Savannah River Ecology Laboratory

Annual Technical Progress Report

2005

Savannah River Ecology Laboratory

Annual Technical Progress Report of Ecological Research

Draft submitted 29 June 2005
Final submitted 19 July 2005

Supported under Cooperative Agreement
DE-FC09-96SR18546
between
The University of Georgia
and the
U.S. Department of Energy
for
The University of Georgia fiscal year ending
June 30, 2005

Paul M. Bertsch, Director

Prepared by Laura Janecek

Savannah River Ecology Laboratory
Drawer E
Aiken, SC 29802
PH (803) 725-2472 FAX 725-3309 E-mail: Janecek@srel.edu
www.uga.edu/srel/

◆◆◆ Table of Contents ◆◆◆

◆◆◆ Savannah River Ecology Laboratory ◆◆◆
FY2005 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 54 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 2005 fiscal year was fulfilled with the publication of 102 journal articles and book chapters by faculty, technical staff, students, and visiting scientists. Two books were also published with SREL personnel as authors. An additional 43 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, radiation ecology, and environmental microbiology.

- SREL researchers, in collaboration with others from Clark Atlanta University, WSRC, and the U.S. Forest Service-Savannah River, developed and implemented an automated vadose zone monitoring system to measure soil moisture and soil matric potential, and to collect lysimeter samples. A telemetry component of the system allows for real-time monitoring of vadose zone conditions in the field from a remote location. Also, the sampling frequency and data retrieval interval can be adjusted without visits to the field site. The system is currently being tested at the Mixed Waste Management Facility on the SRS.
- In a paper published in the journal *Radiation Research*, Dr. Thomas Hinton and colleagues examined whether dose-rate limits established for humans also protect other organisms in the environment, as is currently assumed. They emphasized there is a need to understand how radiation effects are integrated across different levels of biological organization, but to gain such understanding better collaboration is needed between the traditionally separate disciplines of radiation biology and radiation ecology.
- Dr. Paul Bertsch and colleagues received a $239,000 grant from the Department of Energy Office of Science EMSP program to study the link between chemical speciation and bioavailability of uranium and nickel in contaminated sediments. This study, to be conducted in the Tims Branch/Steed Pond system of the Savannah River Site, will provide valuable information on the scientific basis for natural attenuation of similarly contaminated areas.
- SREL researchers received a $500,000 grant from the National Oceanic and Atmospheric Administration (NOAA) to study the role of metal contamination in the proliferation of antibiotic resistance in coastal water-borne pathogens. The three-year award builds upon novel field and laboratory research done at the SRS by Drs. J Vaun McArthur and Ramunas Stepanauskas that suggested microbes resistant to metal contamination may also be resistant to antibiotics.

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

- SREL director Dr. Paul Bertsch received the Soil Science Research Award, a career achievement award from the 11,000-member Soil Science Society of America, the largest professional soil science organization in the world.
- Dr. Andrew Neal was senior author of a publication that was in the top 5% of most-downloaded papers of the 350 published in *Geochimica et Cosmochimica Acta* in the past year. The paper, "Surface structure

effects on direct reduction of iron oxides by *Shewanella oneidensis,*" was done in collaboration with other scientists from Pacific Northwest National Laboratory, Idaho National Laboratory, Montana State University and the U.S. Naval Research Laboratory.

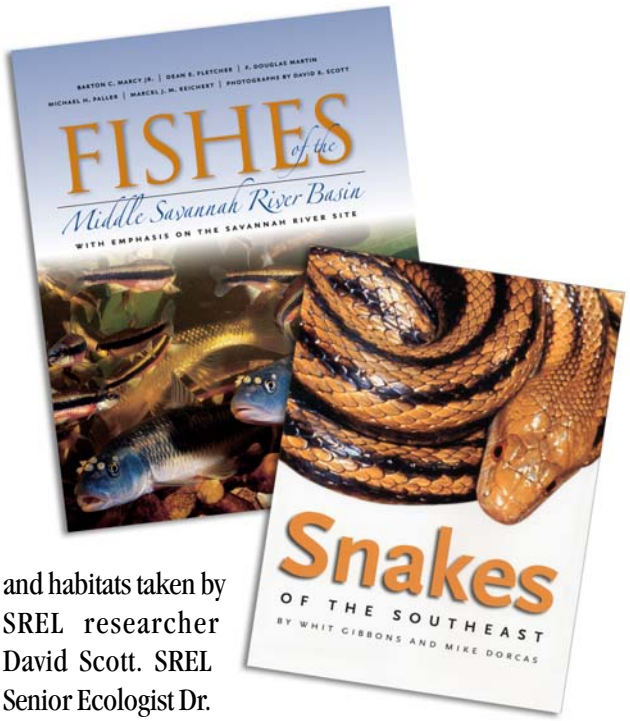
- Dr. Christopher Romanek was recognized for his highly cited research in the geosciences by ISI Essential Science Indicators. Dr. Romanek's record includes 19 papers cited a total of 870 times to date in the field of Geosciences.

During the past year SREL hosted two workshops of national significance. In November, 2004, a workshop on "Inventory and Monitoring of Amphibians and Reptiles" was held at the SREL Conference Center. Co-sponsored by SREL, U.S. Forest Service, and Partners for Amphibian and Reptile Conservation (PARC), this workshop brought together Forest Service personnel from across the country to learn herpetofaunal inventory and monitoring techniques that could be implemented on national forest lands. Over 30 participants took part in this four-day workshop.

In March, 2005, a workshop entitled "Partners along the Fall Line II: Nurturing Ecoregional Partnerships for Preserving Regional Resources and Sharing Data, Scientific Findings, and Technical Approaches" was held at the SREL Conference Center. Sponsored by the Department of Defense SERDP Ecosystem Management Program, the goals of this workshop were to share ecosystem management approaches, address specific opportunities, and nurture ecosystem planning partnerships. Participants representing several state and federal governmental and non-governmental agencies attended this three-day workshop.

2005 saw the retirement of one senior SREL faculty member. Dr. Domy Adriano retired after a 30-year career at SREL, where he concentrated his research mainly on trace elements in the environment. Among other achievements, he published over 200 scientific articles and book chapters and wrote two highly regarded books on trace elements. Dr. Adriano, who retired in April, continues to collaborate on research projects with colleagues at SREL, in the U.S., and abroad.

SREL personnel produced two books during the past year. Researcher Dean Fletcher, with co-authors from Westinghouse Savannah River Company and the South Carolina Department of Natural Resources, published "Fishes of the Middle Savannah River Basin, With Emphasis on the Savannah River Site" in March 2005. This 480-page book features over 200 color photographs of fish species



and habitats taken by SREL researcher David Scott. SREL Senior Ecologist Dr. Whit Gibbons and co-author Dr. Mike Dorcas published "Snakes of the Southeast" in May, 2005. This volume features over 300 color photographs and distribution maps of the 52 snake species found in the southeastern U.S.

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 June 30, 2006. The estimated total cost of this agreement since its inception in 1996 is over $94 million, with DOE contributing about $89 million and the University of Georgia about $4 million. SREL's total operating budgets from DOE and other federal sources in FY02, FY03, FY04, and FY05 were $10.2, $10.2, $9.11, and $8.7 million, respectively. In FY05 SREL received about $7.75M from DOE Office of Science (DOE-SC). The FY06 budget from DOE-SC was projected to be

$0, based upon the President's budget request; SREL had requested $7.978M for FY06 from DOE-SC. However, as a result of discussions with DOE-EM, DOE-SC, NNSA, and UGA, SREL expects the FY06 budget to be $4.3M. SREL also receives almost $798,000 per year from The University of Georgia. During FY05 SREL received $115.6K from DOE and WSRC for specific SRS-related tasks.

Researchers at SREL currently have funding totaling about $2.242M from 33 grants received during FY05, 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. Environmental Protection Agency, the National Science Foundation, and the Department of Defense (DoD). Important grants awarded this year included $115K from the National Institutes of Health to Dr. Travis Glenn; $239K from the DOE-SC Environmental Management Science Program (EMSP) to Dr. Paul Bertsch; and $534K from The National Oceanic and Atmospheric Administration to Drs. Ramunas Stephanauskas and J Vaun McArthur.

As of 30 June 2005 SREL had a permanent staff of about 145 people, nearly all of whom are employees of The University of Georgia. The staff includes 21 research scientists, six 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 10 Ph.D.s in postdoctoral appointments. Research technicians (44), clerical and other support personnel (45), and graduate students (25) comprise the remaining staff categories.

In addition to holding faculty positions in 12 departments at the University of Georgia, various SREL faculty have adjunct status at 18 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 60 editorial or committee positions in national groups and organizations and also serve on several UGA academic and administrative committees. Over 100 lectures, scientific presentations, and posters were presented during the past year at scientific meetings, colleges, and universities, including minority institutions.

In FY05 SREL spent about $100K to replace some existing equipment, including a microwave digester, ultra cold freezer and thermal cyclers, and to purchase a portable X-ray fluorescence analyzer. SREL also purchased a replacement DNA sequencer for its molecular ecology laboratory at a cost of about $180K.

Participants in the SREL Education Program during FY05 came from schools located throughout the United States and included 18 undergraduate students and 37 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 4 graduate students from SREL earned Masters Degrees and 4 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 247 talks, 14 tours, 19 exhibits, and 62 workshops, reaching a total of 64,761 people. Topics of these presentations included biodiversity, the process of science, animal adaptations, plants and wetlands, environmental science and chemistry, local ecosystems and conservation, classification, and careers in ecology and research.

The SREL Distance Learning Program continued to focus its efforts on programming related to the Laboratory's core programs in ecology and environmental science. SREL, in cooperation with and with funding from the UGA College of Pharmacy, has offered a multidisciplinary Master's Degree in Environmental Toxicology via the Georgia Statewide Academic and Medical Systems (GSAMS) network. This is the first advanced degree offered by UGA through any distance learning site in Georgia or South Carolina. Eight students have completed all required coursework and are working on the research component of the degree and four students have graduated from the

program. In an effort to expand its audience, the SREL Outreach Program presented 5 Ecotalks via distance learning to elementary, middle, and high schools in both South Carolina and Georgia. Many Georgia schools have not been able to maintain compatibility with GSAMS and the Georgia audiences are declining.

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 67 scientific meetings and environmental education programs for students, teachers, and the general public this past year, and almost 2,000 people visited the facility.

Representatives of the Laboratory also serve local and statewide communities by organizing a canned goods drive in November, hosting an auction to benefit the South Carolina Chapter of The Nature Conservancy in December, 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. Andrew Neal authored a very popular scientific paper in the journal *Geochimica et Cosmochimica Acta*, an international publication devoted to geochemical issues. Andrew's paper was in the top 25 most-downloaded papers for the period of October 2003 to September 2004. The paper covered the groundbreaking work he and his colleagues are pursuing on the environmentally important process of bacterial iron reduction.

Dr. Paul Bertsch received the Soil Science Research Award, a career achievement award from the Soil Science Society of America that recognizes outstanding research contributions in soil science. Paul also received the D.W. Brooks Award for Excellence in Research, from the College of Agriculture and Environmental Sciences at The University of Georgia.

Graduate student **Meredith Wright**, a student of **Dr. J Vaun McArthur**, was awarded a 2004 Environmental Protection Agency (EPA) STAR Fellowship, valued at over $37,000. Her study is titled "The Transfer and Transport of Antibiotic Resistance in Heavy-Metal Contaminated Streams.

Dr. Christopher Romanek was recognized as a top ten researcher in the field of "Mars Science" for the years 1993-2004 by ISI Essential Science Indicator (http://www.esi-topics.com/mars/index.html).

Dr. Paul Bertsch was appointed to serve on two National Academy of Sciences committees: the U.S. National Committee for Soil Science and the Committee on Earth Resources. Paul was also appointed to a National Academy of Sciences Steering Committee for a Workshop on Frontiers in Soil Science Research, by Executive Director of the Policy and Global Affairs Division of the National Research Council, 2005.

Dr. Domy Adriano was appointed Honorary President of the International Society of Trace Element Biogeochemistry, Vienna, Austria (Autumn 2004).

Dr. Tracy Punshon received the 2004 Editor's Citation for Excellence in Manuscript Review Award from the *Journal of Environmental Quality*, in recognition of her outstanding professional contributions in reviewing papers during 2004.

Graduate student **Sarah Strycharz**, a student of **Dr. Lee Newman**, won a conference award for Best Student Presentation for her poster "Development of a genome-wide screening method to identify gene candidates involved in the degradation of halogenated hydrocarbons using ion chromatography" at the Phytoremediation Session of the Association for Environmental Health Sciences meeting in Amherst, MA.

Graduate student **Chris Winne**, a student of **Dr. Whit Gibbons**, won a travel award from the South Atlantic Chapter of the Society of Wetland Scientists to facilitate his attendance at the Association of Southeastern Biologists annual meeting in Alabama. While at the ASB annual meeting, Chris won the Association of Southeastern Biology Student Research Award in Aquatic Biology for his talk on "Adaptation of a small aquatic snake (*Seminatrix pygaea*) to a dynamic habitat: selection on body size and reproduction after aestivating during drought."

The first SREL Graduate Student Symposium was held in July, 2004; 35 students presented oral or poster presentations. Winners in the platform session included: **Jason Unrine** (first place, $300), **Kimberly Andrews** (second place, $200), and **Jacqueline Litzgus** (third place, $100). Poster session winners included **Angel Kelsey Wall** (first place, $300), **Tracey Tuberville** (second place, $200), and **Meredith Wright** (third place, $100).

Graduate student **Kimberly Andrews**, a student of **Dr. Whit Gibbons**, received the UGA Institute of Ecology's Best Student Paper Award in the applied category. The $500 case prize was awarded for the paper judged to be the best published during the preceding year by an Institute of Ecology graduate student. Her paper was titled "How do highways influence snake movement? Behavioral responses to roads and vehicles;" it has been accepted for publication in the journal *Copeia*.

Graduate students **Gabrielle Graeter** and **J.D. Willson** each received Student Travel Awards from the Society of Wetland Scientists. Both are students of **Dr. Whit Gibbons**.

The **SREL Environmental Outreach Program** was one of two nominations from UGA for a Board of Regents of the University System of Georgia-Best Practices competition.

Dr. Nanthi Bolan, of Massey University, New Zealand, received the M.L. Leamy Award for 2004 from the New Zealand Soil Science Society for most meritorious publications in the last three years (2001-2003). Dr. Bolan was a visiting scientist at SREL for several months during 2004, working primarily with **Dr. Domy Adriano**.

Dr. Kenneth McLeod was appointed to the Science Working Group of the Governor's Task Force on "Coastal Wetland Forest Conservation and Use," for the State of Louisiana.

Undergraduate student **Tom Luhring** won two awards this year. He was the recipient of the Institute of Ecology's Josh Laerm Memorial Outstanding Undergraduate Award. Tom also received the Georgia Museum of Natural History Laerm Memorial Award. Tom participated in the SREL REU undergraduate program in the summer of 2004; his SREL sponsors were **Drs. Whit Gibbons** and **Betsie Rothermel**.

◆◆◆ An Overview of ◆◆◆ Research Themes

SREL receives its DOE funding as a single operating grant to support the activities outlined in the Laboratory's Cooperative Agreement. SREL's management and faculty are responsible for establishing the research programs and plans that implement the terms of the Agreement.

The long-term ecological studies that provide an independent assessment of SRS activities on the environment and demonstrate the relative health of SRS ecosystems have been central to SREL's research throughout its nearly 54-year history. Discussions with DOE-SR administrators in 1999 resulted in a reaffirmation of the importance and value of long-term ecological studies, while at the same time delineating SREL's role in emerging issues related to environmental remediation and restoration.

To detail the research to be conducted with DOE-SC funding, SREL prepares research plans and milestones. Beginning with FY04, research plans have been assembled according to three research themes: **Characterization**, **Ecological Risk and Effects**, and **Remediation and Restoration**—which reflect how the environmental remediation process proceeds. This is done to provide a more useful document for DOE managers and contractors, and to provide researchers with a perspective on how they might identify and address knowledge gaps in each of the themes through scientific research. Numerous knowledge gaps are associated with each theme and some cut across all three themes. For example, processes controlling bioavailability emerge as gaps across all themes; however, the specific gaps and the approaches to dealing with them within the three themes vary based on the level of organization or scale at which they are addressed. The knowledge gaps identified for each theme are not comprehensive, but rather represent those gaps that were addressed in studies planned for FY05.

◆◆◆ Characterization ◆◆◆

Characterization is a necessary first step in determining environmental and health risks and devising remediation and restoration strategies. Characterization has physical, chemical, and biological components, and spans molecular to landscape scales. Although it includes a descriptive component, characterization is more than simply measuring contaminant concentrations in biota or other media, or reporting the presence or quantity of various organisms at contaminated locations. It includes developing an understanding of the processes that control distributions of contaminants, chemical speciation, and bioavailability. Characterization also includes elucidating the environmental and ecological patterns and processes that influence distributions of organisms, biological diversity and function, health, and population processes. Characterization is necessary to construct models of how natural and engineered systems function, both in the presence and absence of environmental perturbations such as anthropogenic contamination. In other words, characterization is best viewed as an activity to better describe and understand particular habitats and systems, both contaminant-stressed and more pristine.

Significant knowledge gaps exist that impair accurate risk assessment, limit remediation and restoration activities, and make cost-effective management decisions difficult or impossible. Only with scientific advancements can informed decisions be made about, for example, (1) whether there is a need to restore or remediate a given site; (2) the state to which a site should be restored; (3) when the process has been completed, and (4) how successful and cost-effective a remediation activity has been. There are significant knowledge gaps that must be closed to achieve the types of characterization necessary to meet remediation and restoration goals. Research at SREL addresses these knowledge gaps 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.

Report on FY05 Characterization Milestones

Question 1:
What are the roles of microorganisms in biogeochemical processes that affect contaminant cycling, speciation, and mobility, and how are these affected by co-contaminants? How do the microbial populations and species diversity affect or contribute to the overall function of the ecosystem?

(A) Use of field flow fractionation to investigate uranium adsorption by *Shewanella*
Investigators: A.L. Neal and B.P. Jackson

We have continued to study the sorption of uranium to *Shewanella* cells and a paper was published recently in *Analytical Chemistry* (SREL reprint 2812) summarizing our results to date. More recently, we have received samples from Dr. Alex Belieav of Pacific Northwest National Laboratory of exopolymers secreted by *Shewanella oneidensis* MR-1 with a high affinity for U. These are believed to be similar to exopolymers we observed during our FFF experimentation. We are currently utilizing High Performance Size Exclusion Chromatography (HP-SEC) to assess the molecular weight of the exopolymer; recent results suggest an approximate size of 200 kDa, but we are aiming to improve our estimate by optimizing the HP-SEC separation.

In preliminary experiments aimed at determining the affect of dissimilatory iron reduction upon the fate of uranium in natural soils, we have been performing experiments using chromate into soils from Savannah River site. These experiments, using *Shewanella putrefaciens* strain CN-32, have focused on the interaction between cells and naturally occurring fulvic acids in surface soils of the Orangeburg series and vadose soils from the Tobacco Road formation. Preliminary experiments indicate that autoclaving does not alter Cr(VI) sorption behavior to the two soils. Very limited abiotic Cr(VI) reduction was observed for either sterile soil upon addition of 0.1 mM Cr(VI), assessed using the diphenylcarbazide method. In contrast, rapid reduction was observed upon inoculation with CN-32. Simultaneously, Fe(II) was higher in treatments inoculated with CN-32 as determined using the ferrozine method. Although the vadose zone soil contained greater quantities of extractable Fe (14.62 ± 0.65 g kg^{-1}, compared to 2.04 ± 0.04 g kg^{-1} in the surface soil) less Fe(II) was identified in solution (0.25 mg L^{-1}) compared to the surface soil (1.6 mg L^{-1}) following 7 days incubation. There is a significant difference between the total organic content of the two soils, the surface soil having 13.5 mg g^{-1} compared to 4.2 mg g^{-1} in the vadose zone soil. Amendment of the vadose zone soil with humic materials extracted from the surface soil effectively increased Fe(II) concentrations to surface soil levels, suggesting that the iron reduction observed in the vadose zone soil could be augmented by humic acids. The presence of humic acids also had a significant effect upon Cr(III) speciation. While in humic acid free vadose zone soil total [Cr] (*i.e.* [Cr(VI) + Cr(III)]) in solution assessed by ICP-Mass Spectrometry was not significantly different from [Cr(VI)], in the surface soil containing significant humic material total [Cr] (2 mg L^{-1}) far exceeded [Cr(VI)] (0.5 mg L^{-1}), suggesting much of the Cr(III) was associated with humic material in the aqueous phase, not as precipitates associated with the soil itself. This has significant implications for the concept of reduced metal species being an acceptable endpoint in surface soils containing high levels of humic materials.

Upon completion of these experiments we aim to study the fate of uranium under identical conditions.

(B) <u>How do radionuclide and heavy metal inputs influence microbial soil populations within a riparian system?</u>

Investigators: C.L. Zhang, G.L. Mills, A.L. Neal, B.P Jackson, and P.M. Bertsch, in collaboration with colleagues at the Savannah River National Laboratory and the Medical University of South Carolina

Past DOE activities have created significant environmental contamination, which can have adverse effect on all forms of life including microorganisms. On the other hand, certain groups of microorganisms are known to enhance the degradation of organic pollutants and the transformation and immobilization of heavy metals and radionuclides. Considerable research has been conducted by DOE to determine the transport and fate of contaminants in soils and groundwater. Technologies have also been developed to enhance biodegradation of chlorinated organics and petroleum hydrocarbons and biotransformation of heavy metals and radionuclides at SRS. However, a deeper understanding of microbial diversity, community structure, and activity is needed to better accomplish DOE missions and respond to new DOE initiatives and environmental bioremediation strategies.

Our initial research focus was on three sites at SRS: (1) Steeds Pond, which is heavily contaminated with metals and radionuclides; (2) Lower Tims Branch, which is less contaminated with metals and radionuclides; (3) an iron creek at D-Area, which is impacted by the seepage of a holding pond for remediation practices. DNA was extracted from soil or sediments using a commercial kit (MoBio Co.). Primers specific for bacteria (27F/1492R) were used for PCR amplification of these DNA samples. The PCR products were screened using denaturing gradient gel electrophoresis (DGGE). The DGGE patterns were compared using statistical methods, which resulted in a similarity tree using the Pearson indices and the un-weighted pair group with arithmetic means

algorithm (UPGMA).

The bands in DGGE lanes were scanned by the Imager System (Alpha Imager 3400) and intensities of bands were measured and the percentages of the bands in the community were calculated using the software. The data were then reorganized and submitted to the database for UPGMA analysis. TreeView software was used to generate a relationship tree. The results showed that about 40 bands in all of five samples can be identified in the urea concentration region from 30% to 55%. Based on the same quantity DNA and references controls, different bands referred to different bacteria species and different intensities referred to different abundances of different populations. Stream sediments and surface soil from Steed Pond showed similar distribution patterns except that the sediment diversity was less evenly distributed than the surface soil. Community evenness for Lower Tims Branch was to some extent short because of the minor dominances of several bands. In the ion creek samples, the sediments and surface mats showed the large likelihood between their diversity patterns and community structures.

