



Savannah River Ecology Laboratory

Annual Technical Progress Report

2006

SAVANNAH RIVER ECOLOGY LABORATORY

ANNUAL TECHNICAL PROGRESS REPORT OF ECOLOGICAL RESEARCH

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Table of Contents

SREL FY2006 OVERVIEW	1
SPECIAL ACCOMPLISHMENTS OF SREL PERSONNEL	5
AN OVERVIEW OF RESEARCH THEMES	7
ENVIRONMENTAL CHARACTERIZATION	8
<i>GOAL: Develop long-term ecological databases to determine whether any changes being observed are the result of natural fluctuations or operational impacts</i>	
Amphibian studies on the SRS	8
Studies of the microbial communities of contaminated and uncontaminated streams	10
Carolina bay restoration studies	11
Sandhills TES population studies	12
Alligator population dynamics	12
GIS-based wildlife literature survey for use in risk assessments	12
The role of SRS wildlife in transferring contaminants off-site	13
 <i>GOAL: Determine the biogeochemical processes that control chemical speciation and mobility of toxic metals, organic contaminants, and radionuclides</i>	
Uranium and nickel speciation in the Steed Pond-Tims Branch system	13
Natural attenuation of PCE/TCE in Pen Branch hyporheic sediments	15
Development of compound-specific isotope ratio analysis	17
Application of surface complexation models to descriptions of contaminant migration in the vadose zone	17
 <i>GOAL: Assess whether sentinel species or other sensors can be used to characterize environmental health</i>	
Studies of the effects of nickel exposures in turtles	18
Studies of the relationship between microbial antibiotic resistance and environmental contamination	20
Alligator population and genetic studies	21
Using ecological indicators to determine disturbance associated with regional land use	21
Using freshwater mollusks to monitor water chemistry	21
Continued research at the Mixed Waste Management Facility	22
ECOLOGICAL RISKS AND EFFECTS	23
<i>GOAL: Determine how changes in contaminant speciation influence dose-response and toxicity relationships</i>	
Exposure of earthworms to sediments from the Steed Pond-Tims Branch ecosystem	23
Exposure of transgenic nematodes to bioavailable metals	24

Dose response relationships of Medaka to gamma irradiation	24
Rates of DNA repair in red blood cells from bluegills exposed to gamma radiation	26
Responses of native plants to elevated soil nickel concentrations	26
Using nitrogen stable isotopes to study how contaminants affect protein metabolism	26
<i>GOAL: Determine the potential effects and interactions from exposure to mixed contaminants</i>	
Determine the impacts of metal contamination on microbial communities	27
Studies of the interaction of nickel and TCE in plants in the Steed Pond-Tims Branch ecosystem	27
Effects of low-dose rate ionizing radiation on frogs and toads	28
Imaging of labeled non-targeted quantum dots within Medaka embryos	28
Assess the influence of nickel and uranium on TCE degradation by bacteria	29
<i>GOAL: Define more clearly the risks from low dose-rate, chronic exposures to radiation</i>	
Radiation-induced untargeted germline mutations in Japanese Medaka	31
REMEDICATION AND RESTORATION	34
<i>GOAL: Identify the traits of native species and populations that best determine their suitability for use in remediation and restoration</i>	
Evaluation of native plants most successful in the restoration of Carolina bays	34
Assess survival of sandhills TES plant species under different land management regimes	35
Studies of plant species most useful for phytoremediation	35
Studies of plant species useful for biodiesel production in the Southeast	36
Studies of carbon sequestration in nutrient-poor soils	36
Studies on the use of native grasses on waste site closure caps	36
Investigations of the effects of flooding on MNA of a metal-contaminated site	36
<i>GOAL: Determine the primary mechanisms by which natural attenuation and engineered remediation processes immobilize contaminants, and identify the appropriate geochemical and biological endpoints to assess sustainability</i>	
Evaluations of the effectiveness of hydroxyl apatite amendments on TCE degradation by microbes	37
Analyses of field-scale tracer data from H-Area subsurface injections experiments	37
RESEARCH SUPPORT PROGRAMS	38
<i>Environmental Health & Safety Program</i>	39
<i>Quality Assurance Program</i>	40
<i>Research Data Archive Activities</i>	40
<i>SREL Undergraduate & Graduate Education Programs</i>	40
<i>Environmental Outreach Program</i>	43
<i>DOE Research Set-Aside Areas</i>	45
EXTERNALLY FUNDED GRANTS	46
PUBLICATIONS	52
SREL ORGANIZATIONAL CHART	58

Savannah River Ecology Laboratory FY2006 Overview

The Savannah River Ecology Laboratory (SREL) is a research unit of The University of Georgia (UGA) and has been conducting ecological research on the Savannah River Site (SRS) near Aiken, South Carolina for almost 55 years. The overall mission of the Laboratory is to acquire and communicate knowledge of ecological processes and principles. SREL conducts fundamental and applied ecological research, as well as education and outreach programs, under a Cooperative Agreement with the U.S. Department of Energy (DOE).

The Laboratory's research mission during the 2006 fiscal year was fulfilled with the publication of 110 journal articles and book chapters by faculty, technical staff, students, and visiting scientists. Two books were also published with SREL faculty members as authors. An additional 72 journal articles have been submitted or are in press. Other noteworthy events took place as faculty members, staff, and graduate students received awards. These are described in the section titled Special Accomplishments of SREL Personnel on page 5.

Notable scientific accomplishments during the past year included work in the areas of environmental remediation and environmental stewardship.

- Results from a collaborative research study were published on the use of dynamic modeling to assess the efficiency of tritium phytoremediation at the Mixed Waste Management Facility. Tritium-contaminated groundwater entering Fourmile Branch through seeps is being retained in a constructed pond and then used to irrigate trees that overly the contaminated groundwater. Managing the application of the contaminated groundwater was evaluated in the context of the amount of rainfall relative to evapotranspiration, seasonality of evapotranspiration, and precipitation variability. Quantification of these relationships allows managers to choose irrigation strategies based on desired long-term system

efficiency. See publication 2959 for more information.

- Sixteen SREL authors contributed to the recently-published book "Ecology and Management of a Forested Landscape, Fifty Years on the Savannah River Site" edited by J.C. Kilgo and J.I. Blake (2005). The book, published by Island Press, summarizes fifty years of research into the biotic communities and native species of the Savannah River Site and the impacts of fifty years of land management on the natural environment of the SRS.
- SREL researchers published results of a conservation project to translocate gopher tortoises from a development site in southern Georgia to the SRS. The gopher tortoise is the only tortoise species inhabiting the southeastern U.S. and is threatened in part of its range. The study found that keeping translocated tortoises penned for up to 12 months at the new site significantly increased site fidelity of the animals and will improve the likelihood of establishing self-sustaining populations. See publication 2903 for more information.

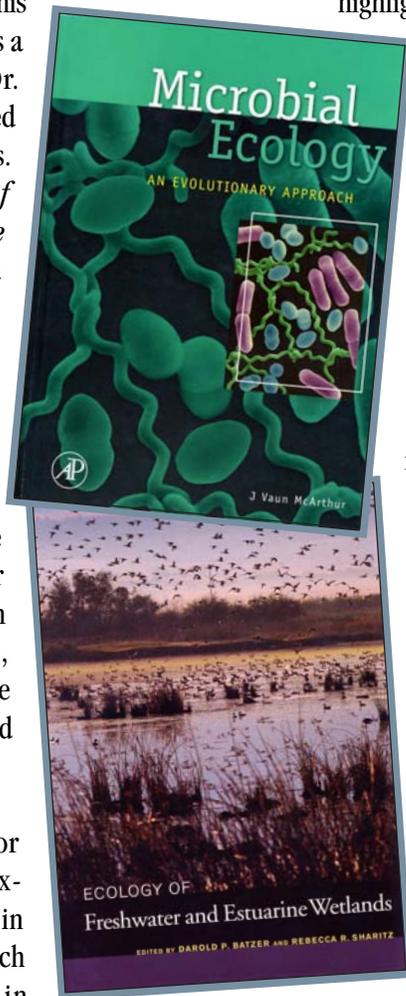


During the past year several faculty had accomplishments worthy of special note:

- **Dr. Thomas Hinton** was promoted in 2006 to Senior Research Scientist. Dr. Hinton, who originally came to SREL in 1992, has established a research program in radioecology. Tom's research interests include transport and fate of radioactive contaminants in aquatic and terrestrial ecosystems, effects of low-level, chronic irradiation on biota, human and ecological effects from exposure to contaminant mixtures, transgenerational effects from contaminant exposure, human and ecological risk analyses, remediation of

radioactively contaminated wetlands, contaminant transport models, and the use of radioactive tracers as a tool for understanding fundamental ecological processes.

- **Dr. Whit Gibbons** received the 2006 Henry S. Fitch Award for Excellence in Herpetology at the annual meeting of the American Society of Ichthyologists and Herpetologists in New Orleans, LA. The award is presented for outstanding contributions to the study of amphibians and reptiles, and is based principally on the quality of the individual's research, with consideration given to educational and service impacts of the individual's career in the field of herpetology.
- Academic Press published a book written by **Dr. J Vaun McArthur** titled *Microbial Ecology: An Evolutionary Approach*. This 432-page book represents a career mile-stone for Dr. McArthur, who has worked at SREL for about 21 years.
- A new textbook, *Ecology of Freshwater and Estuarine Wetlands*, written and edited by Darold P. Batzer and SREL faculty member **Dr. Rebecca R. Sharitz**, was published by the University of California Press in September 2006. This 559-page volume, designed for graduate-level classes, is an up-to-date, authoritative, and accessible survey of the ecology of freshwater and estuarine wetlands.



FY06 was a tumultuous year for SREL. The Laboratory experienced a 42% reduction in base funding from DOE, which necessitated a reduction in research and support staff of about 33%. As a result, eight of 20 faculty members (40%) were lost through retirement

or departures and will not be replaced. Research personnel, expertise, and programs were lost in ecotoxicology, statistics and modeling, aquatic and fisheries biology, wetlands ecology, wildlife biology, and landscape ecology/GIS. Administrative and infrastructure support have been reduced to minimum levels that still ensure operational safety. Graduate student programs have continued, but at a reduced level, with more costs to be paid by UGA or external grants. All journal subscriptions to the SREL library were cancelled, but electronic access continues to be available through UGA.

Despite obvious negative organizational impacts resulting from the budget reduction, there were some positive aspects to SREL's situation. Strong community support highlighted the Lab's importance to local schools and the general public of the area. A strong show of support was also received from the DOE Savannah River Operations Office, resulting in a commitment to maintain current funding levels for the foreseeable future and to renew SREL's five-year Cooperative Agreement with DOE. The new Agreement, which is expected to be finalized soon, will cover the period of 1 October 2006–30 September 2011.

Challenges remain for SREL, including reorganizing research programs to address primarily SRS-specific concerns, and maintaining current research staff and attracting new personnel. SREL researchers are also pursuing additional funding sources to leverage DOE research funds while continuing to focus the Lab's research efforts on projects of interest to the SRS.

2006 saw the retirement of two senior SREL faculty members. Dr. Kenneth McLeod retired after more than 30 years at SREL, where he conducted research in plant ecophysiology. Dr. McLeod published more than 90 scientific articles and was also active with local and regional science fairs. Dr. McLeod will continue to collaborate with SREL and SRS scientists, particularly in research programs related to constructed wetlands and wetland

restoration. Dr. I. Lehr Brisbin, Jr. also retired this year after more than 30 years at SREL. Dr. Brisbin published more than 170 scientific articles and conducted research on vertebrate ecology, radioecology, ecotoxicology, and animal behavior. Dr. Brisbin remains active in research related to animal behavior.

The Savannah River Ecology Laboratory's primary funding source is a Cooperative Agreement between the U.S. Department of Energy and The University of Georgia Research Foundation that currently covers a five-year period from July 1, 2001 through September 30, 2006. The estimated total cost of this agreement since its inception in 1996 is over \$103 million, with DOE contributing over \$98 million and the University of Georgia about \$5.5 million. SREL's total operating budgets from DOE and other federal sources in FY03, FY04, FY05, and FY06 were \$10.2, \$9.11, \$8.7, and \$6.0 million, respectively. In FY06 SREL received \$1M from DOE Office of Science (DOE-SC), \$500K from the National Nuclear Security Administration (NNSA), and \$3M from DOE-EM (Environmental Management). The FY07 budget for SREL is currently projected to be \$4.6M from all DOE sources. SREL also received about \$1M in FY06 from The University of Georgia. During FY06 SREL received \$93K from WSRC for SRS-related tasks.

Researchers at SREL currently have funding totaling about \$2.8M from 32 grants received during FY06, in addition to funds provided by DOE-SR. Sources of grant awards range from private foundations such as the National Fish and Wildlife Foundation to federal agencies such as the U.S. Department of Interior, the National Science Foundation, and the Department of Defense. Major grants received this year included an award of \$363K from the Environmental Protection Agency to Dr. Paul Bertsch; \$94K from the National Science Foundation to Dr. Chris Romanek; and \$189K from the National Oceanic and Atmospheric Administration to Dr. Chuanlun Zhang.

SREL currently has a permanent staff of about 80 people, nearly all of whom are employees of The University of Georgia. The staff includes 14 research scientists, five of whom are co-staffed through tenure-track positions in

various departments at The University of Georgia and one who is co-staffed through a tenure-track position in the School of Public Health of the University of South Carolina. There are another 8 Ph.D.s in postdoctoral appointments. Research technicians (29), clerical and other support personnel (28), and graduate students (17) comprise the remaining staff categories.

In addition to holding faculty positions in 10 departments at the University of Georgia, various SREL faculty have adjunct status at 11 other colleges and universities. Faculty, staff, and students also are active in providing outreach and service to the scientific community. Representatives from the laboratory hold more than 25 editorial or committee positions in national groups and organizations and also serve on several UGA academic and administrative committees. Over 125 lectures, scientific presentations, and posters were presented during the past year at scientific meetings, colleges, and universities, including minority institutions.

In FY06 SREL spent about \$40K to purchase new laboratory equipment, including an auto-titrator and an autoclave. In addition, the Lab purchased a new commercial lawn mower to facilitate grounds maintenance, and video conference equipment to improve interactions with scientific and administrative colleagues.

Participants in the SREL Education Program during FY06 came from schools located throughout the United States and included 7 undergraduate students and 34 graduate students. The graduate students came from 7 different universities in the U.S. and abroad, emphasizing the national and international stature of the SREL program. In the past year 6 graduate students from SREL earned Masters Degrees and 2 earned Doctor of Philosophy Degrees. A National Science Foundation grant from the Research Experiences for Undergraduates Program for a proposal titled "The Impact of Energy Technologies on Natural Environmental Systems" continued to provide funding for the undergraduate program at SREL.

The SREL Outreach Program reaches a different audience in its efforts to communicate scientific awareness to the

general public. During the past year, SREL scheduled 219 talks, 23 tours, 22 exhibits, and 34 workshops, reaching a total of 37,523 people. Topics for these presentations included reptiles, amphibians, southeastern plants and habitats, long-term research, safety, biodiversity, local wetlands and watersheds, conservation, and careers in ecology and research.

The SREL Distance Learning Program transitioned to new technology in 2006. As a result of advances in video conferencing hardware and software and Internet routing improvements, SREL withdrew from the GSAMS (Georgia Statewide Academic and Medical System) video network in favor of an industry standard video-over-IP conferencing system. Our new conferencing system may be used to conduct video conference meetings with any standardized video conference system anywhere in the world. The system provides the ability to project computer-based presentations and demonstrations, VHS or DVD videos, and close-up video feeds from a document camera.

The Conference Center has continued to see wide use, both by SREL personnel and the local community. The facility was used to host a total of 72 meetings and environmental education programs for students, teachers, and the general public this past year, and 2,188 people visited the facility.

Representatives of the Laboratory also serve local and statewide communities by organizing a canned goods drive in May, managing a recycling program, participating generously in the UGA Campaign for Charities, and participating in the regional Heart Walk to benefit the American Heart Association.

Special Accomplishments of SREL Personnel

Dr. Whit Gibbons received the 2006 Henry S. Fitch Award for Excellence in Herpetology at the annual meeting of the American Society of Ichthyologists and Herpetologists in New Orleans, LA. The award is presented for outstanding contributions to the study of amphibians and reptiles, and is based principally on the quality of the individual's research, with consideration given to educational and service impacts of the individual's career in the field of herpetology.

Dr. Thomas Hinton was promoted in 2006 to Senior Research Scientist. Dr. Hinton, who originally came to SREL in 1992, has established a research program in radioecology. Tom's research interests include transport and fate of radioactive contaminants in aquatic and terrestrial ecosystems; effects of low-level, chronic irradiation on biota; human and ecological effects from exposure to contaminant mixtures; transgenerational effects from contaminant exposure; human and ecological risk analyses; remediation of radioactively contaminated wetlands; contaminant transport models; and the use of radioactive tracers as a tool for understanding fundamental ecological processes.

SREL graduate student **Aaliyah Green** received a \$20,000 fellowship from the National Estuarine Research Reserve for her M.S. research on maternal transfer of mercury in Carolina Diamondback Terrapins. **J.D. Willson** received \$1,000 from the Society of Integrative and Comparative Biology for his doctoral research on the effects of snake predation on the expression of alternative life history strategies on salamanders. **Chris Winne** received an award from Sigma Xi for his doctoral research on the relationship between diet and reproduction in watersnakes.

SREL graduate students were well represented at the UGA Institute of Ecology's annual awards banquet on April 28 2006:

- **Meredith Wright** won the Best Student Paper Award

in basic/theoretical ecology.

- **Brian Todd** won the Best Student Paper Award in applied ecology.
- **Gretchen Loeffler Peltier** was awarded a UGA Outstanding Teaching Assistant Award and an Institute of Ecology Distinguished Teaching Award.
- **Carol Flaute** won an Institute of Ecology Teaching Award.
- **Kimberley Andrews** won the Robert Sheldon Memorial Travel Award.

Graduate student **Bill Duval** recently received one of the first Jaworski travel awards from UGA's Department of Plant Biology.

Postdoctoral researchers **Melissa Pilgrim** and **Craig Baker** recently received foreign travel assistance awards from the Office of the Vice President for Research at UGA.

The following graduate students received awards at the Third Annual SREL Graduate Student Symposium, held on 30 June at the SREL Conference Center:

- FIRST PLACE PRESENTATION—**William Duval**, UGA, *Integration in a clonal woody shrub: Responding to change.*
- SECOND PLACE PRESENTATION—**J.D. Willson**, UGA, *Evaluation of trap response in two aquatic snake species using mark-recapture and an experimental release study.*
- HONORABLE MENTION PRESENTATION—**Ellen Breazel**, UGA, *Determining errors in analysis of microsatellite data.*
- FIRST PLACE POSTER—**Elizabeth Burgess**, UGA, *Distribution, diversity, and sulfidogenesis of prokaryotes in the Uzon Caldera.*
- SECOND PLACE POSTER—**Benjamin Neely**, MUSC, *Investigating the effect of manufactured ZnO nanoparticles on *Burkholderia vietnamiensis* PRI*

- HONORABLE MENTION POSTER—**Linda Lee**, UGA, *Floodplain forest regeneration and flood pulse restoration on the Savannah River.*
- HONORABLE MENTION POSTER—**Frantisek Majs**, UGA, *Longitudinal dispersion and tracer migration in a radial flow field.*

SREL graduate student **Meredith Wright** received a National Science Foundation (NSF) award through a program for graduate students called the East Asia Pacific Summer Institute. NSF and its partner agencies in Australia, Japan, Korea, China, and Taiwan co-sponsor graduate students to conduct research for eight weeks in a host laboratory. Ms. Wright will be working at Macquarie University in Sydney from mid-June to mid-August on a research project that will be part of her doctoral dissertation on metal tolerance and antibiotic resistance in bacteria.

Dr. Lee Newman was selected Acting President of the newly formed International Phytotechnology Society. Dr. Newman will serve in this capacity until the first annual meeting of the group, in June 2007.

Research technician **Anna McKee** received a UGA Presidential Graduate Fellowship, a highly competitive award designed to recruit exceptionally qualified students to UGA doctoral programs. A maximum of twelve awards are made annually, and each comes with a stipend of about \$22,000 per year. Anna will begin her graduate studies in the fall, 2006 in the School of Forest Resources with **Travis Glenn** as her co-advisor.

