is producing sublethal effects in fish populations in that system, and/or whether present remediation efforts are reducing those effects. The enzyme, CYP1A, is strongly and rapidly induced in animals exposed to toxic organic pollutants, including PCBs. We hypothesized that CYP1A levels in TB/MR resident fish reflect organic contaminant levels at their site of collection. Research objectives were: 1) To use CYP1A levels in caged fish to evaluate the effectiveness of bioremediation efforts in the TB/MR system, and 2) to determine if CYP1A levels in resident fish in the TB/MR system reflect expected habitat contamination level.

Methodology

As our first step in evaluating PCB-remediation success in the TB/MR system, we measured CYP1A response in naive fish caged in the system. This was necessary because resident fish may not adequately reflect conditions at the site of capture as some species may move between remediated and unremediated areas, while others may have developed resistance to PCB induction of CYP1A. For these reasons, we first evaluated the CYP1A1 response of hatchery-reared, rainbow trout caged at each study site to provide a site-to-site evaluation of the effectiveness of bioremediation efforts in the TB/MR system. Rainbow trout were caged at reference, remediated and unremediated sites in the TB/MR waterway for two weeks in Spring 2002 and evaluated for CYP1A expression level (messenger RNA, CYP1A protein, CYP1A enzyme activity), and gut contents were analyzed to determine what organisms were consumed by the caged trout. To evaluate the response of resident fish species, we measured hepatic CYP1A expression in fish collected from reference, remediated and unremediated sites in the TB/MR waterway. Species were selected based on known sensitivity to CYP1A inducers and on our ability to collect statistically-sufficient numbers of individuals at each site to distinguish site differences in CYP1A response. Resident green sunfish, longear sunfish, creek chub, yellow bullhead, rock bass, spotted bass, and common carp were collected in Fall 2002 and PCB body burdens and CYP1A1 activity evaluated.

Principal Findings and Significance

Our first principal finding suggests that bioremediation efforts to remove polychlorinated biphenyl contamination in the Town Branch waterway were not completely successful. We have three pieces of evidence supporting this conclusion; biological response in fish caged at the site, PCB levels in sediments at the site, and PCB levels in the flesh of resident fish from this site. Rainbow trout caged at the remediated Town Branch site had significantly elevated levels of the biomarker enzyme, CYP1A1, in both their gills and their liver, relative to fish caged upstream at a reference site. Elevated CYP1A expression occurs in response to exposure to inducing chemicals, typically PAHs and PCBs [5, 18]. That CYP1A levels were elevated in the gills of the caged fish, together with evidence of low food consumption by these fish, suggests exposure to contaminants in this system is waterborne. Although not part of our original proposal, we also evaluated levels of PCBs in sediments and resident fish at these sites. Samples of sediment and resident fish were collected by doctoral students in my laboratory, Ben Brammell and Xabier Arzuaga, and by David Price, a doctoral candidate in the laboratory of Dr. Wesley Birge, (Dept of Biology at UK), and analyzed by David Price. These analyses indicated that PCBs are present and bioavailable in the TB/MR system. Highly elevated levels of PCBs remain in the sediments from the remediated section of Town Branch (up to 40 ppm total Aroclor dry wt basis) and have been bioaccumulated to extraordinarily high levels by resident fish collected at this site (up to 98 ppm wet edible flesh, with a median concentration of 24 ppm). These levels are at the high end of those measured in fish from New Bedford Harbor, Massachusetts, considered one of the most highly contaminated PCB Superfund sites in the US. Median PCB levels in edible flesh ranged from 5.5 - 7.4 ppm for New Bedford Harbor flounder species up to 24 ppm median PCB for American eel [17]. PCB levels in fish from the unremediated section of the Mud River ranged from non-detectable up to 20 ppm (median 3.89 ppm), indicating that this site is also a significant source of PCBs for resident species. In comparison, Town Branch reference fish collected upstream of the remediated site had PCB body burdens that were up to 100 times lower than fish from the remediated site, ranging from non-detectable to < 3 ppm (median = 0.56 ppm). Our second main finding was that resident species appear to have developed resistance to at least some of the biological effects of PCBs. Since inducing chemicals are present in this system, as demonstrated by induction of CYP1A in the caged trout, lack of induction in resident species suggests they have developed resistance to the contaminants present in this habitat. Moreover, their PCB body burdens are similar to, and in some cases well above, those known to induce CYP1A in other fish species [1, 8]. The ability of fish to develop resistance to halogenated hydrocarbons, including PCBs and dioxins, has been demonstrated in resident populations of fish in several contaminated sites [2, 6, 7, 12, 15, 16]. To confirm our suspicion that resident species have acquired resistance to PCBs, we collected green sunfish and yellow bullhead catfish in March 2003 and are currently depurating them for use in challenge experiments with PCBs to be conducted in July 2003 (half life of PCBs in fish is 4 months, [13]). By treating depurated resident fish with PCBs we expect to find little to no induction of CYP1A if the fish have developed resistance relative to similarly treated reference fish. In addition to the lack of CYP1A1 inducibility in resistant fish,

these animals have also consistently demonstrated resistance to the harmful effects of these chemicals (reviewed in [6, 9]). If resident fish demonstrate resistance to CYP1A induction in our PCB challenge experiments, we will conduct further studies to explore whether these fish have also developed resistance to the toxic effects of PCBs, specifically, the well-characterized ability of PCBs to alter thyroid hormones [4, 10, 11, 14]. To address this question, we plan to conduct studies examining PCB effects on thyroid hormone in resistant fish under our 2003/2004 USGS 104B grant.

References cited

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2. Bello SM, Franks DG, Stegeman JJ and Hahn ME, Acquired resistance to Ah receptor agonists in a population of Atlantic killifish (*Fundulus heteroclitus*) inhabiting a marine superfund site: In vivo and in vitro studies on the inducibility of xenobiotic metabolizing enzymes. *Toxicol. Sci.* **60**(1): 77-91, 2001.
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7. Elskus AA, Monosson E, McElroy AE, Stegeman JJ and Woltering DS, Altered CYP1A expression in *Fundulus heteroclitus* adults and larvae: a sign of pollutant resistance? *Aquatic Toxicology* **45**(2-3): 99-113, 1999.
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13. Niimi AJ and Oliver BG, Biological half-lives of polychlorinated biphenyl (PCB) congeners in whole fish and muscle of rainbow trout (*Salmo gairdneri*). *Can. J. Fish. Aquat. Sci.* **40**: 1388-1394, 1983.

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Environmentally-induced genes and mechanisms of inheritance: How are the effects of contaminant exposure transferred from one generation to the next?