Bacterial diversity is high at all three sites. The community structure at each location is significantly different, suggesting a geographical variation of bacterial diversity at the SRS. The community structure at Steeds Pond is more similar to that at the Lower Tims Branch than to those at the iron creek. This may reflect environmental differences between the soil and aquatic systems. At the iron creek, black sediment below the iron mat has a higher diversity than the surface mat. This suggests that microbial communities vary in the context of the changing geochemical environment.

Question 2:
How do coupled biological and geochemical processes interact to determine the fate and transport of contaminants in near-surface and surface environments?

(A) Upland land-use effects on downslope conditions

Investigators: S.J. Harper, B.S. Collins, and R.R. Sharitz

Upland land-use can alter forest composition, structure, and biogeochemistry, including movement of materials along geomorphological gradients. For example, forestry practices such as cutting and prescribed fire can result in greater carbon, nitrogen, and sediment movement downslope and inputs of these materials at the upland-wetland interface. In turn, these inputs can influence important drivers of biogeochemical processes, including redox conditions, carbon pools, and nutrient dynamics. Improved understanding of the effects of upland processes on drivers of biogeochemical conditions located downslope will enhance efforts to remediate organic and inorganic contaminants concentrated at the wetland-upland interface.

In FY05 we continued field research along geomorphological gradients to characterize land-use effects on soil characteristics and input of materials, principally nitrogen, at the upland-wetland interface. Over winter 2003-04, we established three sets of sites along the gradient from managed pine forest through slope hardwoods to the wetland-upland interface along Tims Branch. These sites represent the following combinations of land use activities: (a) undisturbed upland, slope, and interface; (b) burned [2004] upland, undisturbed slope, and interface; and (c) burned [2004] upland, thinned [ca. 2000] slope, and interface. Pre- and post-fire sampling of lysimeters, deployed in upland, slope, and upland-wetland interface regions in each set of sites, revealed position along the slope, but not land use, affected NO_3-N concentrations, while land use, but not slope position, affected NH_4-N concentrations. NO_3-N concentrations were highest in slope locations, while NH_4-N was marginally greater in undisturbed sites than in sites with recently burned upland. Mass of organic layer samples was less at the upland-wetland interface than at slope or upland positions. Further, downslope sites leading from burned and cleared uplands had less organic layer mass than those leading from burned or undisturbed uplands. These field data suggest there

is little immediate effect of land-use activities on nitrogen loss/availability in lysimeter samples along the topographic gradient, but prescribed fire and clearing can affect carbon and nitrogen cycling by reducing organic layer pools available for decomposition.

In FY05 we also developed spatially-explicit models using GIS to characterize the physical conditions of the Tims Branch subwatershed on the SRS. Based on a 10-m elevation model and derived slope estimates, we divided the study area (3571 ha) into zones representing upland, slope, and interface areas (65, 12, and 23% of total area, respectively). Model results indicated that these zones differed with regard to dominant soil (Fuquay sand, Vaucluse-Ailey complex, and fluvaquents, respectively) and vegetation types (loblolly/upland hardwoods, open-canopy loblolly, and flood plain sweetgum forest, respectively). Despite these physical differences, all three zones were extensively burned in conjunction with forest management during the past decade of the available historical record (61, 64, and 55%, respectively). Given the prevalence of burning and its importance to nutrient cycling within the ecosystem, further work is needed to determine effects of upland land use practices on biogeochemical drivers at the wetland-upland interface.

(B) Effects of coal fly ash on ecosystems

Investigators: K.W. McLeod, R.R. Sharitz, B.C. Collins, W.A. Hopkins, B.E. Taylor, and B.P. Jackson

Fly ash was released from the D-Area coal-burning power plant into a bottomland forest from 1967 to 1977 as a result of runoff from a filled settling basin. We characterized the extent (length, width, and depth) of fly ash in a 3-dimensional grid at the site. Up to a 2.75 m depth of fly ash was deposited in some areas and total aerial coverage was approximately 40 hectares. Twenty-four hectares held at least 30 cm of fly ash. Arsenic (As) and selenium (Se) were the primary contaminants-of-concern, with mean concentrations in areas, with at least 30 cm of fly ash, of 7 and 4 ppm in the forest floor litter layer, 31 and 6 ppm in the fly ash, and 5 and 3 ppm in the original soil, respectively. Arsenic and selenium concentrations in the ash-contaminated areas were 10 and 2 times greater than in control areas, respectively. Strontium was also elevated (7X) in the ash containing areas. This large scale deposition of the fly ash has also significantly altered the topography of the forested landscape, with ash deposited in low spots and areas with slow water flow rates.

Contaminant concentrations in foliage of herbaceous plants and trees were elevated in the ash plume area (generally by a factor of 2-3 for As and 2-8 for Se), but As and Se concentrations were less than 2 and 4 ppm, respectively. Higher Se concentrations were generally found in tree than herbaceous foliage, while higher As concentrations were more likely found in the herbaceous plants. Earthworms (*Lumbricus rubellus*) and land snails (several species) had elevated levels of both As and Se at sampling sites located on the ash plume. Earthworms contained much higher levels of Se (up to 35 ppm) than snails. Larval amphibians contained more As than adult amphibians, but Se concentrations did not decline with maturing life stage. Elemental concentrations were generally higher in *Bufo terrestris* (southern toad) than *Rana sphenocephala* (southern leopard frog). Concentrations of As, Se, and strontium were up to 11-35 times higher in amphibian metamorphs from the ash area than from an unpolluted area.

While the plant species composition on the ash plume area has shifted to an earlier successional stage, the community characteristics such as basal area and density are similar to those of an adjacent non-contaminated early successional clearcut site. The present vegetation is in transition from the former mature forest community to an impacted and/or recovering community.

The herpetofaunal community is characterized by at least 18 amphibian (14 anuran and 4 salamander species) and 17 reptile (9 snake, 3 lizard, 4 turtle and 1 alligator) species. The diversity is not unusual for this community type. Abundance and species-

richness of soil-dwelling macroinvertebrates (mainly earthworms and land snails) were substantially higher at control sites than at sites located on the ash plume.

Since the plant community is adjusting to the deposited ash, the best management for this site might be monitored natural attenuation, although it is recognized that the metal contaminants will never be converted to less harmful forms and through natural attenuation the contaminants will simply dilute over time into the environment. Then, it must be demonstrated that the migrating ash contaminants are not harming the biota, either individually or by affecting community diversity.

Question 3:
What processes control chemical speciation and mobility of toxic metals, organic contaminants and radionuclides in biota, soil, wetlands, ground water and surface water? What linkages exist between chemical speciation (and other properties of contaminants at the molecular level) and bioavailability, uptake and transfer of contaminants by organisms?

(A) Use of transgenic nematodes to assay heavy metal exposure

Investigators: T.C. Glenn, C.H. Jagoe, B.P. Jackson, P.M. Bertsch, and collaborators from the Idaho National Engineering and Environmental Laboratory (INEEL) and the Universities of Georgia and South Carolina

Work continued on the transgenic nematodes (*Caenorhabditis elegans)* with a metal-specific promoter, metallothionein-2, linked to a Green Fluorescent Protein reporter (mtl-2::GFP), which we have developed and described previously. We have continued development of high throughput assays using plate readers, and a COPAS Biosort (flow cytometer modified to gently handle large particles, such as *C. elegans*). During testing of the COPAS Biosort, we measured fluorescence of unexposed and metal exposed mtl-2::GFP worms. Much of the information below is modified from data gained during a product demonstration and resulting report written by B. Wang of Union Biometrica, who set up the instrument and conducted assays in our labs with worms we provided to him. This information will be important for our resulting publications, and we are likely to invite Dr. Wang to be a co-author.

The worms clearly responded to Cd by producing increased levels of GFP. The treated *mtl*-GFP worms showed a large variance among the individual fluorescence intensities. Indeed, examination of these worms under a fluorescent microscope revealed that although the majority of these worms were highly fluorescent, the rest of them showed low fluorescence levels comparable to that of the untreated ones. The "bright" and "dim" subpopulations of the treated worms can be separated by the COPAS instrument based on these different fluorescence intensity levels. We are now investigating the causes for variance in GFP expression and working to reduce the variance in brightness within our mtl-2::GFP strains.

Spatial distribution and chemical speciation of contaminants

We also used synchrotron X-ray fluorescence to study the spatial distribution of Pb and Cu in *C. elegans* initially exposed to each contaminant in solution. Qualitatively there was a striking difference in the spatial distribution of each contaminant in the nematodes; Cu was relatively homogeneously distributed while Pb was highly localized in the pharynx. The analytical technique was very sensitive to assessing exposure to these metals and in the case of Pb, significant uptake was discernable for aqueous exposure of 2.4 µm. The highly localized nature of Pb uptake and the high concentration of Pb at this hotspot suggested that this was a Pb solid phase. We used µ-X ray diffraction to investigate this hotspot and obtained a diffraction pattern consistent with the Pb phosphate mineral pyromorphite. This work was presented at the Society for Environmental Toxicology and Chemistry 4th World Congress in Portland, October 2004. A manuscript has been prepared, submitted and accepted for publication in *Environmental Science*

and Technology.

(B) Effects of mercury in the environment
Investigators: C.H. Jagoe, W.A. Hopkins, C.S. Romanek, I.L. Brisbin, Jr., and A.L. Bryan Jr.

Although the form of mercury that generally accumulates in fish and wildlife is methyl mercury, most mercury released to the environment by commercial and industrial sources is in inorganic forms. These inorganic species are methylated in soils, waters, and wetlands by microbial processes. Thus, better understanding of the processes that control or influence *in situ* mercury methylation, speciation, and transport is essential to assessing and mitigating environmental mercury contamination. Also, most of the mercury accumulating in fish and wildlife originates in their diet. Therefore, documenting and understanding trophic transfer and trophic relationships within food webs is critical to understanding and managing mercury contamination problems.

In FY 2005, we published a manuscript on mercury concentrations and speciation in amphibian larvae in wetlands, and their food items. Mercury concentrations in larvae from wetlands without obvious local pollution sources were similar to those that caused adverse effects in laboratory experiments. Amphibian larvae from these wetlands also act as prey items for a variety of wildlife, so these mercury concentrations might indicate risks to consumer species. Speciation data were used to calculate bioaccumulation factors for inorganic and methyl mercury in a simple food web composed of periphyton to amphibian larvae. Our results indicated that standard, widely used EPA models do not accurately predict mercury bioaccumulation in these wetland systems.

We published a study in FY05 exploring the relationships between tissue mercury concentrations in benthic bivalves and dissolved mercury concentrations in water. This study examined the relative contributions of small streams and wetlands to mercury in a large river (the Savannah River). *Corbicula* are small, introduced clams. They are sessile filter feeders, so contaminant concentrations in their tissues are highly reflective of local conditions. *Corbicula* living at the mouths of small streams draining wetlands on the South Carolina coastal plain had higher tissue mercury concentrations than those from sites nearby in the main river channel. Methyl mercury concentrations were also higher in tributary stream waters than in the main river channel. These results indicate that small streams draining wetlands on the coastal plain are important sites of mercury methylation, and suggest that such small catchments are of considerable importance in the biogeochemical cycling of mercury on the SRS and, probably, in other southeastern systems.

In concert with other ongoing projects, we have collected fish from SRS reservoirs and offsite locations for analysis of contaminants, including total tissue mercury, for several years. Otoliths from subsamples of these fish were removed and used to measure fish age. Fish size and age data allow calculation of relative growth rates of fish from SRS reservoirs versus offsite locations, and allow for examination of relationships between size, age, and tissue mercury concentrations. A manuscript describing these relationships in reservoirs on the SRS and offsite locations has been submitted for peer-review.

For almost 10 years, we have collected samples of tissues (blood, down, feathers) and food items from wood storks *(Mycteria americana)*, an endangered species that nests in the southeastern U.S. This work was partially funded by the U.S. Fish and Wildlife service. This one-of-a-kind, long-term data set shows that mercury exposure and accumulation in nestling wood storks varies with location and annual rainfall patterns. Parent storks forage in wetlands near nesting colonies and return prey to their nestlings. Mercury concentrations were consistently higher in juvenile birds fed from freshwater wetlands than in those fed from estuarine or salt-water wetlands. This is consistent with the concept that methylation potentials are greater in freshwater systems than in estuarine

systems. Analysis of stable carbon isotopes in tissues provides direct evidence for carbon sources in the diet. Nestlings from colonies where freshwater prey represents the only available carbon source had different $\delta^{13}C$ signatures than those from colonies where saltwater and estuarine prey were available. In coastal colonies, where both fresh and saltwater prey was available, mercury concentrations in nestlings were higher in wet years, when foraging habitat expanded in freshwater marshes filled by rainwater. Shifts in nestling $\delta^{13}C$ signatures between wet and dry years were consistent with this interpretation. A manuscript describing these results is in preparation.

Feather samples have been collected from more than 10 other species of nestling wading birds (including egrets, herons, and ibis) from coastal and inland colonies. This work was partially funded by external grants, and includes a student funded by NOAA. Samples were obtained from inland nesting colonies where parent birds foraged exclusively in fresh water and from birds in coastal colonies where parents foraged in both fresh and estuarine/salt water habitats. Analyses of samples collected in FY05 have been completed. Mercury concentration varies among species, with those feeding at the highest trophic levels (as identified by $\delta^{15}N$ signatures) having the highest mercury concentrations. This work interfaces with studies of trophic relationships and contaminant concentrations in other birds (waterfowl and eagles) as described in other sections of this report.

(C) Earthworms as indicators of uranium and nickel

Investigators: T. Punshon (CRESP), P.M. Bertsch, and B.P. Jackson

The extent to which metal and radionuclide contaminants of soils and sediments pose an environmental hazard depends upon their potential for movement within the environment and their entry into biota. An accurate evaluation of this hazard inevitably involves a determination of bioavailability, because it is now widely accepted that only a fraction of the total contaminant is subject to such movements. Direct measurement of bioavailability in soils and sediments has proved a challenge to environmental science which has so far resisted incorporation into a readily usable test. Current approaches to measuring bioavailability involve chemical and biological tests. Chemical tests involve applying a series of increasingly aggressive extractants and measuring the metal concentration removed by each extraction as a function of the total. Commonly known as a sequential extraction scheme, it was not intended to inform on biological responses. No single fraction or combination of fractions represents that accessible to biota. Although a great many scientific studies have used the scheme to make inferences about speciation and bioavailability, the scheme is actually a measure of chemical lability. Biological tests are considered more appropriate measures of bioavailability because various toxicity and mortality endpoints are related to a definite contaminant concentration. These tests involve exposure of certain test organisms to measured amounts of contaminant in a situation which ideally resembles a field situation. For soils and sediments, in particular, earthworms and nematodes have been used. Our aim in carrying out these earthworm studies is to bridge knowledge gaps between chemical and biological tests, so that we can move toward a greater understanding of bioavailability and derive more accurate measures for risk assessment and remediation.

The contaminant source for the Tims Branch riparian system has been the subject of several key biogeochemical studies at SREL, all of which have found a contrast in the chemical lability of the main contaminants, Ni and U. Geochemical tests have shown higher chemical lability for Ni than U, with the latter being complexed by dissolved organic matter or bound to a colloidal fraction. A survey of the chemical composition of dominant flora corroborated this with the presence of Ni and absence of U within plant tissues.

Our previous work using the U.S. Environmental Protection Agency's Earthworm Subchronic Toxicity test (EST) indicated that the substrate collected in Tims Branch was not toxic to earthworms, and yet they

accumulated extremely high concentrations of U within their tissues (>200 μg g^{-1}), contradicting the core inferences of geochemical tests and evidence from vegetation. Although the EST does not claim to be applicable to U specifically, the data it provided for Ni, a contaminant for which it should be applicable, were also counter to geochemical tests. Nickel bioavailability exceeded that of U by an order of magnitude, but it did not accumulate in earthworm tissues during the EST. Further, U accumulation was strongly affected by components of the artificial soil mixture prescribed in the EST methodology, in particular the level of calcium carbonate incorporated into the soil. Addition of organic matter also suppressed earthworm U accumulation. Our studies indicated that the composition of the artificial soil was just as influential—if not more so—to U accumulation (and by inference, bioavailability) than U concentration of the soil.

The next stage of the research involved examining earthworms using synchrotron X-ray fluorescence spectroscopy (SXRF) available at beamline X26A of the National Synchrotron Light Source (Brookhaven National Laboratory). This non-destructive technique shows the relative abundance and spatial distribution of metals (including U) within environmental samples, and was applied to samples of contaminant-exposed earthworm digestive tract samples (preparation of which is described in the 2004 SREL Annual Technical Progress Report). Collection of compositional maps for earthworms yielded excellent results in FY04 after the EST was repeated using a 50/50 mix of Tims Branch soil and artificial soil, the treatment level in which the highest U accumulation in earthworms was observed. By collecting multiple elemental maps at high resolution (i.e. low pixel: step size ratio), and combining maps to form a composite image of the entire worm, we were able to show the extent of U accumulation in the earthworm gut.

Clearly, U is found only in the tissues within the coelom, and not in the exterior tissues from peritoneum to epidermis. These U-enriched tissues comprise the chloragogue cells, intestinal epithelium, intestine and, tentatively, typhlosole. Uranium distribution is extensive within the crop and gizzard. Choragogeous tissues are of interest in this work, because they are responsible for the formation of mineralized deposits arising from the combination of metals filtered out of the gut with phosphates and carbonates. This is a sequestration mechanism, which protects the earthworm from metal toxicity, and may be the reason behind the low EST mortality but high tissue U concentrations. X-ray fluorescence spectra showed that areas of the earthworm that were particularly enriched with U were also enriched with P and Ca.

Determinations of the speciation of the U accumulated by earthworms has been carried out both by extended X-ray fine structure spectroscopy (EXAFS) and also by size-exclusion chromatography interfaced with inductively coupled plasma mass spectroscopy (SEC-ICP-MS). The oxidation state of the U within the earthworms is known to be in the U(VI) form, although the binding environment of the U is still unknown. By conducting replicated analysis in the EXAFS range of the XAS spectra, and comparing data to known U standards, we are able to determine the binding environment of the U within earthworms. Size exclusion chromatography has been conducted on mineralized granules extracted from earthworm tissues.

The results of the studies conducted on earthworms will be published as a three-paper series, and remaining speciation studies are nearing completion. The first paper will focus on the results of the EST, in particular the effects of calcium carbonate and organic matter, while the second paper will present results from the SXRF and EXAFS studies on abundance, distribution and speciation of U within the earthworm tissues. The final study will present data from size-exclusion ICP-MS of extracted earthworm granules.

(D) Whole-lake experiments on the dynamics of cesium
Investigators: T.G. Hinton and B. Taylor, with collaborators from Colorado State University

2005 Progress: The rich data set derived from our

whole-lake addition of stable cesium to a ^{137}Cs-contaminated pond on the SRS continues to reveal the interesting dynamics of the contaminant. This research is important because whole-lake experiments provide a realistic experimental scale, but are rare and difficult to conduct. Additionally, our data fill a void in the SRS data base by providing information on the first 250 days following contamination by Cs. Two manuscripts were produced from this research in FY05.

The first manuscript (*Pinder III, J.E., et al.. 2005. The influence of a whole-lake addition of stable cesium on the remobilization of aged ^{137}Cs in a contaminated reservoir. J. Environ. Radioactivity 80:225-243;* SREL reprint 2818) quantifies the release of ^{137}Cs from the sediments of the lake following the addition of stable Cs to the water column. Desorption of ^{137}Cs from sediments is an important process that prolongs the bioavailability of the contaminant, enhances the probability of subsequent uptake of the contaminant by biota, and directly relates to the determination of human and ecological risks. European research following the Chernobyl accident examined the remediation potential of adding stable potassium to ^{137}Cs contaminated lakes, but no studies have considered the addition of stable Cs. We documented a 3-fold increase in the dissolved ^{137}Cs inventory of the lake's water column following the addition of 4kg of stable Cs. Several lines of evidence indicated that the source of the increased inventory was a release of ^{137}Cs from the sediments. The dynamics within the water column were modeled with a simple steady-state model of the form: $Y = 3.1 + 6.1\ (1 - e^{(-0.028 * t)})$; where Y predicts the inventory of ^{137}Cs in the water column of the lake and t is the number of days following the addition of the stable Cs.

The second manuscript (*Pinder III, J. E., T. G. Hinton and F. W. Whicker. In press. Foliar uptake of cesium from the water column by aquatic macrophytes. J. Environ. Radioactivity)* examines the impacts of aquatic vegetation on the dynamics of Cs. Specifically, we were interested in the relative importance of foliar uptake of Cs by aquatic plants from the water column versus root uptake of Cs from the contaminated sediments. Although foliar uptake has been previously demonstrated for some species of aquatic plants, most models of the transport and fate of Cs in lakes do not contain components and pathways that consider aquatic plants. We anticipated that this absence might be contributing to a lack of accuracy in some Chernobyl-derived models that predict the long term dynamics of cesium in aquatic systems. We found that aquatic plants can indeed have an important influence on Cs dynamics within a lake, and that foliar uptake was the principal absorption mechanism for four common species of aquatic plants (*Brasenia schreberi, Nymphaea odorata, Myriophyllum spicatum* and *Utricularia inflata*), but was not important in *Typha latifolia, Alternanthera philozeroides* or *Sagittaria latifolia.*

Following the Chernobyl accident ^{137}Cs concentrations in fish were 200 to 20,000 times greater than that in the water. Because freshwater fish are a significant part of the European diet, this lead to ^{137}Cs radiation exposure to humans. Our stable Cs research showed (1) declining concentrations in the water due to uptake by biota and deposition onto sediments, and (2) continuing accumulation in largemouth bass (*Micropterus salmoides*), a common food and game fish. Mathematical modeling was used to estimate that ratios of fish concentrations to water concentrations at equilibrium would be approximately 70,000. This value is greater than those in European lakes. The greater ratio is due to the lower concentrations of potassium in the water of Southeastern lakes.

(E) Chemical speciation in the environment

Investigators: B.P. Jackson, W.A. Hopkins, T. Punshon (CRESP), C.S. Romanek, and P.M. Bertsch

Knowledge of chemical speciation is imperative to understanding mechanisms of toxicity or tolerance to contaminants in organisms. Plants and bacteria can produce extracellular ligands either to enhance uptake of an otherwise unavailable element or to limit uptake of a potential toxicant. We have developed methods

based on interfacing a separation procedure such as field flow fractionation or liquid chromatography with inductively coupled plasma mass spectrometry to investigate trace element speciation in environmental and biological samples in support of a number of research areas at SREL:

Selenium contamination of soils and sediments

SREL studies have focused on Se contamination arising as a result of coal combustion where Se is input into the system in inorganic form either as selenate or selenite. However, plants readily convert inorganic Se to selenomethionine, which is bioavailable to higher organisms and may cause toxicity at elevated concentrations. Additionally, numerous other low molecular weight organoselenium compounds have been identified, although little is known regarding their chemical reactivity and toxicity. We tested a number of different reagents for effectiveness in extracting Se compounds from bullfrogs (*Rana catesbeiana*) collected from a coal fly ash settling basin. These extracts were analyzed by size exclusion chromatography (SEC)-ICP-MS to determine the molecular weight of Se compounds in the extract. If SEC-ICP-MS identified low molecular weight Se compounds (<1000 Da), then the extract was analyzed by ion chromatography (IC)-ICP-MS to identify the compound. SEC-ICP-MS analysis using extractants that do not degrade proteins indicated that Se was a constituent of a high molecular weight compound (>30,000 Da). The use of protease or chitinase as extractants, two enzymes that degrade proteins to the constituent amino-acids, liberated predominantly low molecular weight Se compounds that were identified as selenomethionine by IC-ICP-MS. Future work will focus on determining Se speciation in the sediment and food resources of bullfrogs and in higher trophic organisms. Results from this study will be presented at an international Plasma Mass Spectrometry conference in Durham, England, and a publication will be submitted to a peer reviewed journal in FY05.