SREL graduate student **John Willson** received the Outstanding Student Paper in Herpetology Award from the American Society of Ichthyologists and Herpetologists, Southeastern Division at the Annual Joint Meeting of Ichthyologists and Herpetologists in New Orleans, LA, in July 2006.

The book *Snakes of the Southeast*, by Drs. **Whit Gibbons** and Mike Dorcas, won a National Outdoor Book Award in the Nature Guidebook category.

SREL researcher scientist **Jason Unrine** was elected adjunct professor in Environmental Health Science at UGA.

An Overview of Research Themes

Through a Cooperative Agreement between the Department of Energy and the University of Georgia Research Foundation, SREL provides an independent evaluation of the ecological effects of SRS operations through a program of ecological research, education, and public outreach. This program involves basic and applied environmental research, with emphasis upon expanding the understanding of ecological processes and principles, and upon evaluating the impacts of industrial and land use activities on the environment.

This is accomplished through a broad-based program of field and laboratory research conducted on the SRS and published in the peer-reviewed scientific literature; by providing education and research training for undergraduate and graduate students from colleges and universities throughout the United States and abroad; and

by engaging in community outreach activities and service to professional organizations.

The FY06 SREL research plan responded to a July 1, 2005 letter from the DOE Site Manager (Jeffrey M. Allison) to the SREL Director (Paul M. Bertsch) identifying DOE support for research in three critical areas:

- (1) *environmental characterization,*
- (2) *ecological risks and effects,* and
- (3) *remediation and restoration.*

Research at SREL addresses knowledge gaps in these areas by taking advantage of unique expertise in the environmental sciences and ecology, the unparalleled field research opportunities at the SRS, and the long-term data sets, research tools, and capabilities that SREL has developed over the last half-century.

Environmental Characterization

Characterization is a necessary first step in determining environmental and health risks and in devising appropriate remediation and restoration strategies. Environmental information is also needed to make informed decisions about long-term stewardship and land management, and it is also a critical component of NEPA reports, Records of Decision (ROD), and other regulatory documents. Environmental characterization is more than simply measuring contaminant concentrations in biota or other media, or reporting the presence of organisms at various locations. It includes developing an understanding of the processes that control distributions of contaminants, chemical forms, and their bioavailability. Characterization is also necessary to construct models of how natural and engineered systems function, both in the presence and absence of environmental contamination.

Report on FY06 Environmental Characterization Milestones

GOAL: Develop long-term ecological databases to determine whether any changes being observed are the result of natural fluctuations or operational impacts.

Amphibian Studies on the SRS

INVESTIGATOR: J. WHITFIELD GIBBONS

The long-term Rainbow Bay (RB) study, which has just completed its 27th year, is becoming increasingly valuable as a data set that can be used to examine important questions in amphibian ecology. In recent decades, many amphibian species have declined, some to extinction. Factors suggested as the cause of these declines include habitat destruction and wetland loss, disease, species introductions, climate change and increased UV-B radiation, and pollution. As concerns about global amphibian decline become more pronounced, the RB study allows a comparison of “undisturbed” populations to those elsewhere that may be threatened by these factors.

For example, in 1998 researchers identified a novel fungal pathogen, now known as chytridiomycosis (*Batrachochytrium dendrobatidis*), that has since been linked to mass mortality events and extinctions of amphibian populations in the U.S. and abroad. Chytridiomycosis is known from several locations (past and present) on the SRS. Long-term monitoring at RB and other wetlands on the SRS enabled us to rule out

chytridiomycosis as a likely reason for the local extinction events sometimes observed in particular SRS wetlands. Similarly, the potential effect of climate change on amphibians is of increasing concern. Due to the long-term records on amphibians, rainfall, and wetland hydrology generated by RB and related amphibian studies, we have been able to develop population models that help predict amphibian response to altered climate conditions. Both these studies (effect chytrid fungus on SRS amphibians and response of amphibians to altered hydrology) were published this past year in high-impact journals, and both relied heavily on the RB long-term data (see below for manuscripts related to the RB project in FY06).

We conducted targeted sampling for the presence of chytridiomycosis in larval bullfrogs (*Rana catesbeiana*) in the A-Area remediation wetlands. Of approximately 50 tadpoles sampled, all (100%) were infected with *B. dendrobatidis*. There were no apparent negative effects on the bullfrogs at the larval stage of the life cycle. Whether there is any connection between the prevalence of the fungus and A-Area wetland water quality (e.g., elevated mercury levels) is unknown.

The RB study includes more than the daily monitoring of herpetofauna at the RB Set-Aside Area. Data collected at RB are valuable to describe long-term population trends and patterns, but these data must be supplemented by additional studies in order to understand underlying causes of population fluctuations. Data collected at two additional wetlands on the SRS (Ellenton Bay, EB; Ginger's Bay, GB) have been crucial in this regard, as they represent sites with different characteristics than RB. In addition, we continued to supplement the observational data from RB and other sites with experimental manipulations of species in artificial ponds. During FY06 we exposed different amphibian species to different suites of predators that are found in wetlands that differ in hydrology; i.e., fish in nearly permanent wetlands, invertebrate predators in many intermediate hydrology wetlands, etc. These studies, which are ongoing, will aid in our predictive modeling of amphibian response to altered hydrology. We also continue to conduct targeted sampling at GB and EB. FY06 publications resulting from this work is also listed below.

Ecologists are now recognizing the importance of terrestrial uplands that surround breeding ponds to the semi-aquatic animals that live in these wetlands. Historically we have focused much of our research effort at RB and other wetlands on the aquatic habitat, with little emphasis on the surrounding terrestrial uplands. The RB study and associated modeling efforts have indicated that terrestrial factors have important effects on the survival, dispersal, and migration of juveniles and adults. Much of our effort during the past year has been to better understand these terrestrial processes. Additional research on the influence of terrestrial habitat characteristics on amphibian communities was conducted at GB and four sites associated with the NSF-funded "Land-use effects on amphibians" (LEAP) study.

Terrestrial habitats surrounding isolated wetlands are a critical resource for many pond-breeding amphibian species, yet few studies have examined the terrestrial distribution of post-metamorphic juveniles and adults. We used an encircling drift fence at GB in conjunction with partial fences at 90, 172, and 332 m from the wetland to estimate the distribution of adult marbled salamanders (*Ambystoma opacum*; 3 years) and mole salamanders

(*A. talpoideum*; 1 year), as well the habitat use of juvenile *A. opacum* (1 year). For juvenile *A. opacum*, 80% of newly metamorphosed animals dispersed to Zone 1 (10-89 m from the wetland boundary), 10% to Zone 2 (90-171 m), 6% to Zone 3 (172-331 m), and 4% to Zone 4 (beyond 332 m). Distribution of adult *A. opacum* varied among years, but an average of 27% (range 21-32%) occurred in Zone 3 or beyond in all years. Forty percent of adult *A. talpoideum* occurred in Zone 1, with 15%, 25%, and 20% distributed in Zones 2-4, respectively. In general the form of the distribution of animals was one of two-phase exponential decay. Knowledge of the shape of the terrestrial distribution function is important due the strong influence it has on the buffer zone area required to capture 50% or 95% of the population. Our results indicate that, compared to a normal distribution, a distribution that mimics exponential decay may require a smaller area to protect 50% of the populations of these two ambystomatid salamander species, but a larger area would be needed to protect 95%. These results have important implications for conservation strategies for pond-breeding amphibians.

In the LEAP study, we finished collecting the third year of data on amphibian abundance and movement in response to altered forest management treatments. In addition to continued monitoring of the four LEAP wetlands, we: conducted a study on habitat selection in juvenile marbled salamanders, initiated a experimental study to determine survival of adult ambystomatid salamanders and effects of forest clearcutting, and concluded a study on the survival of juvenile *A. opacum* in altered forests. We will continue to collect data on habitat and environmental changes in altered forest habitats for up to five years post-harvest. We recently published one paper on the response of mole salamanders to forest harvesting techniques, and submitted four papers for review in scientific journals, including one now in press (see below).

Summary:

Long-term studies in relatively undisturbed areas provide invaluable information (such as population trends, effects of climate, etc.) that short-term studies cannot, leading to more sound environmental management. The monitoring of Rainbow Bay reptiles and amphibians continued for its 27th year, as recommended by the SRS Citizens Advisory

Board. Monitoring efforts were accompanied by additional research at other seasonal wetlands and experimental systems, which give us insights into the factors driving population ups and downs. Additional funding was sought in proposals to the Environmental Protection Agency (“*Predicting threshold responses of amphibian communities in isolated wetlands to changes in climate and management practices*,” \$896,773) and the National Science Foundation (“*LTREB: No Wetland is an Island—Assessing the Importance of Population Linkages to Long-term Persistence of Wetland-breeding Amphibians under Scenarios of Modern and Altered Climate*,” \$445,000), although neither was funded in FY06. We will continue to seek outside support for studies at RB, GB, and LEAP wetlands, as these efforts provide vital information on amphibian community dynamics.

Related manuscripts:

- Croshaw, D.A. and D.E. Scott. 2005. Experimental evidence that nest attendance benefits female marbled salamanders (*Ambystoma opacum*) by reducing egg mortality. *American Midland Naturalist* 154:398-411.
- Croshaw, D.A. and D.E. Scott. 2006. Marbled salamanders (*Ambystoma opacum*) choose nest sites at low elevations in a Carolina bay. *Amphibia Reptilia*. *In press*.
- Daszak, P., D.E. Scott, A.M. Kilpatrick, C. Faggioni, J.W. Gibbons, and D. Porter. 2005. Amphibian population declines at the Savannah River Site are linked to hydroperiod, not chytridiomycosis. *Ecology* 86:3232-3237.
- Gibbons, J.W., C.T. Winne, D.E. Scott, J.D. Willson, X. Glaudas, K.M. Andrews, B.D. Todd, L.A. Fedewa, L. Wilkinson, R.N. Tsaliagos, S.J. Harper, J.L. Greene, T.D. Tuberville, B.S. Metts, M.E. Dorcas, J.P. Nestor, C.A. Young, T. Akre, R.N. Reed, K.A. Buhlmann, J. Norman, D.A. Croshaw, C. Hagen, and B.B. Rothermel. 2006. How productive can an isolated wetland be? Remarkable amphibian biomass and abundance. *Conservation Biology*. *In press*.
- Rothermel, B.B., and T.M. Luhring. 2005. Burrow availability and desiccation risk of mole salamanders (*Ambystoma talpoideum*) in harvested versus unharvested forest stands. *Journal of Herpetology*, 39(4):619-626.
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Studies of the Microbial Communities of Contaminated and Uncontaminated Streams

INVESTIGATOR: J VAUN McARTHUR

It has been hypothesized that metal and antibiotic resistance traits may be co-selected for in bacteria, thus we would predict that bacterial exposure to metals would result in increased resistance to both metals and antibiotics. To test this hypothesis, a genotypic study was undertaken to ascertain whether specific resistance determinants are co-selected for in environmental bacteria. The relative

abundances of a metal resistance gene (*arsC*) and an antibiotic resistance gene (*tetAC*) from contaminated and reference sites were assessed using quantitative PCRT (qPCR). DNA was extracted from three stream sites along a gradient of metal contamination, and additionally from four microhabitats within these streams to assess where reservoirs of these genes occur in stream ecosystems. These include DNA from sediments, biofilm on submerged woody debris, digestive tracts of the Asiatic clam (*Corbicula fluminea*), and the water column. To normalize results for between sites and microhabitats comparisons, gene abundances were analyzed as a ratio of gene of interest copy number to 16 S copy number. Results indicate that the ratio of *arsC*:16 S copy number ranged from not detected to 1:10,000 with variation being greater between microhabitats than between sites. The ratio of *tetAC*:16 S copy number ranged from 1:100,000 to 1:50, with variation between microhabitats again being greater than site differences. Results suggest that these metal and antibiotic resistance gene abundances are variable in the environment, but that these genes are generally more abundant in metal-contaminated sites.

Carolina Bay Restoration Studies

INVESTIGATOR: REBECCA SHARITZ

More than 300 isolated depression wetlands (Carolina bays) occur on the Savannah River Site. Once very common throughout the southeastern Coastal Plain province, most Carolina bay wetlands in the region have been drained, cleared, or totally destroyed as a result of land management practices including forestry and agriculture, and by urban and industrial development. The importance of these wetlands as habitat for many regional wetland animal and plant species has been well-documented through long-term SREL studies. It is also recognized that climate-induced fluctuating water levels (including periods of dry as well as inundated conditions) are a critical characteristic of the environment for these unique species. What is not well-known is the natural variability in hydrologic conditions among Carolina bay depression wetlands of different size and location, and whether restoration of such conditions in drained or disturbed depression wetlands will enable re-establishment of the characteristic flora and fauna. Two

studies were conducted in FY06: (1) a 10-year characterization of baseline hydrologic conditions in a selected set of bays differing in size and landscape location on the SRS, and (2) assessment of vegetation changes in herbaceous Carolina bays in response to natural climatic variability, as a baseline for evaluating wetland restoration success.

In spring 1995, 50 Carolina bay depressions on the SRS were selected for hydrologic monitoring. These wetlands are distributed across the different landforms of the SRS and represent the range of size and vegetation conditions that occur in SRS bays. In 2002, the number was reduced to 30 bays for logistic reasons. Frequent measures of water levels over the 10-year period have shown extreme variation among the bays in depth and periodicity of inundation and in frequency of drawdown. Furthermore, wetlands of similar size and in close proximity commonly have very different hydrologic patterns, reducing predictability within clusters of bays. All wetlands were deeply inundated during the EL Niño period of 1998, and all became dry during the drought of 2002. This template of natural variability is providing the needed background for understanding variation in wetland plant and animal species and separating natural variation from potential operational impacts. These data have been used to support studies published recently in *Wetlands* and the *Bulletin of the Torrey Botanical Society*.

The vegetation of ten herbaceous Carolina bays (selected from the 50 in the hydrologic monitoring study) was characterized during a period of average rainfall and again at the end of a four-year drought. Initial vegetation structure affected the change in species composition, as did the water levels. Depression meadow and aquatic pond wetlands showed greater compositional change and increase in abundance of non-wetland plants during the drought than did bays with grass and sedge marsh vegetation. These meadow and pond wetlands had shorter and more variable hydroperiods than did the marshes, which apparently provided opportunities for invasion of upland species into the wetlands. This research was published in the *Bulletin of the Torrey Botanical Society*.

Sandhills TES Population Studies

INVESTIGATOR: REBECCA SHARITZ

Along the southeastern Fall Line region, there are extensive areas of sandhills and related xeric forests that support a unique flora and fauna, including a suite of threatened, endangered and sensitive (TES) plant and animal species. Federal lands along the Fall Line, including the SRS and military installations, are managed to promote open pine woodlands as habitat for the federally endangered red-cockaded woodpecker (RCW). It is not known if management directed towards RCW populations (single-species management) is beneficial, or possibly harmful, for other sandhills TES species. The goals of this research, which is leveraged by funds from the Strategic Environmental Research and Development Program (SERDP), are to evaluate the effects of land management activities on sandhills communities, to assess whether there is a combination of management activities that is suitable for all or most of the sandhills TES species, and to make recommendations for multiple-species management.

Nine TES plant species, listed as Species of Conservation Concern for Georgia and South Carolina, were chosen and 63 populations of these species were sampled. Within each population, TES plant density and reproductive status were recorded, and environmental conditions were characterized. All species occur in sandy soils with very low nutrient levels, however there are significant differences in canopy openness among the TES populations. Soil moisture and soil moisture holding capacity strongly influence the distribution of the TES species. Some species are confined to ultra-xeric soils, while others are transitional to upland pine habitats.

A non-metric multidimensional scaling (NMDS) ordination revealed patterns in community composition and TES species distributions related to land management. Sites with no to minimal land use disturbance contained a greater number of characteristic sandhills species including the TES *Chrysoma pauciflosculoa* (woody goldenrod) and *Stylisma pickeringii* (Pickering's dawnflower). Sites with a history of more frequent fire had a greater abundance of *Nolina Georgiana* (Georgia beargrass), *Liatris segunda* (blazing star), *Carphephorus*

bellidifolius (sandywoods chaffhead) and *Warea cuneifolia* (Carolina pinelandcress). There was a trend of increasing legume dominance with increases in soil moisture and silt content in sites burned five to six years earlier; *Baptisia lanceolata* (lance-leaf wild indigo) and *Phaseolus polystachios* (wild kidney bean) were most abundant in these sites. Continued sampling of marked TES plant populations on the SRS was initiated in summer 2006 to provide a longer-term assessment of response to forest management activities, including thinning and burning.

Alligator Population Dynamics

INVESTIGATOR: TRAVIS GLENN

Efforts for this goal have been minimal. A total of 36 alligators from the SRS were sampled during FY06. Blood samples were added the SREL DNA lab collection. Phenotypic measures (length, weight, etc.) as well as date and location of capture were added to a long-term database of alligators captured on the SRS.

GIS-based Wildlife Literature Survey for Use in Risk Assessments

INVESTIGATOR: CHARLES DAVIS

WSRC continued to contract with SREL to update a literature and GIS database on SRS vertebrate and receptor species for use in SRS risk assessments and for incorporation into the IOU GIS project. As part of these biannual updates, SREL reviewed SREL and USFS-SR publications, reports, theses, and dissertations generated since 2001 and assemble records for all vertebrate species and site locations for the 77 receptor species. These data include: species scientific name, reference citation, geographic location(s) of studies, habitats as described by the references(s), habitat as described by the 1997 habitat map (Pinder 1998), and keywords from the citation(s). Site locations for receptor species were determined and shape files and accompanying metadata were created. Updates to the database tables continued in the Microsoft Access 2000 and Excel formats as directed by WSRC. Abstracts were generated for all new publications and

provided in html format. All information to include database, html documents, metadata, and any new geo-spatial data were provided on CD in html, PDF, and ArcView formats. These data were incorporated into the existing GIS Wildlife Literature Survey database and released to WSRC-ERD for use in risk assessments on the SRS. In addition to these updates, SREL continued with the spatial refinement of the current database and worked to restore and maintain the Excel spreadsheet database associated with the original stand alone ArcView project.

In FY06, WLS Update Versions 14 and 15 (July 2005 to June 2006) were finalized and delivered to WSRC-ERD in CD formats. These updates were produced from 52 publications, including 36 SREL reprints and 16 USFS-SR reports/publications. Based on these publications, 2838 new records were added to the Access databases, 9 new shape files were generated with source information, 52

new abstracts were generated, and 2 new metadata files for the WLS shapefiles were updated. One new receptor species was recommended for adding to the WLS.

The Access databases now have a total of 20130 records coming from 1114 publications. As requested by WSRC-ER, the original stand-alone ArcView project was restored this FY by updating the Wildlife.dbf. This database base is a work in progress and now contains a total of 12382 entries.

The Role of SRS Wildlife in Transferring Contaminants Off-Site

INVESTIGATOR: NONE ASSIGNED

Work to complete this milestone was not initiated due to a reduction in staff and loss of appropriate expertise.

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GOAL: Determine the biogeochemical processes that control chemical speciation and mobility of toxic metals, organic contaminants, and radionuclides.

Uranium and Nickel Speciation in the Steed Pond-Tims Branch System

INVESTIGATOR: PAUL BERTSCH

The sequestration of heavy metal ions, including transition metals, metalloids, and actinides (hereafter, metals) by soils and sediments strongly influences the fertility hazard posed by contaminated environments. Understanding the biogeochemical processes that control the mobility of heavy metals is essential for realistic estimates of human health and ecological risks and prudent remediation and management of contaminated lands. Bulk chemical characterization methods such as total digestions and sequential extractions provide important information but often fall short of describing in adequate detail or with sufficient confidence the physical distribution and chemical speciation of contaminants of concern. Advanced analytical techniques such as IR, Raman, luminescence, and X-ray absorption spectroscopy, are capable of filling this gap. However, when applied at macroscopic scales (i.e., 100 μm or greater), contaminant heterogeneity at the soil

mineral/humic matter assemblage scale is concealed. Consequently, microprobe techniques with spatial resolution on the order of 10's of micrometers and below are essential for obtaining elemental distributions and chemical speciation information for discrete soil mineral phases, particles, and organic structures.