Basic Information

Title:	Environmentally-induced genes and mechanisms of inheritance: How are the effects of contaminant exposure transferred from one generation to the next?
Project Number:	2002KY2B
Start Date:	3/1/2002
End Date:	2/28/2003
Funding Source:	104B
Congressional District:	Sixth Kentucky
Research Category:	Biological Sciences
Focus Category:	Toxic Substances, Water Quality, Surface Water
Descriptors:	prolactin, growth hormone, xenoestrogens, endocrine disruptors
Principal Investigators:	Brian S. Shepherd

Publication

1. Johnson, J., J. Silverstein, B. Small, W. R. Wolters, and B.S. Shepherd, In Press, Disparate Regulation of the Insulin-Like Growth Factor Binding Proteins in an Ictalurid Teleost (*Ictalurus punctatus*), *General and Comparative Endocrinology*.
2. Drennon, K., S. Moriyama, H. Kawauchi, B. Small, J. Silverstein, I. Parhar, and B. Shepherd, Accepted with Revision, Development of an Enzyme-Linked Immunosorbent Assay (ELISA) for the Measurement of Plasma Growth Hormone (GH) Levels in Channel Catfish (*Ictalurus punctatus*): Assessment of Environmental Salinity and GH-Secretagogues on Plasma GH Levels. *General and Comparative Endocrinology*.

Problem and Research Objectives

The pituitary hormones, prolactin (PRL) and growth hormone (GH) are unequivocally involved in vertebrate development and the normal function of tissues and organs. Sex steroids, such as estrogen, control the development of the pituitary gland, its gene expression and the release of hormones (e.g., GH & PRL); however, the neuroendocrine system is further influenced by external factors such as stress, diet, physiological state, and pollutants which have been found to possess biological actions similar to that of estrogen (xenoestrogens). These hormonal mimetics are called "endocrine disrupting chemicals" (EDCs) or "xenoestrogens", because they possess estrogenic activities that can affect endogenous hormones in inappropriate ways. The idea that a single hormone (e.g., PRL) can regulate multiple physiological pathways, suggests that disturbances in its regulation, by an EDC, may negatively impact these pathways in an adult vertebrate and its offspring. We propose that contaminant exposure will not only affect pituitary physiology of the exposed organism, but will also influence the exposed organism's offspring through, as yet, poorly understood mechanisms. In this vein, scientists are increasingly using teleosts to study the effects of pollutants on vertebrate endocrinology as they are sensitive to these chemicals and encounter them routinely and chronically in their environment. To date, studies have mainly focused on the effects of EDCs on physiological end-points, with little emphasis on the impacted endocrine pathways themselves. Interestingly, recent reports suggest the presence of maternal mRNAs (hormone and hormone receptor mRNAs) in the unfertilized eggs of fish and this finding strongly suggests the involvement of these mRNAs in early embryonic development. Despite their presence, the significance and regulation of maternally-derived mRNAs have not been explored. Our aim was to study how estrogenic (e.g., hydroxylated PCBs) pollutants alter maternal endocrine physiology and how such alterations affect maternally-derived mRNAs in the unfertilized eggs of the Yellow Perch (*Perca flavescens*). An understanding of how maternal endocrine physiology affects offspring development will aid in future studies to improve the environmental monitoring and management practices of this important species in areas where endocrine disruption is suspected. To accomplish this, a major objective is the development of research tools that are needed to examine endocrine function in this, and other, important teleost species.

Methods

Using RT-PCR cloning procedures and DNA sequencing, we completed the cloning and characterization of the genes for several important hormones and are continuing to work on others. Total RNA was purified and first strand cDNAs were produced using 5' RACE techniques from pituitary (PRL, GH, SL) and liver (IGF-I) tissue. For cloning, primers were developed based upon conserved regions of known teleost sequences and PCR products were cloned into a plasmid and then transferred into competent *E. coli* cells. Cells were grown on LB agar with kanamycin to select for transformants and then specific clones were chosen, screened and grown in culture for plasmid isolation and DNA sequencing. We are conducting gene expression studies, using Northern Blotting, to identify the transcript size of the mRNAs for the hormones

that we have cloned as well as to determine tissue- and sex-specific differences in patterns of gene expression. In line with our efforts to develop methods to measure plasma hormone levels, we have some quantity of recombinant perch GH and antibody and plan to develop a radioimmunoassay that can be used to measure plasma GH levels in this teleost. We are also continuing our efforts to purify, through a new collaboration, other pituitary hormones (PRL & SL) important to growth and development in this teleost. Hormones will be sequentially purified using gel-filtration and HPLC procedures. Once putative hormone fractions for PRL and SL have been identified, antibodies will be developed in rabbits. We are also continuing field studies and have made significant strides in holding and maintaining yellow perch in our facilities at the University of Kentucky. We are also continuing to collect monthly samples of adult male and female yellow perch in order to examine sex- and season-specific changes in endocrine function and contaminant body burdens. In conjunction with our field studies, we are also sampling channel catfish as positive controls species. Our reason for this is that the perch and catfish inhabit different trophic niches. Furthermore, the catfish is an obligate, benthic-dwelling species, unlike the perch, and is therefore more likely to experience higher body burdens of environmental pollutants.

Principal Findings and Significance

At present, we have full-length cDNA clones for prolactin growth hormone, somatotactin, insulin-like growth factor-I and β -actin and partial clones for a newly identified hormone, termed "Ghrelin", which stimulates GH release and the β -estrogen receptor. Table 1 lists the nucleotide similarities of several other teleosts with the yellow perch PRL, GH, SL, and IGF-I cDNA sequences. Most notable is that yellow perch PRL appears to have a unique deviation from all other known teleost prolactins such that there is a codon gap associated with bases 190-192. In all but one of other known neo-teleost sequences, this codon encodes for the amino acid iso-leucine at position 64.

Table 1. Nucleotide sequence percent similarities for yellow perch PRL, GH, SL and IGF-I cDNAs against a taxonomically diverse group of teleosts. Dashes indicate that cDNA sequences were not available. An (*) designates the sequence with the greatest similarity as indicated by a standard nucleotide Blast search.

Yellow Perch	Black Sea Bream	Red Drum	Euro. Sea Bass	Sea Bream	Catfish	Zebra fish	Carp	Coho Salmon	Chum Salmon	Eel
PRL	-	-	86%*	86%	-	-	65%	-	70%	65%
GH	-	88%*	86%	87%	-	-	57%	63%	63%	-
SL	-	88%*	-	86%	59%	-	-	-	75%	64%
IGF-I	97%*	-	-	96%	-	68%	66%	83%	-	-

Also, in collaboration with Geoff Wallat (Ohio State Extension, Piketon, OH), we continue rearing larval perch in order to examine developmental-specific patterns of gene expression. This effort will allow us to identify and characterize the specific point at which females begin to grow faster than males and the endocrine basis for this difference.