This work has been completed, and was presented at the International Plasma Mass Spectrometry Conference in Durham, England, in September 2004. A manuscript has been accepted for publication in the proceeding volume for the conference. (*Jackson, B.P., et al. In Press. Selenium speciation in amphibian larvae developing in a coal fly ash settling basin. Proceedings of the 9th International Conference on Plasma Source Mass Spectrometry, Durham UK, 2004*).

Arsenic and selenium speciation in soils

Arsenic and selenium have several stable oxidation states and can exist in a number of low molecular weight organic complexes. These different species exhibit differing reactivity, toxicity, and availability, so it is important to quantify As and Se on a species rather than a total element basis. Ion exchange chromatographic methods have been developed to quantify different As and Se species that can occur in environmental samples. This methodology was applied to study residual As and Se in a soil profile five years after application of flue gas desulfurized gypsum stabilized with coal fly ash (both coal combustion residues). Arsenic was found to have not significantly leached from the 0-15 cm soil profile but Se had significantly leached over 5 years. Increased water soluble Se concentrations persisted five years after application. Increased As concentrations were manifest in the ligand exchangeable fraction. The main species detected were arsenate and selenite, for As and Se respectively. Plant uptake of Se was significantly increased in direct relation to initial treatment, with selenium present as selenate in plant tissue extracts collected from invasive plants growing on the treated soils. The one-time soil amendment with coal combustion by-products was shown to have long-term effects on soil As and Se concentrations and increased Se solubility and availability five years after application. The results of this study suggest that repeated application of coal combustion byproducts to soil could be detrimental due to build up of As in the soil profile and the long-term availability of Se. The results of this study were presented at the International Conference on the Biogeochemistry of Trace Elements

in Uppsala, Sweden, in July 2003 and will be published in an upcoming proceedings volume.

This work is complete, the manuscript has been submitted, and the proceedings volume is in press. (*Punshon, T., et al. Arsenic and Selenium Speciation in Aged, FGD-Amended soil. In: Coal Combustion Products and Environmental Issues. Sajwan, K.S., I. Twardowska, T. Punshon, and A.K. Alva (Eds). Springer-Verlag Publishers, New York, USA*).

Trace metal complexation by low molecular weight organic acids

High concentrations of Ni and U exist in the sediments of Steeds Pond and Tims Branch at the SRS. This riparian site is the focus of a number of research projects at SREL studying the solid phase speciation, bioavailability, and trophic transfer of Ni and U. Previous studies have shown that Ni is bioavailable at this site with increased concentrations detected in several plant species. Various low molecular weight organic acids, notably citric acid and histadine, are known to be important complexers of Ni in plants. We have investigated analytical techniques to identify and quantify Ni-organic ligands in the rhizosphere and plant extracts from Steeds Pond/Tims Branch. Using high performance size exclusion columns we have separated Ni-oxalate, -EDTA, -citrate, and -histadine, although the retention time for each species was not solely based on molecular weight. Analysis of a plant extract from Tims Branch showed that Ni was complexed in a low molecular weight compound, most likely Ni-citrate. We are continuing to study the use of SEC-ICP-MS for the separation of low molecular weight metal-organic ligand complexes to characterize the effect of the gel phase on the stability of the metal-ligand complex with the goal of using the technique for unambiguous speciation data of metal-ligand interactions in biological samples.

Preliminary results of Ni and U speciation in plant extracts using these techniques were presented at the 8th International Conference on the Biogeochemistry of Trace Elements, Adelaide, Australia, March 2005. The techniques of using ion chromatography and size exclusion chromatography coupled to ICP-MS to study metal interactions with low molecular weight acids are promising and our preliminary results suggest that further work in this area is warranted.

Laser Ablation ICP-MS

Laser ablation ICP-MS is being used with increased frequency in the environmental sciences because of the spatial control afforded by laser sampling, the reduced sample size requirements, and the ability to sample the solid phase directly without needing to digest the material. This technology was employed to study the trace element content of mussel shells collected from unimpacted and polluted streams on the SRS. Various standardization protocols are being developed to relate the trace element geochemistry of shell carbonate to laboratory standards of similar mineralogy. In addition, a micro-digestion technique was developed to ground truth laser sampling results with ultra small volumes of carbonate that were mechanically removed and dissolved for conventional ICP-MS analysis. Trace element analysis of shell material from the umbo to the outer shell margin will provide a near continuous record of exposure over the time period of shell growth. Stable carbon and oxygen isotope analyses of material collected over an adjacent area provide an independent measure of shell chronology that will be tied to historical records of stream chemistry that reside in various SRS databases. This work will provide information on the factors that influence contaminant exposure and transport in fluvial systems of the SRS.

Laser ablation ICP-MS was also employed to study soil particle resuspension to understory foliage at Tims Branch. High concentrations of U were found in the leaf tissue of understory plants that were not observed in other plant samples collected from the site. We used LA-ICP-MS to directly analyze leaf samples taken from the site. By optimizing the laser power used for the ablation we were able to adjust the time for ablation through the leaf to 1 minute. During this ablation process elemental data were continuously collected

by ICP-MS to create an element profile with depth. Uranium was found to be associated with the leaf surface, consistent with U being bound to sediment particles adhering to the leaf surface. Nickel was more evenly distributed throughout the leaf, consistent with uptake of Ni by the plant. Washing removed 80% of U and 32% of Ni, further substantiating the differential partitioning of these two elements in the understory leaves. The results of this study were published in the *Journal of Environmental Monitoring*.

A number of studies using laser ablation ICP-MS have been on-going during the last year. Work on using mussel shells as an indicator of contamination exposure is continuing. Other studies have used LA-ICP-MS to map the 2-D distribution of Fe, Cu, and Zn in sections of cryofrozen biological soft tissue; the preliminary results on brain thin sections are promising and an analytical methods paper is in preparation. It is anticipated that these methods can be applied to examine contaminant distribution and localization in exposed organisms as a complementary technique to PIXE, SEM, and SXRF.

Field flow Fractionation

Field flow fractionation (FFF) has similarities to chromatography in that it is an analytical separation technique that separates the constituents of a sample based on their hydrodynamic radius. Flow-FFF is a very versatile technique and, depending on the carrier and cross flow rates, it can be used to separate biomolecules or colloidal particles. By coupling flow-FFF to ICP-MS it is possible to determine the elemental constituents of eluting particles in addition to the size information that can be gained from UV detection after calibration of the Fl-FFF technique. We have employed size exclusion chromatography and Fl-FFF coupled to ICP-MS to study the speciation of U in sediment porewaters taken from Steeds Pond. Size exclusion chromatography-ICP-MS revealed that a large fraction of 'soluble' U was excluded from the column, which indicates that U is associated with constituents of the solution phase >30,000 Da. The distribution was bimodal and in addition to the excluded fraction, U was also bound by dissolved organic matter (DOM) of approximately 1000 Da; lower concentrations of Ni were also associated with DOM. Over 80% of soluble U eluted from the SEC column either in the excluded volume or bound by DOM, hence the >0.22 µm fraction that is ostensibly the dissolved fraction actually has very little 'free' uranyl cation in solution. The high Al signal in the excluded peak concurrent with the U peak suggested that this phase may be an inorganic colloid. To investigate this observation further, Fl-FFF was used. This technique was calibrated using sized polystyrene beads to allow accurate sizing of the colloidal particle. Fl-FFF-ICP-MS showed that the colloidal particle was approximately 0.1 µm and contained Al, U, Mn, and Fe.

Flow field flow fractionation (Fl FFF) coupled to ICP-MS was used to study the binding of Ni and U to humic acid and colloidal phases in contaminated sediment porewaters. Results of this study were presented at the 8th International Conference on the Biogeochemistry of Trace Elements, Adelaide, Australia, March 2005. A manuscript of this work was prepared, submitted and published during 2005. (*Jackson, B.P. et al. 2005. Characterization of colloidal and humic-bound Ni and U in the 'dissolved' fraction of contaminated sediment extracts. Environmental Science and Technology. 39, 2478-2485;* SREL reprint 2827).

Flow FFF-ICP-MS was used as an on-line technique to study sorption and pH isotherms of U binding to bacteria. A manuscript of this work was prepared, submitted and published during 2005. *(Jackson,B.P., J.F. Ranville, and A.L. Neal. 2005. Application of flow field flow fractionation-ICP-MS for the study of uranium binding in bacterial cell suspensions. Analytical Chemistry. 77:1393-1397;* SREL reprint 2812*)*.

(F) Use of Monitored Natural Attenuation for chlorinated solvent remediation
Investigators: L.A. Newman and faculty at University of South Carolina

Study of this system is ongoing. We have had some unexpected metabolites show up in the trunks of trees that are being examined on the site, and have started more frequent monitoring of the system to determine what is happening. We have started sampling the deeper aquifer to have more frequent samples to check to chlorinated solvent metabolites. We are also doing DNA extractions, which will be analyzed by microarray to test for the presence of solvent-degrading organisms in the soil and groundwater. This work has been presented by the student at the Association for Environmental Health Sciences annual conference (2004), and the EPA International Phytotechnologies meeting (2005).

Question 4:
How are bioavailability, bioconcentration, and biomagnification related, and what extrinsic and intrinsic properties of systems control these processes?

(A) Modeling cesium dynamics in an SRS cooling pond
Investigators: B.E. Taylor and T.G. Hinton

Radioactive cesium has been an important constituent of the contaminants released accidentally from nuclear reactors. Twenty years after the releases to Pond B on the SRS were stopped, 99% of the radioactivity was due to ^{137}Cs, and 99% of the cesium was in the sediments, where it is adsorbed onto clays. However, this adsorption is reversible, particularly under anoxic conditions (ammonia competes with cesium for binding sites on clays), and about 1% of the cesium was distributed among macrophytes, water, seston, and animals. A group headed by Tom Hinton and John Pinder designed a tracer experiment to elucidate dynamics of cesium in Pond 4, another impoundment on the SRS. Intensive sampling included sediments, water, and biota. This part of the project focused on invertebrates. Our objectives were to measure rates of accumulation by invertebrates and to infer pathways of uptake. In the tracer experiment, we monitored larvae of the phantom midge *Chaoborus punctipennis*, a planktonic predator, and the snail *Helisoma trivolvis*, a littoral consumer. Before the experiment, we sampled the plankton and benthos on a quarterly basis for a year and a half to characterize composition and abundances of the invertebrates. In shallow water, molluscs, including *Helisoma*, were the most abundant macroinvertebrates (median 90 animals·m^{-2}). In deep water, macroinvertebrates were sparse, and *Chaoborus* was the most abundant dipteran (median 4200 animals·m^{-2}). We also collected common taxa of invertebrates and vegetation for analysis of stable isotopes of carbon and nitrogen to provide further information about trophic pathways. Further analysis of these data was completed in FY05.

The experiment revealed rapid uptake and accumulation of cesium (due to regulatory issues, ^{133}Cs, which is stable, was used an analog for ^{137}Cs). Ten days after tracer was added to the epilimnion of the pond in August 1999, cesium concentrations were two orders of magnitude higher in the snail than in water and an order of magnitude higher in *Chaoborus* than in water. Stable isotope data indicated that algae and submerged macrophytes were likely trophic sources for *Helisoma*, even though emergent and floating-leaved macrophytes constitute the bulk of the biomass in the pond. *Chaoborus* were likely feeding mainly on planktonic cladocerans, which feed in turn on planktonic algae and other small particles. Through late fall, concentrations in both taxa changed in parallel with concentrations in the water. However, in spring and summer of the following year, concentrations in both invertebrates doubled, while water concentrations continued generally to decline. Clearly, the concentration of the contaminant in water does not fully govern its availability to the biota. Submissions of two manuscripts from this project are scheduled for FY06.

(B) The potential of red-eared sliders to serve as chronicled indicators of contaminant exposure
Investigators: P.M. Bertsch, B.P. Jackson, T. Hinton, W. Hopkins, and collaborators from the University of South Dakota

With increased interest in the use of indigenous organisms as indicators of environmental

contamination, it has been suggested that turtles would be ideal indicators of metal contamination. They are extremely long-lived, have a wide geographic distribution, occupy a variety of habitats, appear to be relatively tolerant to a range of pollutants, and have shells comprised of bone (apatite), which is a well-known target organ for a number of transition and heavier elements. Furthermore, turtle shells exhibit growth annuli for both the bone and protein (keratin) sheath coating, within which periodic growth deposition bands are discernible. This provides the potential to examine historical information related to metal, metalloid, and radionuclide exposure, akin to dendroanalysis. Our initial studies using spatially resolved synchrotron-based X-ray microanalysis have demonstrated that turtles collected from contaminated environments associated with a former nuclear materials processing facility and a coal combustion waste repository have spatial heterogeneity of Ni, Se, As, and other metals and metalloids, which have been hypothesized to be related to specific exposure events.

Controlled laboratory experiments, using a known contaminant dose over a standard time frame, would offer valuable information supporting the use of turtles as bioindicators. To further explore this idea, 500 hatchling red-eared sliders (*Trachemys scripta elegans*) were obtained in June 2003. The hatchlings were divided evenly into twelve tanks and each tank was assigned to one of four treatment groups. With the exception of the winter months (exotherm inactive periods), turtles have been dosed with nickel citrate ($Ni_3(C_6H_5O_7)_2 \cdot XH_2O$) on a bi-monthly gavaging regime since July 2003. The nickel is administered orally using a transfer pipette, and regurgitation events are recorded. Measurements of individuals (carapace length and width, plastron width and length, and mass) were taken upon initial receipt of turtles and prior to all gavaging events.

All measurements indicate notable growth of surviving turtles regardless of treatment group. Marginal bone fragments, plastron segments, and keratin scute sheaths have been sectioned from individuals in the two highest dosing groups, and control tanks, and analyzed using synchrotron-based X-ray microanalysis at beamline X26-A at the National Synchrotron Light Source (Brookhaven National Laboratory, Upton, NY). Results indicate spatially variable higher nickel concentrations in higher dosing group turtles in comparison to lower and control group individuals, and a gradient of nickel levels is apparent in accordance with dosing. In addition, preliminary ICP-MS and HRTGA analyses have been conducted using marginal scute sections. Additional trials of all analytical techniques, including synchrotron-based microanalysis, ICP-MS (including laser ablation), and HRTGA, are necessary for more conclusive results. The bi-monthly gavaging regime is planned for another growth season (through October 2005), at which time individuals will be sacrificed and additional analyses conducted. After the first round of gavaging, photos were taken of each individual for use in future analyses of fluctuating asymmetry. In addition, DNA strand breakage analyses will be conducted during the winter of 2005-2006. With the combined results of the above techniques, it will be possible to further support the use of turtles as bioindicators through development of a spatial-time distribution of nickel dosing events.

Preliminary results were presented at the Joint Meeting of Ichthyologists and Herpetologists (May 2004). The concept of the experiment was presented, as well as evidence detailing the utility of Synchrotron-based technology in this project and results indicating deposition patterns of contaminants in bone sections. An update will be presented at the Joint Meeting this year (July 2005) which will go into further detail on quantities and patterns of nickel present in accordance with dosing levels, as well as results from HRTGA and ICP-MS analyses.

(C) Processes controlling the distribution of metals in trees inhabiting contaminated environments

Investigators: T. Punshon (CRESP), P.M. Bertsch, B.P. Jackson, S. Harper, and collaborators from the University of Chicago

This project was completed in FY04.

(D) Using gray foxes as indicators of contaminant distributions and trophic level relationships
Investigators: I.L. Brisbin, Jr., C.S. Romanek, B.P. Jackson, and collaborators at the University of South Dakota

During the past year a spatially explicit model was completed for the gray fox (*Urocyon cinereoargenteus*) at the SRS. This model is one of several that have now been developed for various wildlife species at the site for use in ecological risk assessment. This now allows this widely distributed and omnivorous canid to serve as a "receptor species" for studies and evaluations of the fate and effects of site contaminants in the higher trophic levels of natural terrestrial food webs on the site.

Previously collected data describing the relationships between contaminant levels and stable isotope profiles for carbon and nitrogen in gray foxes collected on vs. off of the SRS have now been analyzed. A draft manuscript has been written on these relationships and is now being reviewed prior to being submitted for publication. In addition, using historical and current museum specimens, over 150 hair samples have been collected from gray foxes from throughout their range, along with comparable hair samples from red foxes (*Vulpes fulva*) and coyotes (*Canis latrans*). These hair samples are being analyzed for stable isotopes of carbon and nitrogen to determine whether the trophic relationships of the two fox species have been altered since the time of the unprecedented range expansion of the coyote into the Southeast during the past 20 years.

Widely held opinions have suggested that the coyote may now have become an effective trophic competitor for these other two canid species and that the trophic role of the gray fox at the SRS (and hence its role as a receptor species in ecological risk assessment) may be in the process of being altered as a result of the arrival and establishment of the coyote in the natural terrestrial ecosystems of the SRS.

(E) Patterns and extent of contamination in American alligators from the southeastern U.S.
Investigators: I.L. Brisbin, Jr., C.H. Jagoe, T.C. Glenn, and C.S. Romanek

This work had not been projected to continue through FY05. However an opportunity was provided to submit an invited paper for publication summarizing our 35+ years of study of alligator populations on the SRS. This paper emphasized studies evaluating the effects of reservoir impoundment and management upon the alligator population inhabiting the Par Pond reactor cooling reservoir. A partial reservoir drawdown in 1991-1994 proved to be the major source of negative impacts upon the population while radionuclide contamination of alligator meat was not of concern.

As is the case elsewhere throughout the alligator's range in the Southeast, however, levels of mercury contamination of alligator meat could, under some circumstances, exceed those generally considered safe for human consumption. Two papers describing this problem have now been prepared and submitted for presentation at a national environmental toxicology meeting, and both continue to be prepared for publication. In one of these papers, mercury levels in meat from SRS alligators were lower than those collected from an area near a polluted Superfund site in south Georgia, while lowest mercury levels were found in meat of alligators from Louisiana and alligator farms.

Question 5:
What types of mathematical or statistical models best describe contaminant distributions and the process of contaminant uptake and accumulation in biota and their habitats? Does the selection of the best model make a difference in terms of decisions about how (or if) to remediate contaminated areas?

(A) Modeling spatial distributions of cesium in reservoir sediments
Investigators: S.J. Harper, T.G. Hinton, and M.D. Wilson

Relatively little is known about the spatial distribution of radionuclides within cooling reservoirs. To characterize and predict the distribution and dynamics of such contaminants, a spatially-explicit modeling approach is needed. Surveys on the SRS indicate that the vast majority of ^{137}Cs contamination is confined to the sediment of former cooling reservoirs, though tremendous variability in concentrations is observed among sample locations. Much of this variation is likely attributable to underlying spatial heterogeneity in sediment characteristics, as strong gradients in depth, slope, and soil texture can exist in these man-made systems. Given this variability, statistical power analyses indicate that it could take thousands of sediment samples to detect significant changes in radionuclide inventories over time due to remediation efforts or natural attenuation. Thus, while the vast majority of radionuclide contamination is contained within sediment, it is very difficult to quantify spatial distributions and document subsequent declines in contamination. Advances in the field of hydroacoustics afford a technological solution to this problem. Hydroacoustic sampling provides enhanced statistical power to determine concentration distributions by accounting for the spatial variability in factors known to influence radionuclide dynamics, including bathymetric features (depth, slope) and sediment characteristics (soil texture, water content, organic matter).

The merit of this spatial modeling approach was demonstrated for ^{137}Cs in Pond B at the SRS. We used multiple transducer frequencies during extensive hydroacoustic surveys to provide detailed spatial information for bathymetric features (e.g., depth, distance from bank, and slope), lake-bottom sediment class types, and submerged aquatic vegetation. Based upon these maps, point-based sediment samples were collected across observed ranges in depth, sediment type, and slope. Laboratory analyses of these samples were conducted to quantify ^{137}Cs concentrations, moisture content, organic content, and texture (percent sand, silt, and clay) of sediments. Significant linear and nonlinear relationships were found, and were especially strong among soil moisture, organic content, and ^{137}Cs. Extensive sampling of these correlative factors via hydroacoustics allowed a full-coverage map of ^{137}Cs to be generated. In FY05 we strengthened and validated the model developed for Pond B by repeating our detailed hydroacoustic surveys and laboratory analyses. Results indicated that hydroacoustic-derived predictors of bathymetric and sediment characteristics were very reliable and consistent among sample dates. In addition, results indicated significant redistribution of ^{137}Cs in surficial sediments occurred due to focusing near the bottom of slopes. Resuspension and redistribution due to effects of wind or other extrinsic factors was not detected over this relatively short time period. Continuation of this research could provide greater understanding of the distributions of radionuclides within reservoirs, and thus contribute towards better resource management. Such models should prove useful for documenting and predicting contamination declines, predicting responses of contaminants to reservoir draw down or environmental change, and enhancing estimates of exposure risks for target fishes and wildlife species that utilize these systems.

(B) Use of non-normal statistical models to analyze environmental contaminant data

Investigators: C.H. Jagoe, M.D. Wilson, I.L. Brisbin, Jr., T.C. Glenn, and colleagues from The University of Georgia and the National Institutes of Health

Many statistical models assume that variables are normally distributed. These models are powerful and well developed, but assumptions of normality are frequently not met in environmental sampling. In fact, frequency distributions of contaminants in organisms often appear non-normal, and may be highly skewed. Selection of appropriate data analysis techniques requires knowledge of underlying distributions and their properties. Probability-based risk assessment models are also affected by the underlying frequency distributions of the input data. For example, widely used modeling tools such as Crystal Ball (Decisioneering, Inc.) typically require specification of the frequency distribution of variables for probabilistic simulations. We have published several

papers describing radiocesium distributions in biota, and demonstrated that these are best fitted using non-normal models (lognormal or Weibull). This work extends this analysis to other contaminants, particularly heavy metals such as mercury. We identified representative data sets that included tissue contaminant concentrations from both vertebrates and invertebrates, from on the SRS and from other areas. Data were tested for normality and lognormality using Lillefors tests. Contaminant distributions were non-normal in all the data sets tested to date. In some cases, the data were lognormally distributed. This project interfaces with work on bootstrap goodness of fit test reported in the effects and risk assessment under section 9. A publication related to this work, demonstrating a preferred method for calculating average radiation dose to groups of individuals in currently in press in the *Journal of Radiological Protection*. We anticipate that these studies will yield additional manuscripts for submission later this year. We will continue such goodness of fit analyses in FY06, and incorporate additional data from existing data sets.

Question 6:
Can sentinel species be used as surrogates to determine environmental health, and can this information be used to determine whether remediation is needed in a given area?