Electron microscopy and electron microprobe analysis (EMPA) are environmental characterization workhorses but suffer from limitations imposed by sample preparation requirements, the use of high vacuum in the sample chamber, limited penetration depths, and reduced sensitivity for higher Z elements. Moreover, speciation information is not available from most electron beam techniques, and electron beam damage to the sample can be substantial. The development of synchrotron-based X-ray microprobes for environmental analysis has overcome many of these limitations.

On the Savannah River Site (SRS), a mixture of heavy metals have accumulated in wetland and riparian sediments rich

in natural organic matter and iron (hydr)oxide minerals. The sediments lie along a several kilometer stretch of Tims Branch, which received direct discharges of wastewater from metallurgical facilities involved in the manufacture of Al clad (depleted and natural) U reactor targets used in Pu production. Substantial quantities of U, Ni, and Al were released to the environment, along with lesser quantities of Zn, Cu, Pb, and Cr. The nature and extent of contamination poses a number of challenges for DOE in terms of long-term stewardship. The presence of U, Ni, and the other metals is of direct concern as remobilization and transport offsite via erosion remains a serious issue. Traditional remediation strategies such as “dig and haul” and capping would destroy a functioning and valuable ecosystem and would exact a high cost, both monetarily and in terms of risk, due to their labor intensive and invasive nature. Such approaches are also contrary to DOE guidelines (10 CFR, Part 1022) for compliance with Executive Orders 11988 and 11990 established for the protection and management of wetlands and floodplains.

Previous characterization, using indirect methods such as sequential extractions, indicated U availability is dominated by its strong association with dissolved and solid phase organic matter. Nickel lability appeared to be controlled chiefly by Fe oxide phases and by discrete, refractory forms possibly linked to contaminant source terms (Sowder et al., 2003). Evidence for reduction of the U(VI) to U(IV) form has not been observed in either sediments from the field or in biologically active laboratory microcosms in spite of conditions favorable for U reduction.

Ongoing characterization of U and Ni bioaccumulation within and transfer between trophic levels (including soil, detritivores, plants, insectivores, herbivores, and omnivores) indicates that Ni is much more bioavailable and mobile than U, which is present predominately as the uranyl (UO₂²⁺) dioxo-cation. Nickel accumulation in wetland plants and small mammal tissues exceeded that of U by 2–4 orders of magnitude (Punshon et al., 2003a; Punshon et al., 2003b), which is consistent with geochemical observations (Sowder et al., 2003).

In this study, we apply the capabilities provided by the microprobe facilities at the National Synchrotron Light

Source and the Advanced Photon Source to characterize elemental distributions and chemical speciation of U and Ni in contaminated riparian sediments on the SRS. These methods, when combined with information obtained from traditional bulk chemical characterization techniques and from EMPA, allow for a much greater understanding of the biogeochemical control and cycling of heavy metals in a dynamic and complex environment than previously possible.

Optical microscopy revealed a number of visible features consistent with detrital organic structures at various stages of decomposition. Electron microprobe analysis showed significant correlations for Ca with S and P with R² of 0.89 and 0.69 respectively in the organic rich regions. Silicon was significantly correlated with Ti (R² = 0.57, p < 0.0001). Aluminum concentrations fell into two categories, at low Si (<1% wt/wt), Al was significantly correlated with Ca (R² = 0.95, p < 0.0001), while for Si >7% wt/wt, the Al: Si ratio was 1.01 ± 0.22, consistent with the dominant kaolinitic clay mineralogy of these sediments. The EM and optical microscopy results indicated that Ca was an excellent proxy for organic matter and Ti for mineral matter in the sediment thin sections. Synchrotron X-ray fluorescence elemental maps of areas identified by optical microscopy showed localized high Ni concentrations occurred in high Fe regions and high U concentrations in high Ca regions. Areas of concurrent high Ni and U were filtered according to 90th percentile values of Ca and Ti. Two distributions in the data were then apparent with high U corresponding to high Ca regions and high Ni corresponding to high Ti regions. Xanes analysis indicated that U was present as U(VI) in the organic regions. These microscopic studies support macroscopic observations of U affinity for organic matter and Ni affinity for inorganic sorption reactions. Moreover, these data support the contention that decaying and detrital organic matter is a sink for U in this riparian system

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Natural Attenuation of PCE/TCE in Pen Branch Hyporheic Sediments

INVESTIGATORS: GARY MILLS, CHUANLUN ZHANG, AND CHRISTOPHER ROMANEK

The CMP groundwater plume originates below the Chemical, Metals, and Pesticide (CMP) pits waste site on the SRS. Between 1971 and 1979, chlorinated solvents, metals, pesticides, and electrical parts containing PCBs

were dumped into unlined pits at the site. In 1984, the waste materials were excavated and the site was back-filled with soil, including the installation of a shallow (3 ft.) plastic liner to minimize surface water infiltration. However, the dense chlorinated solvents have penetrated the 90 ft. vadose zone beneath the pits and remain a source of contamination to the underlying Upper Three Runs Aquifer groundwater. The contaminated groundwater plume is moving north and intersects a section of Pen Branch Creek between Road C and Youman's Road. The contaminants of concern in the CMP groundwater plume are tetrachloroethylene (PCE), trichloroethylene (TCE) and, to lesser extent, carbon tetrachloride (CT; 1).

Natural physical, chemical, and biological processes often act to reduce the concentration, mobility, and toxicity of contaminants in the environment. Monitored natural attenuation (MNA) assesses the contribution of these processes in the cleanup of contaminated soils and groundwater. MNA is often considered as part of an overall remediation plan that includes the use of engineered

treatment technologies to achieve acceptable levels of risk to human health and the environment. MNA of chlorinated solvents is most effective in oxygen-deficient (reducing) environments where microorganisms utilize iron and sulfate instead of oxygen during respiration while simultaneously removing chlorine atoms from compounds like PCE, TCE, and CT in a process called reductive dechlorination. Saturated floodplain soils and sediments adjoining streams generally provide conditions favoring reductive dechlorination and have been the focus

of several MNA studies at the SRS, including PCE/TCE plumes migrating toward Four Mile Branch Creek and Castor Creek. However, no studies have explicitly examined natural attenuation processes within the subsurface stream sediments that are

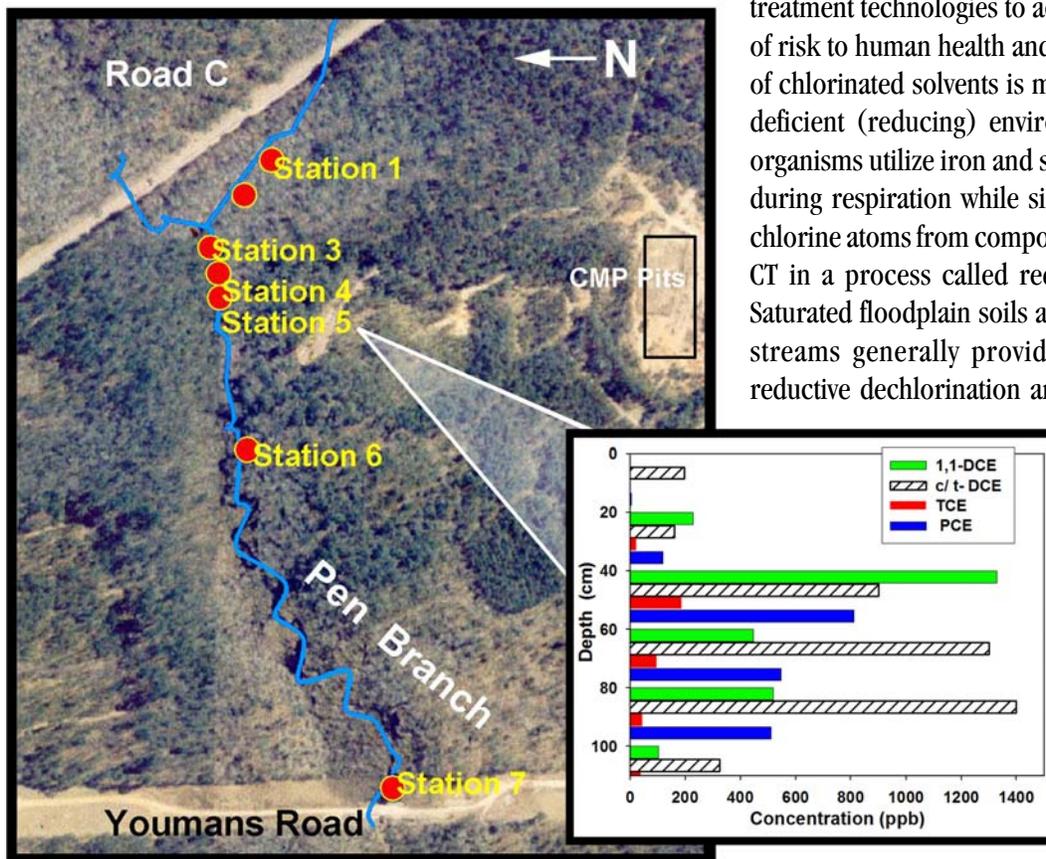


Figure 1: Sampling locations on Pen Branch Creek. Inset graph shows the contaminant profile for one core from Site 5.

directly linked to stream surface water. This region is called the hyporheic zone and has been shown to play a critical role in controlling the flux of groundwater solutes to surface waters (2-4). With regard to MNA, it is the final interface before contaminants outcrop into regulated receiving waters.

The goal of this study is to examine the role of MNA processes within the hyporheic zone in mitigating the flux of PCE, TCE, and CT from the CMP groundwater plume into Pen Branch Creek. The specific tasks are to determine the distribution of source contaminants and their degradation products in the hyporheic zone within the expected region of plume outcropping in Pen Branch Creek. Relevant geochemical and microbial MNA parameters are also measured to provide the necessary environmental context to draw inferences regarding the predominate processes controlling the contaminant distribution. The study is a collaborative effort with Dr. John William and undergraduate interns from South Carolina State University. Seven sampling locations in Pen Branch Creek between Road-C and Youman's Road (Fig. 1) were established in July 2005 based on existing data from well water monitoring and passive diffusion samplers in the lower flood plain as well as the predicted plume flow from a numerical transport model (1). Hyporheic sediment cores were collected by augering within a PVC pipe, which isolated the cores from the stream surface waters. Sediment sections in roughly 10 cm intervals were sampled from the sediment-water interface to the Tan Clay-Lower Clay layer at a depth of 90-110 cm. Triplicate cores were taken from both proximal and distal locations in the stream channel at each sampling site during the summer and fall in 2005 and then re-sampled in the summer 2006, resulting in nearly 700 volatile organic carbon (VOC) analyses. PCE and TCE contamination was detected at all sites except for Site 7 near Youman's Road. PCE values ranged from <0.1 to 1242 ppb and from <0.4 to 506 ppb for TCE. Both PCE and TCE concentrations were highest at Site 5, with progressively decreasing values observed at sites up and down stream from this location. The observed PCE and TCE concentrations were compared with values predicted by the numerical model developed for the CMP groundwater plume.

Overall, the region of plume movement into the stream agrees very well; however, measured concentrations are in some cases much higher than predicted. Isomers of dichloroethylene (cis-DCE, trans-DCE, 1,1 DCE), derived from the microbial degradation of both PCE and TCE, were also detected in the presence of the source contaminants. Concentrations of these products often exceeded values for PCE and TCE, indicating significant natural attenuation via microbial reductive dechlorination. As expected there is considerable heterogeneity between sites and between cores at the same site. A contaminant profile for one core at Site 5 is shown in the insert for Fig. 1. This does not present a typical profile but rather captures features observed in many of the cores, especially at the more contaminated locations (Sites 3 and 5). Generally, the highest contaminant concentrations were found between 40 and 60 cm, suggesting this region as the primary zone of plume infiltration. Since degradation products usually exceeded the source contaminants PCE and TCE, significant microbial degradation takes place within the floodplain sediments. However, it also indicates that rates of natural attenuation processes within the floodplain are insufficient to completely remove contamination. From this mid-depth region of infiltration, contaminant concentrations decrease toward the stream-sediment interface (0-10 cm). This is in part likely due to mixing with stream surface water in shallow sediments but since the ratio of PCE/TCE to DCE also decreases, this suggests further microbial degradation within the stream hyporheic zone.

A surprising finding for this system is that 1,1 DCE is often the primary degradation product. In most studies, cis-DCE with much smaller amounts of trans-DCE are the principal products observed for microbial reductive dechlorination, leading subsequently to the formation of vinyl chloride and carbon dioxide. A recent study has shown that an isolate of *Dehalococcoides* from a contaminated site reduced TCE to ethylene exclusively through 1,1 DCE (5). This pathway has not been widely reported and will be further explored in the CMP-Pen Branch system. Separate core samples from Site 3 and 5 have been extracted for microbial DNA and lipid biomarker analysis. These analyses are nearly complete and will provide information on the microbial communities active within the hyporheic sediments as well as further insight on the observed distribution of source

contaminants and their degradation products.

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Development of Compound-Specific Isotope Ratio Analysis

INVESTIGATORS: CHRISTOPHER ROMANEK, CHUANLUN ZHANG, AND GARY MILLS

The stable isotope composition of natural and anthropogenic materials provides insights into the origin and modification of carbon and nitrogen bearing compounds in terrestrial (soil) and aquatic (sediment) ecosystems over time. The knowledge gained is amplified when bulk materials are separated into their component compounds prior to isotopic analysis. Toward this end, the stable isotope laboratory at the Savannah River Ecology Laboratory has been developing protocols for the pre-purification and carbon isotope analysis of microbial lipids from soils and sediment samples by gas chromatography-C-isotope ratio mass spectrometry (GC-C-IRMS). The first carbon isotope measurements of phospholipid fatty acid methyl esters are complete and protocols are being developed to analyze the neutral lipid and glycolipid

fractions. Standard reference materials of known carbon isotope content are being developed for all three lipid fractions to facilitate the collection of calibrated analyses. This methodology will be used to obtain carbon isotopic data on the microbial lipids isolated from the hyporheic sediment in Pen Branch Creek. These results will help elucidate the primary carbon processing pathways employed by microorganisms in these sediments and provide further insight into the biogeochemical parameters controlling the degradation rates of PCE and TCE in this system.

Application of Surface Complexation Models to Descriptions of Contaminant Migration in the Vadose Zone

INVESTIGATORS: JOHN SEAMAN AND ANDREW NEAL

The effectiveness of enhanced *in situ* remediation approaches depends largely on the ability to deliver and stimulate the growth of dissimilatory metal reducing (DMR) bacteria within the zone of interest while maintaining formation integrity, especially in close proximity to recharge wells. A series of batch and column studies are underway to evaluate the ability to enhance contaminant metal reduction within the highly weathered soils and aquifer sediments of the Southeastern U.S., with special attention placed on the impact of nutrient amendments on the mobility of Cr(VI) and other redox sensitive contaminants. In the batch experiments, complete Cr(VI) reduction in the inoculated treatments for both soils was observed within the first four days of incubation, with higher levels of Fe(II) solubilized with extended incubation. For the subsurface material, essentially all of the reduced Cr, i.e., Cr(III), was sorbed; however, a significant fraction of the reduced Cr remained soluble in the surface soil treatments despite the buffered pH, suggesting that metal complexation by dissolved organics may be occurring. In the absence of *Shewanella putrefaciens* CN32, similar levels of Cr(VI) sorption were observed for both soils, in sharp contrast to preliminary batch and column studies that observed greater Cr(VI) sorption in the Fe-oxide rich vadose sediment, compared to the surface soil, suggesting that abiotic reduction of Cr(VI) occurred within the surface soil.

In the column experiments, greater Cr(VI) sorption as indicated by retardation was observed for the oxide rich vadose sediment when compared with the surface soil. Only the carbonate buffer was effective at increasing the pore solution pH to a range suitable for CN32; however, both of the buffers enhanced Cr(VI) mobility by either reducing the positive charge associated with amphoteric minerals (i.e., Fe and Al oxides) or competing for the remaining anion sorption sites. In addition, both buffer solutions caused significant clay dispersion for the surface soil, as indicated by effluent turbidity. In contrast to the batch results, high levels of Cr(VI) recovery indicated little potential for reduction in the absence of the inoculum.

To date, batch and column studies indicate that *in situ* reduction may be an effective means of remediating groundwater systems contaminated with redox sensitive contaminants such as U and Cr. However, the current batch experiments suggest that the effectiveness is greatly influenced by the composition and mineralogy of the soil in question. In addition, preliminary column experiments indicate that buffered nutrient solutions often required to stimulate the microbial reduction may enhance the migration of contaminants by altering surface chemical processes and inducing clay dispersion that could adversely impact formation integrity and hydraulic conductivity.



GOAL: Assess whether sentinel species or other sensors can be used to characterize environmental health.

Studies of the Effects of Nickel Exposures in Turtles

INVESTIGATOR: PAUL BERTSCH

The timing and mass release associated with contamination events within ecosystems are often unknown, which limits the ability to estimate exposure histories and evaluate ecological risk. There has been interest in recent years in using indigenous organisms as indicators of environmental contamination, with a specific emphasis on long-lived biota that have distinguishable features associated with periodic growth. These long-lived organisms in particular can be useful as biomonitors for reconstructing contaminant exposure histories (i.e., Hunter et al. 1997; Punshon et al. 2005; Seltzer and Berry 2005). Additionally, such biomonitors hold great potential to be used in long-term environmental surveillance monitoring and research activities, as well as to evaluate the efficacy of natural attenuation, or *in situ* stabilization or bioremediation activities.

Evidence of the timing of historical contaminant exposure to organisms is particularly relevant in situations such as that at the SRS, where uranium and nickel (>40,000kg) were deposited from the mid 1950's through mid 1980's. A spillway breach in 1984 generated a release of this material into a relatively unimpacted ecosystem. Subsequent environmental impact monitoring has been

conducted on a variety of taxa and in several ecosystems, including research focusing on trophic level transfer of contaminants. An outcome of this research includes evidence that dendroanalysis can provide a temporal record of geochemical changes. Study of the growth rings of the black willow (*Salix nigra* L.) from this contaminated area on the SRS proved useful as a method for assessing elemental presence in ground water within an environment (Punshon et al. 2005).

Turtles serve as an attractive bioindicator species because they are extremely long-lived organisms, appear to be relatively tolerant to a range of pollutants, and their shell is comprised of bone (apatite), which is a well known target organ for various transition and heavier elements.

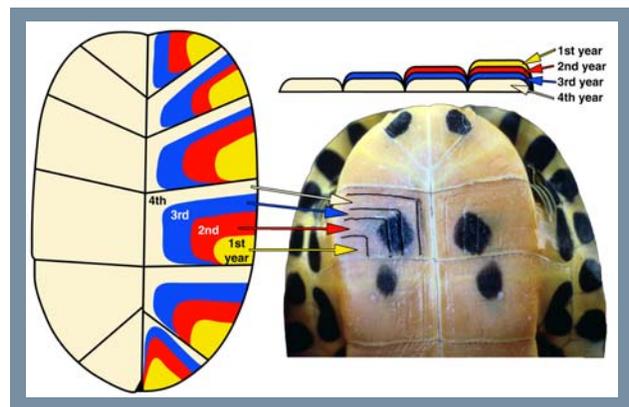


Figure 2: Growth patterns of turtle scutes.