Occurrence and distribution of mercury in Mammoth Cave National Park

Basic Information

Title:	Occurrence and distribution of mercury in Mammoth Cave National Park
Project Number:	2002KY4B
Start Date:	3/1/2002
End Date:	12/31/2003
Funding Source:	104B
Congressional District:	Kentucky Second
Research Category:	Water Quality
Focus Category:	Groundwater, Sediments, Solute Transport
Descriptors:	atmospheric deposition, karst systems, bioaccumulation
Principal Investigators:	Cathleen Joyce Webb

Publication

1. Webb, Cathleen J., Gretchen E. Berryman, Melissa A. Petty, Glenda H. Jones, and Stephen V. Hartman, 2003, Occurrence and Distribution of Mercury in Mammoth Cave National Park - Phase 0, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, Kentucky, p. 59-60.

Problem and Research Objectives

Atmospheric deposition of mercury from power plant emissions, a major input of mercury into the environment, is coming under closer scrutiny by regulatory agencies. With increasing demand for power, applications for many new coal-fired power plants are currently being considered. Consequently, an understanding of the existing levels of mercury is critical, particularly in a karst aquifer system (such as in South-central Kentucky) where transport of contaminants can be rapid. Mammoth Cave National Park (MCNP) is affected by the atmospheric deposition of mercury, contributed to by coal burning power plants. With over twenty new power plant applications under consideration in the Commonwealth of Kentucky, significant increases in mercury deposition to the park and surrounding area can be expected.

The overall vision and scope of this project were to understand the physical and geochemical processes that govern the fate and transport of mercury in a karstic aquifer system. Mercury mobility in surface water and ground water are of great concern because of toxic effects on the environment. Mercury is a persistent, bioaccumulative toxin, with significant impacts on aquatic species, such as mussels. The specific research project examines mercury transport in ground water and surface water in Mammoth Cave National Park. Water sampling results will be complemented by an investigation of the extent of bioaccumulation of mercury in fish and mussels and the levels and distribution of mercury in fish and mussels will be correlated with concentrations of mercury measured in the atmosphere, water, and sediments of the study site.

The specific objectives of this work were to: (1) establish the extent, occurrence, and distribution of mercury in groundwater, surface water and sediments in Mammoth Cave National Park, (2) determine the levels of mercury (a persistent bioaccumulative toxin) in fish and mussels in the Park in order to compare the mercury levels in fish and mussels to the health, diversity, population, and reproductive status of the species, and (3) investigate the fate and transport characteristics of mercury in a karst aquifer system. Objectives 1 and 2 have been actively pursued over the course of this project. Experimental work for objective 3 will be completed during summer 2003.

Methodology

Water samples were collected monthly and analyzed for mercury. Sediment samples were collected and analyzed on a seasonal basis. Rainfall was measured on a daily basis. Mercury analysis was done using Leeman Hydra atomic absorption (Hydra AA) spectrometry. The samples are digested using the Leeman Hydra Prep to convert all forms of mercury into Hg^{2+} and to eliminate the existence of any organic substance.

Procedure for sample digestion:

- 1) Sample mass determined and placed into a sample cup.
- 2) 2.25 mL concentrated AquaRegia (3:1 HCl: HNO_3) was added.
- 3) The sample cup was heated at 95 °C for 2 minutes in a water bath.

- 4) After the sample cooled, 6.75 mL 5% KMnO₄ was added.
- 5) 4.5 mL deionized (DI) water was added.
- 6) A second aliquot of DI water (4.5 mL) water was added.
- 7) The sample was heated in the water bath at 95 °C for 30 minutes.
- 8) After the sample cooled, 3.6 mL 12%:12% NaCl:hydroxylamine sulfate was added.

Procedure for sample analysis:

The concentration of mercury (ppt for water samples and ng/g for sediment samples) was determined by Leeman Hydra Atomic Absorption:

- 1) The spectrometer was calibrated with standard solutions.
- 2) The sample was withdrawn by a pump and mixed with the 10% SnCl₂:10% HCl solution, which ensures Hg²⁺ was reduced to Hg⁰, a volatile species.
- 3) In a liquid/gas separator, the mixed solution was bubbled by ultra high purity nitrogen and the mercury vapor was carried to pass through a dryer to dehumidify the gaseous mixture. The dry mercury vapor then entered a dual beam optical cell, which had been optimized for fast response time and high sensitivity. A mercury lamp controlled by the error signal of the reference beam delivered a stable source of emission at 254nm. Absorbance by the mercury cold vapor was measured using a solid state detector with a wide dynamic range.

The analytical results were organized into tables and graphs based on sample site location, mercury concentration, and the sampling month.

Samples of drum, a long-lived bottom feeding species, large mouth bass, and *Corbicula fluminea* the Asiatic clam were homogenized and analyzed for mercury using the same methods as sediment. Muscle tissue and liver from both types of fish were analyzed. The mercury concentrations of these water species were compared to other freshwater species according to the U.S. Food and Drug Administration Center for Food Safety and Applied nutrition Office of Seafood, 2001.

Principal Findings and Significance

Results of sampling and analysis of the water, sediment, and aquatic life of the park indicate detectable levels of mercury. As expected, mercury levels in water are generally low (0 - ~20 ppt) since mercury preferentially binds to sediments and organic material. Ugly Creek Spring and Bush Island sampling locations show peak levels of mercury in the 30 – 40 ppt range. Seasonal changes and precipitation levels affected the concentration of mercury that was detected in the water. Samples collected on days with high precipitation showed higher levels of mercury. Mercury concentrations observed in sediments ranged between 0 – 100 ppb. Pike Spring, Mile 205.7 Spring, and Big Spring all show consistent high levels of mercury in sediments (30 – 70 ppb range). These sites are all located in the upstream portion (Green River) of the sampling area. The 0.233 ppm mercury found in the muscle of the Drum fish illustrates the potential threat of mercury to the food chain. These fish are long-lived bottom feeders, and are vulnerable

to bioaccumulation of mercury from aquatic organisms, ground water, and sediment layers in MCNP. The observed mercury levels in Drum are comparable to those seen in other species that are considered within the safety limits of the U.S. Food and Drug Administration, but may indicate the potential for future increases that could pose a threat to the ecosystem.

Impacts of surface mine valley fills on downstream peak flows in eastern Kentucky

Basic Information

Title:	Impacts of surface mine valley fills on downstream peak flows in eastern Kentucky
Project Number:	2002KY6B
Start Date:	3/1/2002
End Date:	2/28/2003
Funding Source:	104B
Congressional District:	Sixth
Research Category:	Climate and Hydrologic Processes
Focus Category:	Floods, Surface Water, Hydrology
Descriptors:	mountaintop removal, coal mining, flood frequency and magnitudes
Principal Investigators:	Jonathan Phillips

Publication

1. Phillips, Jonathan D., Submitted, Impacts of Surface Mine Valley Fills on Headwater Floods in Eastern Kentucky , Environmental Geology, 43 p.