(A) Using sentinel species to monitor the effects of environmental contaminants on biota
Investigators: I.L. Brisbin, Jr., C.H. Jagoe, and C.S. Romanek

With the refocusing of the SREL mission to confine field work on contaminants to several specific sites on the SRS, it has been determined that none of the potential sentinel species that were being considered during the previous year of this work would be appropriate for use in such areas. Accordingly, plans to release and study sentinel populations in contaminated habitats on the SRS have now been placed in abeyance. However, an opportunity was provided to prepare an invited paper for publication describing long-term studies designed to evaluate the potential for radio-telemetered eastern box turtles (*Terrapene carolina*) to serve as a multiply recapturable free-ranging surrogate to evaluate contaminant bioavailability in contaminated SRS habitats. Because of the large size and long time frame of the data base involved, this paper was able, we believe for the first time ever, to estimate cohort survival simultaneously by two independent methods: standard mark-and-recapture analysis and a modified mark-and-recapture method based on long-term documentation of the survival and fates of turtles carrying radio transmitters. This paper has now been completed and submitted to the editors of the invited publication.

(B) Examination of a small mammal community in the contaminated Tims Branch watershed
Investigators: I.L. Brisbin, Jr., C.S. Romanek, C.H. Jagoe, B.P. Jackson, and collaborators at the University of South Dakota

A Master's thesis has now been completed and successfully defended dealing with the relationship of heavy metal contamination to trophic position (as revealed by stable isotope analyses) within small mammal communities inhabiting contaminated habitats on the SRS and off-site control areas. This thesis, *The Use of Small Mammals as Indicators of Heavy Metal Bioavailability in a Contaminated Riparian Zone* (University of Georgia, 2003), is now in the process of being edited into a format for submission for publication in a peer-reviewed toxicology journal. This process should be complete and the paper submitted by the end of summer, 2005, since an off-site collaborator from the University of South Dakota is spending part of the summer at the SRS and will be working with the other authors on this paper. This thesis research found no statistically significant relationship between trophic position and levels of metal body burdens in the small mammals sampled. This suggests that some environmental, demographic, or perhaps seasonal factor is working to confound what would normally be expected to be a pattern of increasing levels of metals contamination at higher trophic levels.

(C) Analyses of contaminant levels in feral swine from the SRS

Investigators: I.L. Brisbin, Jr., C.S. Romanek, B.P. Jackson, and collaborators at the University of South Dakota.

During the past year heavy metal analyses were completed on feral swine muscle samples for which radiocesium levels had already been determined. The majority of stable isotope analyses have now also been completed and the remainder should be completed within the next few months. At that time final data analyses will be undertaken and a manuscript will be prepared for publication. In the meantime a paper describing a spatially explicit model for the wild hog as a receptor species for ecological risk assessment activities at the SRS has been revised with regard to reviewers' comments and resubmitted for consideration for publication. In addition, an invited paper has been completed and submitted for publication describing the importance of long-term data bases in the development of management plans for free-ranging wild hogs. This paper was presented during the previous year and will now appear as part of the published proceedings of an SRS-sponsored symposium on the ecology and management of wild hogs.

(D) Herpetofaunal diversity on the SRS

Investigators: J.W. Gibbons and collaborators

Long-term data on the herpetofaunal distribution and abundance patterns on the SRS will prove to be helpful in addressing remediation and restoration goals. By continuing to collect long-term data and then using those data sets in conjunction with GIS coverage of SRS waste sites, we can develop simple models to estimate how many species occur in waste sites of particular size and habitat characteristics.

Long-term data have been collected at many sites on the SRS—most noteworthy are Rainbow Bay (RB; continuous data since 1978), Ellenton Bay (EB; 20 yrs of data since 1975), Ginger's Bay (GB; yearly data since 1986). These continuous or nearly continuous records are supplemented by shorter duration studies that encompass a broader range of habitats, such as snake sightings on SRS roads. In recent years we have also used outside funding from the National Science Foundation (NSF) and other agencies and foundations to sample additional SRS locales (e.g., four Carolina bay wetlands that are part of the NSF "Land Use Effects on Amphibian Populations" study) and additional species (e.g., studies of gopher tortoise relocation funded by the Department of Defense and the U.S. Forest Service).

In FY 2005 we continued sampling efforts at RB (which is the longest running amphibian study in the world) for all herp species and at GB and EB for select species. At RB to date more than 600,000 captures of 75 reptile and amphibian species have been recorded. These data were integral to two manuscripts currently in press (*Taylor, B.E., D.E. Scott, and J.W. Gibbons. Coupling catastrophic reproductive failure and terrestrial survival: Implications for management of pond-breeding salamanders. Conservation Biology*; and *Dixon, P. and J.J.K. Pechmann. A Statistical Test to Show Negligible Trend. Ecology*) and one submitted to *Ecology* (*Daszak, P., et al. Chytridiomycosis does not cause population declines in long-term monitored amphibian populations at the Savannah River Site*). Recent capture data from EB during FY 2004 were used to prepare and submit the manuscript *How Productive Can an Isolated Wetland Be? Remarkable Amphibian Biomass and Abundance* by J. W. Gibbons et al.

Accurate assessment of distribution and abundance data for all species of herpetofauna requires extensive compilation and consolidation of all data sets collected since 1951. In FY05 we continued gathering historical data from SRS inventory and research projects, verifying data set records, and reformatting the data as necessary so that individual files are compatible. We currently have completed the SRS turtle dataset through FY05 (beginning with captures in 1967; 12 species, 35,084 captures and recaptures). Several large snake data sets have been reformatted and

merged that include 7,419 captures of 34 species from 1967 through 1988. We have also added several smaller data sets, including ones from 1951-1955, and from 1989-2004, to bring the snake records above 16,000 captures. More than one million amphibian captures from RB, EB, and GB are currently being compiled in addition to other amphibian projects being obtained through direct contact with former SREL faculty and students. The consolidated database will include captures of all representative SRS herpetofauna, but several rare, unusual, and potential receptor species have been selected for in-depth study of demographics, habitat preferences, and distribution patterns. These include the spotted turtle, gopher frog, tiger salamander, pine snake, and southern hognose snake, all of which have been recognized as sensitive species in need of protection at the federal level. Other species will be added to this list as finalized datasets are analyzed.

Question 7:
What effective techniques permit studies of bacterial community structure and function without isolation? How can these techniques be applied to studies of contaminant dynamics or other environmental problems?

(A) Fatty acids as an indicator of microbial diversity in environmental samples
Investigators: C.L. Zhang, G.L. Mills, and C.S. Romanek

Membrane phospholipids are one of the essential components of microbes that function at the interface between the intracellular biochemical cycling and the external environments. Phospholipids are sensitive to environmental changes and may vary in relative abundance and structures. These features can be recognized as biomarkers, which, along with their isotopic composition, can provide information about their chemical taxonomy, type of carbon cycling and environmental nutrition. The SRS has been an important place to observe the impacts of heavy metals and radionuclides on local ecology. This study aimed to determine environmental factors that may have impacts on the microbial cell membrane.

Surface soil, pond sediment, stream mud, and sand samples were collected from Beaver Pond, Beaver Dam, and soil at the Lower Tims Branch on the SRS. Samples were freeze-dried before lipid extraction. A fraction of the sample was extracted by a mixture of chloroform-methanol-H_2O for total lipids. The total lipids were fractioned into polar, neutral, and glycol lipids by organic solvents with different polarities on a silicic column. The polar fraction of lipids was further methylated for gas chromatographic or gas chromatographic-mass spectroscopy analyses. Information about lipid profiles and single lipid molecular structures was analyzed. The carbon isotopic compositions of single molecular fatty acids were also measured.

Generally, the SRS samples have unusually high numbers of phospholipid fatty acids (PLFA). More than 50 fatty acids with different structures and carbon numbers could be discriminated. Among them, the strain chains C_{16}, C_{18} are most common. Terminal branched and unsaturated lipids were also significant. Monounsaturated C_{16} and C_{18} were common in all samples, with some samples containing a small fraction of monounsaturated C_{17} chains. Iron-reducing, sulfate-reducing, and photoautotrophic microorganisms were expected to contribute to the lipid pool in these samples. The carbon-isotope profiles indicated considerable variation between fatty acids within one sample as well as between samples. The Beaver Pond and Creek mud have lower ^{13}C than the soil samples, which may indicate more extensive *in situ* carbon cycling in the aquatic environment than in the soil environment.

This is an ongoing project. The phospholipid data will finally be combined to the molecular data to allow us to have a better understanding of carbon flow in this environment. Consequently the microbial metabolism of Fe and S will also be elucidated. These links are important in microbial remediation because the iron (oxyhydr) oxides and iron sulfur compounds are important carriers of radionuclides, whose trans-

formation requires coupling between carbon, sulfur, and iron cycling.

(B) Screening microbial communities to determine the effects of contaminant exposure on antibiotic resistance

Investigators: JV. McArthur, T.C. Glenn, and C.H. Jagoe

Industrial contamination of aquatic systems is a major problem on DOE properties. Included in this contamination are significant amounts of heavy metals and organic pollutants. From past research we have shown that bacterial exposure to heavy metal contamination at field concentrations appears to select for increased incidences of antibiotic resistance. We have sought to determine whether indirect selection can result in increased levels of antibiotic resistance and to elucidate the mechanisms involved. Considering that <1-10% of bacteria in nature can be cultured, we have developed culture-independent methods based on flow cytometry that allow the screening of complete microbial assemblages. Existing cultivation techniques target only a minor fraction of natural bacteria. Therefore it is most likely that plating experiments fail to account for and to identify most of bacteria resistant to antibiotics and heavy metals in natural samples. To overcome this methodological limitation, we have developed a combination of cutting-edge culture-independent techniques to separate and identify resistant and sensitive bacterial cells. We have begun a series of experiments investigating the role of macroinvertebrates as "hot spots" of microbial activity to harbor and promote antibiotic resistance. Meredith Wright, a Ph.D. student working on this project, has received an EPA Star Fellowship to study the role of macroinvertebrates in the spread of antibiotic resistance in bacteria. She has found that bacteria associated with *Corbicula*, an introduced bivalve, in contaminated streams on the SRS have higher proportions of antibiotic resistance than do *Corbicula* in uncontaminated streams.

Another graduate student, Ginger Humphries, is using culture-independent techniques based on flow cytometry to study interactions of mercury and selenium exposure in bacteria from Savannah River water. In eukaryotes, small amounts of selenium can reduce or ameliorate mercury toxicity. This study will test whether selenium has similar effects on mercury toxicity in prokaryote communities, and whether selenium reduces the development of antibiotic resistance associated with mercury exposure. This project thus links both characterization of the development of antibiotic resistance with a basic toxicological study.

(C) Evaluating the antibiotic resistance of known waterborne pathogens *E. coli* and *Vibrio* sp. in the environment

Investigators: JV. McArthur, T.C. Glenn, and C.H. Jagoe

We sampled sediment and water from Shipyard Creek, Charleston, SC in March and June and the ACE Basin in June and isolated *E. coli* and vibrios using standard methods. These cultures will be screened for antibiotic resistant by the NOAA Charleston lab. DNA extracted from all of the isolates and from bacteria in the sediments has been amplified by PCR. Integrase primers have been used to detect occurrence of the integrase gene. In addition to the microbiological sampling we have collected samples from the sediment and water at each site for analysis of metal content, dissolved organic carbon, antibiotic content, and nutrient analysis.

Question 8:

How do seasonal variations or episodic perturbations influence contaminant speciation, bioavailability and accumulation?

(A) Bioavailability of metals in relation to wetland hydroperiod

Investigators: C.H. Jagoe, A.L. Bryan Jr., and I.L. Brisbin Jr.

Both natural and man-made wetland systems are subject to periodic flooding and drying. Such flooding

and drying can be due to seasonal variations, environmental factors such as beaver dams or erosion, or human activities such as wetland restoration or dam construction. Intermittent flooding, whether frequent of infrequent, can influence metal speciation. Previous work, including studies at SRS locations, suggested that mercury bioavailability and accumulation is related to hydroperiod (relative amount of time that a wetland contains water) and intermittent flooding of soils and sediments. In preliminary laboratory experiments with crayfish (*Procambarus clarkii*), repeated flooding and drying of mesocosms increased bioavailability of some metals. In FY05 we continued collection of samples of biota from Carolina bays (intermittently flooded wetlands on the coastal plain), where previous studies had demonstrated a relationship between bay hydroperiod and fish mercury concentrations. Surveys of amphibian larvae did not indicate similar relationships. Differences in accumulation patterns among fish and amphibians may reflect differences in the form of mercury accumulated by these organisms. This reflects the importance of metal speciation to bioavailability. Plans to place crayfish in bays with different hydroperiods to compare measure metal uptake over time were postponed indefinitely in FY05 because of the realignment of SREL research activities. We developed a proposal to look at effects of temporary impoundments on the dynamics of contaminants in a contaminated stream. This proposal seeks to expand studies on hydroperiod to examine the role of impoundments in contaminant sequestration, speciation, and mobilization. We began sampling native biota from free-flowing and impounded sections of streams on SRS with historic inputs of contaminants to gather preliminary data for the proposed activities. Results of these studies will allow evaluation of potential impacts of wetland remediation projects, particularly those where water levels vary annually or seasonally.

Question 9:
How can conservation plans for amphibian populations provide insurance against rare but catastrophic events?

(A) Modeling population dynamics of an amphibian species to encourage sound natural resource management

Investigators: B. Taylor and J.W. Gibbons

The fundamental challenge of population ecology is to identify and quantify the factors needed for reliable understanding and sound management. Processes critical to the success of a population may operate at spatial and temporal scales outside the scope of ordinary field studies. The long history of ecological studies at the SRS provides data that can be used to address such issues. Wide variation in reproductive success is common among amphibians that breed in seasonal ponds, but persistence of adults can buffer against these fluctuations, particularly for long-lived species such as the marbled salamander (*Ambystoma opacum*). We hypothesized that the frequent episodes of catastrophic failure observed in a 24-year field study at Rainbow Bay in South Carolina would enhance the importance of high terrestrial survival. To assess the magnitude of these responses, we adapted an age-structured, individual-based model with density-dependent growth and survival of larvae. The model was based on extensive data from local field studies and experiments. At Rainbow Bay, reproductive success was poor (<1 metamorph per breeding female) in nearly half of the 22 years that the species bred; complete failure occurred in 6 of 22 years, when pond hydroperiod was <2 mo. To estimate the density-independent component of survival in the pond stages (eggs and larvae), we fitted stock-recruitment curves from the model to field data for years of good reproduction. Initial conditions in the simulations described the colonization observed at Rainbow Bay. We measured effects of catastrophic reproductive failure, occurring randomly with probability 0.5 per year, by comparing simulations with catastrophic failure and good survival in the pond stage to simulations with equivalent average survival in the pond stage. With consistently good survival in the pond

stages, the simulated population required survival probabilities in the upland stages (juveniles and adults) near 0.5 per year to persist and near 0.8 per year to achieve the increases observed at Rainbow Bay. Catastrophic failure created additional fluctuations in the population, raised the thresholds of survival required for persistence, and caused extinction under conditions that were otherwise favorable. The marbled salamander at Rainbow Bay is not at great risk of extinction due to catastrophic failure, but the risk increases dramatically if life span is decreased or frequency of failure is increased. Any reduction in terrestrial survival will have deleterious consequences by reducing the breeding populations at equilibrium, even if it does not jeopardize persistence. The model for the marbled salamander provides assessments of risk that can be applied to poorly-studied species with similar life histories, such as the endangered flatwoods salamander (*A. cingulatum*).

A manuscript by Taylor, Scott, and Gibbons describing this work is in press in *Conservation Biology*.

◆◆◆ Ecological Risks and Effects ◆◆◆

Investigators in the Ecological Risks and Effects Research Theme at SREL conducted a wide variety of research during FY05 that will assist DOE in making better-informed decisions about remediation and land management. This research attempts to reduce many of the knowledge gaps currently associated with ecological risk analyses and the effects that contaminants have on biota. In FY05 researchers associated with this theme conducted research on:

- measuring the impact of mercury speciation on amphibians
- understanding trophic transfers of contaminants in terrestrial ecosystems
- integrating contaminant effects among different levels of biological organization
- developing biomarkers to measure transgenerational risks from contaminant exposures
- developing fish models for understanding key issues in ecological risk analyses
- understanding how the spatial and temporal variation of contamination alters risks
- examining potential negative impacts to biota due to indirect effects of remediation
- determining levels of sensitivity among biota exposed to organic contaminants
- determining the importance of fish and waterfowl as vectors for contaminant dispersal
- quantifying contaminant exposures to endangered species on the SRS
- determining interactive effects of mixed contaminants
- examining risks from chronic low-dose rate irradiation
- using stable isotopes as biomarkers of contaminant effects
- measuring maternal transfers of contaminants
- determining if contaminant exposure promotes antibiotic resistance in bacteria
- improving the statistical analyses of ecological risk data sets

Report on FY05 Risks and Effects Milestones

Question 1:
How does contaminant speciation influence bioavailability, and how do changes in bioavailability alter contaminant dose-response and toxicity relationships?

A. Impacts of mercury speciation on amphibians
Investigators: C.H. Jagoe and W.A. Hopkins

The possible effects of dietary mercury exposure at environmentally realistic concentrations on developing amphibians are poorly understood. Existing data suggest that amphibians can accumulate mercury from their diet while feeding in wetlands, but it is unclear whether this exposure negatively impacts amphibian populations. We published a paper in 2005 quantifying the trophic transfer of inorganic and organic mercury species from *aufwuchs* (periphyton) to amphibians. In two other papers published in FY05, we reported dietary mercury accumulation in laboratory feeding studies and showed that this exposure resulted in effects on larval development and life history traits important to amphibian population dynamics. Southern leopard frog (*Rana sphenocephala*) larvae were exposed to experimental diets formulated to contain mercury concentrations and species similar to those in *aufwuchs* from aquatic habitats

contaminated by atmospheric deposition. Observations on mortality, incidence of malformation, and larval growth and development were made for 254 days. Increased mortality, malformations, and changes in growth and development were observed at mercury concentrations (1,500-3,300 ng Hg/g DW) similar to the highest concentrations in amphibian diets expected from atmospheric deposition. The results of this study are more ecologically realistic than results obtained from aqueous mercury exposure in previous studies. Our results indicate that dietary mercury exposure in habitats primarily contaminated by atmospheric deposition has the potential to cause adverse effects in amphibian larvae. A new study was begun using a similar experimental design (frog larvae were fed experimental diets with ecologically realistic mercury concentrations), to examine changes in behavior with Hg accumulation. Mercury is neurotoxic, so changes in nervous functions affecting locomotion, sensory perception, or memory should be manifested as changes in behavior. We hypothesize that behavioral changes represent a sensitive indicator of mercury toxicity, and that behavioral changes might impact ecologically relevant activities such as locating food, feeding, and predator avoidance. This study will be completed in early FY06 and results prepared for publication.

B. Trophic transfers in contaminated terrestrial systems

Investigators: W.A. Hopkins and B.P. Jackson

Additional research is needed to understand how contaminant speciation affects bioavailability in terrestrial organisms, particularly in relation to trophic transfer (often the most important route of exposure in terrestrial organisms). In FY04 we developed methods for exposing terrestrial organisms to dietary trace elements under conditions that allow quantification of trophic transfer, chemical speciation, and biological effects at multiple trophic levels. This research was completed in FY04 and a manuscript detailing our findings was published in FY05 (*Hopkins, W.A., et al. 2005. Transfer of Se from prey to predators in a simulated terrestrial chain. Environmental Pollution 134:447-456;* SREL reprint 2811). Additional manuscripts are in preparation that describes changes in chemical speciation that occur depending upon the fate of transported selenium.

Question 2:

How do contaminant effects integrate over different levels of biological organization (i.e., how much molecular and cellular damage is required before effects become significant to individuals, populations, and communities)?

(A) Integrating effects over different levels of biological organizations

Investigators: T.G. Hinton, T.C. Glenn, and W.A. Hopkins, with additional collaborators from Colorado State University and The University of Georgia

Two manuscripts were published in FY05 relative to the linking of molecular level effects from radiological exposure to population-level endpoints. The first manuscript (*Hinton, T.G., et al. 2004. Radiological effects on the environment: A need to question old paradigms and enhance collaboration among radiation biologists and radiation ecologists;* SREL reprint 2791) provides a historical perspective of the current paradigm that does not explicitly protect nonhuman biota from radiation, but instead relies on the concept that if dose limits are set to protect humans, then the environment is automatically protected as well. The paper summarizes recent international questioning of this paradigm and points out the controversial aspects of three different frameworks being proposed to protect the environment from ionizing radiation, including that proposed by DOE. We emphasize that to properly address radiation protection of the environment we need to understand how effects are integrated across different levels of biological organization. To accomplish this, an enhanced collaboration is needed among the traditionally separate disciplines of radiation biology and radiation ecology.

The second manuscript (*Hinton, T.G. and F. Bréchignac. In press. A case against biomarkers as*

they are currently used in radioecological risk analyses: A problem of linkage. IRSN Collection Series, Suppl. 1, vol. 39. EDP Sciences, Paris) identifies the problem of biomarkers in ecological risk analyses generally lacking a linkage between molecular-level effects and quantifiable impacts observed in individuals and populations. The paper stresses that from an ecological risk analysis perspective, the challenge is to evaluate whether changes in contaminant levels constitute a significant risk to a population. That challenge is even more daunting when biomarkers of damage are used as a surrogate indicator of a population effect. Although the biomarker may be a very sensitive and early warning sign of a possible effect, the applicability of such damage to ecological risk can only be assessed once the connection has been made to changes in individual resource allocation-based life histories that are coupled to population dynamics.

In collaboration with colleagues at the Medical College of Georgia, we conducted preliminary studies of adaptive response (AR) with ionizing radiation (IR) as the stressor. Adaptive response is a phenomenon in which an organism's response to a contaminant is reduced if it has been previously exposed to a priming dose. The concept has been demonstrated many times *in vitro*, but rarely *in vivo*, and has implications to radiation workers and the dose response curves upon which regulations governing radiological exposures are based. Medaka fish and SREL's Low Dose-rate Irradiation Facility are ideally suited to examine the phenomenon.