They also display discernible growth rings in their scutes (Figs. 2 and 3), which could provide a chronological record similar to dendroanalysis. In addition, segments of marginal scutes can be removed without permanent damage to the organism, allowing for non-destructive sampling. A common southeastern species of turtle with wide applicability is the yellow-bellied slider (*Trachemys scripta scripta*), which is found from southeastern Virginia through northern Florida (Conant and Collins 1998). Preliminary SXRF analyses on thin sections of shell samples from an individual collected from a pond within the contaminated site and from a control turtle of the same species collected in an uncontaminated environment were conducted at the National Synchrotron Light Source (NSLS; Brookhaven National Laboratory, Upton, New York). Significantly elevated Ni concentrations were observed in the contaminated shell, co-associated with elevated Fe and Cu. Spectra were also collected along the bone section progressing from old tissue (representing when the turtle was young) to new tissue (when the turtle was old), and a marked decrease in bone Ni concentrations, expanding over three orders of magnitude, were observed for the contaminated shell. In contrast, metal concentrations within the control shell remained relatively constant (Hunter et al. 1997; Sutton et al. 2002). It is known that *T. scripta* has a changing diet pattern with age, with a shift from a predominantly carnivorous diet when young to a predominantly herbivorous diet when older. This shift in food preference may explain the observed trends in metal concentrations where maximum exposure would be anticipated during the carnivorous feeding periods (Clark

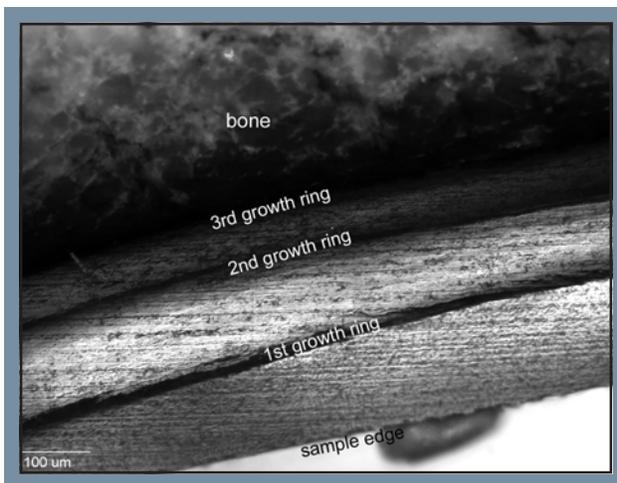


Figure 3: Growth rings in turtle scutes.

and Gibbons 1969). A similar methodological approach was taken using laser ablation ICP-MS to study keratin scute sections of desert tortoises (*Gopherus agassizii*) that had undergone environmental arsenic exposure due to anthropogenic influence. A pattern of chronological elemental uptake was observed in this species that supports the use of this type of analysis as a method for assessing long-term exposure (Seltzer and Berry 2005). However, there remains uncertainty surrounding mechanisms leading to observed elemental distribution patterns in animals collected from the field, limiting the power of using this information for retrospective monitoring or for dose reconstruction.

A critical step in fusing the link between indicator species such as turtles and contamination events is the necessity of a controlled dosing experiment. In June 2003, five hundred hatchling red-eared sliders (*T. scripta elegans*) were obtained from a private breeding facility, and have been reared in cattle tanks located at the Savannah River Ecology Laboratory of The University of Georgia on the SRS. Beginning in July 2003, individuals were orally gavaged with nickel citrate at concentrations (in triplicate) of 0 (control), 20 mgL⁻¹, 65 mgL⁻¹, and 300 mgL⁻¹ Ni during active growth on a bi-monthly basis. These concentrations were selected to represent both biologically realistic and extreme exposure conditions. Growth measurements (plastron length and width, cara-pace length and width, and mass) of all individuals were taken prior to each gavaging. No significant relationship has been observed between mortality and treatment group, or growth rate and treatment group. Marginal and plastron scutes (keratin and bone) from individuals in

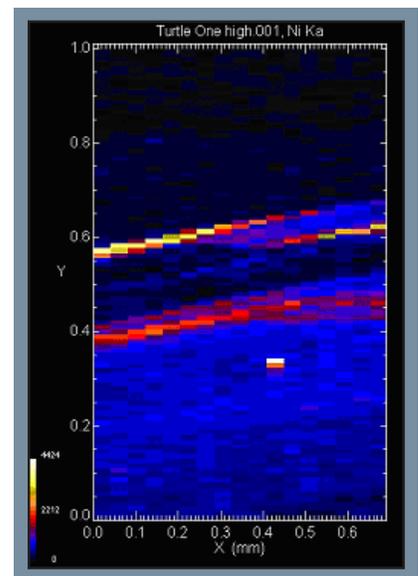


Figure 4: Disposition of nickel in growth rings of turtle shells.

each treatment group have been analyzed using an SXRF microprobe at NSLS (beamline X26A) with both line scans and 2-D maps being generated. Deposition of nickel within growth rings is apparent, with a Ni concentration gradient corresponding to dose. In addition, the older growth ring typically contains higher concentrations of nickel in comparison to more recently placed rings (Fig. 4). This potentially depicts the relationship between turtle size/growth and ability to process contaminant loads, and spatial mapping is being conducted to examine the differences in concentration between annuli.

The results of our controlled experiment thus far demonstrate the potential utility of turtles as sentinel species for use as a biomonitor/bioindicator for a range of ecological risk applications and for long-term surveillance and monitoring activities. Currently, we are limited by the beam size available at X-26A (nominal beam size of ~10 mm). Although distinct patterns of Ni deposition are discernible, additional investigation using a smaller beam would potentially reveal more structure and detail within annuli, perhaps allowing unambiguous conclusions regarding the timing of exposure relative to element deposition and remobilization in the scute. In addition, a greater sensitivity is needed for conducting micro-XANES and EXAFS in order to elucidate first shell atoms and coordination numbers to assess shell mineralization patterns. We propose to conduct additional studies with smaller beam size and greater x-ray flux at the Advanced Photon Source, Argonne National Lab.

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Studies of the Relationship Between Microbial Antibiotic Resistance and Environmental Contamination

INVESTIGATOR: J VAUN McARTHUR

Various enteric bacteria have been used as indicators of water-borne pathogens. We isolated *Escherichia coli* from several marine and estuarine locations and screened these organisms for levels of antibiotic resistance to determine whether there was relationship between levels of antibiotic resistance and known contamination. Fluoroquinolones are an important class of antimicrobial agent used for the treatment of invasive infections in numerous clinical and veterinary settings. We isolated and characterized a highly ciprofloxacin- and moxifloxacin-resistant *Escherichia coli* strain from Ship Yard Creek, Charleston, South Carolina; a site with a long history of metal contamination. Toxicity experiments demonstrated that this strain exhibited extreme resistance to ciprofloxacin (MIC 200 µg/ml) and moxifloxacin (MIC 120 µg/ml) as well as high-level resistances to over a dozen other antibiotics, including moxacin, ampicillin, kanamycin and chlorophenicol, that exceed all reported levels of antibiotic resistance. FT-IFR analysis of cells grown in the presence of high concentrations of ciprofloxacin demonstrated the induction of a proteinaceous cellular exudate, suggesting that this isolate may utilize a novel or hitherto

uncharacterized mechanism of fluoroquinolone resistance. Significantly, this strain was isolated from a site with no obvious sources of antibiotic contamination, thus the development of front-line antibiotic resistance in an established human pathogen outside of a clinical setting is somewhat unexpected. This constitutes the first report of moxifloxacin and ciprofloxacin resistance from an environmentally derived *E. coli* isolate.

Alligator Population and Genetic Studies

INVESTIGATOR: TRAVIS GLENN

A three-year contract with the Louisiana Department of Wildlife and Fisheries was concluded during FY06. We studied the mating system and reproductive patterns of American alligators (*Alligator mississippiensis*), assessing multiple paternity and nesting demographics using microsatellite DNA loci. Eggs were sampled from wild American alligator nests on the Rockefeller Wildlife Refuge (RWR) in southwest Louisiana (1997-2005); females guarding the nests were also sampled. Females and offspring were genotyped using five polymorphic microsatellite loci. Genotypes of the hatchlings were consistent with the guarding females being the mothers of their respective clutches when females were present to be sampled. Multiple paternity was found in more than one third of the clutches at RWR. Nests in the same location in successive years were used to determine if females are using the same nesting location in multiple years and if offspring from the same nest have the same parents in successive years (i.e., to determine if alligators keep the same mates for multiple years). Two of the recaptured females at RWR mated with the same male in different years, two mated with different males in different years, and one female mated with one male the first year captured and then produced offspring the following year with that same male and an additional male.

A new panel of tetranucleotide microsatellite DNA loci was developed and used to determine 10255 genotypes from 101 clutches with a total of 2295 alligators provided by Dr. Ruth Elsey and staff of the RWR. These data are being used to develop new models for mutation rates in American alligators. Eggs from RWR were also taken to collaborators

at the Universities of Southern California and South Carolina. The eggs were used for experiments to learn more about the developmental biology of alligators. These observations and experiments will contribute to a broader understanding of alligator reproductive strategies and how they can be used as environmental sentinels.

Using Ecological Indicators to Determine Disturbance Associated with Regional Land Use

INVESTIGATOR: BEVERLY COLLINS

Indicators developed at Fort Benning were measured in an SRS forest stand within the Advanced Tactical Training Area (ATTA). The ATTA site has been prescribe-burned and was thinned recently, but has had no military or heavy vehicle use. Canopy openness, depth of the soil A layer, soil compaction, tree basal area and density, and ground cover were surveyed during summer, 2006. The SRS site was compared to 40 sites at Ft. Benning that spanned disturbance classes from low (class 1) to high (class 10). The ATTA site fell within disturbance classes 1-3 (low disturbance) for soil A layer depth and compaction. Compared to 'typical' mixed pine hardwood stands at Ft. Benning with lighter or heavier military use, the SRS site had a wider range of soil A layer depths and compaction, due primarily to skidder tracks. The SRS site also had more open canopy and a less abundant ground layer. The ATTA site thus appears to have a low disturbance index, but the indicators were not wholly comparable between the SRS and Ft. Benning due to differences in vegetation.

Using Freshwater Mollusks to Monitor Water Chemistry

INVESTIGATOR: CHRISTOPHER ROMANEK

Freshwater bivalves precipitate shells that accrete new material over time. As such, the shells may function like a tape recorder, storing environmental information over the life of an individual. This information, which is stored in the form of trace element and stable isotope ratios, could be valuable by providing historical records of water chemistry when environmental samples are not available. Further, the shells may potentially record water quality data

at a temporal resolution that would be cost prohibitive to acquire using conventional sampling protocols. Potential information that may be retrieved from the shells include: temperature, precipitation and discharge data, and biological oxygen demand (respiration/photosynthesis rates), as well as the aqueous concentration of a variety of trace metals (e.g. manganese and copper).

Our research has focused on six sites on the SRS: (1) Meyers Branch, (2) Pen Branch, and (3) Upper Three Runs Creeks, which are not impacted by site activities, (4) Lower Three Runs Creek downstream of the Par Pond cooling water reservoir, (5) Tims Branch, which has variable metal contamination (e.g., Ni, U), and (6) Beaver Dam Creek, which contains elevated levels of trace elements associated with coal pile leachate (e.g., Al, As, Cd, Cr, Co, Cu, Fe, Ni, Se, Zn). Freshwater bivalves, *Elliptio complanata*, were collected from site streams and their shells were analyzed at high resolution by micro-milling and isotope ratio mass spectrometry (IRMS) for $\delta^{18}\text{O}$, $\delta^{13}\text{C}$, and δD values and by laser ablation ICP-MS for the concentrations of Mn, Cu, Sr, and Ba in the shells.

In FY2006, we published a paper in *Chemical Geology* (Carroll *et al.*, 2006: "The relationship between the hydrogen and oxygen isotopes of freshwater bivalve shells and their home streams") that documents the relationship between δD values in freshwater bivalve shells and the surrounding waters they inhabit. Based on the predictable relationship that exists between δD and $\delta^{18}\text{O}$ values for meteoric waters, the δD values of shell material will aid in the interpretation of $\delta^{18}\text{O}$ values for temperature and precipitation information. Two additional manuscripts are in preparation that report on the trace element records of *Elliptio complanata* collected from various streams on the Savannah River Site. In these papers, we demonstrate that shell concentrations of Mn, Sr, and Ba often reflect aqueous concentrations, although in highly impacted environments (e.g. Beaver Dam Creek) this relationship requires additional information on organismal health for it to be useful. The results suggest that freshwater bivalves may be reliable monitors of environmental conditions in most environments. Finally, this research forms the basis for a dissertation by Ms. Monica Carroll, who will be graduating at the end of 2006.

Continued Research at the Mixed Waste Management Facility

INVESTIGATOR: JOHN SEAMAN

The automated weather station and irrigation-pond depth sensor were installed at the MWME. Semiannual reports documenting evapo-transpiration efficiency estimates based on soil core samples and the Cornell model were submitted to SGCP. Development and testing of the automated vadose monitoring systems continues. The sampling system is currently being modified so that one ISCO sampler can be used to collect and store samples from multiple suction lysimeters, each with a dedicated vacuum pump to control the sampling tension. This modification required additional flow control valves and a major change to the system programming to coordinate suction development to pull water into the lysimeter and then subsequent release of the vacuum tension and purging required to deliver the sample to the ISCO fraction collector.

Ecological Risks and Effects

Estimated risks and effects determine the need for remediation and restoration efforts, while perceived risks and effects determine the public's acceptance and support of DOE policies and actions. Estimating ecological risks and effects on the basis of sound science helps to ensure that good decisions are made by reducing uncertainties associated with complex environmental processes. A 1999 report from the National Academy of Sciences stated that *“Ecological risks are better characterized at the Savannah River Site than at any other DOE installation, due in part to the designation of the site as a National Environmental Research Park and the presence of the Savannah River Ecology Laboratory.”*

Report on FY06 Risks and Effects Milestones

GOAL: Determine how changes in contaminant speciation influence dose-response and toxicity relationships.

Exposure of Earthworms to Sediments from the Steed Pond-Tims Branch Ecosystem

INVESTIGATOR: PAUL BERTSCH

The Tims Branch/Steed Pond system is heavily contaminated with U, Ni, and other metals that were released during operation of the M-Area nuclear materials metallurgical facility used to fabricate U targets for the production of Pu. Ongoing studies are examining the ecological risk associated with the massive contamination dispersed in riparian sediments along several km of the Tims Branch corridor. The EPA's Earthworm Subchronic Toxicity test (EST) forms part of the Ecological Effects Test Guidelines, allowing the characterization of potential ecological risk from soil contamination by exposing earthworms to various incremental mixtures of the test soil over a period of 28 days, and measuring mortality or bioaccumulation. Such toxicity tests play an increasingly important role in how regulatory agencies estimate ecological risk by attempting to measure bioavailability rather than relying on total metal concentrations in soils or sediments. Preliminary studies have demonstrated that U and Ni accumulation from contaminated Tims Branch sediments by the model earthworm *Eisenia foetida* are

significantly greater than those from control soils. Of particular interest was the very high bioaccumulation of U relative to Ni, an observation inconsistent with what we have observed for a number of other receptor species. This observation has implications for regulatory action for this site, given the importance of earthworms for establishing soil screening levels. Synchrotron-based X-ray fluorescence analyses of earthworm sections have demonstrated localized regions of high U concentrations

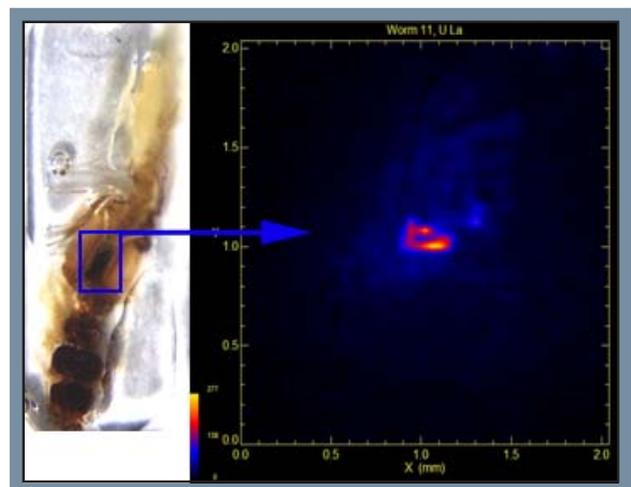


Figure 5: Localized regions of high uranium concentration in an earthworm digestive tract.

in the chloragogenous tissue located on either side of the digestive tract (Fig. 5). Preliminary micro-XRD data provide evidence that the U is present as a uranyl phosphate mineral phase consistent with the formation of calcium pyrophosphate granules produced by chloragocytes. This chemical form of U is known to be insoluble, suggesting a biological sequestration mechanism. In upcoming studies we will be evaluating the potential ecological significance of this observation.

Exposure of Transgenic Nematodes to Bioavailable Metals

INVESTIGATORS: PAUL BERTSCH, TRAVIS GLENN, AND ANDREW NEAL

Detection and quantification of contaminants in water and soil at environmental levels continues to be of paramount importance in all areas of human activities, especially in those where waste is generated, treated and disposed-of, or released to the environment. Bioassays have become a standard method for monitoring hazardous waste, and numerous approaches describe the use of invertebrates as sentinels for environmental health. In this study we report a rapid, inexpensive bioassay for heavy metal contaminants.

We identified and selected a stable transgenic line of the nematode *Caenorhabditis elegans* that carries an integrated promoter from the *C. elegans* metallothionein-2 gene (*mtl-2*), which controls the transcription of the green fluorescence protein (GFP). This line produced fluorescence in response to heavy metals such as cadmium, zinc, mercury, lead, and nickel. This fluorescence is dose dependent and can be directly detected with a spectrofluorometer, providing a fast and sensitive assay for exposure to bioavailable heavy metals. The system requires as little as 4 hours of exposure for higher concentrations (data not shown) and up to 24 hours for lower ones. In single metal assays, a positive correlation between the concentration of the metal and fluorescent emission was demonstrated for many the heavy metals tested (Cd, Cu, Hg, Ni, and Zn; but not As or Pb). In preliminary experiments we have demonstrated that toxicity of Zn^{2+} to *C. elegans* is related to the free ion activity rather than total metal concentration. Future studies will examine

whether this phenomenon extends to other metals and to ligands of varying stability and chemical lability. The worms also seem to secrete a fluorophore into metal containing exposure media, even when the *mtl-2::GFP* is not expressed (e.g., Pb exposure). We have some evidence that this fluorophore is not GFP; further characterization of this phenomenon is ongoing. Ongoing work is evaluating these worms to assess bioavailability and toxicity of manufactured nanoparticles.

Results to date demonstrate the applicability of these transgenic nematodes for fast and inexpensive detection of heavy metals in aquatic media and to provide unique information about the risks and effects of contaminants commonly found in the soils of DOE remediation sites.

Dose Response Relationships of Medaka to Gamma Irradiation

INVESTIGATORS: DANIEL COUGHLIN, TRAVIS GLENN, THOMAS HINTON, OLGA TSYUSKO, AND YI YI

Determining dose-response relationships for various measures is important for understanding biological effects and for determining the lowest dose at which effects can be measured. Dose response relationships may vary with different doses (being linear among some doses and non-linear among others), dose rates, radiation type and energy, and among individuals (because of differing genetic susceptibility and environmental factors). Below, we describe experiments using acute irradiation of Medaka to determine the dose response relationships between mutation rates of microsatellite DNA loci and the radiation doses applied. We conducted a range-finding dose response experiment to estimate the magnitude of individual variation in response, assayed by germline mutations of microsatellite DNA loci.

In this experiment we studied germline mutations in Japanese Medaka (*Oryzias latipes*) exposed to four different doses of ionizing radiation. Our goal was to evaluate the frequency of germline mutations in Medaka when exposed to known amounts of radiation and to construct a dose-response curve for these relationships. We were interested in detecting the lowest dose at which

effects can be observed and in individual genetic responses of the studied Medaka families to radiation exposure. Nine highly variable microsatellite loci (mutation rates of 10^{-2}) identified in previous studies were used. Prior to the exposure, medaka pairs were bred, and 96 hatchlings per pair were collected to serve as controls for microsatellite analyses. The same Medaka breeding pairs (two per treatment) were exposed later to four doses of acute ionizing radiation (0.1, 0.5, 2.5, and 5 Gy) and then allowed to breed again. DNA was extracted, microsatellite loci were amplified, and the genotypes were identified for the parents and all hatchlings produced before and after the exposure. Mutation frequencies were calculated for each locus and compared among all treatments and controls. We expected increases in the germline mutation rates with increasing dose to the parents. We also expected variation in the response (i.e., differing genetic sensitivity) among families, but tested against the null model of no differences among families. Until recently, when working with other vertebrate model species, such as mice, transgenerational effects induced by radiation were obtained by pooling data from several families because the study organisms, mice, can produce only a modest number of offspring. We were able to estimate individual genetic susceptibility to radiation exposure by using a model organism, Medaka fish, capable of producing many more offspring than mice.