Problem and Research Objectives

The potential impacts of valley fills associated with mountaintop removal/valley fill (MTR/VF) coal mining on downstream flooding in the coalfields of eastern Kentucky and adjacent states have been a subject of both public debate and scientific uncertainty. Previous studies, and basic hydrologic principles, indicate that flood frequencies and magnitudes might be increased or decreased downstream of valley fills. This study explored three aspects of that issue.

Methodology

First, bankful channel dimensions downstream of VF sites were measured and compared to those of similar unmined basins to see if channel hydraulic geometry had adjusted to a new post-fill flow regime. Second, hydrologic models were applied to assess relative runoff production and surface and subsurface flow detention times in the eastern Kentucky coalfields, using input parameters reflecting the range of typical hydrologic conditions at valley fills and undisturbed low-order valleys. Finally, the likelihood of short-range variability of storm precipitation was evaluated by applying the state probability function to NEXRAD radar estimates of precipitation for two 2001 storms which produced flash floods in eastern Kentucky.

Principal Findings and Significance

There was no systematic evidence of changes in bankful channel dimensions downstream of MTR/VF sites. However, most sites were essentially bedrock channels. Even if changes in flow regime have occurred, there has likely been insufficient time for a morphological response in these channels. Channel geomorphology is not a reliable indicator of changes in flow regimes in this situation. While mobile-bed, alluvial channels are able to respond morphologically to changes in flow regime relatively rapidly, the vast majority of channels affected or potentially affected by valley fills in eastern Kentucky are bedrock, or characterized by a thin veneer of sediment above bedrock. The response time for such channels is too long to make channel dimensions and hydraulic geometry a useful tool for addressing the problem of this study.

Results of the hydrologic modeling show that there is a clear risk of increased flood potential (greater runoff production and less surface flow detention) following MTR/VF operations, and suggest that, on balance, valley fills are more likely to increase than to decrease flood potential. However, there is a wide range and variability of outcomes, both qualitatively and quantitatively. Reduced as well as increased flood risks are possible, and the degree of either may vary markedly. The effects of MTR/VF mining on downstream peak flows are highly contingent on local pre- and post-mining conditions, and it would be unwise to apply generalizations to specific sites.

Finally, the occurrence of flash floods downstream of MTR/VF operations when nearby unmined areas did not flood or had less severe floods has frequently been explained in terms of locally greater precipitation. The spatial structure of precipitation from two storms causing flash flooding in 2001 indicates that, at the scale of the analysis (pixel size of approximately 2 km) large local variations in storm precipitation are unlikely. However, local spatial variability in storm precipitation and in runoff response is a well-known phenomenon, and it is likely that in at least some cases flash floods in both VF and unmined headwater basins can be attributed to highly localized precipitation and microscale meteorological phenomena.

The results of this study, including the review of previous work, reflect the difficulties often encountered in developing generalizations in hydrology and fluvial geomorphology. There are numerous variables and controls that affect hydrologic response, and significant variation in those controls and variables. Additionally, drainage basins are strongly influenced by historical factors ranging from their geological evolution to contemporary land management (such as mining and reclamation practices). Thus, it is difficult to state with confidence that MTR/VF does, or does not, increase or even tend to increase peak flows downstream. The situation is analogous to studies of downstream impacts of dams, where it has been shown that many outcomes are possible, that these outcomes are highly contingent on local conditions, and that prediction is not feasible except on a case-by-case basis.

We can say that MTR/VF mining can increase downstream flood risks, and that there is a very high probability that this has occurred and will occur downstream of some fills. On balance, results suggest that an increase in peak flows is more likely than a decrease or no change. However, our results also show that there is likely to be a great deal of variation, and that at a significant number of sites no increase in peak flows or flood risk will occur.

The variability in runoff and flow detention changes at VF sites also suggests that monitoring-based and comparative-watershed studies are likely to be of limited use in providing general or regional-scale answers into the issue of valley fill effects on peak flows, unless a large number of basins can be instrumented. Otherwise, the idiosyncratic nature of hydrologic response makes extrapolation of results problematic. This suggests that future work should be event-based and historical. That is, the question of valley fill effects of downstream peak flows should be approached via the analysis of specific flood events, and historical reconstruction of flow regimes, to specifically address the before- and after-mining flow regimes.

Investigations of flood complaints have generally found that when flood impacts can be directly attributed to mining, drainage and water control structures have been improperly designed, constructed, or maintained by the operators. This, coupled with the variable and highly contingent nature of the hydrologic response of VF sites, suggests that regulatory monitoring and enforcement is critical to reducing downstream flood risk.

Where have all the yellow perch (*Perca flavescens*) gone: Are endocrine disruptors (Xenoestrogens) involved?

Basic Information

Title:	Where have all the yellow perch (<i>Perca flavescens</i>) gone: Are endocrine disruptors (Xenoestrogens) involved?
Project Number:	2001KY2341B
Start Date:	3/1/2001
End Date:	8/31/2002
Funding Source:	104B
Congressional District:	Sixth
Research Category:	Biological Sciences
Focus Category:	Conservation, Methods, Models
Descriptors:	reproduction, endocrinology, physiology, teleost
Principal Investigators:	Brian S. Shepherd

Publication

1. Lynn, Scott G. and Brian S. Shepherd, 2002, Herbicide Body Burdens and Endocrine Correlates in Yellow Perch (*Perca flavescens*) from Old Woman Creek (OWC) National Estuarine Research Reserve and Lake Erie, in Proceedings of the Ohio Valley Chapter of the Society of Environmental Toxicology and Chemistry (SETAC) Symposium entitled "Molecular Approaches to Toxicological Questions", University of Louisville, Louisville, KY, May 16-17, 2002.

Problem and Research Objectives

Growth, development and reproduction, in all vertebrates, are regulated through the orderly, but complex release of the pituitary hormones, growth hormone (GH) and prolactin (PRL). Adding to this complexity are pollutants that mimic/alter the actions of endogenous hormones. These hormonal mimetics are called "endocrine disrupting chemicals" (EDCs) or "xenoestrogens", because they possess estrogenic activities that can affect endogenous hormones in inappropriate ways. To date, studies have mainly focused on the effects of EDCs on physiological end-points, with little emphasis on the impacted endocrine pathways themselves. To understand the sub-lethal impacts of EDC exposure in aquatic vertebrates, an approach focused on determining the mechanism(s) of endocrine disruption is required. The study of an ecologically-and economically-important organism that responds uniquely to estrogen would facilitate our understanding in many ways. In this regard, our aim is to develop the yellow perch (*Perca flavescens*) as a model to study the effects of estrogenic EDCs on teleost endocrine physiology. An understanding of the endocrine physiology of this teleost will aid in future studies to improve the environmental monitoring and management practices of this important species in areas where endocrine disruption is suspected. To accomplish this, our immediate objective is to develop the molecular endocrine tools needed to investigate the endocrine mechanisms of development/growth in this teleost. Once characterized, the sub-lethal effects of EDCs on teleost physiology can then be explored.