The first step was to determine the dose response curve for IR exposure of the medaka embryo. The main, or "challenge" dose of ionizing radiation was delivered at the pre-early gastrula stage (medaka stage 12, approximately 10-12 hours post fertilization, hpf). Following the challenge dose, embryos are incubated for about 40 h to allow onset of apoptosis. At that time (medaka stage 25), organ systems are present, including the brain, heart, circulatory system, digestive tract, and kidney. Considerable morphologic differentiation has occurred, and spatial distribution of apoptotic cells can be assessed relative to anatomical landmarks. Sporadic apoptosis occurs in the absence of IR exposure as part of normal development (0 mGy). Little or no change in this low background level of apoptosis was evident at 150 mGy. At the next higher dose of 500 mGy, a monolayer of apoptotic cells became visible in the region lining the ventricle of the brain, particularly toward the rostral surface. Apoptosis was more pronounced at 1.5 Gy, encompassing the entire surface of the ventricle. At doses of 5 Gy and 15 Gy, apoptosis becomes evident throughout the head of the embryo. Some morphologic abnormality (eye reduction) occurs at 15 Gy, reflecting extensive cell death. The ventricular surface is a region of active cell proliferation that at slightly later times in development forms the "ventricular zone", a region of intense cell proliferation and neuronal birth. The occurrence of radiation-induced apoptosis in this region is consistent with the differential sensitivity of rapidly dividing cells to radiation. The principal target of IR is DNA, and the dose of IR needed to produce a given effect scales linearly with genome size. The medaka genome is about five times smaller than the human genome and two to three times smaller than that of the zebrafish. The 150 mGy to 15 Gy range used here is thus equivalent to about 30 mGy to 3 Gy for humans. To provide context, the low end of the range, adjusted to the equivalent human exposure and for radiation quality, is 10-fold higher than the annual natural background exposure (3 mSv; 1 mGy=1 mSv for ^{137}Cs), less than the annual NRC dose limit for radiation workers (50 mSv), and less than the dose from a single whole-body CT scan (50-150 mSv). The results thus illustrate a strength of the medaka embryo system, which is the presence of a measurable response at doses that approach actual exposure scenarios.

We next examined an adaptive response evoked by exposure to priming doses of radiation, followed by larger challenge doses. To maximize the chances of seeing an effect, two priming doses were used, one delivered to the parents (priming dose 1) and another, much lower dose, delivered directly to the very early embryos (priming dose 2). For priming dose 1, a

breeding population was exposed to ^{137}Cs radiation, for 6 days at 0, 4, or 40 mGy d^{-1} (0, 24, or 240 mGy total dose). On day 6, newly deposited embryos were collected and exposed briefly to priming dose 2 (0, 0.15, 0.3, 1.5, or 3.0 mGy as indicated). Embryos were transported to the Medical College of Georgia and incubated until they reached stage 12. Embryos were then challenged with an acute 1.5 Gy dose of ^{137}Cs irradiation, incubated until stage 25 to allow for onset of apoptosis and TUNEL-stained to reveal apoptotic cells.

Consistent with previous results, embryos that received the challenge dose in the absence of priming had apoptotic cells at the boundary of the ventricle and telencephalon, in the hindbrain, and in the tail. Control embryos that did not receive the challenge dose showed only the expected background level of apoptosis. In contrast, offspring of parents that received a 240 mGy (40 mGy per day) priming dose were resistant to the challenge dose. There was little or no increase in apoptosis above the spontaneous level, and no sign of the increased apoptosis at the ventricular surface that characterizes radiation-injured embryos. The other treatment groups showed suboptimal AR. Among embryos receiving the challenge dose, there was less apoptosis than in the unprimed group. The primed embryos showed very little of the apoptosis at the ventricular-telencephalon boundary that is diagnostic of radiation injury induced by stage 12 challenge. Embryos did, however, show a somewhat elevated level of apoptotic cells dispersed throughout the head region. This more dispersed pattern of apoptosis was seen even in the absence of challenge. We speculate that elevated, but dispersed, apoptosis may reflect injury from priming dose 2. Further experiments, where parents and embryos are irradiated separately, will be required to test this hypothesis.

In summary, clear evidence for AR was seen at 240 mGy, equivalent to about 50 mGy in humans. This dose is expected to produce only 0.5-1 Double Strand Breaks/cell. Suboptimal AR was seen also at a 10-fold lower dose, sufficient to induce only one Double Strand Break per 10 or 20 cells, cumulatively over a 6 d priming period. Results indicate that the medaka embryo model can be used to study and quantify adaptive response induced at very low doses and dose rates.

We also submitted three proposals in FY05 to DOE's Low Dose Program. All three proposals used medaka as the model organism and SREL's Low Dose-rate Irradiation Facility. The first two proposals received good reviews but did not score high enough to garner funds. The third proposal was fully funded, and the research is targeted to begin in late July 2005.

(B) Development of biomarkers to measure trans-generational risks from contaminant exposure

Investigators: T.C. Glenn, T.G. Hinton, C.H. Jagoe, and I.L. Brisbin, Jr.

This fiscal year, we emphasized work in the medaka fish. We have previously identified STR loci from traditional laboratory methods of constructing enriched libraries. We also searched Genbank for STR loci in medaka sequences. Raw and modestly processed whole-genome shotgun (WGS) sequence data of the medaka have recently become available from sequencing done in Japan (e.g., http://shigen.lab.nig.ac.jp/medaka/genome/top.jsp). We searched the available WGS data for all possible STR DNA sequences. We have discovered 70,245 unique sequences with at least one microsatellite repeat. Tetra- and penta-nucleotide repeats are abundant. A reasonably large number of loci were discovered with the same core repeats at the most often studied ESTR loci in mice. Approximately one third of the primer pairs that we have designed yielded scorable polymorphic loci (26 loci of a total of 72). About 50% of tetra and penta-nucleotide repeat loci identified from the WGS data have yielded scorable polymorphic loci. The 26 candidate loci yielded 2-11 alleles per locus from a survey of 19 fish in the SREL breeding colony. These were used to identify mutant alleles that were then used in determination of effects of low dose-rate irradiation.

We screened the 26 candidate primer pairs among parents and 200 offspring from control breeding pairs to identify loci with the highest mutation rates. We have identified 7 loci in which an average of at least 1% of the offspring contain mutant (non-parental) alleles.

We have also screened the 7 loci with observed mutations in the control breeding pairs, along with 2 other loci that could be easily incorporated into the multi-locus genotyping panels, in medaka that had been irradiated at the Low Dose-rate Irradiation Facility (LoDIF) at SREL. All medaka in the irradiated group were exposed as juveniles to 68 mGy/d from a ^{137}Cs source. They were then housed unirradiated for several months. For 7 days immediately prior to breeding, the males were irradiated again. Offspring deriving from eggs obtained 30 days post-irradiation (i.e., sperm derived from irradiated spermatogonia) were collected. A total of 188 offspring from 4 breeding pairs were analyzed. Offspring from the irradiated parents have elevated mutation frequencies relative to unirradiated controls ($p=0.03$; Fisher's Exact Test: parental vs. non-parental alleles, control vs. exposed). Similar results are obtained, and the same conclusion is drawn from using alleles or numbers of offspring with mutations. It is clear that two of the loci (#21 and #45) are responsible for most of the response observed. Additional experiments to determine the consistency of this response are underway.

Although the Fisher's Exact Test is used most commonly in published studies of this type, it does not account for differences among families. To account for variation among families, mutation rates in the exposed and control groups were compared using generalized estimating equations to fit a Poisson regression model that accounted for multiple measurements on fish within several families in SAS. In this analysis, the number of non-parental alleles in each individual was modeled as a Poisson variable with treatment (exposed vs. control) as the main factor, and family nested within treatment as the source of repeated measurements. The mean number of mutations for the control fish was 0.080 and this was significantly less than the mean number of mutations in exposed fish (0.149; $p = 0.018$).

Thus, we have now demonstrated in medaka: (1) large numbers of STR loci are available, (2) in control breeding pairs about 10% of the loci yield ≥1% offspring with mutant alleles, and (3) STR loci respond in the expected direction due to low dose-rate irradiation. We are, therefore, poised to determine the response of STR mutations in medaka under a variety of experimental conditions.

We also completed work for 7 publications, including SREL reprints 2782, 2838, 2839, 2840, 2841, and 2870, in addition to one paper that is press (*Crosbaw, D.A., N.A. Schable, M.B. Peters, and T.C. Glenn. In press. Isolation and characterization of microsatellite DNA loci from Ambystoma salamanders. Conservation Genetics*.

(C) Development of fish models for understanding human and ecological risks

Investigators: T.G. Hinton, T.C. Glenn, W.A. Hopkins, and collaborators from The University of Georgia

See report under section 2A above.

Question 3:

How do spatial and temporal variation in contamination, variation introduced by remediation practices, and species-specific differences in ecology and physiology alter risks to humans and other biota?

(A) Waterfowl as vectors of contaminant dispersal

Investigators: I.L. Brisbin, Jr., C.H. Jagoe, and C.S. Romanek

In order to adequately assess the potential of migratory waterfowl to serve as long-distance vectors of SRS contaminants to the food chain of the hunting public, more specific information is needed concerning the exact ecological/trophic roles of these birds in SRS food chains and the specific origins/destinations of migratory movements of the birds which winter in SRS wetlands and thus are potentially vulnerable to

exposure to site contaminant releases. During the past year we addressed these issues through analyses of previously collected SRS waterfowl samples for contaminant burdens (radiocesium and mercury) and associated levels of stable isotopes of hydrogen, carbon, and nitrogen in the same individuals. These analyses were conducted on samples of 26 American coots (*Fulica americana*), 16 lesser scaup (*Aythya affinis*), and 16 ring-necked ducks (*Aythya collaris*).

Hydrogen isotope analyses of muscle samples indicated that while away from the SRS, coots tended to spend time at more northerly latitudes (further away from the SRS) than did lesser scaup. Mercury concentrations in both muscle and liver increased from coots (largely herbivorous) through ring-necked ducks (more omnivorous) with highest levels occurring in lesser scaup (more carnivorous), thus supporting the idea of general biomagnification with increasing contamination levels at increasingly higher trophic levels. Levels of stable isotopes of nitrogen however failed to confirm that lesser scaup were necessarily feeding at higher trophic levels than the other two species, suggesting the need for more in-depth dietary analyses for this species while wintering at the SRS site. Neither mercury nor radiocesium contamination levels showed any discernible relationship to latitudinal origin of the birds as suggested by hydrogen stable isotope profiles. However, differing frequency distributions of hydrogen stable isotopes in muscle vs. liver samples within the same species indicated the need to consider relative rates of isotope turnover within different tissues before, during, and after the period of migration.

As was the case during the previous year, a nearly complete absence of the formerly abundant American coot from the abandoned SRS cooling reservoirs during the winter of 2004-2005 again prevented the collection of an annual sample of this species as part of a long-term study of radoicesium contamination levels in wintering SRS waterfowl. Coots have been routinely used over the years as the "worst possible case" indicator for radiocesium because of the elevated levels of this contaminant that are routinely shown by this species when it is present on the SRS.

(B) Risk associated with remediation
Investigator: L.A. Newman

The student working on this project has completed her work and has written up the paper on the results. We have seen that exposure to TCE by *C. elegans* at concentrations approximating that seen on a remediation site did not result in increased lethality or a decrease in mobility. Exposure of earthworms did not result in any external affects, and the worms did not show a physiologically relevant increase in the levels of metabolites.

The work with *C. elegans* will be continued, looking at other parameters, such as reproductive success and feeding patterns with TCE. These parameters, as well as movement and lethality, will also be studied for other solvents. This work is being done in conjunction with faculty at the University of South Carolina-Aiken.

(C) Determining the most sensitive species to organic contaminants
Investigators: W. Hopkins and scientists from USGS-Patuxent Wildlife Center and Michigan State University

For the risk assessment process to be maximally effective, it is important to understand which receptor organisms are most sensitive to the effects of environmental contamination. It has been repeatedly suggested that reptiles are the most sensitive vertebrates to organic contaminants due to their poorly developed detoxification system, but little empirical evidence supports this contention. In collaboration with scientists from USGS-Patuxent Wildlife Center and Michigan State University, we completed two studies in FY04 that characterized aspects of the cytochrome P450 system in snakes (family Colubridae). This research was completed in FY05 and two manuscripts detailing our findings have been completed (one under internal review, the other in review at *Toxicol Applied Pharmacol*). We anticipate that both will be published in FY06.

(D) Fish as vectors of contaminant dispersal

Investigators: D. Fletcher and collaborators (M. Paller, Savannah River National Laboratory; J. Isely, Clemson University; W. Littrell and S. Dyer, WSRC-SGCP)

Consumption of contaminated fish by anglers is potentially an important offsite pathway for human exposure to ^{137}Cs originating from the SRS. To better understand this problem, a radiotelemetry study was conducted from June 1999 through April 2003 to characterize the movements of potentially contaminated largemouth bass in Steel Creek, a restricted-access SRS stream, and the publicly accessible Savannah River. Results from the radio tracking study indicated that mobility varied among individual largemouth bass and was affected by season, water level, and habitat. Largemouth bass usually established comparatively small home ranges, but often left them and sometimes traveled long distances, especially during February through May, their expected pre-spawning and spawning periods. High water levels during this time appeared to produce the greatest number of long distance movements by largemouth bass. After long distance movements, bass frequently set up new home ranges; however in the Savannah River, homing to the Steel Creek area was also observed.

In spite of the occasional long distance movements, largemouth bass tended to be relatively sedentary much of the time. This was particularly true in upper Steel Creek, where movement was more restricted and home ranges smaller than in lower Steel Creek and the Savannah River. As a result, upper Steel Creek contributed few largemouth bass to the Savannah River. Most of the interchange of largemouth bass between Steel Creek and the Savannah River was restricted to the lower reaches of Steel Creek, especially that on the Savannah River floodplain. Seasonal and diurnal interchange occurred between these habitats. The tendency to occupy relatively small home ranges much of the time also restricted largemouth bass dispersal in the Savannah River. Approximately 90% of the largemouth bass relocations in the Savannah River were within 10 km of the mouth of Steel Creek.

A study of the escapement of fishes from SRS reservoirs identified critical influences of outlet structure type and lake levels on movement of fishes though outlet structures. Species composition of escapement catch also varied temporally. Fish that passed through the dams likely presented a larger ecological risk than human risk because of their size and species composition. However, escapement during critical high water discharges remains to be ascertained.

A mark-recapture population survey conducted in conjunction with the radiotelemetry study showed that after two summers of hypolimnetic releases from L Lake and several years of reduced stream discharge, small numbers of largemouth bass were resident in Steel Creek. Although bass populations rebounded when warm water discharge from L Lake was restored, the small number of bass present in Steel Creek at the conclusion of our study was likely contributing relatively few individuals to the Savannah River. A comparison of the total amount of ^{137}Cs transported annually from Steel Creek by largemouth bass and the amount transported by water and suspended sediments clearly showed the predominance of the latter in terms of total quantity of ^{137}Cs moved from the SRS into public areas. This conclusion was supported by a published dispersal model (*J. Environ. Radioactivity*, 2005), which showed that ^{137}Cs levels in Savannah River largemouth bass predicted on the basis of largemouth bass dispersal from SRS streams were below actual levels measured in Savannah River largemouth bass. Although this discrepancy could result from erroneous assumptions in the dispersal model, an alternative explanation is that some ^{137}Cs is accumulated by largemouth bass within the river as a result of the discharge of ^{137}Cs in water and sediment from SRS creeks. We conclude that during our study, the emigration of largemouth bass from Savannah River Site streams was unlikely to be responsible for long distance dispersal of substantial radiological contamination in the Savannah River.

(E) Endangered species using contaminated sites
Investigators: A.L. Bryan Jr., I.L. Brisbin, Jr., and C.H. Jagoe

To document Bald Eagle utilization and behavior on the SRS, we conducted studies which resulted in a manuscript regarding eagle food habits, feeding rates, and nesting behavior (*Bryan, A.L. Jr., et al.. In Press. Behavior and food habits of breeding Bald Eagles in inland South Carolina. Southeastern Naturalist*). This manuscript provides baseline information necessary for predictive models of dietary contaminant exposure. Models concerning contaminant exposure via the diet by nesting eagles on the SRS have been constructed and subsequent manuscripts are in preparatory stages, with submission to appropriate scientific journals scheduled for the latter half of calendar 2005

Studies regarding contaminant exposure (mercury) to another endangered species, the Wood Stork (*Mycteria americana*), relative to variation in wet and dry seasons are addressed under Characterization 3(B).

Question 4:
What are the potential interactions (additive, synergistic, antagonistic) from exposures to mixed (radioactive and non-radioactive) contaminants, as well as other environmental stressors?

(A) Complex interactions between biotic and abiotic factors and assessments of risk
Investigators: W. Hopkins, T. Hinton, and scientists from USGS-Columbia Environmental Research Center

See report under section 2A above. In addition, SREL's Low Dose-rate Irradiation Facility was the core for a proposal that was submitted to the National Institutes of Environmental Health Sciences (NIEHS). The proposal is very relevant to contaminant issues on all DOE sites: "Contaminant Mixtures: Multigenerational Effects from Chronic Exposures." The proposal, which involves 12 research groups from three countries, requested $13M from the NIEHS over a 5-year period. Successful applicants will be notified in the fall of 2005.

Question 5:
What are the risks from low dose-rate, chronic exposures to radiation?

(A) Determining the risk from chronic low-dose radiation exposure
Investigator: T. Hinton

See report under section 2A above.

Question 6:
What are the most cost-effective, yet still valid, biomarkers of meaningful impacts to biota?

(A) Development of biomarkers to asses the effects of contaminants on biota
Investigators: C. Romanek, C.H. Jagoe, and W. Hopkins

Chronic exposure to contaminants causes stress in organisms, which often manifests itself as increased metabolic demand because energy is used to: (1) metabolize, sequester, or excrete contaminants, (2) repair contaminant-induced damage, and (3) maintain homeostasis. Because more energy is used for these functions, less is available for other activities such as growth and reproduction. Biochemical markers, or biomarkers, are commonly used to indicate the effects of contaminants on impacted organisms as they potentially provide an early warning of ecological damage to exposed biota. Conventional biomarkers like metallothionein or glutathione indicate exposure to various pollutants, but better measures of increased energetic costs of pollutant exposure are needed. Our preliminary studies have shown that changes in the ratio of the stable isotopes of nitrogen in tissues of fish and birds exposed to toxic metals (e.g. Hg) provide this measure. These changes indicate that rates of protein turnover increase with

exposure to pollutants. We have shown that nitrogen isotope shifts in proteinaceous tissues precede changes in metallothionein and glutathione biomarkers when animals are exposed to contaminants (e.g. Hg). However, the exact causes and relationships to protein turnover remain to be clarified.

In FY2005, we published a manuscript in *Environmental Science & Technology* (Shaw-Allen et al., *In press*) that documents these findings. Increases in nitrogen isotope ratios were observed in the whole liver and the acid soluble fraction of the liver of animals fed diets relatively high in Hg compared to a reference group. Meanwhile, significant differences were not detected in hepatic levels of the metal-binding biomarkers metallothionein and glutathione. These shifts are due to increased rates of protein breakdown relative to synthesis and reflect a fundamental shift in protein metabolism. Because the shift in the acid-soluble fraction was stronger than the bulk liver, the increase is probably driven by an increase in bodily derived amino acids in the free amino acid pool.

This work forms the basis for continued research to develop the techniques to analyze the nitrogen isotope ratios of specific biomarkers and the amino acid residues that comprise them. It is our hope that nitrogen isotopes can be used to determine the metabolic pathways and fluxes that are altered by exposure to contaminants (e.g. Hg). Presently, our laboratory is developing procedures to hydrolyze proteins and retain amino acid groups that are sensitive to oxidation such as cysteine and methionine. These amino acids contain sulfur and are active sites for mercury binding. Progress has been made in the development of techniques related to compound specific isotope ratio analysis. GC-C-IRMS instrumentation has been used to separate derivitized carbon-bearing compounds and analyze them individually for their carbon isotope composition. This work will be extended to include the derivitization of amino acids for nitrogen isotope analysis to further elucidate how contaminant exposure affects organisms, populations and communities in impacted environments.

Question 7:
What are the mechanisms and consequences of trophic and maternal transfer of contaminants?

(A) Maternal transfer of contaminants in amphibians
Investigators: W. Hopkins and B. Jackson, with scientists from University of Maryland-Chesapeake Biological Laboratory

Although there is now general consensus that many amphibian populations around the globe are declining at alarming rates, the cause of most declines remains unknown. Environmental contamination is one of several factors implicated in declines, and may be particularly important for sensitive developmental life stages. Developmental pathways in embryonic amphibians can be altered when embryos are exposed to contaminants via two primary mechanisms: uptake from their surroundings (e.g., water), and transfer from mother to offspring. To date, no studies have examined maternal transfer of contaminants in amphibians, despite its devastating effects on development in every other major vertebrate lineage. In FY05, we determined that adult female narrow mouth toads (*Gastrophryne carolinensis*) breeding near a near a coal burning power plant transfer high concentrations of Se and Sr to their eggs. Contaminated eggs produced 20% fewer viable offspring due to decreased hatching success and an increased prevalence of developmental malformations. Our study demonstrated that maternal transfer is an important route of contaminant exposure in amphibians and warrants future study.

In FY05 we presented our findings at the annual meeting of the Ecological Society of America and the World Congress of the Society of Environmental Toxicology and Chemistry. The manuscript describing this work is under internal review and will be submitted to *Environmental Science & Technology* in early FY06.

(B) Indirect effects of contaminants
Investigators: W. Hopkins, D. Scott, S. Harper, B. Jackson, and B. Metts

Recent evidence suggests that the effects of contaminants on wildlife often exceed those estimated in laboratory toxicity tests because other environmental factors (e.g., predators, resource abundance) can influence contaminant toxicity. We conducted a series of three outdoor mesocosm experiments with either pesticides or complex mixtures of metals to examine the effects that these contaminants have on complex assemblages of aquatic organisms. In the first of these studies, we manipulated pesticide (carbaryl) concentration (control, 3.5 mg/L, 7.0 mg/L) and larval density (low and high) of two species of *Ambystoma* salamanders to examine how the natural stress of competition might influence contaminant toxicity. Carbaryl negatively affected salamander snout-vent length (SVL), growth rate, lipid reserves, time to metamorphosis, percent survival, and percent metamorphosis. The intensity of this effect on SVL, lipid reserves, and growth rate was influenced by density. Additionally, the two species responded differently to the treatments. The effects of carbaryl and increased density on percent metamorphosis were nearly additive, but were generally less than additive on other variables. The negative effects of chemical contamination on salamanders were likely due to pesticide-induced reductions of food resources, as zooplankton abundance decreased by as much as 97% following carbaryl application. Our first study demonstrated the importance of the interactive effects that chemical contamination and natural environmental factors have on salamander assemblages. The second two studies used a similar approach to determine whether complex mixtures of metals had similar detrimental effects on zooplankton assemblages and if predators reliant upon zooplankton would be adversely impacted. Preliminary results suggest that metal contamination does decrease zooplankton abundance, but not nearly to the extent that pesticides do. Effects on salamander assemblages also appear less pronounced, but may still be sufficient to reduce the number of viable recruits to terrestrial habitats. Interestingly, concentrations of metals accumulated in salamander tissues were extremely high, suggesting that they may act as important vectors of contamination from aquatic to terrestrial habitats.

The first of these studies was published (*Metts, B.S., et al. 2005. Interaction of an insecticide with larval density in pond-breeding salamanders (Ambystoma). Freshwater Biology 50:685-696;* SREL reprint 2815). Data are currently being statistically analyzed from the latter two studies and manuscripts will be submitted to peer-reviewed journals in FY06.

(C) Use of stable isotopes to elucidate exposure pathways
Investigators: C. Romanek, C.H. Jagoe, I.L. Brisbin, Jr., W. Hopkins, and B. Jackson

This project was completed in FY04.

Question 8:
What are the risks associated with the indirect selection for antibiotic resistance by microbial exposure to heavy metals?