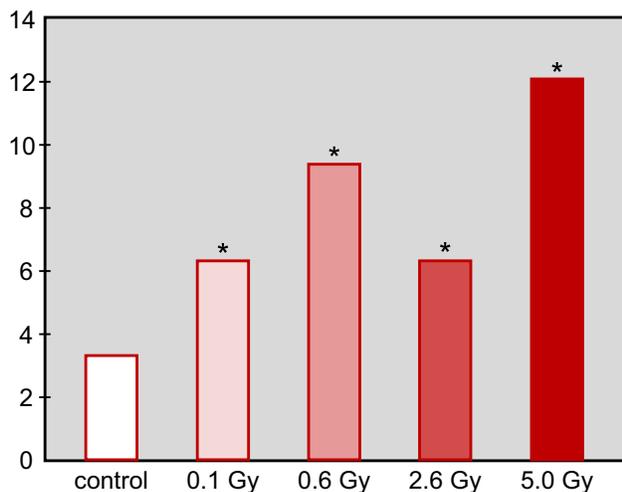


Figure 6: Percentage of mutations in Medaka offspring produced before and after their parents were exposed to 0.1, 0.5, 2.5, and 5Gy from a ^{60}Co source.

Our preliminary results suggest that mutations are significantly elevated in hatchlings of Medaka after their parents were exposed to any of the four radiation doses, when compared to the mutation frequencies of the offspring of the same parents before exposure (Fisher's exact test $P < 0.05$). Germline mutation rates increased significantly even at the lowest dose of 0.1 Gy (Fig. 6) raising concerns for the induction of germline mutation rates by environmentally-relevant radiation doses. These data provide critical points for a dose-response curve.

The percentage of mutations at 2.5 Gy, even though significantly higher than in control, is less than would be expected with a linear dose-response relationship. The lower percentage of mutations at this dose is likely due to lower susceptibility to irradiation of one family (#4), which did not show any differences in the mutation rates for hatchlings before or after their parents were exposed to 2.5Gy. Within each treatment the families showed different numbers of mutations induced by ionizing radiation, with one being more sensitive than the other families. This is the first study that allows us to differentiate among the individual genetic responses of each family to radiation exposure.

A large amount of variation was also detected among loci in the number of mutant alleles. Significant increases in mutation frequencies were observed at five out of the nine loci surveyed in the offspring of exposed parents, indicating that these loci are responsible for most of the responses observed for each treatment.

Current experiments focus on characterizing additional families to better understand: variance in response among individuals, response at different stages during the spermatogenesis (i.e., in offspring deriving from exposed sperm, spermatids, or spermatocytes), response of chronic vs. acute exposure, and transgenerational effects.

Rates of DNA Repair in Red Blood Cells from Bluegills Exposed to Gamma Radiation

INVESTIGATORS: DANIEL COUGHLIN, TRAVIS GLENN, THOMAS HINTON, OLGA TSYUSKO, AND YI YI

Radiation has the potential to cause DNA strand breaks, many of which are repaired. Repair rates can also vary among cell types. Knowledge about repair dynamics can be important for understanding cellular responses to radiation-induced DNA damage, standardizing sampling protocols to maximize the detection of responses, and explaining variation in responses among individuals. We determined the time required to repair DNA damage in red blood cells from bluegills (*Lepomis macrochirus*).

Fish were exposed to 3 Gy from a ^{60}Co source. Series of blood samples were collected from the same individuals before the exposure, and at 1, 30, and 90 minutes after exposure. DNA damage within red blood cells was detected using the Alkaline Comet Assay. Three DNA damage parameters were measured (moment of comet's tail, percentage of DNA migrated into the tail, and the tail length).

All three parameters responded in a similar way to the exposure. Significant damage in the blood cells was observed immediately after, as well as 30 minutes following the irradiation. The level of damage observed 30 minutes after exposure was on average 20% less than the amount of damage measured immediately after the irradiation. The amount of damage detected 90 min. post-irradiation was not

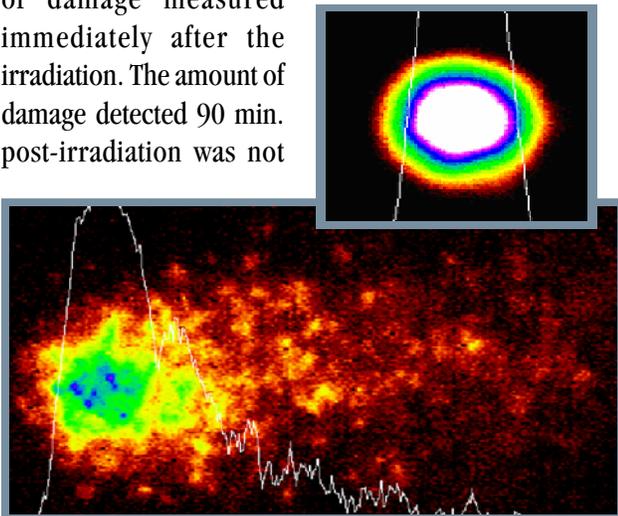


Figure 7: Results of a Comet Assay, showing a normal cell (top) compared to a damaged cell (bottom).

significantly different from that detected in controls, suggesting that the majority of the damage detectable by the Comet Assay was repaired within 90 min.

These results suggest that DNA repair processes for bluegill red blood cells exposed to ionizing radiation are relatively slow compared to many other tissues from different species, but are still fast relative to cell turn-over rates as well as the amount of time required to perform the comet assay on a large number of samples. Rapid repair rates, if not properly accounted for, are a potential source of bias in estimating the amount of DNA damage from exposure to ionizing radiation using the Alkaline Comet Assay.

Responses of Native Plants to Elevated Soil Nickel Concentrations

INVESTIGATOR: BEVERLY COLLINS

Pinus taeda (loblolly pine) seedlings were grown over two seasons in the field, along Tims Branch, in sites with and without elevated Ni. Plants also were grown in the greenhouse under flooded or unflooded conditions and with no, 35 ppm, or 70 ppm Ni added to the soil. In the field, plant ecophysiological measures, including photosynthesis, stomatal conductance, and internal CO_2 , and growth (plant height, diameter) did not differ between pines planted on “hot spots” and those planted on “cold spots.” In the greenhouse, photosynthesis and stomatal conductance was lower for plants growing in 35 ppm and 70 ppm Ni, but plant height and diameter did not differ. Flooding decreased all ecophysiological measures and plant height. Three of the five pots that were flooded and had the highest level of nickel added died during the flooding period. To date, these results suggest elevated Ni, particularly when combined with flooding, contributes to stress and reduced performance of loblolly pine seedlings.

Using Nitrogen Stable Isotopes to Study how Contaminants Affect Protein Metabolism

INVESTIGATOR: CHRISTOPHER ROMANEK

The nitrogen isotope composition of biological tissues provides key insights into the source of nitrogen in the

diet and the pathways by which N-bearing molecules (e.g. amino acids and proteins) are modified for growth and metabolism. Microorganisms commonly fix nitrogen (as N_2) from the atmosphere or they exploit processes (e.g., ammonification, nitrification, denitrification) that involve nitrogen redox gradients to extract energy from the environment. On the other hand, primary producers (e.g. plants/algae) utilize dissolved ammonium or nitrate as nutrients and consumers (heterotrophs) acquire nitrogen primarily through the consumption of protein.

Because nitrogen isotopes are fractionated as they are passed up a food chain, natural abundance distribution patterns may be used to estimate the trophic position of consumers in an ecosystem. In addition, deviations from the expected relationships can be used to determine if stressors (e.g. environmental or natural) are affecting physiological processes such as protein turnover. For example, previous work in our laboratory documented the effect of Hg exposure on the nitrogen isotope

composition of consumer tissues (Shaw-Allen et al. 2005. Shifts in relative tissue $\delta^{15}N$ values in snowy egret nestlings with dietary mercury exposure: A marker for increased protein degradation. *Environ. Sci. & Technol.* 39, 4226-4233). This work has been extended in FY06 to include additional bird (Cerulean Warbler, *Dendroica caerulescens*) and fish species (large-mouth bass, *Micropterus salmoides*).

Concomitant with these studies, our lab is developing compound-specific isotope ratio analysis as a method to determine the nitrogen and carbon isotope composition of individual amino acids that comprise protein. At this stage, work primarily focuses on protocol development for the quantitative separation and chemical analysis of intact amino acids (e.g. essential amino acids such as phenylalanine) that may provide key insights into nitrogen sources and protein turnover in natural and impacted ecosystems.

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GOAL: Determine the potential effects and interactions from exposure to mixed contaminants.

Determine the Impacts of Metal Contamination on Microbial Communities

INVESTIGATOR: J VAUN McARTHUR

There is growing concern that metal contamination acts as a selective agent in the attenuation and proliferation of antibiotic resistance. Of particular concern, metal contamination may represent a recalcitrant and globally distributed selection pressure. A comparative analysis of the antibiotic resistance profiles of 437 environmentally-isolated *Escherichia coli* strains, representing two metal contaminated coastal sites (Ship Yard Creek, Charleston, SC and the LCP Chemical Site, Brunswick, GA, USA) and an uncontaminated reference site (ACE basin, SC, USA) was performed. Isolates from the metal-contaminated sites, Ship Yard Creek and Brunswick, exhibited resistances to three or more structurally diverse antibiotics in 53% and 40% of screened isolates, respectively, compared to just 29% from the uncontaminated reference site. Furthermore, a small number of isolates from the metal contaminated

sites exhibited extreme multi-antibiotic resistance (to 9 or more antibiotics) that exceeds all previously reported levels of antibiotic resistance currently published in the scientific literature. Significantly, this small subset of multi-resistant strains was also extremely resistant to a number of front-line antimicrobials, which include moxifloxacin and ciprofloxacin. The detection of extreme multi-antibiotic resistance in pathogenic bacteria isolated from environments devoid of significant sources of antibiotic contamination is of considerable concern. Metal contaminated environments may subsequently contain a sizeable pool of antibiotic resistance determinants capable of horizontal transfer via commensals into clinical settings.

Studies of the Interaction of Nickel and TCE in Plants in the Steed Pond-Tims Branch Ecosystem

INVESTIGATOR: LEE NEWMAN

In an area such as Tims Branch, there is a long-standing

contamination of nickel. Migration of a chlorinated solvent plume will be moving trichloroethylene into the area. There are three possible outcomes for the interactions between the contaminants and the plants: (1) the presence of the nickel will interfere with the metabolism of trichloroethylene (TCE) in the plants, (2) the presence of TCE will affect the plant root cell membranes, altering nickel uptake, and (3) the movement of TCE into the area will affect soil chemistry and make the nickel more bioavailable.

We have been exposing hybrid poplar plants to varying concentrations of trichloroethylene and nickel in hydroponic conditions to determine if outcomes 1 or 2 are likely to occur. Preliminary data indicate that the presence of nickel affects the formation of metabolites in the leaves and roots of the plant.

Data regarding changes in nickel uptake with exposure to TCE are less conclusive. The original nickel concentrations chosen elicited toxicity responses in the plants, and thus are not reliable.

We will be expanding this work to look at less toxic doses of nickel to the plants to better mimic the Tims Branch system. We will also be increasing the number of plants per condition to obtain better statistical reliability.

Effects of Low-dose Rate Ionizing Radiation on Frogs and Toads

INVESTIGATORS: DANIEL COUGHLIN, TRAVIS GLENN, THOMAS HINTON, DAVID SCOTT, KAROLINA STARK, AND OLGA TSYUSKO

Recently, the international radiation protection guidelines changed from emphasis on protection of humans, with an implied protection of biota, to explicit guidance for plants and animals. However, debate exists concerning appropriate endpoints to measure when ensuring protection of biota. Amphibians are an interesting model for examining radiation exposure to biota because many species spend a large part of their life in wetlands where radioactive ^{137}Cs can accumulate, and thus they have potential to be chronically exposed. Currently, there is a lack of knowledge on the level of protection needed for

amphibians, especially regarding chronic low-dose radiation exposure. We were particularly interested in the combined effect of multiple stressors and thus designed these experiments to test whether the stress associated with a greater density of organisms impacted effects from irradiation.

We conducted radiation effect studies in an outdoor low-dose rate irradiation facility with ^{137}Cs sources on several amphibian species (spadefoot toads – *Scaphiopus holbrookii*, southern toads – *Bufo terrestris*, and bullfrogs – *Rana catesbeiana*). Amphibians were exposed to dose rates of 0.1, 2, 20, and 200 mGy/day. Due to their complex life cycle we were particularly interested in their radiosensitivity during egg development, the larval period, and the metamorphic period. Hatching success of eggs, larval survival, and age and body size at metamorphosis were measured at two different animal densities (0.7 and 2 individuals/liter).

A dose rate up to 200 mGy/day did not affect hatching success, larval survival, length of development, and body size at metamorphosis for these amphibian species. In contrast, higher density (2 vs. 0.7 individuals/liter) did significantly decrease the body size of *S. holbrookii* at metamorphosis. In addition, a subsample was analyzed for DNA strand breaks in the red blood cells using the alkaline single cell electrophoresis (Comet Assay). *Rana catesbeiana* showed a significantly higher percentage of DNA damage than *B. terrestris* and *S. holbrookii*, suggesting species differences in radiosensitivity that correlated to genome size of the organisms. These results provide valuable information to further understanding the effects of chronic low-dose radiation on amphibians.

Imaging of Labeled Non-Targeted Quantum Dots within Medaka Embryos

INVESTIGATORS: KOICHI AIZAWA, DANIEL COUGHLIN, THOMAS HINTON, ANDREW NEAL, AND SARAH WALLACE

Nanotechnology is a swiftly developing industry that is expected to revolutionize a number of fields, from cosmetics to cancer detection and treatment. While research into the potential uses of nanotechnology seems

to be expanding exponentially, research into the toxicological effects of such particles has been limited. The potential human and ecological risks from exposure to nanoparticles are uncertain. This study was intended to characterize the movement and behavior of non-targeted quantum dots (QDs), a type of nanoparticle, in the developing embryo of Medaka, *Oryzias latipes*. By better understanding how quantum dots interact within a living system, it is possible to better assess the risks of biologically active or toxic nanoparticles. The study was designed to focus on *in vivo* imaging technology, and on how the movement and accumulation of quantum dots in embryonic structures might allow insight into potential toxicological risks. By observing where quantum dots accumulate in living systems, and by characterizing their movement and behavior inside a developing embryo, we hope to better understand the toxicological risks presented by more reactive and potentially toxic nanoparticles. The study was conducted as part of SREL's undergraduate research program. Sarah Wallace, a sophomore at Hartwick College, Oneonta, NY, conducted the experiments during her 10-week undergraduate experience at SREL.

Medaka (*Oryzias latipes*) embryos were injected with non-targeted and streptavidin-conjugated QDs, and observed over the course of development. Medaka eggs

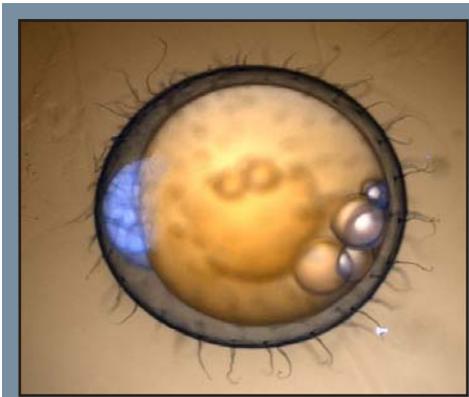
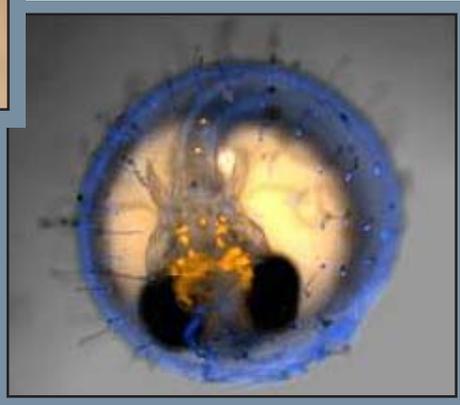


Figure 8. [top] Developmental stage 10 of the Medaka embryo (~7 hours post-fertilization) showing the blastodermal cells in blue, sitting on top of the egg yolk. Oil droplets are seen on the right side of the egg.

Figure 9. [right] Developmental stage 30 of the Medaka embryo (~4 days post-fertilization) showing two dark eyes, body cavity, notochord, tail, and pectoral fins.



the transport and fate of the fluorescent particles within a developing vertebrate. The various stages of egg development in medaka are well documented, thus, abnormalities due to contaminant exposure can be quantified.

A large difference in mortality was observed between injected and non-injected eggs. Control embryos had a mortality rate of 50%, while all injected groups had mortality rates above 70%. The medaka embryos were easily imaged throughout development (Figs. 8 and 9). The QDs, however, aggregated and did not disperse throughout the developing embryo as expected.

A second series of experiments used centrifugation and sonification of the QD solution prior to micro-injecting it into the 2-stage cell of the newly fertilized medaka egg. The additional sample preparation did not improve QD dispersal within the embryo. Subsequent conversations with the QD manufacturer revealed that aggregation was a problem that many researchers were experiencing. Although additional work with the vendor is on-going to solve the aggregation problem, this research has demonstrated that the medaka embryo may be a viable model for determining developmental problems from nanoparticle exposures.

are transparent and thus make a wonderful model for studying

Assess the Influence of Nickel and Uranium on TCE Degradation by Bacteria

INVESTIGATOR: PAUL BERTSCH

Binary and ternary mixtures of contaminant classes (e.g., metals, radionuclides, chlorinated hydrocarbons) have been identified at 64% and 49% of U.S. Department of Energy (DOE) facility waste sites, respectively. Although microorganisms can degrade many organic contaminants, co-contaminant metals inhibit processes required for attenuation and bioremediation, although few

studies have addressed their impacts. Thus, a major challenge in developing remediation strategies for mixed waste sites includes understanding how the presence of metals can affect microbial processes involved in contaminant degradation or sequestration.

For over three decades the Tims Branch watershed, located on the DOE's Savannah River Site (SRS; Aiken, SC), received metal-contaminated wastewater resulting from production of U-Al alloy fuel and depleted U targets. This waste was comprised primarily of U and Ni with lesser amounts of other metals. Additionally, an ~8 km² subsurface plume of chlorinated organic solvents, primarily trichloroethylene (TCE) and tetrachloroethylene, originating from this processing facility has begun to outcrop within riparian sediments along the Tims Branch corridor. Previous studies have examined the chemical and biological availability of Ni and U at this site. Based on our previous studies employing sequential chemical extractions of contaminated sediments, uptake by indigenous plants, and the isolation of Ni-tolerant bacteria from within the Tims Branch corridor, Ni appears to be more available to a number of bioreceptors than U. Considering the known toxic effect of these metals, they could potentially hinder intrinsic degradation of TCE at this site.

Hydroxylapatite (HA) $[\text{Ca}_{10}(\text{P})_4)_6(\text{OH})_2]$ is recognized for its ability to sequester lead (Pb) and other metals, including U and Ni, to reduce metal toxicity. HA sequesters cationic metals (e.g., Cd, Zn) by cation exchange with Ca, surface complexation, or precipitation as metal phosphates. Surface complexation of U was found to be the dominant reaction followed by U-phosphate precipitation. Our

previous studies demonstrate that HA reduces the soluble and chemically labile fractions of U and Ni in contaminated sediments. In this study we evaluate Ni and U toxicity to *Burkholderia vietnamiensis* PR1₃₀₁ (PR1), a constitutive TCE-degrader that has been used in both microcosm and field studies. Furthermore, we examined the efficacy of a sequestration agent (HA) for reducing co-contaminant metal toxicity and the resulting enhancement of microbial degradation of TCE.