Methods

Molecular tools are being developed to characterize the hormonal pathways responsible for growth and development in yellow perch. We have obtained partial cDNA clones for PRL, GH (Courtesy of F. Goetz, Univ. of Notre Dame) and the estrogen receptor genes. RT-PCR procedures (5'-RACE) and automated DNA sequencing have been employed to clone and verify cDNA sequences. We also have partial cDNA clones for the pituitary hormone somatolactin (SL) and the insulin-like growth factors (IGFs I & II). Once full-length cDNAs are obtained, the sex-, tissue- and developmental-specific expression of these genes will be examined in yellow perch. In addition to our molecular studies, we are also working to purify native perch GH and PRL and to generate primary antibodies to these hormones. This will permit the development of assays to measure blood GH & PRL levels and receptor abundance, distribution and affinity in perch throughout the life-cycle and in those exposed to EDCs.

Principal Findings and Significance

We have a full-length cDNA clone for perch GH (courtesy of F. Goetz, Univ. of Notre Dame), and partial clones for PRL and estrogen receptors- α & β . We have also cloned the hormones, somatolactin (SL: pituitary hormone), IGF-I & -II and will clone perch P-450 aromatase. We continue to obtain the full-length cDNAs for these clones. We have also collected pituitaries from approximately 6,000 animals for purification of

native perch GH and PRL.

This work was to be completed in collaboration with another lab, but this did not work out. We began rearing larval perch in order to examine developmental-specific patterns of gene expression. An understanding of the underlying endocrine physiology of yellow perch growth and reproduction will aid in future studies to improve the environmental monitoring, management practices, aquaculture and restoration of this important species.

Developmental stability as an indicator of amphibian population health and environmental degradation

Basic Information

Title:	Developmental stability as an indicator of amphibian population health and environmental degradation
Project Number:	2001KY2441B
Start Date:	3/1/2001
End Date:	5/31/2002
Funding Source:	104B
Congressional District:	First
Research Category:	Biological Sciences
Focus Category:	Water Quality, Conservation, Toxic Substances
Descriptors:	bioindicators, conservation biology, pollutants, monitoring
Principal Investigators:	Howard H. Whiteman

Publication

1. Meredith, Christy and Howard Whiteman, 2002, Lethal and sublethal effects of increasing nitrate concentration on *Ambystoma mexicanum* embryos and larvae. Annual Sigma Xi Poster Competition, Murray State University.
2. Whiteman, Howard and Bommanna Loganathan, 2003, Developmental Stability in Amphibians as a Biological Indicator of Chemical Contamination and Other Environmental Stressors, Presented at the EPA/EPSCoR Special Conference on Biomarkers, May 12, 2003, Lexington, Kentucky.

Problem and Research Objectives

One of the most important, yet most difficult, tasks associated with conservation of any organism is the identification of populations subject to stress before such stress has a detrimental effect (Clarke 1995). This is particularly true of amphibians; the global decline of amphibians is considered a disturbing indicator of environmental degradation because it may forebode of cascading ecological effects, as well as raising health concerns about human populations (Wyman 1990, Wake 1998). Amphibians are ideal biological indicators, because their semi-permeable epidermis and complex life cycle expose them to multiple stressors in both aquatic and terrestrial environments (Wyman 1990). Because of this, amphibians should be among the first vertebrates affected by anthropogenic stressors in either of these environments (Stebbins and Cohen 1995). Furthermore, some of the same stressors affecting amphibians are known to have negative effects on other species, including humans (e.g., PCBs, UV light, etc.; Wake 1998, Carey 2000). Biologists thus need an early-warning system that could identify environmentally-stressed animals before the stressor causes population and/or regional harm. Such an indicator should be able to measure stress-induced effects before drastic changes in morphology take place which would subsequently decrease the organism's survival and reproductive abilities. One such indicator is obtained by measuring developmental stability (DS), the ability of an organism to develop normally under a range of environmental conditions (Waddington 1942, Clarke 1995). The objective of the current project was to utilize developmental stability as an indicator of amphibian stress and habitat quality from temporary ponds in Kentucky that vary in land use, water quality and other anthropogenic disturbance.

Methodology

During 2001 we continued our collection and imaging analysis of bullfrog (*Rana catesbiana*) larvae and 12 eastern newt (*Notophthalmus viridescens*) adult males. After transporting amphibians to MSU, each individual was anesthetized using tricaine methylchloride (MS-222), and measured for snout-vent length (mm) and mass (g). Each individual was then photographed with a Pixera Professional digital camera connected to a PC. After imaging was complete, animals were submerged in aged water to revive them and released back to their pond of capture. Measurements of DS concentrated on morphological structures directly related to amphibian fitness. Each individual was measured three separate times in order to statistically analyze measurement error (Palmer 1994). Temperature, pH, conductivity, dissolved oxygen, and alkalinity was measured at each pond with portable meters and orthophosphates and nitrate/nitrite were measured using a Lachat Nutrient Analyzer at MSU's Hancock Biological Station (HBS). Currently statistical analyses are being conducted to correlate habitat variables with levels of asymmetry in each species.

Experiments were modified slightly from the grant proposal. We reared *Ambystoma mexicanum* embryos and larvae under various nitrate concentrations. We used this species, an endangered Mexican salamander, because we were concerned that utilizing embryos from spotted salamanders,

A. maculatum, would have been unwieldy and might lead to spurious results. The latter species has large, gelatinous egg masses in which cutting single embryos for experiments is difficult and can lead to increased mortality. *A. mexicanum* is closely related to *A. maculatum* as well as other Kentucky congeners (*A. tigrinum*, *A. opacum*, *A. talpoideum*) and thus provides an indicator of potential native species response. In addition, *A. mexicanum* is utilized extensively by developmental biologists and can be readily reared in the laboratory, allowing for its potential future use in toxicology labs in university, government (EPA) and corporate settings. We reared embryos in various levels of nitrate, a common agricultural pollutant, and assessed mortality as well as sublethal effects. Surviving larvae were then moved to the same or different nitrate treatment to determine the effects of increasing, decreasing, or constant levels of nitrate on larval growth and development. After rearing for three weeks, larvae were photographed as above.