(A) Importance of heavy metal exposure in promoting antibiotic resistance in bacteria
Investigators: JV. McArthur, C.H. Jagoe, and T.C. Glenn

We have established that native aquatic bacteria that are exposed to heavy metal contamination on the Savannah River Site have increased levels of antibiotic-resistance towards commonly used antibiotics. We have effectively shown that indirect selection caused by exposure to metals actually increases the proportion of bacterioplankton carrying antibiotic resistant traits. Likewise, bacterioplankton exposed to antibiotic show increased levels of metal resistance. We seek to determine how wide-spread this phenomenon is by increasing our survey of new habitats. Major accomplishments of FY05 include:

Performed a microcosm experiment using coastal water collected from the ACE Basin to determine the

indirect selection of antibiotic resistant bacteria when exposed to heavy metals.

1. Amended triplicate bottles with varying concentrations of mercuric chloride, cadmium chloride and potassium dichromate.
2. Performed flow cytometric analysis to quantify total number of bacteria at the beginning and end of the experiment.
3. Cultured nearly 300 bacterial isolates that were screened for antibiotic and metal resistance.
4. DNA from all isolates was amplified by PCR and sequenced.
5. Samples also collected from the microcosms for analysis of metal content, dissolved organic carbon, antibiotic content, and nutrient analysis.

Gene transfer experiments:

1. Tested GFP (green fluorescent plasmid) marked recipients for use in gene transfer experiments.
2. Performed filter mating experiments with GFP marked recipients and bacteria exposed to metals from the sediment and water of the D Area ash settling basins.
3. Performed a GFP-plasmid transfer experiment at the Skidaway Institute of Oceanography to develop a flow-cytometric assay for high throughput determination of model plasmid transfer rates under various conditions such as metal exposure.

(B) Mechanisms that link heavy metal and antibiotic resistance

Investigators: JV. McArthur, C.H. Jagoe, and T.C. Glenn

Bacterial traits that have environmental importance are often carried on mobile elements called plasmids. It is not clear how, when, and how often plasmid DNA is exchanged among bacteria in the environment. We seek to determine the risks associated with plasmid transfer of plasmids carrying antibiotic resistance genes in native populations of bacteria. Major accomplishments of FY05 include:

- Re-tested resistance of 300 *Aeromonas* isolates from bullheads (*Ameiurus* spp.) and sediment from Four Mile Creek and Meyers Branch to chloramphenicol, gentamycin, kanamycin, streptomycin, and tetracycline.
- Extracted plasmids from 150 *Aeromonas* isolates from bullheads, bass, and sediment from Four Mile Creek, Meyers Branch, and Par Pond and obtained 6 plasmids. Grew up and extracted plasmids from 43 previously frozen isolates from the cadmium-tetracycline and nickel-ampicillin microcosm experiments. These isolates were resistant to 3 or more selective agents (antibiotics or metals).
- Collected sediment and water from the Savannah River, extracted DNA, and amplified using 6 ORI specific primer sets specific to a variety of plasmid incompatibility groups. Got amplification with primers specific to the trfA2 region of the IncP plasmid group in both water and sediment.
- Set up microcosm experiment with Savannah River water and sediment spiked with cadmium and nickel.
- Plasmids have been isolated from environmental bacteria. The number and size of plasmids has been measured. A sub-sample of plasmids is being transformed into laboratory strains of *E. coli* for further characterization.
- Initial gene transfer experiments have been conducted between indigenous bacteria (gene donors) from the ash settling basins of a coal-fired power plant and a *Pseudomonas* sp. B13-GFP2 strain (gene recipient). These experiments indicate that ash-settling basins contain horizontally transferable genes encoding resistances to tetracycline, gentamycin, streptomycin, and mercury. Such gene transfer experiments will be a major focus of FY05 research and further characterization of the mobile elements identified from these experiments will be the focus of additional research in FY05 and FY06.

Question 9:
What statistical techniques provide the most power and highest confidence when estimating total dose or exposure?

(A) Bootstrap methods
Investigators: M.D. Wilson, T.G. Hinton, and C.H. Jagoe

Several data sets were tested using a traditional goodness-of-fit test, (Lilliefors test for normality) before and after log transformation, as well as using a bootstrap goodness-of-fit test. A peer-reviewed journal article was published in the *Journal of Radiological Protection* that illustrated the bootstrap hypothesis testing and power analysis on the Sellafield (UK) data set.

The bootstrap goodness-of-fit test was developed and applied to several data sets. The Bootstrap goodness-of-fit test was positive when the Lilliefors test was positive, indicating that the bootstrap method produced robust estimates of the distributions. However, the benefit of this test is that a test of the distribution of the mean can be applied. This is beneficial because it is most often the distribution of the mean that is of interest in hypothesis testing. If a data set fails distributional tests, but the mean can be shown to pass, then classical distributional theory can be applied to conduct inference and hypothesis testing.

For the data sets failing both normal and log-normal, the bootstrap test was applied to both the raw data and the log-transformed data. Some additional data sets were shown to have log-normal means by the results of the bootstrap test. The data sets that are identifiable as normal or lognormal will be used to confirm results of the non-parametric methods, which should prove to be consistent with methods based on distributional assumptions for data sets which meet these assumptions. This work is integrated with work on the distributional properties of contaminant data sets described in the Characterization section, question 5.

(B) Bayesian methods
Investigators: M.D. Wilson, T.G. Hinton, and C.H. Jagoe

The software package WinBUGS has been installed and elementary code has been written. Work currently focuses on learning the use of WinBUGS and the specific implementation of both Bayesian mixture model estimation and traditional Bayesian methods when prior distributions can be specified.

(C) Mixture models
Investigators: M.D. Wilson, T.G. Hinton, and C.H. Jagoe

We have begun writing and testing code in the mathematical programming language, Matlab to estimate the parameters of a mixture of normal and log-normal distributions on simulated data where the true parameters are known. This method is quite computationally intensive and developing code is not straightforward. This work continues.

Because of recommendations from DOE, new proposals were developed. This work was incorporated into a proposal to characterize radiocesium on the SRS. This work proposes using stable isotope analysis to identify the position of an organism in a food chain and to use mixture model methods to characterize the degree of mixing between normal and log-normal.

◆◆◆Remediation and Restoration◆◆◆

The SREL Remediation and Restoration Group conducts multidisciplinary research designed to assist DOE in the development, evaluation, and stakeholder acceptance of remediation and restoration efforts that are protective of human as well as ecosystem health. The following discussion summarizes research accomplishments for FY05.

Report on FY05 Remediation and Restoration Milestones

Question 1:
How can solute transport and retention within the variably saturated vadose zone, which serves as the long-term source for many contaminated systems, be incorporated within conceptual and predictive models of contaminant migration?

(A) Field studies of vadose zone solute transport cycling models
Investigators: J.C. Seaman and collaborators from Clark Atlanta and Cornell Universities

We continued the development and refinement of vadose transport model within the HYDRUS1D code for describing tritium movement through the soil profile, including parameterization for tritium losses due to evapotranspiration. We determined water retention properties for a number of distinct soil horizons reflecting the typical soil pedon observed within the irrigation site using the UFA system. Two separate field scale tracer experiments were conducted (i.e., pulse tritium applications) and we continued sampling additional irrigation plots as part of collaboration with Cornell University (Drs. Riha and Rebel) and the U.S. Forest Service-Savannah River (Drs. Blake, Hitchcock, and Barton). Laboratory experiments were initiated to determine the utility of Surface Complexation Modeling (SCM) to variably saturated systems. SCM addresses some of the inherent uncertainties in the Kd approach to predicting solute partitioning through the use of a limited number of independent parameters that can account for changes in partitioning under variable geochemical conditions. In SCM, contaminant sorption behavior is described in terms of generic surface functional groups, with formation constants, reaction stoichiometries, and in some cases sorption site densities determined by fitting batch sorption data, factors that may change with fluctuating moisture conditions.

Question 2:
What are the primary hydrogeochemical processes that determine contaminant migration under the kinetically controlled, heterogeneous conditions encountered in the field?

(A) Development of an automated vadose monitoring system
Investigators: J.C. Seaman and collaborators from Clark Atlanta University and DYNAMAX, Inc.

During FY05 we installed radiotelemetry equipment, incorporated their function within the AVM data logging/instrument control program, and developed the initial web-based data reporting system (http://loggernet) with the help of Jeff Harris and Deno Karapatakis. We also installed an additional set of soil moisture and tension sensors on the second irrigation plot, for which custody was returned to S&GCP so that

it may be included in the normal irrigation schedule used for the majority of plots. The original AVM soil lysimeter pumping system proved unreliable under further field testing, failing to consistently pull the required vacuum to draw sample into the lysimeter. To address this shortcoming, the system was redesigned to include an additional vacuum pump separate from the ISCO pumping system that will now be dedicated to moving the sample from the lysimeter to the fraction collector and purging the sampling lines as necessary. A prototype of the new system has proven effective under limited laboratory testing. The revised patent application, including the new radiotelemetry capabilities, will be submitted following successful field testing of the new AVM design.

Question 3:
What hydrogeochemical factors control permeable reactive barrier function?

(A) *In situ* redox manipulation studies
Investigators: G.L. Mills, J.C. Seaman, and A. Neal

Over the past decade, permeable reactive barriers have been developed as an alternative to traditional pump and treat systems for groundwater remediation. This technology involves construction of permanent, semi-permanent, or replaceable units across the flow path of a dissolved phase contaminant plume. As the contaminated groundwater moves through the permeable reactive zone, contaminants are immobilized or degraded and uncontaminated groundwater emerges down gradient. The reactive materials employed within the barrier should: (1) be non-toxic, (2) remain effective for immobilizing or degrading contaminants for several years, (3) be sufficiently insoluble to remain in place, (4) retain permeability following reaction, and (5) be cost-effective relative to other treatment technologies. *In-situ* redox manipulation (ISRM) using sodium dithionite to create a subsurface reactive barrier for the treatment of reducible groundwater contaminants, including PCE, and Cr, is being implemented at DOE's Hanford Site. To date, the ISRM approach has not been widely tested on highly-weathered geological materials like the Upper Coastal Plain sediments present on the SRS. The fact that dithionite is commonly used in the extraction of Fe oxides suggests that such a treatment may be deleterious to the physical integrity of the sediments, an aspect that has not been adequately addressed in previous studies. Therefore, the objective of this study is to use dynamic column techniques to evaluate the effectiveness of dithionite reduced SRS aquifer materials in degrading or immobilizing CCl_4, PCE, TCE, and Cr(VI) by chemical reduction focusing on the behavior of Fe and the physical and hydrological properties of the aquifer sediments.

The installation of a chemically reducing reactive barrier using dithionite solution includes an injection phase during which dithionite permeates the treatment zone, a reaction phase that reduces the Fe within the aquifer sediments, and an extraction phase, when unreacted dithionite is removed from the system. Results from the column experiments indicated that significant amounts of Fe are mobilized only after injection of 12 pore volumes of dithionite solution (34.5 mmol $S_2O_4^{2-}$; pH 11). The mobilized Fe is predominately from oxide, carbonate, and sulfide minerals. Structurally bound and exchangeable Fe^{2+} changed little after 2 pore volumes. A study on the effect of reaction time on Fe mobility showed significant Fe^{2+} solubilization and transport after 10 hours that the Fe was derived from the oxide, carbonate, and sulfide phases.

The reducing capacity of the dithionite treated aquifer sediments was assessed by determining the amount of soluble Cr(VI) sequestered from groundwater within the reduced sediments. The results indicate that the injection of 12 pore volumes of dithionite solution and a subsequent reaction time of 6 hours were the optimum conditions for creating an effective treatment barrier while minimizing the dissolution of Fe into the effluent remediated groundwater. No significant decrease in hydraulic conductivity or increase in groundwater turbidity (i.e., colloid mobility) were observed using these conditions, suggesting that these conditions would be suitable for treatment systems

using ISRM in South Eastern Coastal Plain sediments.

Question 4:
What are the primary mechanisms by which amendments control immobilization of contaminants, and what are the appropriate geochemical and biological endpoints to evaluate the stability of immobilized contaminants?

(A) Immobilization of contaminants through the addition of amendments

Investigators: T.G. Hinton, P. Bertsch, H. Dion, B. Koo, C. Romanek, R. Sharitz and collaborators from Savannah River National Laboratory (D. Kaplan, A. Knox)

Three remediation-oriented manuscripts were published in FY05 from our research on the Savannah River Site. In the first manuscript (*Hinton, T.G., et al. 2005. Phytoextraction of uranium and thorium by native trees in a contaminated wetland. J. Radioanalytical and Nuclear Chem. 264:417-422;* SREL reprint 2829) we explored the potential of native trees to remediate a uranium- and thorium-contaminated wetland. This work examined the first step of phytoimmobilization by determining the ability of native trees to extract U and Th from a contaminated wetland located on the SRS. The focus on evaluating native plants was prompted by the desire not to introduce foreign species into the ecologically sensitive area and because intrusive remediation approaches are not feasible in legislatively protected wetlands. Based on measurements of the annual biomass of leaves and their contaminant concentrations, we estimated the reduction in soil contamination over time. Significant differences among tree species were found, with tupelo (*Nyssa sylvatica*) and sweetgum (*Liquidambar styraciflua*) having a significantly greater capacity to remove U and Th from the soil than the other tree species found on the site. Phytoextraction rate constants were developed and revealed that although U and Th phytoextraction was exceptionally high at the site—an order of magnitude greater than published literature would predict—the community of native trees would lower the soil inventory of U and Th by only 1% over the next 100 years.

A second manuscript (*Kaplan, D. I., et al. 2005. Cesium-137 partitioning to wetland sediments and uptake by plants. J. Radioanalytical and Nuclear Chem. 264:393-399;* SREL reprint 2830) examined the partitioning of ^{137}Cs in wetland sediments and plants in a contaminated canal used to discharge effluents from the R-reactor in the 1960s on the SRS. The physical location of ^{137}Cs as a function of depth within the sediment, as well as among sediment components (clay, sand, organic matter) revealed that the maximum ^{137}Cs concentrations generally occurred 2.5 to 7.5 cm below the sediment surface. About 60% of the total ^{137}Cs inventory in each sampled sediment core existed at the organic matter-mineral interface. Sequential extractions revealed that most of the ^{137}Cs in this interface layer was bound by amorphous Fe-oxyhydroxides or organic matter. Almost half of the ^{137}Cs was weakly held to these soils, compared to only 10% similarly held in sediments from the Hanford Site. The Hanford sediments contain a higher percentage of illite clays, and illite strongly binds Cs. The lack of strong binding on the SRS sediments was also observed in the propensity for plants to take up high percentages of ^{137}Cs from the sediments. These data reveal yet again that site specific sampling is needed to understand the dynamics and resulting risks associated with cesium contaminated environments.

The third manuscript (*Dion, H.M., et al. 2005. Cesium-137 in floodplain sediments of the Lower Three Runs Creek on the DOE Savannah River Site. J. Radioanalytical and Nuclear Chem. 264:481-488;* SREL reprint 2842) explored ^{137}Cs in sediments of a floodplain contaminated by releases from the SRS. Examination of ^{137}Cs within different sediment textural classes revealed that the highest concentrations of ^{137}Cs (Bq kg^{-1}) were in the clay fractions of the sediments. Clay, however, comprised a tiny percentage (< 2%) of the sediment. Sand was the dominant component (79 to 99%). Although the concentration of ^{137}Cs in the sand fraction was four times less that in the clay, the much larger abundance of sand resulted in it

containing the largest percentage of the creek's total ^{137}Cs inventory (total Bq). Thus, the dynamics of Cs within the watershed are likely influenced more by the sand fraction than the clay. This observation is counter-intuitive to the widely held belief that the environmental behavior of Cs is governed by clay minerals. The sand-sized fraction contained specific sorption sites, which may be due to nanostructural mica present as coatings on sand-sized particles. Bulk mineralogy was inadequate to discern the presence of these mica phases. This research suggests that ^{137}Cs transport in these highly weathered, sand dominated sediments may be governed by mechanisms not previously explored.

(B) Testing immobilization capacity of soil amendments for use in phytoextraction
Investigators: D.C. Adriano and collaborators from Universitaat fur Bodenkultur, Vienna, Austria

This project was completed in FY04.

(C) The role of biosolids in the remediation of coal reject/ fly ash
Investigators: D.C. Adriano, B. Koo, T. Punshon, and collaborators from the University of Kentucky

This project was completed in FY04.

(D) Effects of hydroxylapatite on the toxicity of nickel and uranium to microorganisms
Investigators: P.M. Bertsch, B.P. Jackson, T. Punshon (CRESP), and collaborators at the Medical University of South Carolina and the University of Chicago

We have demonstrated a pH-dependent Ni-toxicity to a constitutive TCE degrader, *Burkholderia cepacia* $PR1_{301}$. PR1 is able to grow at higher concentrations of Ni at pH 5 (17 mM) as compared to pH 6 (3.4 mM) and pH 7 (0.85 mM). This trend was not observed in the Ni-resistant strains *Wautersia metallidurans* CH34 and 31A, which have well-studied Ni efflux transporters. While possessing similar efflux transporters, different trends were noted between CH34 and 31A. CH34 was most resistant to Ni at pH 6 (12.8 mM) and least resistant at pH 7 (8.5 mM) while 31A was most resistant at pH 6 (34.1 mM) but least resistant at pH 5 (17 mM). To determine if the transporters were influenced by pH-dependent Ni-toxicity, two transconjugants were created by Dr. D. van der Lelie (Brookhaven National Laboratory) by inserting the *ncc-nre* Ni-resistance genes from 31A into PR1 and BuST, another constitutive TCE degrader created from *B. cepacia* G4 (the parent strain of PR1). Insertion of these efflux transporters increased Ni resistance in PR1:ncc-nre as compared to PR1. Growth was observed at 51.1, 17 and 1.7 mM Ni at pH 5, 6 and 7, respectively, displaying at least a two-fold level of increase in Ni resistance as compared to PR1. BuST:ncc-nre was able to grow in the presence of at least 51.1, 17 and 3.4 mM Ni at pH 5, 6 and 7, respectively. Both transconjugants displayed the same pH-dependent trend in Ni toxicity as observed with PR1. Further work is underway to determine the mechanism of Ni-tolerance displayed by PR1. Ni accumulation by PR1 does not explain changes in Ni toxicity with change of pH as PR1 accumulated 1.49 ($\pm$ 0.25), 1.12 ($\pm$ 0.09) and 3.88 ($\pm$ 0.93) mg Ni^{-1} g dry weight at pH 5, 6 and 7, respectively. Examination of PR1 culture supernatants by size exclusion chromatography coupled to inductively coupled plasma mass spectrometry to detect potential Ni-binding substances detected a small (<500 Da) Ni-binding substance. This same peak was detected in abiotic controls, suggesting that it is a medium component and not produced by PR1. Two-dimensional gel electrophoresis has been used to examine changes in protein expression in PR1 after exposure to Ni at pH 5 and 7. A chaperonin protein, GroEL, which is involved in protein folding/refolding, was identified by peptide mass fingerprint analysis. GroEL showed the highest level of expression at pH 5 after a 3 h exposure to 3.4 mM Ni. An additional 12 proteins showing altered levels of expression under the conditions examined have recently been identified by protein sequencing using a tandem mass spectrometer. Ni transport assays are currently underway to determine if Ni is transported out of the

cell and if pH influences Ni transport. In addition, genomic DNA of PR1 and four Gram-positive, Ni-tolerant isolates from Steed Pond, a U and Ni contaminated wetland at the SRS, will be examined using a metals-related microarray in the laboratory of Dr. J. Zhou at Oak Ridge National Laboratory. The microarray consists of almost 2000 genes dealing with metal homeostasis (i.e., transport, resistance, sequestration, etc.) with approximately 60 of these relating to Ni.

Publications:

Van Nostrand, J.D., A.G. Sowder, P.M. Bertsch, and P.J. Morris. *In press*. The effect of pH on the toxicity of nickel and other divalent metals to *Burkholderia cepacia* $PR1_{301}$. E*nviron. Toxicol. Chem.*

Presentations:

Van Nostrand, J.D., A. Sowder, J. Arthur, P.M. Bertsch, and P.J. Morris. 2005. Ni tolerance in *Burkholderia cepacia* $PR1_{301}$, American Society for Microbiology General Meeting, Atlanta, GA, (poster presentation).

Van Nostrand, J.D., J. Arthur, P.M. Bertsch, and P.J. Morris. 2004. Protein expression changes in *B. cepacia* $PR1_{301}$ with exposure to nickel at pH 5 and 7. EPA STAR Graduate Fellowship Conference, Washington, DC, (poster presentation).

Van Nostrand, J.D., J. Arthur, P.M. Bertsch, and P.J. Morris. 2004. Changes in protein expression in *B. cepacia* $PR1_{301}$ after exposure to nickel at pH 5 and 7. Student Research Day, Medical University of South Carolina, Charleston, SC, (poster presentation).

Question 5:

Can plant genes involved in the remediation of groundwater contaminants be identified?

(A) Searching for genes involved in the degradation of halogenated solvents

Investigators: L.A. Newman and researchers at the University of Washington

Work is continuing on this project. We are currently screening a cDNA library from tobacco, which is known to metabolize TCE at a higher rate that *Arabidopsis*. This screening is being done by looking for the release of bromide ions from the metabolism of ethylene dibromide. This compound is transformed by the same enzymes involved in TCE degradation, and the bromide can be tracked easily in suspension cultures where chloride cannot be tracked.

We have also identified five P450 genes from *Arabidopsis* which have the same reaction domain as seen in P450 2E1, which is the major TCE degrading enzyme in mammals. These genes have been cloned out of *Arabidopsis* and linked to strong constitutive promoters, and inserted back into both *Arabidopsis* and tobacco. We have multiple transformants in tobacco, and have transformed *Arabidopsis* and are waiting for those plants to germinate. Once we have sufficient plant material, we will screen for changes in metabolic patterns as well as sensitivity to high levels of TCE. We also have *Arabidopsis* lines that have had the genes knocked out, and we are currently screening them for changes in TCE degradation patterns. This work has been presented by the student at the Association for Environmental Health Sciences annual conference (2004), and the EPA International Phytotechnologies meeting (2005). The poster at the AEHS conference won the Best Student Presentation award for the conference.

Question 6:

How do microbes affect metal speciation transformations, and what is the sustainability of these transformations *in situ*?

(A) Comparing microbial communities in the rhizosphere vs. bulk soil

Investigators: C.L. Zhang, D. Adriano, and B. Koo

It is hypothesized that root exudates stimulate microbial growth and affect community structure in the rhizosphere. These microorganisms serve as focal points for metal accumulation and immobilization

using cell walls as templates for adsorption and transformation. The overarching goal is to determine the microbial community structure affected by root exudates, and co-existing heavy metals and radionuclides and to determine the role microorganisms play in the speciation, uptake, and immobilization of co-contaminant metals and radionuclides in the rhizosphere. We characterized the difference in microbial community structures between the bulk soil and the root hair in the same sample location. Samples were collected from the Lower Tims Branch on the SRS. DNAs were extracted from these samples using a commercial kit and primers specific for Bacteria (27F/1492R) were used for PCR amplification of these DNA samples. The PCR products were screened using Denaturing Gradient Gel Electrophoresis (DGGE). Individual bands in the DGGE gel were excised and purified and the DNA fragments were sequenced. All the sequences were then clustered and analyzed phylogenetically using software packages. Our preliminary results indicate the difference in microbial diversity is small between the bulk soil and the root hair for a particular sample location. This study was terminated and our efforts have been focusing on other areas of research in microbial ecology at the Savannah River Site.