Burkholderia vietnamiensis PR1₃₀₁ grew at 34.1 and 1.7 mM Ni at pH 5 and 7, respectively, with 0.01 g mL⁻¹ HA as compared to 17 and 0.85 mM Ni without HA. PR1 grew at 4.2 mM U at pH 5 and 7 with 0.01 g mL⁻¹ HA as compared to 1.1 mM U without HA. A similar decrease in the toxicity of Ni and U in combination was observed with HA. The ability of PR1 to degrade TCE in the presence of 0.85, 1.7, and 3.4 mM Ni and with 0.42 and 1.1 mM U was examined. The presence of TCE resulted in a decreased tolerance of PR1 to Ni and U; however, HA facilitated TCE degradation in the presence of Ni and U, effectively doubling the metal concentrations at which TCE degradation proceeded. These data suggest that metal sequestration via HA amendments may offer a feasible approach to reducing metal toxicity to microorganisms at mixed waste sites, thereby enhancing the degradation of co-contaminant organics.

Publications:

Van Nostrand et al, 2006. Reduction of Nickel and Uranium Toxicity and Enhanced Trichloroethylene Degradation to *Burkholderia vietnamiensis* PR1₃₀₁ with Hydroxylapatite Amendment. *In review*. Environmental Science & Technology.

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GOAL: Define more clearly the risks from low dose-rate, chronic exposures to radiation.

Radiation-Induced Untargeted Germline Mutations in Japanese Medaka

INVESTIGATORS: DANIEL COUGHLIN, TRAVIS GLENN, THOMAS HINTON, D. MAIN, R. PODOLSKY, OLGA TSYUSKO, AND YI YI

The overall intent of our research is to better understand the effects of chronic contaminant exposure, such as ionizing radiation, on the induction of germ-line mutations and germ-line genome instability. The objective of this project was to identify microsatellite loci with high background mutation rates, and then to use these loci to compare mutation rates between offspring of control and irradiated Medaka fish.

We identified microsatellite loci using traditional laboratory methods of constructing enriched libraries. We also searched Genbank for medaka sequences containing microsatellites. Whole-genome shotgun (WGS) sequence data of Medaka have recently become available from sequencing done in Japan. We searched the available WGS data for all possible microsatellite DNA sequences with 1-5 bp repeat units using the script CUGISSR, which searches for sequences according to user-defined criteria. We identified 70245 unique sequences with at least one microsatellite repeat. There are various types of microsatellites (from di- to penta-nucleotides) in the medaka genome, with tetra-nucleotide repeats being most abundant (45.5%) and penta-nucleotide repeats being least abundant but still plentiful (9.1%). A reasonably large number of loci were also discovered with the same core repeats that occur in the most often studied ESTR loci in mice: Hm-2 contains an (ACGG)*n* repeat and Ms6-Hm contains an (AGGGC)*n* repeat. In the Medaka genome (ACGG)*n* occurred 30 times and (AGGGC)*n* occurred 6 times.

PCR primers were designed from the sequences flanking the microsatellite region. An engineered sequence (5'-CAGTCGGGGCGTCATCA) was then added to the 5' end of one primer in each primer pair to allow use of a third primer in the PCR (with the engineered sequence) that is fluorescently labeled for detection on a DNA analyzer/sequencer. PCR amplifications were performed and 72

primer pairs were tested.

Of 72 primer pairs tested, 26 yielded scorable polymorphic loci. About 50% of tetra- and penta-nucleotide repeat loci identified from the WGS data have produced polymorphic loci of high quality. We screened the 26 candidate primer pairs among parents and 200 offspring from four control breeding pairs to identify loci with the highest mutation rates. We identified seven loci in which an average of at least 1% of the offspring had mutant (non-parental) alleles. We also added two other highly variable loci that were easily incorporated into the two multi-locus genotyping panels. We used these nine loci for genotyping Medaka progeny from irradiated parents. DNA was extracted from one-day old hatchlings and from fin clips of the parental fish using a Chelex method. A total of 188 offspring from four exposed breeding Medaka pairs were analyzed.

To determine if chronic radiation exposure can increase mutation frequencies in these 9 loci, six-week old female subadult Medaka were chronically irradiated to ¹³⁷Cs at a dose-rate of 68 mGy/day in SREL's low dose-rate irradiation facility for 45 days (total dose=3 Gy), removed from irradiation during the winter (169 days), and then bred with chronically irradiated males. Exposure of the males also began as subadults (six-weeks old) and continued through maturation to the same dose rate, but intermittently for a longer period, resulting in a total dose of 10.4 Gy. Although males were exposed intermittently, they were irradiated continuously for seven days prior to breeding. The dose regimes were dictated by another experiment, and were not designed specifically for this microsatellite analysis. Offspring were obtained from eggs collected 30 days post-irradiation to ensure that the sperm was derived from irradiated spermatogonia rather than a later stage of spermatogenesis.

Overall, 1784 parental alleles and 16 non-parental alleles (mutations) were found within the progeny (N=200) of the control Medaka families, whereas 1664 parental alleles and 28 non-parental alleles (mutations) were found within the progeny (N=188) of the irradiated families. This gives the estimates for mutation rates in control and exposed

medaka as 0.89×10^{-2} and 1.65×10^{-2} per locus per offspring, respectively.

In our study, mutations were observed at all nine loci surveyed in the offspring of exposed parents. The mean number of mutations for the offspring of exposed families was 0.149 ± 0.044 , which was significantly higher than that of the control (0.080 ± 0.028). The results were statistically significant when variation among families was not accounted for (Fisher's exact test $P = 0.03$) and are even more significant when this variation was taken into consideration ($P = 0.018$).

We estimated the number of mutational events required to obtain the increase in microsatellite mutation frequencies that we observed in hatchlings of the exposed medaka. The calculations were conducted using the highest radiation dose to conservatively determine whether this increase in mutation frequency can be due to direct interaction of the radiation with DNA in the parental medaka. We calculated that there were about 2.7×10^4 mutational events per genome. By multiplying the highest dose (10 Gy), the genome size, and an estimate of double-strand breaks (DSBs) induced by irradiation ($\sim 1 \times 10^{-8}$ DSB $\text{bp}^{-1} \text{Gy}^{-1}$), the number of DSB per genome expected from the targeted radiation effects was estimated at 85 and 37, respectively. Thus, the observed mutation rate at microsatellite loci is more than 300 to 700 times higher than that expected from DSBs induced from the radiation.

Microsatellite loci may also be affected by damage other than DSBs. Cluster damage represents another source of significant damage that is difficult for cells to repair, with DSBs accounting for 20-30% of such damage, but this still gives a relatively low number of events per cell (i.e., $85 \times 5 = 425$ vs. 2.7×10^4). The total number of single-strand breaks (SSBs) induced by radiation ($\sim 1.88 \times 10^{-7}$ SSB $\text{bp}^{-1} \text{Gy}^{-1}$) for medaka exposed to 10 Gy will be 1600. The total number of damaged sites, even including easily repaired damage, may reach ~ 1000 sites Gy^{-1} per genome for medaka. Thus, all estimates for the expected number of DSBs, SSBs, and damaged sites for medaka exposed to 10 Gy are still well below what we observe. This finding indicates that the mutations we observed were due to a combination of direct, targeted effects and indirect, non-

targeted mechanisms.

Considerable variation occurred among families and loci in the number of mutant alleles. Two loci (21 and 45) showed striking increases in mutation frequency, whereas the rest were similar in both groups, indicating that these two loci are responsible for most of the response observed. These loci are not unusual compared to the other loci regarding GC content of the repeat units. It is interesting that two of the exposed families each had four mutated alleles at locus 21 or 45, and this caused the highest mutation rates at these loci. The other two families showed a more even distribution of non-paternal alleles across loci. This finding suggests different degrees of sensitivity to radiation for different loci in different families.

The mutational spectrum differed among loci. Mutations occurred at loci 21 and 45 as a result of gain or loss of not more than 1-3 repeats whereas at three other loci (2, 35, and 58) the number of repeats deleted or added was larger (up to 10). Also, locus 45 showed only a loss of repeats. These differences in the mutational spectrum may partially explain the highest mutation rate for these two loci. However, three loci (52, 59, and 60) did not lose or gain more than two repeats but their mutation rate was lower than that for loci 21 and 45.

In conclusion, this study demonstrated that medaka have a large number of microsatellite loci and that about 10% of the loci surveyed have a high mutational background (higher than 10^{-2} per locus per gamete). Overall, the microsatellite loci responded in the direction expected from chronic radiation exposure, confirming that Medaka can serve as an efficient and general model for studying contaminant-induced mutations. The magnitude of the mutational response, however, was greater than what would be expected from direct DNA damage, and suggests that indirect, non-targeted mechanism(s) contributed significantly to the mutations. Even with relatively small sample sizes, our ability to detect significant increases in mutation rates at the microsatellite loci, as well as differential response to radiation among individual families, demonstrate the potential of microsatellites as markers for monitoring germline mutations within the Medaka model. Other fish models, such as zebrafish, are also

excellent candidates for similar research. Additional research is needed to determine the consistency of response among loci, families, and radiation exposures; as well as the extent to which genomic context determines the response of individual loci, and whether results can be generalized across the genome.

Remediation and Restoration

The knowledge and expertise based at SREL are ideally suited to address the remediation and restoration of large land areas contaminated with relatively low levels of metals, organics, and radionuclides. SREL conducts multidisciplinary research designed to assist in the development, evaluation and stakeholder acceptance of remediation and restoration efforts that protect human and ecosystem health. Fundamental to the success of various bioremediation, natural attenuation, and *in situ* remediation applications is an understanding of the underlying scientific principles on which they are based.

Report on FY06 Remediation and Restoration Milestones

GOAL: Identify the traits of native species and populations that best determine their suitability for use in remediation and restoration.

Evaluation of Native Plants Most Successful in Restoration of Carolina Bays

INVESTIGATOR: REBECCA SHARITZ

In 1998, the SRS Carolina Bay Restoration Project was initiated by SREL, the U.S. Forest Service, the U.S. Fish and Wildlife Service, and several universities. Sixteen severely degraded Carolina bays were chosen for experimental restoration, and DOE-SR will receive credits to its wetland mitigation bank for this project. After pre-treatment studies in 1998-2000, the hydrology of these bays was restored in 2000-2001 by plugging drainage ditches. Vegetation treatments included clear-cut removal of invasive woody species and planting of wetland herbaceous or forest species into the basins. It was anticipated that wetland species present as seeds in the soil (seed bank) would germinate and become established once soil, light, and moisture conditions were appropriate (passive revegetation).

Results provided some evidence that passive revegetation can be a successful restoration strategy for these wetlands. After removal of forest dominated by facultative woody species, the restored wetlands quickly developed a dense

cover of herbaceous species, roughly half of which were wetlands species. In addition, seed banks contributed many wetland species to the restored vegetation. Common wetland species were emergent sedges and forbs whose establishment is favored by shallow water or water drawdown. By the third year of post restoration, vegetation analysis revealed a significant increase in obligate and facultative wetland species and a decline in overall plant species richness. However, hydrologic recovery was slowed by unpredictable drought conditions that allowed non-wetland upland species to become established and to persist. Deep and long ponding in later years reduced vegetative cover and suppressed upland herbaceous species. Thus, the potential exists for a self-designed vegetative cover dominated by wetland plants, depending upon the final hydrologic regimes that become established in individual wetlands. The results of the first three years of this study have been published recently in *Restoration Ecology and Ecological Restoration*.

Assess Survival of Sandhills TES Plant Species Under Different Land Management Regimes

INVESTIGATOR: REBECCA SHARITZ

Sandhills that occur along the Fall Line region of the southeastern Coastal Plain contain a suite of threatened, endangered and sensitive (TES) plant species. The responses of these TES plants to habitat disturbances are not known, and forest management practices in this region have the potential to destroy populations, causing them to become even more rare. Conservation of these TES species requires an understanding of their responses to specific disturbance conditions, and their potential for transplantation from highly disturbed to less disturbed sites. The effects of disturbance associated with forest management and military training activities (as occur on numerous DoD installations along the Fall Line), are being evaluated in an experimental study leveraged by funds from the Strategic Environmental Research and Development Program (SERDP). Experimental gardens were established to mimic high disturbance (motorized vehicle use, burning or mechanical forest understory removal), low disturbance (foot traffic, foxhole digging), and no disturbance. Four perennial sandhills TES (*Baptisia lanceolata* (lance-leaf wild indigo), *Carphephorus bellidifolius* (sandywoods chaffhead), *Nolina Georgiana* (Georgia beargrass), *Stylisma pickeringii*) (Pickering's daisy) were selected for planting because their life forms are characteristic of many of the sandhills TES plants.

At the end of the first growing season, the species differed in response to the disturbance treatments. *Baptisia* and *Carphephorus* had higher survival in the most highly disturbed sites, *Nolina* survived best in the undisturbed sites, and *Stylisma* fared equally well across all treatments. All species had higher growth rates in the more open canopy areas of the highly disturbed sites. Continued sampling of these experimental populations for several more years is needed and planned for a full assessment of the individual species' survival and reproductive success across the range of disturbances. This information will be valuable to resource managers at federal installations along the Fall Line.

Studies of Plant Species Most Useful for Phytoremediation

INVESTIGATOR: LEE NEWMAN

We have completed several studies looking at native species of plants to determine those with the best suitability for both remediation and restoration purposes.

We have completed and sent out the manuscript (Journal of Environmental Quality) on the study that examined native SE deciduous trees and compared them to a remediation standard, the hybrid poplar. We found that sweet gum trees are comparable to poplar in both volumes of water/contaminant uptake and metabolic potential. The native willow species did poorly and displayed a toxic response to the trichloroethylene (TCE) not seen in the other species.

We have completed and sent out the manuscript (Journal of Environmental Quality) on the study looking at native SE coniferous trees and comparing the rapidly growing Leyland cypress to hybrid poplar. None of these trees had the water/contaminant uptake potential of the poplar, but the longleaf pine seemed to be quite competent at metabolizing the TCE.

These two studies show that native plants have equal potential for uptake and degradation of TCE. While the coniferous trees do not have the water uptake potential, they have the advantage of being active year round.

We have also studied switch cane, a native grass species used for streamside restoration and *Arundo*, a non-native that is useful for biofuel production. Both plants showed the ability to take up and metabolize TCE into expected metabolites within the leaf tissue. We are currently working on this data to get it ready for publication.

The graduate student who was working on the Pen Branch study successfully defended her graduate thesis on this work in Spring 2006. We are currently working on the data and plan to be submitting the manuscript this fall to a peer-reviewed scientific journal.

Studies of Plant Species Useful for Biodiesel Production in the Southeast

INVESTIGATOR: LEE NEWMAN

We have been growing several species of plants, including Brassica, sunflower, soybean, and castor bean using three fertilizer regimes to determine the best plant/fertilizer to produce biodiesel. We used native soil, conventional fertilizer, or biosolids for the fertilizer, and so far all plants have responded best with the biosolids. This would allow for the use of a waste product for the production of fuel. We have also determined that the species of sunflower used is important, and there is a susceptibility to fungal infections in humid climates. We are currently harvesting the majority of the plants (some Brassica will be grown as a winter crop) and determining the amount of seed produced. If possible, we will also be looking at oil production levels. We will also determine the amount of biomass produced, as this material has potential to be used for either direct power generation through burning or ethanol production.

Studies of Carbon Sequestration in Nutrient-Poor Soils

INVESTIGATOR: LEE NEWMAN

We did a preliminary study to look at how growing conditions affect carbon sequestration in soils in the form of glomalin production. Results are very preliminary, but in nutrient-poor soils such as found in the Southeast, it appears that water is the limiting factor, not nutrients as originally suspected. We will be working on the analysis of the remaining samples collected this year to determine if this holds across other species.

Studies on the Use of Native Grasses on Waste Site Closure Caps

INVESTIGATOR: BEVERLY COLLINS

Native grasses, *Andropogon virginicus* and *Sorghastrum nutans*, were grown in the greenhouse in pots with typical closure cap soil with and without fertilizer and with once- or twice-weekly watering. In both species, fertilizer and fertilizer combined with watering increased stem length

and mass and root mass. Fertilizer also increased root length of *Andropogon*. Under fertilized and watered conditions, root length of *Andropogon* and *Sorghastrum* reached 65 cm and 54 cm, respectively. The most stressed conditions (no fertilizer and once-weekly watering) produced *Andropogon* roots that averaged only 13 cm depth, but had less effect on *Sorghastrum*; even under the stressed conditions, *Sorghastrum* roots reached 44cm. These results suggest that grass roots would quickly fill rooting space on closure caps with fertilizer amendment and supplemental watering. Under passive management, *Sorghastrum* may be preferred to *Andropogon* because of the more aggressive rooting strategy.

Investigations of the Effects of Flooding on MNA of a Metal-Contaminated Site

INVESTIGATOR: BEVERLY COLLINS

Mycorrhizal colonization on roots of *Pinus taeda* (loblolly pine) and *Andropogon virginicus* (broomsedge grass) was surveyed along the slope at the A-01 wetland along Tims Branch. Samples were taken at the wetland edge (0 m), 10 m, 30 m, 50 m, 70 m, 90 m, and 110 m. Mycorrhizae colonization was quantified using the gridline intersect method on 5 cm root segments. The percentage of colonized roots was calculated from the sum of the horizontal and vertical intersections with colonization divided by the sum of the horizontal and vertical intersections without colonization. Mycorrhizal colonization (%) differed significantly with elevation for both *Andropogon* ($p < 0.0001$) and *Pinus* ($p < 0.0001$). For both species, colonization was greater near the wetland and decreased upslope. Phosphatase activity could not be determined for the samples, but the colonization results suggest mycorrhizae are more abundant and the potential for phosphatase-mediated effects on metals in the rhizosphere are greater in the moist soils near the wetland.



GOAL: Determine the primary mechanisms by which natural attenuation and engineered remediation processes immobilize contaminants, and identify the appropriate geochemical and biological endpoints to assess sustainability.

Effectiveness of Hydroxyl Apatite Amendments on TCE Degradation by Microbes

INVESTIGATOR: PAUL BERTSCH

This research is described on page 29, as part of the following project: “Assess the influence of nickel and uranium on TCE degradation by bacteria.”

Analyses of Field-Scale Tracer Data from H-Area Subsurface Injection Experiments

INVESTIGATOR: JOHN SEAMAN

Hydrodynamic dispersion, the combined effects of chemical diffusion and differences in solute path length and flow velocity, is an important factor controlling contaminant migration in the subsurface environment. However, few comprehensive three-dimensional data sets exist for critically evaluating the impact of travel distance and site heterogeneity on solute dispersion. Therefore, a series of field-scale experiments using tritiated water ($^3\text{H}_2\text{O}$), bromide (Br^-), and two fluorobenzoates (2,4 and 2,6 Di-FBA) as tracers was conducted in the water-table aquifer on the U.S. Department of Energy’s Savannah River Site (Seaman et al., 2006). Longitudinal dispersivity and travel times for $^3\text{H}_2\text{O}$ breakthrough were estimated by fitting the field data to analytical approximations of the advection-dispersion equation (ADE), one derived for 1-D uniform flow and the second for diverging radial flow. Dispersivity varied greatly between wells located at similar transport distances and even between zones within a given well. The radial flow equation generally described tritium breakthrough better than the uniform flow solution, as indicated by the coefficient of determination, r^2 , yielding lower dispersivity while accounting for breakthrough tailing inherent to radial flow conditions. Complex multiple-peak breakthrough patterns observed within certain sampling zones were replicated in subsequent tracer experiments, and a strong correlation was observed between dispersivity and arrival times observed from one experiment to the next, indicative of the general reproducibility of the tracer

results. Temporal moment analysis was used to evaluate tracer migration rate as an indicator of variations in hydraulic conductivity and flow velocity, as well as mass recovery and retardation for the ionic solutes compared to $^3\text{H}_2\text{O}$. Retardation factors for Br^- ranged from 0.99 to 1.67 with no clear trend with respect to transport distance; however, Br^- mass recovery decreased with distance, suggesting that the retardation data are biased in terms of early arrival because of limited detection and an insufficient monitoring duration. Similar results were observed for the FBA tracers. Despite tracer retardation and incomplete mass recovery, both ADE models were able to reasonably describe the anion data without accounting for sorption reactions, indicating that chemical interactions with the geologic matrix may be interpreted in terms of a physical transport process, i.e., flow velocity, path length, pore connectivity, multiple flow domains, dispersivity, etc.

Publications:

Seaman, J.C., M. Wilson, P.M. Bertsch, J. Singer, F. Majs, and S.A. Aburime. 2006. Tracer migration in a radial flow field: Longitudinal dispersivity and anionic tracer retardation. *Vadose Zone Journal*. *In review*.