Principal Findings and Significance

All field images have been measured, and we are analyzing the results statistically. Our preliminary analyses of bullfrog tadpoles showed significant correlations with anthropogenic stress. Experimental results showed that salamander eggs are fairly robust against increasing concentrations of nitrate, whereas larvae are highly susceptible at nitrate levels commonly experienced in natural farm ponds. Mortality rate was much higher in larvae than eggs at the same nitrate concentrations, perhaps because nitrate affects metabolic processes that are not yet functioning in developing embryos. In addition, both embryos and larvae showed significant sublethal effects, in terms of time to hatching, size at hatching, and larval growth rate.

Bacterial ratios and neural networks for modeling Kentucky River water quality

Basic Information

Title:	Bacterial ratios and neural networks for modeling Kentucky River water quality
Project Number:	2001KY2781B
Start Date:	3/1/2001
End Date:	5/31/2002
Funding Source:	104B
Congressional District:	Sixth
Research Category:	Water Quality
Focus Category:	Water Quality, Models, Surface Water
Descriptors:	bacteria, ratios, indicators
Principal Investigators:	Gail Montgomery Brion, Srinivasa Lingireddy

Publication

1. Neiman, Jonathan , 2002, Novel Bacterial Ratio for Predicting Fecal Age, MS Thesis, Department of Civil Engineering, University of Kentucky, Lexington, KY.
2. Brion, G.M. and S. Lingireddy, 2002, Artificial Neural Network Modeling: A Summary of Successful Applications Relative to Microbial Water Quality, in Proceedings Joint CSCE/EWRI Environmental Conference, Niagara Falls, Canada, July 2002.
3. Nieman, J. and G.M. Brion, 2002, Novel Bacterial Ratio to Predict Fecal Age, In Proceedings Joint CSCE/EWRI Environmental Conference, Niagara Falls, Canada, July 2002.
4. Brion, G.M., T.R. Neelakantan, and S. Lingireddy, 2001, New Tools to Define the Impact of Stormwater on Receiving Surface Waters, in Proceedings ASCE Environmental Water Resources Institute Congress, Orlando, Florida, May 2001.
5. Brion, G.M., and S. Lingireddy, 2002, Artificial Neural Network Modeling: A Summary of Successful Applications Relative to Microbial Water Quality, in Proceedings 3rd World Congress of the International Water Association, Health-Related Water Microbiology Symposium, Melbourne, Australia, April.
6. Neiman, J. and G.M. Brion, 2002, Novel Bacterial Ratio for Predicting Fecal Age, in Proceedings 3rd World Congress of the International Water Association, Health-Related Water Microbiology Symposium, Melbourne, Australia, April.

Problem and Research Objectives

Our nation's rivers as well as our local water supply the Kentucky River are overburdened with pathogen indicators, and presumably pathogens. Existing indicator systems fail to identify the source and age of fecal contamination thereby limiting their usefulness as risk assessment tools. Watershed managers, water utilities, public health microbiologists, and regulators need new indicator systems; systems that provide more specific information about fecal contamination to assess the pathogen risk in source water and implement changes to management practices and/or treatment methods when required. The Kentucky American Water (KAW) Company is one of the few facilities that has monitored their intake for a range of bacterial indicators. The database KAW has kept is incomplete, with the peak numbers of bacteria often missing due to inadequate dilution. Published research by the PIs has proven using multi-parameter databases and advanced neural network programming can predict peak microbial concentrations. The main objective of this research is to backfill the missing data in the KAW database, which is crucial for developing reliable indicator systems.

Methodology

A feed-forward neural network will be trained on historical but complete (those with no missing/incomplete records) observations from KAW database to predict a range classification for a single parameter from the other available parameters. Once trained, the model will be asked to predict the range of concentration of bacteria for the missing observations based upon the pattern and interrelationships it has learned between other water quality parameters (pH, alkalinity, turbidity, etc.) The neural network model predictions for bacterial concentrations will be checked against the results of the simultaneous laboratory survival studies and new data from KAW collected during spring, summer, and early fall peak events.

Principal Findings and Significance

In addition to providing further insight into the abilities of neural network models in classifying fecal contaminant sources and in backfilling missing data, the research is expected to strengthen the previous findings on bacterial ratio (Atypical Coliforms/ Total Coliforms or AC/TC) as a reliable indicator of age of fecal source. Principal findings reported at conferences triggered interest in this research and have resulted in collaboration with two International groups: 1. Department of Microbiology, University of Barcelona, and 2. Institute of Environmental Science & Research Limited, Christchurch Science Center, NEW ZEALAND, of which the second group has already confirmed our research findings on AC/TC ratios on one of their databases.

Information Transfer Program

The Institute's Information transfer program has numerous components. The Environmental Systems Seminar Series is managed with assistance from the Institute. Graduate students working toward the certificate are required to participate in the seminar for two semesters. The presentations are also open to the general public. The theme of the series for the 2001-2002 academic year was "The Many Lives of the Kentucky River." Presentations during the reporting period included:

Town Branch: The forgotten heart of Lexington, Zina Merkin, Town Branch Trail, Inc.

Aqua-farming in Kentucky, J. Tidwell, President of the World Aquaculture Society

Water works wonders: Recreational fisheries in Kentucky, Benjy Kinman, Kentucky Department of Fish and Wildlife Resources

Water in Kentucky : Challenges and solutions for the future, James Kipp, Kentucky Water Resources Research Institute

The Kentucky Water Resources Annual Symposium was held February 20, 2003. This one-day symposium allowed individuals from universities, government agencies, and the private sector to present information on completed and ongoing research and management activities. Twenty-two platform presentations were featured in two concurrent sessions. Eight poster presentations were also included in the program. A plenary session on identification of research needs allowed participants to interact and network in small groups. There were 88 registrants for the symposium. Abstracts were printed as a proceedings volume and distributed to all participants.

Proceedings Kentucky Water Resources Annual Symposium, 2003, Kentucky Water Resources Research Institute, February 20, 2003, Lexington, Kentucky, 60 p.