Question 7:
What traits of 'native' plants and populations determine their suitability for use in remediation and site restoration?

(A) Use of fluorometry in appraising plant health during phytoremediation
Investigator: K.W. McLeod

Phytoextraction, the use of plants to remove contaminants from soil, is widely recognized as more cost-effective than excavation and disposal. Lead is a contaminant of concern that is very costly and difficult to remove from the environment. When lead is taken up in leaves, it causes damage to the photosynthetic apparatus. This damage causes an increase in fluorescence, which can be detected using a fluorometer. But other plant stresses can also cause changes in fluorometric measurements and these secondary stresses must be also recognized. The objectives of this recent experiment were: (1) to determine if lead stress can be detected in kenaf (*Hibiscus cannabinus L.*) before visible symptoms occur, and (2) compare two different fluorometers. If fluorometry can detect stress before visible symptoms are apparent, then possibly the soil chemical environment can be controlled to maximize contaminant uptake, minimize plant damage, and ultimately maximize contaminant extraction.

Kenaf plants were grown in potting soil artificially contaminated with lead. A constant concentration of acetic acid was used to acidify the soil (necessary to extract the lead), while a variable concentration of chelate (EDTA, ethylenediamine tetraacetic acid) was used to bind the metal. A LICOR 6400 portable photosynthesis system fitted with a leaf chamber fluorometer and the CFM-636973 fluorometer were used to take fluorometric measurements at specific intervals after chelate/acid addition. Rfd, an indicator of stress, was well correlated between both machines ($r=0.83$, $n=162$). It was possible to detect stress before the appearance of visible stress (spotting) on the leaves with either fluorometer. However, because lead stress caused severe leaf senescence to occur within 48 h after treatment, fluorometric analysis did not give sufficient advance time to permit modification of the soil contaminant environment and prevent leaf death.

(B) Evaluating native woody plants for efficiency of phytoremediation of chlorinated solvents
Investigators: L.A. Newman and researchers from the U.S. Forest Service and the University of Washington

We have completed the study on the hardwoods. While the poplars have always been used as a 'standard' for remediation potential, we found that sweetgum and sycamore performed as well if not better as far as water uptake rates. We also saw higher levels of metabolites in these plants than in the poplar. Regulators we spoke with showed a much greater interest in evergreen

species as an alternative to poplar, so we looked at a variety of evergreen plants rather than the herbaceous plants. As might be expected, there were much lower uptake rates in the evergreen species. This work has been presented by the student at the Association for Environmental Health Sciences annual conference (2004), and the EPA International Phytotechnologies meeting (2005). We plan to run the herbaceous plants this fall, when greenhouse temperatures have moderated.

(C) Comparing poplar cultivars for use in phyto-remediation in the Southeast
Investigators: L.A. Newman and researchers from the U.S. Forest Service

Field work has been completed for this project. We looked at parameters including survival, height and truck diameter, as well as insect and disease resistance. We found that there was a great deal of variation between the plants, but that three were consistently better suited for growth in the Southeast. This work has been written up as two articles and submitted to *Biomass and Bioenergy*, and have been accepted for publication. We will be collecting hardwood cuttings this fall from these clones and growing them in the greenhouse to determine their water uptake efficiencies.

Question 8:
Can natural processes, such as succession, be directed or accelerated to restore sustainable vegetation on impacted or remediation sites, including closure caps, floodplains, and isolated wetlands?

(A) Use of native vegetation on waste site closure caps
Investigators: B.S. Collins and K.W. McLeod

In October 2003, we initiated research to examine establishment and maintenance of alternative vegetation on closure caps. Currently, caps on the SRS are vegetated by seeding or sodding turf grasses, which must be maintained by frequent mowing. In spring and summer 2004, we surveyed existing caps to identify extant vegetation. In addition, we began an experiment to compare vegetation establishment using alternative species (a common native grass [broomsedge, *Andropogon virginicus*] vs. a turf grass [centipede], sown with or without a naturalized legume [*Lespedeza cuneata*] that is common in oldfields on the SRS) planted in mesocosms constructed to mimic closure caps. Additional treatments, including watering, fertilizer, lime, mulch, and season of planting (spring vs. summer) were applied to test their effects on vegetation establishment. We also began a field plot study to determine if turf grass or native broomsedge vegetation is more susceptible to invasion by undesirable deep-rooted species. Plots were randomly assigned to mowing (mowing, no mowing) and loblolly pine addition (seeding, no seeding) treatments. The vegetation survey revealed that 'natural' oldfield herbaceous vegetation and woody species found in oldfields, sandhills, pine plantations, and upland pine forests can establish within the planted grass community on closure caps. The presence of the legume, mulch, and consistent watering each resulted in significantly higher vegetation cover in the experimental cap communities established in spring 2004; for summer 2004 plantings, consistent watering did not increase vegetative cover. With consistent watering, sections sown with turf grass seed had lower vegetation cover than other watered combinations, though still much higher cover than any non-watered combination. Through winter and spring, 2005, no loblolly seeds had germinated in plots where they were sown. We are continuing to monitor these plots. Overall, the initial results indicate 'natural' vegetation is a suitable alternative to turf grass communities on closure caps, although consistent watering and presence of a legume may increase cover with spring-planted native grass or turf grass vegetation.

(B) Succession in disturbed floodplain wetlands and sandhills
Investigators: R R. Sharitz, K.W. McLeod, S.J. Harper, and B.S. Collins

From 1992 to 1994, species trials were initiated in

the Fourmile Branch delta to investigate the best methods of reestablishing tree species in a severely disturbed, thermally-affected stream delta. Treatments examined included planting stock type, habitat, tree shelters, root pruning, and competition controls. Survival of most species, as determined in 1994 or 1996 and 2003, changed little over the past decade, and was not strongly affected by the treatments within a trial, except for root pruning. Trees in many treatments have grown tremendously, but individuals with no competition controls generally grew more slowly. For example, baldcypress (*Taxodium distichum* Richard) has had a high survival rate, regardless of whether they were planted as bareroot or balled-and-burlapped saplings, and have grown to 8-12 meters in height. Overcup oak (*Quercus lyrata* Walter), water hickory (*Carya aquatica* (Michaux f.) Nuttall), Nuttall oak (*Q. nuttallii* Palmer), and willow oak (*Q. phellos* L.) planted in later trials, also had adequate survival rates and have grown to 5+ meters. Low mortality rates after the initial 3-4 years suggest that these species are appropriate for restoration. In contrast, survival of water tupelo (*Nyssa aquatica* L.) and green ash (*Fraxinus pennsylvanica* Marshall) have continually declined over time, making these species less suitable for successful restoration. These results were presented at the Thirteenth Biennial Southern Silviculture Research Conference and will be published in the proceedings of this meeting.

Sandhills, nutrient-poor habitats with sandy, xeric soils, support a unique flora and fauna, including a suite of threatened, endangered, and sensitive (TES) plant and animal species. Many of these habitats have been disturbed by former and current land-use practices and by SRS management activities. We used high-resolution satellite images and aerial photography in a geographic information system (GIS) database to extract sandhills from surrounding pine woodlands and managed forests of the SRS. These sandhills sites were surveyed for vegetation and soil characteristics (moisture, texture, pH, and nutrients) and were compared with sandhills habitats from other federal installations in the region. During FY04 we completed TES plant surveys and lab analyses of soils. We now are using this information to develop preliminary TES habitat models. In addition, we have planted focal TES plants into experimental gardens to examine the effects of land uses (logging, human foot and vehicle traffic) on plant survival and growth. We also have implemented an experiment to study the effects of forest management (fire, herbicide, shredding) on a sandhills community.

(C) Survey of plants colonizing coal ash basins
Investigators: L.A. Newman and faculty from the University of Kentucky

We have completed the survey of the coal reject basin, looking at types, locations, and frequency of vegetation, metal levels in the growth matrix, and metal levels in the plants. What we found was that early succession plants such as blackberry and bayberry did well on the site, and were found in multiple areas, even those with low pH. More sensitive plants such as poplar were only found in areas where pH levels were more stabilized. None of the plants studied showed metal tissue levels that would characterize them as accumulator plants, and thus potential issues with food chain transfer do not appear to be an issue. This work is in the process of being written up for publication.

(D) Restoration of isolated depression wetlands
Investigators: R.R. Sharitz and B.E. Taylor

Many Carolina bays and other isolated wetland ponds on the SRS were ditched, cleared, and otherwise altered by landowners prior to the establishment of the SRS. In 1998, the 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 are undergoing 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. In FY04, the third year of post-restoration, vegetation analysis revealed an increase in obligate and facultative wetland species to 50% (compared with 28% prior to restoration treatments), coupled with a decline in overall plant species richness as invasive upland species were extirpated. Comparison of pre- and post-restoration invertebrate assemblages revealed increases of 5-10 or more microcrustacean species in most ponds where hydroperiod was affected substantially. Other predicted effects (increase of species richness with conversion from forested to herbaceous vegetation, loss of ephemeral habitat specialists with increased hydroperiod) have not yet been observed. Post-treatment monitoring of the vegetation and invertebrates was completed, providing the data needed for assessment of restoration success.

Vegetation research was supported by a grant from USDA Forest Service. Papers characterizing plant communities of reference depression wetlands were published in *Wetlands* and *Journal of the Torrey Botanical Society*. A manuscript on vegetation response to restoration treatments was submitted in FY05.

Research on aquatic invertebrates was also supported by a grant from USDA Forest Service. Bimonthly invertebrate monitoring (3 years pre-treatment; 4 years post-treatment) was completed in December 2005. Sample processing is complete for micro-crustaceans, nearly complete for macroinvertebrates. Stable isotope studies to compare trophic position of invertebrates between forested and herbaceous wetlands will be completed in FY06. We published a paper in *Journal of the North American Benthological Society* that uses the pre-treatment macroinvertebrate data in a comparison of wetland communities on a continental-scale gradient (Canada to Florida). We published an invited research report in *Ecological Restoration* showing the rapid response of microcrustacean species richness in the treatment wetlands. We submitted a manuscript to *Wetlands* on trajectories of change in microcrustacean assemblages in Bay 93, another restored wetland, over an 8-year post-treatment period. We also published two general accounts of aquatic invertebrates in the SRS: a National Environmental Research Park report on microcrustaceans of the SRS and a chapter on aquatic invertebrates of the SRS.

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

Environmental Health and Safety Program

Donald R. Mosser and Vivian G. Dicks

The Savannah River Ecology Laboratory continues to operate successfully under the work-smart safety and environmental standards that resulted from SREL's participation in DOE's 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 maintains a commitment to two full-time employee positions dedicated to the support of the SREL Environmental Health and Safety (EH&S) Program. Approximately 23 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 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. New personnel safety and environmental orientation was presented to approximately 40 individuals during the past year. Additionally, training was provided for Chemical Coordinators and hazardous waste workers. WSRC provided Radiological Worker and radiation generating device training to SREL personnel as necessary.

SREL's internal computer network was used to provide targeted safety information to specific work groups within the laboratory. Lessons learned and health and safety topics were distributed via e-mail throughout the year. Safety training literature was also made available in break rooms and hallway literature racks.

Facility inspections remain a cornerstone of the SREL Safety Program. SREL personnel conducted regularly scheduled facility inspections. SREL also 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. Safety Services personnel reviewed and approved more than 265 chemical purchases. SREL continues to support the SRS Environmental Management System as a signatory to the SRS Environmental Management System Policy Statement.

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 234 pounds of excess hazardous laboratory chemicals and laboratory hazardous wastes to a hazardous waste disposal firm (May 2005).

In support of SRS safety programs, SREL participated in the development of corrective action plans related to the SRS Lessons Learned program and the development of the new SRS visitor control programs. As part of the new SRS visitor control program, SREL participated in a sitewide review of each organization's work activities and associated work hazards as they relate to visitors and subcontractors. SREL continues to move forward with implementation of the new SRS visitor control requirements.

Distance Learning Program

Laura Janecek and Marie Hamilton

The Savannah River Ecology Laboratory maintains a state-of-the-art Distance Learning (DL) facility that delivers two-way audio and visual transmissions via a T1 line. This facility is part of the Georgia Statewide Academic and Medical Systems (GSAMS), a cooperative and collaborative distance education network in the state of Georgia with about 300 interactive audio and videoconference classrooms. SREL's DL Facility provides the capability to communicate with other distance learning users throughout the country. SREL uses DL for classroom instruction for a Master's degree program, other graduate courses, outreach presentations, graduate student committee meetings, faculty meetings, and staff briefings. The primary distance learning program

for SREL is the multidisciplinary Master of Science degree in Environmental Toxicology, offered in cooperation with the UGA School of Pharmacy. This is the first degree offered by UGA through any distance learning site. Eight students have completed all required coursework and are working on the research component of the degree, and four students have graduated from the program. Because of increased costs associated with administration of this degree program, it is likely that it will no longer be offered after FY05.

The SREL Outreach Program has been using DL technology to extend their programming because they can reach multiple classrooms as well as minimize travel and animal handling time by using this facility. Outreach personnel presented 5 lectures on various ecological topics to students in South Carolina and Georgia K-12 schools. Many Georgia schools have not been able to maintain compatibility with GSAMS and the Georgia audiences are declining.

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 FY05 and the Central Archive Data Repository now has information covering over 511 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 FY05. 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 and Graduate Education Program

Travis Glenn

The objective of the SREL Education Program is to promote professional development and enhance environmental awareness among undergraduate and graduate students through research participation and training programs with emphasis on conducting ecological research important to the Savannah River Site mission. Undergraduate and graduate student participants in FY05 are listed in Table 1 (pages 54-55).

The SREL Education Program has averaged 18 undergraduate students per year since 1968. These students, from over 275 different colleges and universities, have been co-authors on 150+ peer reviewed research publications; more than 200 of these students have gone on to pursue careers in science. The Undergraduate-Research Experience for Undergraduates, funded by the National Science Foundation, sponsored 11 students this past year. In addition, we sponsored three student funded by NOAA, two students funded by SREL, and two funded by South Carolina State University.

Since 1967, an average of six students a year have

completed graduate studies at SREL and over 315 dissertations and theses have been written. During FY05, eight students completed their degree requirements (four M.S. and four 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, our 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 FY05:

Heather Brant	M.S.	University of Georgia	2004	Charles Jagoe
Sarah Drake	M.S.	University of Georgia	2004	Rebecca Sharitz
Susanne Hauswaldt	Ph.D.	University of South Carolina	2004	Travis Glenn
Olga Tsyusko	Ph.D.	University of Georgia	2004	Michael Smith/Travis Glenn
Susan Turner	Ph.D.	University of Georgia	2004	Rebecca Sharitz
Angel Kelsey Wall	M.S.	University of Georgia	2004	Charles Jagoe/John Seaman
Neera Young	M.S.	University of Georgia	2004	Michael Smith
Yong Jin Lee	Ph.D.	University of Georgia	2005	Christopher Romanek

Table 1A. SREL Undergraduate Student Program Participants

Student	Academic Institution	Faculty Advisor
Adam David Borden	Louisiana State University, LA	Steven Harper
Danielle Denise Britt	University of South Carolina, Aiken	Gary Mills
Caleb Ryan Caton	University of South Dakota, Vermillion	I Lehr Brisbin
Latasha Yolanda Cofer	Voorhees College, SC	Travis Glenn
Anthony Brian DeMarco	University of Georgia, Athens	John Seaman
Celeste Tara Holz-Schietinger	The Evergreen State University, WA	Travis Glenn
Travis Johnson	Reinhart College, GA	Charles Jagoe/J Vaun McArthur
Ramirez Ezell Lewis	Fort Valley State University, GA	Charles Jagoe/J Vaun McArthur
Rachel Diane Mahan	University of Missouri, Columbia	J. Whitfield Gibbons
Deidra Marie McCray	South Carolina State University	Gary Mills
Nina Marie Menichino	University of Wales, Bangor, United Kingdom	Andrew Neal
Andrea LaToya Richardson	South Carolina State University	J Vaun McArthur
Karen Jean Schlatter	McGill University, Montreal, Canada	J. Whitfield Gibbons
Alexa Michel Schmitz	Oberlin College, OH	Lee Newman
Jennifer Marlene Summerlin	Augusta State University, GA	Charles Jagoe
Tara Denice Sumter	South Carolina State University	Gary Mills
Jennifer Marie Thomsen	Clemson University, SC	Christopher Romanek
Marshall Therdrick Washington	Claflin University, SC	Charles Jagoe/J Vaun McArthur

Table 1B. SREL Graduate Student Program Participants

Student	Degree	Institution	Faculty Advisor
Kimberly Andrews	Ph.D.	University of Georgia, Athens	J. Whitfield Gibbons
Elizabeth Burgess	Ph.D.	University of Georgia, Athens	Andrew Neal
Monica Carroll	Ph.D.	University of Georgia, Athens	Christopher Romanek
*Mara Cea	Ph.D.	La Frontera University, Chile	Gary Mills
*Alcides Cintra	Ph.D.	Universidade Estadual Paulista, Brazil	Domy Adriano
Dana Cook	Ph.D.	University of Georgia, Athens	Andrew Neal
Sarah DuRant	M.S.	University of Georgia, Athens	William Hopkins
William Duval	Ph.D.	University of Georgia, Athens	Rebecca Sharitz
Carol Flaute	M.S.	University of Georgia, Athens	Steve Harper
Gabrielle Graeter	M.S.	University of Georgia, Athens	J. Whitfield Gibbons
Aaliyah Green	M.S.	University of Georgia, Athens	Charles Jagoe
Ellen Hepfer	M.S.	University of Georgia, Athens	Machelle Wilson
Ryan Holem	M.S.	University of Georgia, Athens	William Hopkins
Cori-Alice Holladay	M.S.	Appalachian State University, NC	Beverly Collins
Glenn Kirkland	M.S.	University of Georgia, Athens	I Lehr Brisbin
Yong Jin Lee	Ph.D.	University of Georgia, Athens	Christopher Romanek
Frantisek Majs	Ph.D.	University of Georgia, Athens	John Seaman
*Mirela Matioc	Ph.D.	University of Georgia, Athens	Travis Glenn
*Clint Page	Ph.D.	University of Georgia, Athens	Andrew Neal
Gretchen Peltier	Ph.D.	University of Georgia, Athens	William Hopkins
John Peterson	M.S.	Auburn University, Alabama	William Hopkins
Steven Schaff	Ph.D.	University of Georgia, Athens	Kenneth McLeod
Julian Singer	Ph.D.	University of Georgia, Athens	John Seaman
Amy Squire	M.S.	University of Georgia, Athens	Rebecca Sharitz
Karolina Stark	Ph.D.	University of Stockholm, Sweden	Thomas Hinton
*Steven Stoddard	Ph.D.	University of Illinois	Rebecca Sharitz
Brian Todd	Ph.D.	University of Georgia, Athens	J. Whitfield Gibbons
Olga Tsyusko	Ph.D.	University of Georgia, Athens	Michael Smith/Travis Glenn
Joy Van Nostrand	Ph.D.	Medical University of South Carolina	Paul Bertsch
Qin Wang	Ph.D.	University of Georgia, Athens	Machelle Wilson
Arlena Wartell	Ph.D.	University of Georgia, Athens	Travis Glenn
Jamie Williams	M.S.	University of Georgia, Athens	Barbara 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

** Short-term students – did not complete degree while part of the SREL Education Program*

Environmental Outreach

Kenneth McLeod

As a unit of The University of Georgia, the Savannah River Ecology Laboratory has the tripartite missions of research, outreach, and education. These three missions are complimentary and have strongly contributed to SREL's position as a respected, unbiased source of information in the Central Savannah River Area. The intellectual independence of the academician assures the local community of objective research on the impacts of site operations on SRS and regional ecosystems. Added to this independence is the dedication and enthusiasm of the SREL Environmental Outreach Program, which can be easily assessed by their Vision – *To translate ecological knowledge into public passion for science* – and Mission – *To increase the understanding of science by communicating ecological knowledge to the public through educational programs, products, publications, and the media.*

SREL uses information from its own research efforts to educate the public locally, regionally, and nationally. 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.

The Outreach Program is designed to enhance SREL's overall mission of acquiring and communicating environmental knowledge and addresses the U.S. Department of Energy's (DOE) current focus on environmental issues. Public education, especially for K-12 audiences, is accomplished through a variety of programs.

- During the past year, SREL scheduled 247 talks, 14 tours, 19 exhibits, and 62 workshops, reaching a total of 64,761 people. Topics for these presentations included biodiversity, the process of science, animal adaptation, plants and wetlands, chemistry and environmental science, local ecosystems and conservation, classification, environmental stewardship, and careers in ecology and research.
- Over 325 4th and 5th grade students and 16 teachers at three Aiken County and one Lexington County elementary schools participated in 5 individual class science workshops and a daylong field trip. Scientific inquiry and process skills were stressed; students worked in groups, conducted experiments, and recorded and analyzed data.
- School groups enjoy field trips to the Laboratory's Conference Center to participate in the Ecologist-for-a-Day program.
- Teachers are trained in methods of teaching ecology during workshops and are provided with materials produced by the Outreach staff.
- Outreach speakers have made 5 presentations to multiple classrooms through the use of the Distance Learning Facility. Many Georgia schools have not been able to maintain compatibility with GSAMS and the Georgia audiences are declining.
- An internal laboratory newsletter, *The Grape Vine*, is distributed electronically, 12 times per year.

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. These presentations, offered in schools, emphasize hands-on scientific learning using activities in the environmental sciences. "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. Participants get an opportunity to work with SREL researchers catching, marking, and measuring various species of reptiles, amphibians, small mammals, and invertebrates. In addition, the Outreach Program offers tours of SREL facilities and surrounding field sites, as well as exhibits and workshops for the general public.

During the past year SREL completed-its new "research-to-classroom" hands-on science program for elementary school students. With funding from the American Honda Foundation (AHF) and partial funding from The Christensen Fund , SREL presented five workshops to every 5th grade class at East Aiken and Greendale Elementary schools, and to every 4th grade class at J.D. Lever Elementary and Midland Valley Charter Schools in Aiken

County; workshops were also presented to two 4th grade classes at Batesburg-Leesville Elementary School in Lexington County. In each two-hour classroom session, SREL staff showcased one or two SREL researchers conducting studies in subject areas related to the topic of the day, and students learned about on-going research by scientists working in their community. Students then performed hands-on activities that reinforced the content, as well as scientific inquiry and process skills. All activities were linked in some way to local habitats as well as to South Carolina and national science standards. At the conclusion of the program, all students participated in a daylong field trip and acted as "junior scientists" by using the skills taught in the classroom in an outdoor setting. Funding for this program was not renewed for FY06 by The American Honda Foundation, so SREL is seeking funding to continue this popular program from other sources.

Associated with the AHF funding, SREL continued a website (www.kidsdoscience.org) that provides all the necessary materials for the 10 hands-on activities used in the classroom workshops. This website serves as an excellent resource for any teacher who may want to use the activities/materials. Also as part of the AHF funding, additional programs were also given to general audiences such as civic, scout, and church groups, as well as at community and regional events. Many of these programs continued to emphasize "the methods of science," but additional topics included watershed processes and pollutants, groundwater contamination, phytoremediation, radioecology, and creation and restoration of seasonal wetlands.