Research Support Programs

Several SREL programs provide critical support to the research, outreach, and education missions of the Laboratory. These support programs include:

- Environmental Health and Safety Program
- Quality Assurance Program
- Research Data Archive Activities
- SREL Undergraduate and Graduate Education Programs
- Environmental Outreach Program
- DOE Research Set-Aside Areas

Environmental Health & Safety Program

DONALD R. MOSSER, EH&S MANAGER

The Savannah River Ecology Laboratory (SREL) continues to operate successfully under the work-smart safety and environmental standards that resulted from SREL's participation in U.S. Department of Energy's (DOE) Necessary and Sufficient process. These standards continue to address the hazards associated with SREL operations by permitting a focused effort on the health and safety issues most pertinent to SREL operations. SREL supports and promotes an integrated approach to SRS environmental health and safety issues as a signatory to the SRS Workplace Safety, Health and Security Policy and the SRS Environmental Management System Policy Statement.

SREL maintains a commitment of one full-time position (SREL EH&S Manager) dedicated to the support of the SREL EH&S Program. Approximately 14 laboratory research technicians also provide support to the SREL EH&S Program by serving as laboratory Chemical Coordinators. Chemical Coordinators are responsible for maintaining chemical inventory information and providing support in the identification, accumulation, and storage of hazardous wastes. In addition, SREL maintains a Health and Safety Committee designated to meet quarterly to evaluate safety program performance and make safety program and policy recommendations to the SREL EH&S Manager and the SREL Director.

In an effort to increase the efficiency and effectiveness of the SREL EH&S Program, an emphasis continues to be placed on safety and environmental training of SREL personnel. All new SREL personnel receive a two-hour SREL specific orientation on the topic of SREL safety and environmental programs, policies, and procedures in addition to the SRS required General Employee Training (GET). New SREL personnel also receive job specific safety training provided for by their SREL supervisor. Approximately 24 new SREL personnel received this required training during FY2006. Additionally, SREL personnel received EH&S related training during FY2006 in the following functional areas as their job tasks required:

- Chemical Coordinator Training – chemical inventories

and hazardous waste generation and management

- Radiological Training – Radiological Worker Training, Radioactive Sealed Source User Training, and Radiation Generating Device training

SREL's internal computer network was used to provide targeted safety information to specific groups in the laboratory. Approximately 29 targeted Lessons Learned bulletins were distributed SREL personnel during FY2006. Additionally, relevant SRS environmental health and safety information and bulletins distributed the SRS e-mail system were reviewed and forwarded to SREL personnel as required. The SREL EH&S Manager functions as an interface with other SRS organizations in receiving and distributing applicable Lessons Learned information. By integrating with other SRS organizations to share Lessons Learned information, SREL takes advantage of the collective experience and improvements identified by other organizations for similar work processes and controls at SREL.

The SREL EH&S Manager reviewed and approved approximately 242 hazardous chemical procurement requests for hazard potential identification and waste minimization efforts. Waste minimization and chemical disposal issues continue to be emphasized to increase efficiency and cost effectiveness. Waste minimization techniques such as source reduction and bench-top treatment continue to be incorporated into experimental protocols, reducing the burden associated with waste disposal procedures while supporting SREL's pollution prevention efforts. In cooperation with WSRC Solid Waste Division, SREL successfully shipped approximately 726 pounds total of excess hazardous laboratory chemicals and laboratory hazardous wastes to a hazardous waste disposal firm (November 2005, May 2006). Although this represents an increase over FY2005 total hazardous waste generated, the increase is attributed to the generation of waste chemical stocks due to the discontinuation of several SREL research programs at the end of FY2005.

SREL workers reported four recordable work related injuries/illnesses during FY2006. All injuries/illnesses were reported immediately to the designated DOE-SR oversight personnel and subsequently evaluated by the SREL EH&S

Manager and the SREL Health and Safety Committee for required corrective actions. None of the injuries in FY2006 resulted in lost work days or restricted work days, but were recordable due to medical treatment received by the injured employees. A common causal factor among all the recordable injuries/illnesses was employee behavior related to exceeding personal performance abilities, i.e., employees were attempting to perform too much work within the available time for the job. SREL distributed a lessons learned document to personnel on August 14, 2006 which addressed and highlighted the issues of recognizing personal performance limitations, adequate work planning and scheduling, and prompt work cessation upon acknowledgement of an initial injury such as in muscle, back, limb, or joint injuries. SREL continues to be vigilant in its conduct of work and in promoting a safe working environment in order to prevent work related injuries and illnesses.

SREL conducted assessments in the areas of chemical and radiological air emissions, community right-to-know, and the Georgia Right-to-Know law in compliance with state and federal requirements. SREL also participated in the SRS's annual, comprehensive review and declaration process for Integrated Safety Management Systems (ISMS). As part of the annual ISMS declaration, SREL developed an Integrated Safety Management System Description Document and identified its annual safety goals and areas for safety performance improvements. SREL received no Notices of Violation in FY2006 as the result of external or internal reviews, inspections, or assessments.

Quality Assurance Program

LAURA JANECEK

SREL has continued to maintain a formal, U.S. Department of Energy (DOE)-approved Quality Assurance (QA) program. The program is devoted to assuring the continuing quality of SREL research. These SREL "Good Research Practices" highlight research concepts and context, research logistics, and the conduct of research and are available to all SREL personnel on the Lab's intranet web site. All new Laboratory research personnel are

required to familiarize themselves with this material prior to beginning work at SREL.

Research Data Archive Activities

LAURA JANECEK AND DEBBIE REESE

Responsible management of research data holdings plays an important role in preserving the SREL's corporate memory. Since 1989, SREL has been actively building a centralized repository of research data files and the associated "metadata" necessary to make these data fully accessible. The goals of SREL's Research Data Archive activity are to avoid the inadvertent loss of data and to use advanced electronic computer/communication technology, including the use of computer networks and the Internet, to provide access to important data as efficiently as possible. Inclusion of new and historical research information into the SREL data archives continued during FY06 and the Central Archive Data Repository now has information covering over 515 separate studies.

The web-based SREL data archive system that allows users to upload metadata information and actual data files directly from their office desktop computers continued to work well during FY06. Anyone at SREL or on the SRS can search for data using this new web-based system, however decisions about releasing original data to third parties are retained by the principal investigators.

SREL Undergraduate & Graduate Education Program

TRAVIS GLENN

The objectives of the SREL Education Program are to (1) recruit and develop additional professionals to the environmental sciences, with an emphasis on recruitment from under-represented minority groups, and (2) enhance environmental awareness and research opportunities among undergraduate and graduate students with emphasis on conducting ecological research important to the Savannah River Site mission. Undergraduate and

graduate student participants in FY06 are listed in Table 1 (page 42).

The SREL Education Program has a long history of training undergraduate students. Undergraduate students from more than 275 different colleges and universities have co-authored more than 150 peer reviewed research publications and more than 200 of these students have gone on to pursue careers in science. Recent downsizing has resulted in a significant reduction in the number of students sponsored relative to previous years. The Undergraduate-Research Experience for Undergraduates, funded by the National Science Foundation, sponsored six students this past year. In addition, we sponsored one student funded by SREL and two NNSA-sponsored summer interns from Morris College, Sumter, SC.

SREL also has a long history of funding graduate students. Since 1967, an average of six students per year have completed graduate studies at SREL, resulting in a total of more than 325 dissertations and theses. During FY06, eight students completed their degree requirements (six M.S. and two Ph.D.). Since 1985, our graduate students have won over 200 awards from regional, national, and international competitions at numerous professional societies and foundations. During the past year, SREL graduate students continued to compete successfully for various national and regional awards. Some of these are listed in the section on Special Accomplishments (page 5).

SREL Graduate Students Completing Degree Requirements in FY06:

<u>Student</u>		<u>University</u>		<u>Faculty Advisor</u>
Alcides Cintra	Ph.D	Universidade Estadual Paulista, Brazil	2005	Domy C. Adriano
Sarah DuRant	M.S.	University of Georgia	2005	William A. Hopkins
Gabrielle Graeter	M.S.	University of Georgia	2005	J. Whitfield Gibbons
Ryan Holem	M.S.	University of Georgia	2005	William A. Hopkins
Catherine King	M.S.	University of Georgia	2005	J Vaun McArthur
Amy Squire	M.S.	University of Georgia	2005	Rebecca R. Sharitz
Dean Croshaw	M.S.	University of New Orleans	2006	Travis C. Glenn
Joy Van Nostrand	Ph.D.	Medical University of SC, Charleston	2006	Paul M. Bertsch

Table 1A. SREL Undergraduate Student Program Participants

<u>Student</u>	<u>Academic Institution</u>	<u>Faculty Advisor</u>
Samary Amaro-Garcia	University of Puerto Rico, Mayaguez	Andrew L. Neal
Gillian Connolly	Earlham College, IN	Lee A. Newman
Andrew Durso	University of Georgia, Athens	J. Whitfield Gibbons
Heidi Hatcher	Appalachian State University, NC	J Vaun McArthur
Rebecca Maska	Bradley University, IL	Lee A. Newman
Bradley Temple	University of South Carolina, Aiken	Lee A. Newman
John Vaughn	Morris College, Sumter, SC	John Seaman
Sarah Wallace	Hartwick College, NY	Thomas G. Hinton & Andrew L. Neal
Jerrica Washington	Morris College, Sumter, SC	John Seaman

Table 1B. SREL Graduate Student Program Participants

<u>Student</u>	<u>Degree</u>	<u>Institution</u>	<u>Faculty Advisor</u>
Kimberly Andrews	Ph.D.	University of Georgia, Athens	J. Whitfield Gibbons
Ellen Breazel	M.S.	University of Georgia, Athens	Machelle D. Wilson
Elizabeth Burgess	Ph.D.	University of Georgia, Athens	Andrew L. Neal
Monica Carroll	Ph.D.	University of Georgia, Athens	Christopher S. Romanek
Alcides Cintra	Ph.D.	Universidade Estadual Paulista, Brazil	Domy C. Adriano
Dana Cook	Ph.D.	University of Georgia, Athens	Andrew L. Neal
Dean Croshaw	M.S.	University of New Orleans	Travis C. Glenn
Jaclin DuRant	M.S.	University of Georgia, Athens	Rebecca R. Sharitz
Sarah DuRant	M.S.	University of Georgia, Athens	William A. Hopkins
William Duval	Ph.D.	University of Georgia, Athens	Rebecca R. Sharitz
Carol Flaute	M.S.	University of Georgia, Athens	Steven J. Harper
Gabrielle Graeter	M.S.	University of Georgia, Athens	J. Whitfield Gibbons
Aaliyah Green	M.S.	University of Georgia, Athens	Charles H. Jagoe
Ryan Holem	M.S.	University of Georgia, Athens	William A. Hopkins
Cori-Alice Holladay	M.S.	Appalachian State University, NC	Beverly S. Collins
Glenn Kirkland	M.S.	University of Georgia, Athens	I Lehr Brisbin
Linda Lee	M.S.	University of Georgia, Athens	Rebecca R. Sharitz
Thomas Luhring	M.S.	University of Georgia, Athens	J. Whitfield Gibbons
Frantisek Majcs	Ph.D.	University of Georgia, Athens	John C. Seaman
Steven Schaff	Ph.D.	University of Georgia, Athens	Kenneth W. McLeod
Julian Singer	Ph.D.	University of Georgia, Athens	John C. Seaman
Amy Squire	M.S.	University of Georgia, Athens	Rebecca R. Sharitz
Karolina Stark	Ph.D.	University of Stockholm, Sweden	Thomas G. Hinton
Steven Stoddard	Ph.D.	University of Illinois	Rebecca R. Sharitz
Brian Todd	Ph.D.	University of Georgia, Athens	J. Whitfield Gibbons
Joy Van Nostrand	Ph.D.	Medical University of South Carolina	Paul M. Bertsch
Qin Wang	Ph.D.	University of Georgia, Athens	Machelle D. Wilson
Arlena Wartell	Ph.D.	University of Georgia, Athens	Travis C. Glenn
Jamie Williams	M.S.	University of Georgia, Athens	Barbara E. Taylor
John Willson	Ph.D.	University of Georgia, Athens	J. Whitfield Gibbons
Christopher Winne	Ph.D.	University of Georgia, Athens	J. Whitfield Gibbons
Meredith Wright	Ph.D.	University of Georgia, Athens	J Vaun McArthur
Qi Ye	Ph.D.	University of Georgia, Athens	Chuanlun Zhang
Weidong Zhao	Ph.D.	University of Georgia, Athens	Chuanlun Zhang

Environmental Outreach

J. WHITFIELD GIBBONS

GOAL: Maintain public outreach and communication programs to enhance the public's understanding of environmental issues affecting the SRS and to increase general ecological awareness.

The Savannah River Ecology Laboratory (SREL) Outreach Program uses information from SREL research efforts to educate the public locally, regionally, and nationally. The Outreach Program is designed to enhance SREL's overall mission of acquiring and communicating environmental knowledge and highlight the U.S. Department of Energy's (DOE) focus on environmental issues. Issues as diverse as amphibian and reptile population declines, potential responses of organisms to contamination, distribution and abundance of sensitive species, monitored natural attenuation, and dispersal of organisms from radioactively or chemically contaminated sites all are important beyond SREL.

By the end of FY05 the Outreach Program lost five employees (3.5 internally funded and 1.5 externally funded) due to budget reductions and program termination. Consequently, the Outreach Program efforts were necessarily reduced in FY06.

Public education during FY06, especially for K-12 audiences, was accomplished through a variety of programs and materials.

- During the past 15 months SREL scheduled 219 talks, 23 tours, 22 exhibits, and 34 workshops, reaching a total of 37,523 people. Topics for these presentations included reptiles, amphibians, southeastern plants and habitats, long-term research, safety, biodiversity, local wetlands and watersheds, conservation, and careers in ecology and research. Presentation numbers for talks and workshops declined from the previous year by 30% and 66% respectively due to the reduction in staff and the elimination of funding from the American Honda Foundation.
- Student groups from 24 schools enjoyed field trips to the Laboratory's Conference Center to participate in

the Ecologist-for-a-Day program.

- Twelve Aiken County teachers participated in a 2-day workshop on southeastern habitats, in which they were trained in methods of teaching ecology and were provided with materials produced by the Outreach staff.

Outreach programs include: *Ecotalk*, an opportunity for students to have nature brought into their classroom for a face-to-face lesson on a variety of live animals found in local habitats; *Ecologist for a Day* visits allow students to spend the day in the field gaining hands-on knowledge of the plants and animals of the unique Upper Three Runs Creek area; civic group presentations; and ecological tours. All school programs incorporate science standards and curriculum for particular school districts. In many of these programs participants get an opportunity to work with SREL staff catching, marking, and measuring various species of reptiles, amphibians, small mammals, and invertebrates. In addition, the Outreach Program offers tours of SREL facilities, as well as exhibits and workshops for the general public.

SREL hopes to continue its "research-to-classroom" hands-on science program for elementary school students, which was developed over three years with funding from the American Honda Foundation (AHF) and The Christensen Fund. Three proposals were written and submitted as follow-ups to the program ("Improving teaching skills through an emphasis on local habitats" to the Environmental Protection Agency, \$73,438; "Upper Three Runs Creek-Community Education about the World's Most Biodiverse Stream" to the Environmental Protection Agency Region IV, \$46,431; "Improving teaching skills through an emphasis on hands-on inquiry-based science" to the AHF; \$22,127). Although none have been funded at this time, the Outreach Program continues to seek additional money to help offset the funding cuts in FY06.

During the past year SREL received funding from the Five-Star Restoration Matching Grants Program administered by the National Fish and Wildlife Foundation (NFWF) and the U.S. Environmental Protection Agency (EPA). The grant, entitled "Watershed Restoration and Education in Aiken County, South Carolina," will focus on restoring habitat in

an Aiken County Open Land Trust (ACOLT) parcel. Community volunteers began removing invasive plant species at the project site in April, and will plant native species in fall 2006. Watershed education includes classroom presentations at local schools, regional teacher workshops, and forums designed for landowners and businesses that have the highest potential to impact local streams. Project partners with SREL include the city of Aiken Planning Department, Aiken County Planning Department, AICOLT, Aiken Sunrise Rotary Club, and Millbrook Elementary School. An Outreach tri-fold brochure to be produced as part of this grant will describe the watersheds of Aiken County and highlight will the rich biodiversity of the Upper Three Runs Creek system.

Websites associated with SREL's Outreach program continue to be popular. The main SREL Outreach site (<http://www.uga.edu/srel/outreach.htm>) receives numerous hits, as it has links to the popular *Ecologist for a Day* program, Outreach fact sheets and products, and the Ecoviews newspaper column. SREL also continues the website for *Kids Do Science* (www.kidsdoscience.org) that provides all the necessary materials for 10 hands-on activities developed as part of the hands-on science program with the AHE. This site is frequented by teachers from throughout the country who use the materials in their own classes. Also, in August 2006 SREL reinstated the popular SPARC (Student Partners in Amphibian and Reptile Conservation) website, which had been created for and part of the national PARC website until its redesign in 2004. The SPARC "virtual walk of the Southeast" is expected to become popular again once it is "rediscovered" by the major search engines.

SREL is a founding member of the Central Savannah River Area Environmental Science Education Cooperative (CSRA ESEC), which sponsors an annual EcoMeet as well as other science-related programs throughout the year. This year's EcoMeet had 125 students from regional middle schools participating in an ecological science-bowl at the Silver Bluff Audubon Sanctuary.

SREL Outreach members and faculty have also provided expertise to the Aiken County Watershed Alliance (ACWA), the watershed group associated with AICOLT and a partner in the NFWF grant. Additionally, SREL continues to support

the Central Savannah River Area Regional Science and Engineering Fair, Inc., an organization that serves an 18-county area of South Carolina and Georgia. Five SREL faculty members assisted by judging local/regional science fairs during the past year.

SREL distributes thousands of copies of educational products and materials nationwide to schools, organizations, and the general public. Educational materials include two six-foot-long full-color posters describing the importance of wetlands to reptiles and amphibians, along with teachers' guides. The full-color brochure *Snakes of Georgia and South Carolina* (currently in its fifth printing) has proved to be an extremely successful educational product that reflects positively on DOE and the SRS. The book has been placed at no charge in every public library in Georgia and South Carolina and is also widely distributed at no cost to hospital emergency rooms, veterinary clinics, ambulance services, classrooms, scout leaders, and to various other organizations such as the Boys and Girls Clubs in Aiken and Augusta. Articles referencing the book have appeared in numerous newspapers and magazines including publications in Florida and Texas.

The Outreach Program also continued to distribute educational materials including fliers on *Carnivorous Plants and Their Habitats*; *An Amphibian's Eye View of Wetlands*; and *Is it a Water Moccasin?*; a children's comic book entitled *Stepping into Ecology: the Ecological Adventures of Mud E. Boot*; a sticker on *Chemistry – it's all about the nature of things*, and the *Metric System Rap* bookmark, as well as the numerous fact sheets available through the website. All of these products have been extremely popular and thousands of copies have been distributed during the past year. Previously created full-color fact sheets and research "snapshots" on a wide variety of research topics were distributed as well.

Funding cuts in 2005-06 resulted in the loss of SREL's Public Relations Coordinator position (i.e., the person responsible for distribution of news releases on a variety of topics to selected media affiliates, officials of DOE, and The University of Georgia). Although we no longer submit press releases to media outlets, the Outreach Program continues

to respond to inquiries from the press, directing reporters to the most appropriate researchers for their stories. In addition, SREL encourages researchers to initiate press contacts and submit research information to appropriate audiences. In 2005-06 SREL researchers provided information to such diverse outlets as the *BBC*, *the Orangeburg Times and Democrat*, *USGA's Wildlife Links* magazine, and a Denver police forensics laboratory, as well as local news outlets in the Southeast such as *The Alpharetta Revue and News*, *The Aiken Standard*, *The Atlanta Journal-Constitution*, *The Augusta Chronicle*, *Bluffton Today*, *The Athens Banner-Herald*, *The Brunswick News*, *The State*, and *The Times Standard*. Topics in the news included: animal behavior, SREL research specialties such as stable isotopes and 'extremophile' work, environmental analysis using high-tech instrumentation, environmental impacts related to contaminants, and SREL researcher profiles.