The Association of State Dam Safety Officials (ASDSO) is a national, non-profit association dedicated to the improvement of dam safety through research, education and communications. The national office is located in Lexington, Kentucky and the unit is affiliated administratively with the University of Kentucky through the Water Resources Research Institute. In addition to regional technical seminars held throughout the country, ASDSO also maintains a clearinghouse of books, videos, articles, and CD-ROMs on subjects related to dam safety. The association web site is: <http://www.damsafety.org>

Training opportunities provided during the reporting period included: March 22, 2002 - Dam Owner Workshop, Eureka, MO; April 26, 2002 - Dam Owner Workshop, Phoenix, AZ; April 29-May 1 - Tailing Dams 2002 Conference, Las Vegas, NV; May 2-3, 2002, West Regional Technical Seminar, Las Vegas, NV; June 2-5, 2002, South Regional Conference, Atlanta, GA; September 8-11 - Dam Safety 2002 ASDSO Annual Conference, Tampa, FL

The Ohio River Basin Commission seeks to improve the water resources programs and related land programs of its member states. As an interstate body, the commission endeavors to contribute to the formulation of a comprehensive, coherent, and coordinated national water policy that recognizes interstate water issues and the primary role of the states in water resources planning and management. The ORBC cosponsors the Institute's Annual Symposium. Its office is located in Lexington, Kentucky and the unit is affiliated administratively with the University of Kentucky through the Water Resources Research

Institute.

The Superfund Basic Research Program at the University of Kentucky (supported by NIEHS) is collaborating with educators and outreach personnel to develop community-based programs. Educational programs and materials related to the nutritional implications of Superfund site chemicals are a major focus. The KWRRRI Director served as the Co-Project Leader for Core C: Superfund Outreach Program for Kentucky. An Information Coordinator was employed by the Institute for the project. Efforts were directed toward assisting existing community action groups in the vicinity of the Paducah Gaseous Diffusion Plant with emphasis on garden produce.

The 2002 Kentucky Nonpoint Source Conference was held September 23, 2002 at Lake Barkley State Resort Park in Cadiz, Kentucky. Goals of the meeting were to stimulate technology transfer and education related to nonpoint source pollution control. There were 64 registrants for the meeting. Forty-seven participants also attended the field trip to the Homestead Coal Mine Reclamation Project in Hopkins County that evening. The Institute served as a co-sponsor for the meeting and field trip and was responsible for providing logistical planning and support including publicity, mailings, hotel arrangements, program development, catering/meeting room arrangements, and audio/visual equipment. The abstracts and agenda were compiled into a conference program manual.

2002 Kentucky Nonpoint Source Conference, 2002, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, Kentucky, 144 p.

The EPA Regions 3, 4, 6 & 7 and States and Tribes ("Quad Region") Annual Nonpoint Source Conference was held September 23-27, 2002 at Lake Barkley State Resort Park in Cadiz, Kentucky. The meeting was sponsored by the Federal Environmental Protection Agency. The Institute assisted with meeting arrangements and helped compile the conference manual. A special Education, Information and Outreach Workshop kicked off the conference and was followed by topical sessions provided by the 29 states and tribes of these four regions on channel design, agriculture, watershed planning, best management practices, management evaluation systems, stormwater Phase II rules, state revolving funds, and nonpoint source program effectiveness. There were 96 pre-registrants for the 2002 Quad Regions Annual Conference.

2002 Quad Region Annual Nonpoint Source Conference, 2002, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, Kentucky, 65 p.

The Kentucky Water Resources Research Institute co-sponsored several additional lectures, seminars, and displays.

Greg Jennings, March 6, 2002, Stream Restoration Case Studies and Lessons Learned in North Carolina, Natural Resource Science and Conservation Distinguished Lecture Series.

David Hyndman, August 28, 2002, Efficient large-scale bioremediation in a heterogeneous aquifer: the Schoolcraft bioaugmentation experiment, National Ground Water Association Darcy Lecture.

Earth Science Week Open House, October 16, 2002 in conjunction with the Kentucky Geological Survey and the University of Kentucky Department of Geological Sciences.

Jean Bahr, February 13, 2003, Groundwater as an ecosystem resource, GSA Birdsall-Dreiss Distinguished Lecture.

Jean Bahr, February 14, 2003, Geochemical heterogeneity of groundwater in contaminated and uncontaminated aquifers, GSA Birdsall-Dreiss Distinguished Lecture #2.

Linking land use to water quality in the Muddy Creek subbasin, Kentucky River Watershed

Basic Information

Title:	Linking land use to water quality in the Muddy Creek subbasin, Kentucky River Watershed
Project Number:	2002KY7B
Start Date:	3/1/2002
End Date:	10/31/2003
Funding Source:	104B
Congressional District:	Sixth
Research Category:	Not Applicable
Focus Category:	Management and Planning, Water Quality, Education
Descriptors:	watershed planning, geographic information systems, non point pollution
Principal Investigators:	Alice Jones, Danita Maynard LaSage, Diane E. Vance, Mark Wiljanen

Publication

1. Sturgill, JoAnn, Jennifer Curra, Kathryn Takacs, and Danita LaSage, 2002, Characterization of Water Quality in an Eastern Kentucky Stream, in GSA Abstracts with Programs, Volume (34), Number 6. ISSN 0016-7592.
2. Jones, Alice L., Danita LaSage, and Mark Wiljanen, 2003, Linking Land Use to Water Quality in the Muddy Creek Subbasin, Kentucky River Watershed, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, Kentucky, p. 57-58.

Problem and Research Objectives

The purpose of this project is to investigate whether watershed data currently collected by regulatory and other agencies is sufficient to characterize the relationship between land use and water quality in a 4th order watershed, and to identify gaps that exist between currently available data and the information needed to adequately characterize this relationship.

The study area is the Muddy Creek subbasin of the Kentucky River Watershed, located in south-central Kentucky in northeastern Madison County. Muddy Creek enters the Kentucky River upstream of Fort Boonesborough, and its watershed covers about 45,000 acres to the east of Richmond and extending south to include much of the Bluegrass Army Depot.

Identified threats to water quality in the subbasin include pathogens from agricultural sources or ineffective septic systems, extensive sedimentation, potential cumulative downstream stormwater impacts from expanding suburban development from the Richmond area, and potential contamination from the U.S. Army Bluegrass Depot which is a storage facility for conventional and chemical weapons including nerve gas, and is also leased as grazing land to local cattle operators.

Methodology

In the Spring of 2002, students in Dr. Alice Jones's (Geography) Environmental Land Use Planning course assembled a GIS database of map layers available from public sources including soils, drainage, topography, geology, and land use of the area. The class also "ground-truthed" these layers by performing field surveys of these layers on an area of the Bluegrass Army Depot. They then used an overlay analysis to identify critical terrestrial habitat areas within the watershed and threats to these habitats.

Meanwhile, under the supervision of Danita LaSage (Earth Sciences), student researchers collected monthly water samples at eight sites along the length of the stream. In the initial proposal, the Summer 2002 samples were to be collected by students in a "Chemistry Methods for Teachers" class taught by Dr. Diane Vance (Chemistry). However, the class was cancelled, so the samples were taken, instead by three earth sciences students under LaSage's supervision.

Following the initial Spring 2002 GIS assembly period, Jack Phillips under the supervision of Dr. Mark Wiljanen, continued to synthesize and analyze the GIS information and added the water testing information. He then examined the data looking for spatial relationships between land use that could be linked with water quality.