DOE Research Set-Aside Areas

CHARLES E. DAVIS

As a National Environmental Research Park (NERP), the SRS currently has 30 Areas (14,100 acres/5,706 ha) set aside for long term ecological research. These areas are located in 43 of the Site's 89 timber resource compartments and have approximately 270 miles (435 km) of posted boundary line. SREL has custodial responsibility of these areas and manages them in cooperation with the USFS-SR. Due to funding cuts in FY06, SREL's support for the Set-Aside Program has been reduced significantly. Implementation of management treatments in those Set-Asides that have an approved Site-Use Permit continued.

Management of the Set-Aside Areas

During the first quarter of FY06, SREL continued work on management plans for the Craig Pond/Sarracenia Bay Set-Aside (Area No. 17), UGA Old Lab Site (Area No. 2), and Ginger's Bay Set-Aside (Area No. 19). Pine beetle infestation in an ice damaged stand slated for regeneration and conversion to a longleaf/wiregrass community in the Flamingo Bay Set-Aside was recommended for harvest in

the winter of 2007. The thinning treatment proposed in the Ginger's Bay Set-Aside was cancelled due to predicted unacceptable logging damage and lack of future coordination. The first thinning treatment in the Dry Bay Set-Aside was revisited for future coordination needs. In the Rainbow Bay Set-Aside, researchers decided to leave Site legacy culvert piping in place to provide continued habitat for organisms.

The additional acreage that was proposed to be added to the Oak Hickory Forest (Area No. 12) was approved this FY and 80 acres (32.4 ha) of upland hardwoods and isolated wetland communities will be posted for inclusion into the Set-Aside Program. It was suspected that the Beech Hardwood Set-Aside (Area No. 6) is being impacted by lead (Pb) contaminants from an upslope 1950s pistol range (re: ECODS-G-5) and monitoring was initiated by WSRC; to date SREL has been unable to obtain these results. Prescribed winter season burning coordination continued between SREL and the USFS-SR to reduce potential impacts to Set-Asides.

Research in Set-Aside Areas

Set-Asides continued to be used by researchers this FY both as research study sites and as reference sites for collections of uncontaminated plants, animals, soils, or water. Twenty three publications were published this year with study sites that included Set-Asides. Many publications continue to be devoted to research in depressional wetland Set-Asides. A number of publications used Set-Aside areas as controls when evaluating ecological risks, contaminant transport, and site remediation.

Externally Funded Grants

PI	Domy Adriano
Project Title	SGER: Regulation of Metal Bioavailability in Floodplain Continuum by Carbon and Sulfur Cycling
Funding Agency	National Science Foundation
Budget	\$99,906
Period	August 1, 2003–July 31, 2005
PI	Paul M. Bertsch
Project Title	Linking Chemical Speciation, Desorption Kinetics, and Bioavailability of U and Ni in Aged-Contaminated Sediments: A Scientific Basis for Natural Attenuation and Risk Assessment
Funding Agency	US Department of Energy/Office of Science EMSP
Budget	\$239,000
Period	February 1, 2005–July 31, 2006
PI	Paul M. Bertsch
Project Title	Tidal creek materials loading for the SC-GA LU-CES Program
Funding Agency	SC Sea Grant Consortium
Budget	\$60,514
Period	July 1, 2004–June 30, 2006
PI	Paul M. Bertsch
Project Title	Bioavailability, toxicity and trophic transfer of manufactured ZnO nanoparticles: A view from the bottom
Funding Agency	US Environmental Protection Agency
Budget	\$363,680
Period	July 1, 2005–September 30, 2008
PI	Paul M. Bertsch
Project Title	Watershed restoration and education in Aiken County, South Carolina
Funding Agency	National Fish and Wildlife Foundation
Budget	\$15,000
Period	October 1, 2005–September 30, 2007
PI	I. Lehr Brisbin, Jr.
Project Title	Ecological Studies of Birds in the Vicinity of the Augusta Regional Airport at Bush Field and the Messerly Wastewater Treatment Plant
Funding Agency	The Augusta-Richmond County Consolidated Government
Budget	\$154,915
Period	October 1, 2003–September 30, 2005
PI	I. Lehr Brisbin, Jr.
Project Title	Ecological Studies of Birds in the Vicinity of the Augusta Regional Airport at Bush Field and the Messerly Wastewater Treatment Plant
Funding Agency	The Augusta-Richmond County Consolidated Government
Budget	\$95,834
Period	October 1, 2005–December 30, 2006
PI	I. Lehr Brisbin, Jr.
Project Title	Mercury Studies on Wading Birds on the Georgia Coast
Funding Agency	Savannah Presbytery MK Pentecost Fund
Budget	\$15,938
Period	July 1, 2004–December 31, 2006

PI A. Lawrence Bryan, Jr.
 Project Title Determination of Use of Aquatic Roadside Habitats for Foraging by Endangered Wood Storks (*Mycteria Americana*)
 Funding Agency Georgia Department of Transportation
 Budget \$24,964
 Period April 18, 2005–April 17, 2006

PI A. Lawrence Bryan, Jr.
 Project Title Determination of Wood Stork Breeding Success in Georgia in 2004
 Funding Agency US Fish and Wildlife Service
 Budget \$55,164
 Period April 1, 2004–September 30, 2009

PI A. Lawrence Bryan, Jr.
 Project Title Determination of Core Foraging Areas for Wood Stork Nesting Colonies in Northern Florida
 Funding Agency US Fish and Wildlife Service
 Budget \$30,000
 Period March 1, 2006–September 30, 2009

PI A. Lawrence Bryan, Jr.
 Project Title Determination of Wood Stork Colony Breeding Success on Kings Bay Naval Submarine Base
 Funding Agency US Fish and Wildlife Service
 Budget \$8,414
 Period March 1, 2006–September 30, 2009

PI Kurt Buhlmann
 Project Title GIS Mapping Project with Turtles
 Funding Agency World Wildlife Fund
 Budget \$2,000
 Period January 1, 2006–May 30, 2006

PI Beverly Collins
 Project Title On-site Field Study Coordination, Data Acquisition, Data Analysis, Monitoring Support, and Technology Integration Assistance in Support of Continuing Ecological Studies at Ft. Benning, Georgia
 Funding Agency US Department of Defense
 Budget \$181,462
 Period June 1, 2005–August 15, 2006

PI J. Whitfield Gibbons
 Project Title Development of Habitat Guidelines for Herpetofauna
 Funding Agency USDA Forest Service
 Budget \$53,400 in FY04
 Period corresponds to Cooperative Agreement (expires September 30, 2005)

PI J. Whitfield Gibbons
 Project Title Cooperative Agreement: The Inventory Report for the Southeast Coastal Network
 Funding Agency USDI National Park Service
 Budget total award of \$262,770
 Period April 1, 2001–February 24, 2006

PI J. Whitfield Gibbons
 Project Title Collaborative Research: Land-use Practices and Persistence of Amphibian Populations
 Funding Agency National Science Foundation
 Budget \$230,164
 Period May 15, 2003–April 30, 2008

PI J. Whitfield Gibbons
 Project Title Website Maintenance and Scientific Oversight of the North American Reporting Center for Amphibian Malformations (NARCAM)
 Funding Agency US Department of Interior (USGS)
 Budget \$60,023
 Period April 1, 2006–March 31, 2007

PI J. Whitfield Gibbons
 Project Title Turtle Management on National Wildlife Refuges
 Funding Agency US Department of Interior
 Budget \$60,000
 Period March 1, 2004–December 15, 2005

PI J. Whitfield Gibbons
 Project Title Synthesis Report and Scientific Literature Review on Impacts of Roads on Reptiles and Amphibians
 Funding Agency US Department of Transportation
 Budget \$45,000
 Period September 10, 2004–August 31, 2006

PI J. Whitfield Gibbons
 Project Title Incidence of Chytrid Fungal Parasitism in Southeastern National Parks
 Funding Agency US Department of Interior
 Budget \$15,300
 Period September 13, 2004–September 30, 2006

PI J. Whitfield Gibbons
 Project Title Support of the PARC Website
 Funding Agency National Fish and Wildlife Foundation
 Budget \$3,243
 Period April 1, 2005–June 30, 2006

PI J. Whitfield Gibbons
 Project Title Feasibility of Translocation as a Management Tool for Diamondback Rattlesnakes
 Funding Agency SC Department of Natural Resources
 Budget \$32,000
 Period January 1, 2006–September 30, 2006

PI J. Whitfield Gibbons
 Project Title Ecology of the Gopher Tortoise (*Gopherus polyphemus*) in South Carolina
 Funding Agency SC Department of Natural Resources
 Budget \$9,800
 Period January 1, 2006–September 30, 2006

PI J. Whitfield Gibbons
 Project Title Wildlife Highway Crossing Study: Investigation of the Use of Highway Culverts by Wildlife—Preconstruction Phase (Highway Widening)
 Funding Agency US Department of Interior (USGS)
 Budget \$53,556
 Period February 17, 2006–September 30, 2006

PI J. Whitfield Gibbons
 Project Title Incidence of Chytrid Fungal Parasitism in SECN Parks II
 Funding Agency CESU-Piedmont/National Parks Service
 Budget \$31,243

Period	May 1, 2006–December 31, 2007
PI	J. Whitfield Gibbons
Project Title	Modeling Extinction Risk of Native and Translocated Gopher Tortoise Populations: Developing a Decision Tree for Managing “At Risk” Populations
Funding Agency	Dept. of the Army
Budget	\$43,699
Period	April 27, 2006–July 31, 2007
PI	Travis Glenn
Project Title	DNA Research to Support Management of American Alligators in Louisiana
Funding Agency	Louisiana Department of Wildlife and Fisheries
Budget	\$30,000
Period	September 1, 2003–June 30, 2006
PI	Travis Glenn
Project Title	Development of <i>Peromyscus</i> genomics
Funding Agency	University of South Carolina
Budget	\$251,153
Period	August 1, 2004–July 31, 2006
PI	Thomas Hinton
Project Title	Transgenerational Effects of Chronic Low-Dose Irradiation in a Medaka Fish Model System
Funding Agency	Department of Energy Low Dose Program
Budget	\$192,006 for SREL to date
Period	July 1, 2005–June 30, 2008
PI	Charles H. Jagoe
Project Title	Environment Cooperative Science Center: Regional Studies in Sustainable Management of Coastal and Marine Habitats for Decision Making
Funding Agency	South Carolina State University
Budget	\$172,444
Period	January 1, 2001–September 30, 2005
PI	Charles H. Jagoe
Project Title	REU-Effects of Energy Technologies on Environmental Systems
Funding Agency	National Science Foundation
Budget	\$175,400
Period	May 1, 2005–April 30, 2007
PI	Charles H. Jagoe
Project Title	Maternal Transfer of Mercury in Carolina Diamondback Terrapins
Funding Agency	US Department of Commerce/NOAA
Budget	\$20,000
Period	June 1, 2006–May 31, 2007
PI	J Vaun McArthur
Project Title	Fellowship for Meredith Wright: Transfer and Transport of Antibiotic Reistance
Funding Agency	US Environmental Protection Agency
Budget	\$19,859
Period	August 13, 2004–August 13, 2007
PI	Andrew Neal
Project Title	Molecular Mechanisms of Bacterial Attachment to Fe(III)-Oxide Surfaces

Funding Agency	US Department of Energy
Budget	\$453,295
Period	June 1, 2005–September 14, 2007
PI	Christopher S. Romanek
Project Title	Collaborative Research: Holocene Shell Accumulation from the Southeast Brazilian Bight: Multi-Centennial Dynamics of Oceanographic, Environmental, and Ecological Changes
Funding Agency	National Science Foundation
Budget	\$94,776
Period	July 1, 2006–June 30, 2009
PI	Betsy Rothermel
Project Title	Assessing the Susceptibility of North American Plethodontid Salamanders to Chytrid Fungal Infection
Funding Agency	World Conservation Union
Budget	\$2,000
Period	April 1, 2006–March 31, 2007
PI	John C. Seaman
Project Title	Tritium Distribution, Mixing, and Transport at the Tritiated Water Management Facility
Funding Agency	USDA Forest Service
Budget	\$88,160
Period	April 15, 2003–December 31, 2006
PI	John C. Seaman
Project Title	Application of Surface Complexation Models to Predicting Actinide Fate and Transport in Variably Saturated Systems
Funding Agency	Clark Atlanta University
Budget	\$20,193
Period	October 1, 2005–September 30, 2006
PI	Rebecca Sharitz
Project Title	Impacts of Military Training and Land Management in Threatened and Endangered Species in the Southeastern Fall Line/Sandhills Community
Funding Agency	Strategic Environmental Research and Development Program (SERDP)
Budget	\$939,523 total (\$41,404 in FY06)
Period	2002–2006
PI	Rebecca R. Sharitz
Project Title	Effects of Altered Flows in the Congaree River on the Floodplain of the Congaree Swamp National Monument
Funding Agency	Cooperative Ecosystem Study Unit-Piedmont
Budget	\$50,005
Period	September 10, 2003–September 30, 2006
PI	Rebecca R. Sharitz
Project Title	Vegetation Establishment Success in Restored Carolina Bay Depressions on the SRS
Funding Agency	USDA Forest Service
Budget	\$35,500
Period	September 1, 2003–May 31, 2006
PI	Rebecca R. Sharitz
Project Title	Determine the Long-term Effects of Wind Disturbance on the Old-growth Forests and Lianas of the Congaree National Park
Funding Agency	Cooperative Ecosystem Study Unit-Piedmont
Budget	\$49,491
Period	October 1, 2005–September 30, 2007

PI	Rebecca R. Sharitz
Project Title	Proposed Floodplain Plant and Invertebrate Studies for the Savannah River Flows Project (2005-2007)
Funding Agency	The Nature Conservancy
Budget	\$25,000
Period	August 1, 2005–July 31, 2007
PI	Rebecca R. Sharitz
Project Title	On-site Field Studies and Long-term Monitoring Required for BRAC Implementation, Environmental Compliance, Technology Integration Assistance in Support of Installation Response to Developing Environmental Requirements, and Assistance with Developing New Installation Environmental Reporting Models at Ft. Benning, Georgia
Funding Agency	Department of Defense (CESU-Gulf)
Budget	\$302,909
Period	September 16, 2006–August 15, 2007
PI	Ramunas Stepanauskas
Project Title	The Role of Metal Contamination in the Proliferation of Antibiotic Resistance in Coastal Water-Borne Pathogens
Funding Agency	US Department of Commerce/NOAA
Budget	\$534,311
Period	September 1, 2004–August 31, 2007
PI	Carl Strojjan
Project Title	Technical Review/Comments–SRS Environmental Report for 2004
Funding Agency	Education Research and Development Association (ERDA)
Budget	\$4,454
Period	January 18, 2005–July 17, 2006
PI	Barbara E. Taylor
Project Title	Stream Studies on the Savannah River Site, Aiken County, South Carolina
Funding Agency	University of Kentucky/USDA-Forest service
Budget	\$153,946
Period	February 1, 2006–September 30, 2007
PI	Chuanlun Zhang
Project Title	Carbon Isotope Fractionations Associated with Bacterial Methane Oxidation: Implications for Carbonate Buildups at Hydrocarbon Seeps
Funding Agency	American Chemical Society
Budget	\$54,358
Period	August 1, 2002–August 31, 2005
PI	Chuanlun Zhang
Project Title	Microbial Interactions and Processes: Diversity, Function, and Biogeochemical Consequences of Chemolithoautotrophic Archaea in Nevada Hot Springs
Funding Agency	National Science Foundation
Budget	\$357,032
Period	May 1, 2004–July 31, 2007
PI	Chuanlun Zhang
Project Title	Genomic and Lipid Biomarker Monitoring of Microbial Communities Affecting the Formation and Degradation of Gas Hydrate in the Gulf of Mexico: Implications for Microbial Ecology and Global Climate Change
Funding Agency	University of Mississippi
Budget	\$189,533
Period	September 1, 2005–August 31, 2006

Publications

Books Published in FY06

- McArthur, J Vaun. *Microbial Ecology: An Evolutionary Approach*. 2006. Academic Press. 432 pp.
- Batzer, Darold P. and Rebecca R. Sharitz. *Ecology of Freshwater and Estuarine Wetlands*. 2006. University of California Press. 559 pp.

Journal Articles Published in FY06

- | | | | |
|------|--|------|---|
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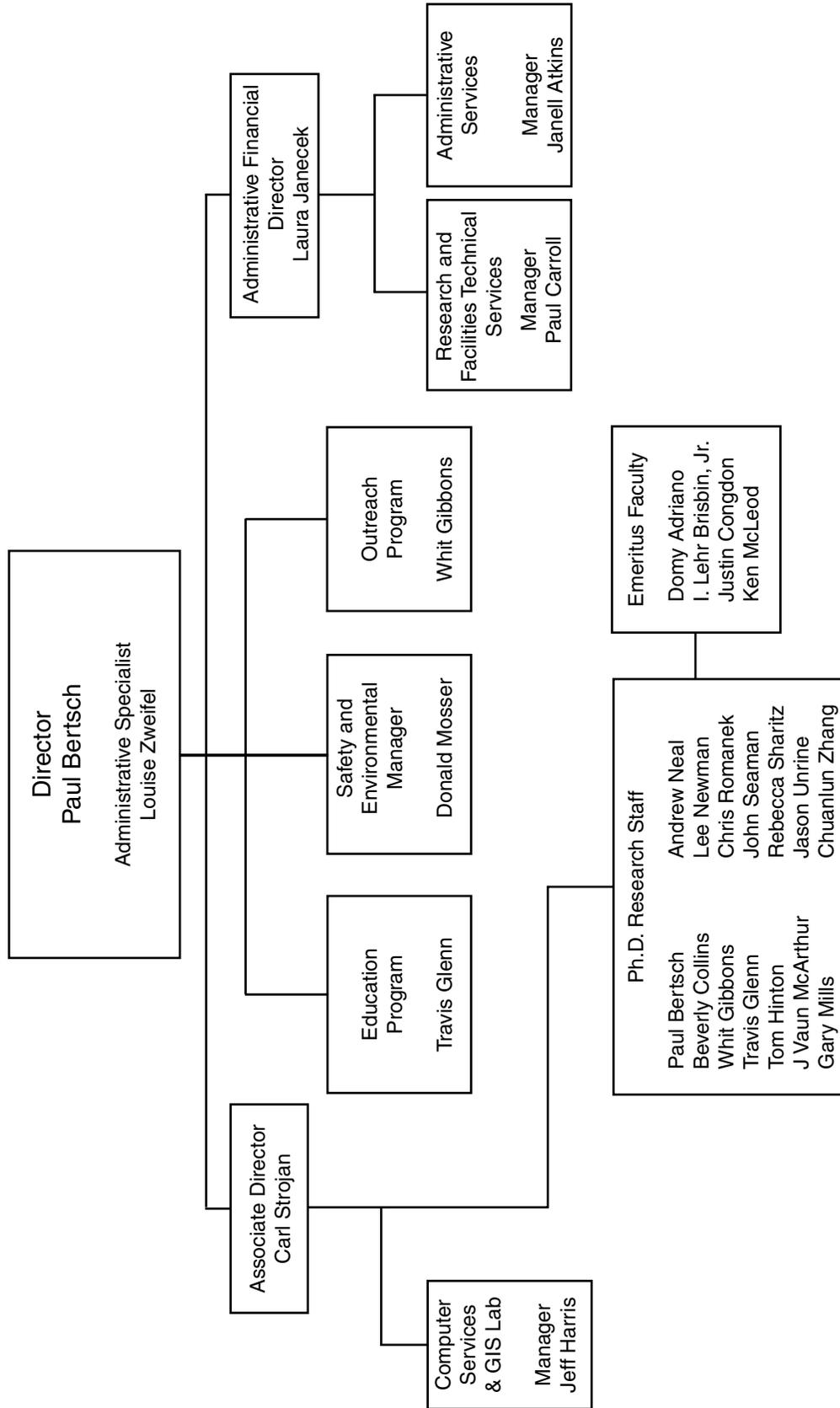
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SREL Organizational Chart

Savannah River Ecology Laboratory Organization Chart





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