In the early Spring of 2002, further field checking of the land use layer was performed by two wildlife management students under the supervision of Fish and Wildlife Conservation Biologist Tom Edwards.

Principal Findings and Significance

There are four areas where current information is insufficient to characterize stream structure and health:

1. *Critical pollution problems.* There are indications of critical pollution problems in the upper reaches of the watershed — particularly pathogenic activity indicated by total fecal coliform counts. Other parameters of concern include low dissolved oxygen levels (below 5 ppm) in both the study samples and those collected by Watershed Watch volunteers and high levels of orthophosphate in more than 2/3 of the samples, most likely associated with agricultural impacts.
2. *A lack of stream hydrology and discharge information.* Even the most basic information on stream hydrology and discharge rates is absent at the 4th-order watershed scale. There is no permanent discharge monitoring station anywhere in the watershed, and available topographic maps of the region are 30 to 50 years old.
3. *A lack of ecological and biological information in both the aquatic and terrestrial ecosystems.* The only aquatic biological information available was informal site assessments performed during the initial selection process. These assessments indicate severe degradation from sedimentation, and a lack of macroinvertebrate activity, particularly in the upper reaches of the watershed—which is consistent with the critical pathogenic and phosphorous pollution problems discussed previously. During the spring of 2003, Dr. Guenter Shuster’s graduate aquatic biology class used four Muddy Creek sites for the field portion of the class. Analysis of the data is still in process.
4. *Land use/Land cover scale refinement issue.* The best available land use/land cover layer reflecting distinct vegetation and ecosystem structures as well as developed landscapes is the GAP analysis developed by the Kentucky Department of Fish and Wildlife at a 30-meter pixel resolution. While this resolution is adequate for gross assessments, field surveys indicated that the two most frequent GAP land uses throughout the watershed—“Pasture/Grassland” and “Agricultural/Other” bear little relationship with what is found on the ground. Field studies indicate that areas with intensive cattle grazing are found in areas classified as “Pasture/Grassland” and “Agricultural/Other” but at the same time, large uninterrupted areas of undisturbed prairie grasses with no livestock are also found in these same GAP categories. Since the most critical water problems—pathogen and sediments—are associated with livestock operations, the inability to distinguish grazed pastureland from other grasslands makes tying land use to water quality from currently existing land use data difficult.

Significance

These preliminary results indicate that data collection by regulatory and other agencies is too irregular and sporadic to adequately characterize the relationship between land use and water quality in a typical fourth-order watershed—a landscape scale at which it would be reasonable to expect that land management practices should produce observable results in water quality.

As state's watershed management efforts shift focus from the broad basin-wide scale to the local-level subbasin scale, it is essential to determine whether the information currently collected by regulatory and other agencies is sufficient to characterize the relationship between land use and water quality in these smaller subbasins.

This project resulted in cooperative efforts involving students and faculty in Earth Sciences, Geography, Chemistry, and Wildlife Management at Eastern Kentucky University. In addition, members of the general public were drawn into the effort by distributing water testing kits to community volunteers as a part of the Year of Clean Water Monitoring Day, October 18, 2002. Twelve volunteers collected samples at a total of 21 sites throughout the county through this linkage.

The project generated several additional offshoot collaborations that are significant in that they may lead to continued interdisciplinary, interinstitutional, and interagency collaborations.

1. Water quality testing was conducted on the Eastern Kentucky University Meadowbrook Farm. As a result of arranging access to the farm for regular water testing, Farm Manager Michael Judge initiated efforts to improve water quality on the farm land including enrolling a 3-mile section along Muddy Creek into the federal Conservation Reserve Program.
2. The Spring 2003 graduate Aquatic Biology class at Eastern Kentucky University (Dr. Guenter A. Schuster) used Muddy Creek as its study area and performed macroinvertebrate analysis at four of the project's eight sampling sites. This activity began closing the information gap on biological indicators of water quality in the study area.
3. Dr. Gail Brion (Civil Engineering) of the University of Kentucky Environmental Research and Training Laboratory performed tests for nitrates and other water-quality indicators at no cost to the project. She has expressed an interest in continuing to develop collaboration between Eastern Kentucky University and the University of Kentucky on issues related to water quality.
4. Mapping of several grassland sites related to field checking of the GIS layers led to increased interest by management at the Bluegrass Army Depot in encouraging regeneration of remnant prairie grasses on that site and implementing best management practices to protect water quality in the vicinity of the Depot.

Student Support

Student Support					
Category	Section 104 Base Grant	Section 104 RCGP Award	NIWR-USGS Internship	Supplemental Awards	Total
Undergraduate	12	0	0	0	12
Masters	2	0	0	0	2
Ph.D.	3	0	0	0	3
Post-Doc.	0	0	0	0	0
Total	17	0	0	0	17

Notable Awards and Achievements

2002KY4B - Gretchen E. Berryman received an Honorable Mention Award (Second Place) at the 2003 Western Kentucky University/Sigma Xi Undergraduate Research Symposium for her poster entitled: "Occurrence and Distribution of Mercury in Mammoth Cave National Park (Phase 0-1)."

In addition, the initial effort supported by this project resulted in the preparation and funding of a follow-on proposal entitled: "Evaluation of Mercury Bioaccumulation in the Green River Ecosystem." This continuation project will be supported by the National Park Service under a joint program with the USGS. The three-year project (\$236,105) will run from October 1, 2003 through September 30, 2006.

2002KY7B - The Eastern Kentucky University, University Research Fund granted an award of \$4,881 for continued student support during the extension period (through October 2003) for the project entitled: "Linking Land Use to Water Quality in the Muddy Creek Subbasin, Kentucky River Watershed."

2000KY2B and 2001KY2781B - Gail M. Brion and Srinivasa Lingireddy. Initial efforts supported by these grants have led to successful funding for continuation and expansion of this work. "Using Neural Networks to Create New Indices and Classification Schemes" EPA Grant Number: R829784 has been funded for the period July 1, 2002 through June 30, 2005 (\$523,938). The grant was awarded through the STAR Program (Science to Achieve Results) which employs a competitive solicitation and independent peer review. The project will work on further developing an early warning tool for surface water treatment plants to detect unsafe levels of bacteria in order to help improve drinking water safety and reduce risks to America's public water systems.

Publications from Prior Projects

1. 2000KY1B ("Natural attenuation of trichloroethene in wetland soils and paleowetland sediments") - Articles in Refereed Scientific Journals - Etienne, N., D. L. Butler, A. E. Fryar, and M. S. Coyne, 2001, Trichloroethene in Wetland Soils and Paleowetland semiments, Bioremediation Journal, 5(1), p. 27-50.