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 28 teams from regional middle schools participating in an ecological science-bowl. Next year, the Richmond County (Georgia) Board of Education will require all county middle schools to participate in the EcoMeet. Participation in ESEC has provided the opportunity to showcase SREL at Augusta's Fort Discovery Science Museum and also has provided an opportunity for speakers from the Lab to address groups at Fort Discovery.

SREL Outreach members have also provided expertise to the Aiken County Watershed Alliance (ACWA), a local community group, and the Central Savannah River Area Regional Science and Engineering Fair, Inc., an organization that serves an 18-county area of South Carolina and Georgia. In addition, Outreach members educated local and state leaders on the ecological value of seasonal wetlands, and accompanied personnel of the Southern Environmental Law Center on visits to regional newspapers to highlight the importance of seasonal wetlands.

Thousands of copies of educational products and materials are distributed 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 *Chemistry – it's all about the nature of things* sticker, the Metric System Rap bookmark; and an emergency services calendar that depicts plants of the SRS (produced in cooperation with Westinghouse Savannah River Company). All of these products have been extremely popular and thousands of copies have been distributed during the past year. Also distributed was *The National Environmental Research*

Park at Savannah River Site: Serving an Essential Mission for 25 Years. The National Environmental Research Park (NERP) brochure highlights 25 years of research associated with the NERP program at the Savannah River Site. Full-color fact sheets and research "snapshots" on a wide variety of research topics are published and distributed as well.

The public relations component of the Outreach Program includes the distribution of news releases on a variety of topics to selected media affiliates, officials of DOE, and The University of Georgia. In FY05 SREL researchers provided information to such diverse media outlets as *Science, New York Times, The Washington Post, Fortune, MSNBC, National Geographic News, The Times-Union, Nature (Jacksonville, FL) The Christian Science Monitor, The Chronicle of Higher Education,* the *Associated Press, Reptiles, The Geology Society News, Skirt (a woman's weekly), Wildlife Magazine, National Wildlife, Outdoor Life, Georgia Magazine, The Vivarium, Hi* (an Arabic language magazine),and many local news outlets in the Southeast such as *The Atlanta Journal-Constitution, The Augusta Chronicle, The Charlotte Observer, The Athens Banner-Herald, Beaufort Gazette, The Charleston Post and Courier, The State, The Aiken Standard, Savannah Morning News, The Island Packet,* and the *Asheville Citizen News.* Online coverage has included such sites as Charlotte.com, Audubon, Environmental News Network, Lowcountry Now, EurekAlert, Topix.net. Access NorthGA.com, Ascribe, dateline.columbia, GoUpstate.com and others. Topics in the news ranged from animal behavior to environmental impacts.

The Public Relations office screens most inquiries from the press, directing reporters to the most appropriate researchers for their stories. In addition, SREL initiates press contacts, maintains a website for reporters and submits releases and stories to appropriate websites and magazines, including alumni magazines.

DOE Research Set-Aside Areas

Charles E. Davis

The Savannah River Site (SRS) is a National Environmental Research Park (NERP) and its large land area and controlled public access provide a diverse and protected outdoor laboratory where researchers study the environmental impacts of the SRS's industrial and forest management operations. Because these studies are usually long-term, they require relatively undisturbed areas as "control" sites where reference, baseline data can be obtained. Known as Research Set-Aside Areas, these reference sites have been withdrawn from the SRS's commercial forest and set aside by the Department of Energy (DOE) primarily for non-manipulative ecological research and educational outreach activities. These areas also serve as "reserve" areas that represent excellent examples of both the typical and unique plant communities indigenous to the SRS while providing critical habitat for the Site's threatened, endangered, or sensitive (TES) flora and fauna. Currently, there are 30 Set-Aside Areas on the SRS that collectively account for approximately 14,100 acres (5,706 ha), or 7% of the Site. Individually, they range in size from 10 acres (4.05 ha) to 7,400 acres (2.995 ha), and are located in 43 of the Site's 89 timber resource compartments. There are approximately 270 miles (435 km) of posted boundary line associated with these Set-Aside Areas.

Periodic inspections of Set-Aside boundary postings are conducted by SREL where potential land-use conflicts or impacts are anticipated. SREL and the USFS-SR cooperate in verifying and updating Set-Aside boundary lines that are concordant with prescribed forest stand treatments. To date, boundaries for 12 of the 30 Set-Aside Areas have been updated using GPS (Global Positioning System) technology; significant portions of the E. P. Odum Wetland (Area No. 30) and Ruth Patrick/Meyers Branch (Area No. 11) Set-Asides have been completed as well. Because this GIS boundary layer continues to be a dynamic, working layer, SREL provides updates of the Set-Aside boundary layer to the USFS-SR upon request. SREL updated this boundary layer, including the metadata, and released it internally in FY05.

Management of the Set-Aside Areas

SREL's Set-Aside Research Coordinator is responsible for developing, writing, and implementing stewardship management plans for each of the 30 Set-Asides. Set-Aside Areas that have an anticipated management need in the near future (e.g., plantation first thinning, adjacent property management concern) are scheduled for management plan development. Management plans for Set-Asides are generally developed by a team of individuals from various SRS organizations. When necessary, researchers with individual expertise or with a long-term research interest in a Set-Aside may be requested to participate as a core team member.

During the past year, SREL worked on five management plans, completing plans for Rainbow Bay Amphibian Reserve Set-Aside Area (Area No. 16) and the Risher Pond Set-Aside. 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) were also worked on. These plans were expanded this year to address researcher safety, nuisance flora and fauna, and ecolitter issues.

Some accomplishments for the Set-Aside program for this FY included marking 17 acres (6.9 ha) of pine for removal as a first thinning treatment in the Rainbow Bay Set-Aside. A major milestone was accomplished this year when a 30-acre (12.2 ha) pine thinning treatment was conducted in the Flamingo Bay Set-Aside (Area No. 21), marking the actual implementation of a management plan. In the Ginger's Bay Set-Aside, a pine stand was approved for first thin marking pending Set-Aside Task Group approval. Although researchers requested that Steel Creek Bay (Area No. 08) and Ellenton Bay (Area No. 01) be burned this past year, the treatments were not done due to renewed rains. In the Craig Pond/Sarracenia Bay Set-Aside, a defensive fire line was installed within the Set-Aside as a measure of control when Chem Nuclear conducted a prescribed burn in the spring. The road running through Ellenton Bay was cleared of vegetation to facilitate tours; researchers initiated trapping to control beaver and otters in this Set-Aside because of the impact these animals were having on herpetofaunal research. Finally, additional acreage (yet to be determined) was added to the Oak Hickory Forest (Area No. 12) and the Upper Three Runs Creek Set-Asides following a USFS-SR ID team recommendation. It was suspected that the Beech Hardwood Set-Aside (Area No. 6) is being impacted by Pb contaminants from an upslope 1950s pistol range and monitoring was initiated by WSRC.

Research in Set-Aside Areas

Set-Asides continued to be increasingly used by researchers this FY both as research study sites and as reference sites for collections of uncontaminated plants, animals, soils, or water. Nine theses and dissertations were published this year with study sites that included Set-Asides. Many publications were devoted to research in depressional wetland Set-Asides. A new plant study was initiated this FY in which two Set-Aside Areas are being used as controls to evaluate impacts from military training and forest management on TES species. A number of publications used Set-Aside areas as controls when evaluating ecological risks, contaminant transport, and site remediation.

Groups other than SREL also used Set-Aside Areas. SRNL used organisms collected in Set-Asides to test methods of evaluating remediation and restoration actions as well as the development of terrestrial bio-assessment protocols at DOE sites. Archaeologists with the USC-Savannah River Archaeologist Research Program expanded their investigations at the Flamingo Bay Set-Aside this year. In cooperative efforts, SREL and USFS-SR researchers continued to study coarse woody debris decomposition, softmast production in bottomland hardwood forests, and the role of fleshy fruit production, consumption, and dispersal on promoting biological diversity.

FY05 SREL documents, publications, theses, and dissertations that used DOE Set-Aside Areas

Thirty-two documents were published this year reporting activity and research from the Set-Aside Areas. Recent SREL and other SRS agency documents, scientific publications, theses, and dissertations that used or referenced DOE Set-Aside Areas during FY05 include:

Andrews, K. 2004. Interspecific comparisons of behavioral responses of southeastern snakes to roads. M.S. Thesis. University of Georgia, Athens, Georgia, USA.

Batzer, D.P., S.E. Dietz-Brantley, B.E. Taylor and A.E. DeBiase. 2005. Evaluating regional differences in macroinvertebrate communities from forested depressional wetlands across eastern and central North America. Journal of North America Benthological Society 24:403-414.

Bertsch, P.M. and C.L. Strojan. 2004. Science in support of assessing ecological risk and implementing novel remediation strategies at the Savannah River Site, SC. Waste Management 2004 Conference, February 29-March 4, 2004, Tucson, AZ.

Burbage, L.E. 2004. Environmental gradients and plant species distributions with Carolina bay wetlands. M. S. Thesis. University of Georgia, Athens, Georgia, USA.

Comer, C.E. 2005. An analysis of spatial and genetic population structure in white-tailed deer and implications for management. Ph.D. Dissertation. University of Georgia, Athens, Georgia, USA.

Collins, B.S., JV. McArthur and R.R. Sharitz. 2004. Plant effects on microbial assemblages and remediation of acidic coal pile runoff in mesocosm treatment wetlands. Ecological Engineering 23:107-115.

Davis, C.E. 2005. Stewardship management plans for the Rainbow Bay Amphibian Reserve Area Set-Aside on the Savannah River Site Site. Savannah River Ecology Laboratory, University of Georgia, Aiken, SC.

Davis, C.E. 2005. Stewardship management plans for the Risher Pond Set-Aside on the Savannah River Site Site. Savannah River Ecology Laboratory, University of Georgia, Aiken, SC.

De Steven, D., and M.M. Toner. 2004 Vegetation of Upper Coastal Plain depression wetlands: Environmental templates and wetland dynamics within a landscape framework. Wetlands 24:23-42.

Harmon, S.M., J.K. King, J.B. Gladden, G.T. Chandler and L.A. Newman. 2005. Mercury body burdens in *Gambusia holbrooki* and *Erimyzon sucetta* in a wetland mesocosm amended with sulfate. Chemosphere 59:227-233.

Harper, S.J. and R.R. Sharitz.2005. Delineating Sandhills communities: The use of advanced techniques to extract features from satellite imagery. *In* Bettinger, P.C.C Hyldahl, S.D. Danskin, J. Zhu, Y. Zhang, W.G. Hubbaard, T. Lowe, M. Wimberly, and B. Jackson. (eds.) Proceedings of the 4th Southern Forestry and Natural Resources GIS Conference, December 16-17, 2004. Athens, GA. Warnell School of Forest Resources, University of Georgia, Athens GA.

Hopkins, W.A., C.T. Winne and S.E. DuRant. 2004. Differential swimming performance of two natricine snakes exposed to a cholinesterase-inhibiting pesticide. Environmental Pollution 133:531-540.

Jenkins, C.A. 2004. Investigating growth patterns in *Kinosternom subrubrum* species of turtle. M.S. Thesis. University of North Carolina at Chapel Hill. Chapel Hill, North Carolina, USA.

Jin, V.L. 2004. Soluable organic nitrogen in plant and soil processes in the upper coastal plain. Ph.D. Dissertation. University of Georgia, Athens, Georgia, USA.

Kelsey-Wall, A. 2004. Evaluation of a Tritium Irrigation Site: A Field Study, Assessment of the Biological Half-Life and evaluation of Oxidative Stress Effects in Mice with Low-Level, Oral Exposure to Tritium. M.S. Thesis. University of Georgia, Athens, Georgia, USA.

Kelsey-Wall, A., J.C. Seaman, C.H. Jagoe, C.E. Dallas and K.F. Gaines. 2005. Rodents as receptor species at a tritium disposal site. Journal of Environmental Radioactivity 82:95-104.

Kilgo, J.C. and J.I Blake. (*In press*). Ecology and management of a forested landscape: Fifty years of natural resource stewardship on the Savannah River Site. A publication of the U.S Forest Service-Savannah River.

Kwit, C., D.J. Levey and C.H. Greenberg. 2004.Contagious seed dispersal beneath heterospecific fruiting trees and its consequences. Oikos. 107:303-308.

Marcy, B.C., D.E. Fletcher, F.D. Martin, M.H. Paller, and M.J.M. Reichert. Fishes of the Middle Savannah River Basin: With Emphasis on the Savannah River Site. University of Georgia Press.

Metts, B.S., W.A. Hopkins and J.P. Nestor. 2005. Interaction of an insecticide with larval density in pond-breeding salamanders (*Ambystoma*). Freshwater Biology 50:685-696.

Mulhouse, J.M. 2004. Vegetation change in herbaceous Carolina bays of the Upper Coastal Plain: Dynamics during drought. M.S. Thesis. University of Georgia, Athens, Georgia, USA.

Paller, M.H., C.H. Jagoe, H. Bennett, H.A. Brant and J.A. Bowers. 2004. Influence of methylmercury from tributary streams on mercury levels in Savannah River Asiatic clams. Science of the Total Environment 325:209-219.

Paller, M.H., D.E. Fletcher, T. Jones, S.A. Dyer, J.J. Isely and J.W. Littrell. 2005. Potential of largemouth bass as vectors of 137Cs dispersal. Journal of Environmental Radioactivity 80:27-43.

Parresol, B.R. 2004. Point and fixed plot sampling inventory estimates at the Savannah River Site, South Carolina. Report. USDA Forest Service, Savannah River, Aiken, SC.

Rinehart, B.D. 2004. The use of small mammals as indicators of heavy metal bioavailability in a contaminated riparian zone. M.S. Thesis. University of Georgia, Athens, Georgia, USA.

Roe, J.H., W.A. Hopkins and B.P. Jackson. 2005. Species- and stage-specific differences in trace element tissue concentrations in amphibians: implications for the disposal of coal-combustion wastes. Environmental Pollution 136:353-363.

Sever, D.M. and W.A. Hopkins. 2004. Oviductal sperm storage in the ground skink *scincella laterale* holbrook (Reptilia: scincidae). Journal of Experimental Zoology 301A:599-611.

Taylor, B. E. and A. E. DeBiase. 2005. Are microcrustaceans useful for assessing success of wetland pond restoration? (South Carolina). Ecological Restoration 23:56-57.

Turner, S.A. 2005. Facilitation and competition between a nitrogen-fixing perennial legume, *Lespedeza cuneata*, and an annual, *Heterotheca subaxillaris*, in a South Carolina old field. Ph.D. dissertation. University of Georgia, Athens, Georgia, USA.

Unrine, J.M., C.H. Jagoe, A.C. Brinton, H.A. Brant and N.T. Garvin. 2005. Dietary mercury exposure and bioaccumulation in amphibian larvae inhabiting Carolina bay wetlands.

Environmental Pollution 135:245-253.

Willson, J.D., C.T. Winne and L.A. Fedewa. 2005. Unveiling escape and capture rates of aquatic snakes and salamanders (*Siren* spp. and *Amphiuma means*) in commercial funnel traps. Journal of Freshwater Ecology 20:397-404.

Winne, C.T., M.D. Dorcas and S.M. Poppy. 2005. Population structure, body size, and seasonal activity of black swamp snakes (*Seminatrix pygaea*). Southeastern Naturalist 4:1-14.

◆◆◆ 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	Examination of Coastal Aquaculture Effluent and Receiving Water Quality Throughout the Tidal Cycle
Funding Agency	US Department of Commerce/NOAA
Budget	$94,094
Period	July 1, 2003–May 31, 2005

PI	Paul M. Bertsch
Project Title	Tidal Creek Materials Loading for the South Carolina-Georgia LU-CES Program
Funding Agency	South Carolina Sea Grant Consortium
Budget	$67,255
Period	July 1, 2003–November 30, 2004

PI	Paul M. Bertsch
Project Title	Tidal Creek Materials Loading for the South Carolina-Georgia LU-CES Program
Funding Agency	South Carolina Sea Grant Consortium
Budget	$60,514
Period	July 1, 2004–June 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	$154,915
Period	October 1, 2003–September 30, 2005

PI	I. Lehr Brisbin, Jr.
Project Title	CLEARMADD
Funding Agency	Centers for Disease Control and Prevention
Budget	$25,000
Period	August 1, 2002–July 31, 2005

PI	I. Lehr Brisbin, Jr.
Project Title	Mercury studies on wading birds on the Georgia coast
Funding Agency	Savannah Presbytery MK Penetcostal Fund
Budget	$8,200
Period	July 1, 2004–May 31, 2005

PI	A. Lawrence Bryan, Jr.
Project Title	Determination of Wood Stork breeding success in Georgia in 2004
Funding Agency	USDI Fish and Wildlife Service
Budget	$8,325
Period	April 1, 2004–September 30, 2004

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	Beverly Collins
Project Title	Thresholds of Disturbance: Land Management Effects on Vegetation and Nitrogen Dynamics
Funding Agency	Strategic Environmental Research and Development Program (SERDP)
Budget	$1,331,765 total ($33,500 in FY05)
Period	January 1, 2000–February 28, 2005

PI	Beverly Collins
Project Title	SEMP Integration Program
Funding Agency	Strategic Environmental Research and Development Program (SERDP)
Budget	$20,000 in FY05
Period	July 1, 2003–June 30, 2005

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
Funding Agency	US Department of Defense
Budget	$90,000
Period	June 1, 2005–October 31, 2005

PI	J. Whitfield Gibbons
Project Title	PARC Program
Funding Agency	USDA Forest Service
Budget	$39,000 in FY05
Period	July 2003–June 2005

PI	J. Whitfield Gibbons
Project Title	Effects of Forestry Practices on the Gopher Tortoise
Funding Agency	Strategic Environmental Research and Development Program (SERDP)
Budget	$50,000
Period	January 2004–December 2004

PI	J. Whitfield Gibbons
Project Title	PARC Program
Funding Agency	USDI Fish and Wildlife Service
Budget	$8,730 in FY04
Period	January 2004–December 2004

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	Development, Production, and Distribution of Environmental Education Materials for Indigo Snake Protection
Funding Agency	USDI Fish and Wildlife Service
Budget	$39,916
Period	May 1, 2001–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–April 24, 2006

PI	J. Whitfield Gibbons and William Hopkins
Project Title	Sublethal Effects of Pesticide Exposure
Funding Agency	US Golf Association
Budget	$87,600 total award
Period	February 1, 2001–January 31, 2005

PI	J. Whitfield Gibbons
Project Title	A Primary Understanding of Our Environment: Teaching Kids the Science of Ecology
Funding Agency	The Christensen Fund
Budget	$105,000
Period	August 1, 2002–August 1, 2005

PI	J. Whitfield Gibbons
Project Title	Inventory of Herpetofauna for the Appalachian Highlands and Cumberland Piedmont Networks of the National Park Service
Funding Agency	USDI National Park Service
Budget	$37,226
Period	September 9, 2002–December 31, 2005

PI	J. Whitfield Gibbons
Project Title	A Primary Understanding of Our Environment: Teaching Kids the Science of Ecology
Funding Agency	American Honda Foundation
Budget	$69,080
Period	August 1, 2003–November 30, 2004

PI	J. Whitfield Gibbons
Project Title	Collaborative Research: Land-use Practices and Persistence of Amphibian Populations
Funding Agency	National Science Foundation
Budget	$149,903
Period	May 15, 2003–April 30, 2006

PI	J. Whitfield Gibbons
Project Title	Operation of the NARCAM Website
Funding Agency	US Department of Interior
Budget	$85,000
Period	August 1, 2003–August 31, 2005

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–September 26, 2005

PI	J. Whitfield Gibbons
Project Title	Incidence of Chytrid Fungal Parasitism in Southeastern National Parks
Funding Agency	US Department of Interior
Budget	$10,000
Period	September 13, 2004–September 40, 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	History, Ecology, and Demography of the Gopher Tortoise at Tillman Sandridge Preserve (TSP) and Public Service Authority
Funding Agency	SC Department of Natural Resources
Budget	$9,600
Period	May 1, 2005–October 31, 2005

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	Molecular Phylogeny of North American Spiranthes Orchids
Funding Agency	American Orchid Society
Budget	$6,450
Period	January 1, 2004–December 31, 2005

PI	Travis Glenn
Project Title	Support for Turtle Conservation
Funding Agency	National Fish and Wildlife Foundation
Budget	$14,206
Period	July 1, 2003–June 30, 2005

PI	Travis Glenn
Project Title	Development of Peromyscus genomics
Funding Agency	University of South Carolina
Budget	$115,028
Period	August 1, 2004–July 31, 2005

PI	Thomas Hinton and Travis Glenn
Project Title	Transgenerational Effects of Chronic Low-Dose Irradiation in a Medaka Fish Model System
Funding Agency	Department of Energy Low Dose Program
Budget	$1,033,705 total ($128,373 for SREL in FY05; award pending)
Period	March 1, 2005–February 28, 2008

PI	William Hopkins
Project Title	Modeling the Individual and Interactive Risks to an Amphibian Population Resulting from Breeding Site Contamination and Terrestrial Habitat Loss
Funding Agency	US Environmental Protection Agency/University of Maryland
Budget	$68,730
Period	December 19, 2001–December 18, 2004

PI	William Hopkins
Project Title	Effects of Maternal Transfer of Contaminants on Amphibian Development and Fitness
Funding Agency	The Eppley Foundation for Research, Inc.
Budget	$11,500
Period	February 11, 2004–February 10, 2005

PI	Charles H. Jagoe and J Vaun McArthur
Project Title	REU: The Impact of Energy Technologies on Natural Environmental Systems
Funding Agency	National Science Foundation
Budget	$193,800 total award
Period	May 1, 2002–April 30, 2005

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 total award
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	$175,000
Period	May 1, 2005–April 30, 2007

PI	Andrew Neal
Project Title	Uranium Speciation on Sediment and Grouted Surfaces
Funding Agency	Bechtel BWXT Idaho, LLC
Budget	$15,180
Period	August 6, 2003–August 15, 2005

PI	Christopher S. Romanek
Project Title	Controlled Growth of Biologic and Abiotic Carbonates and Fe-oxides
Funding Agency	National Aeronautics and Space Administration
Budget	$50,000 total award
Period	May 14, 1999–July 31, 2004

PI	Christopher S. Romanek
Project Title	Alumni Initiatives Award between SREL and University of Granada
Funding Agency	Council for International Exchange of Scholars
Budget	$11,720
Period	June 1, 2003–May 31, 2005

PI	Christopher S. Romanek
Project Title	Aging of Coral Specimens from NOAA-SCDNR 2003 Ocean Exploration Mission to the Charleston Bump
Funding Agency	South Carolina Department of Natural Resources
Budget	$11,596

Period	September 1, 2003–August 31, 2004
PI	Christopher S. Romanek
Project Title	Aging of Coral Specimens from NOAA-SCDNR Project: "From the Estuary to the Abyss: Exploring along the Latitude 31-30 Transect"
Funding Agency	South Carolina Department of Natural Resources
Budget	$8,400
Period	January 23, 2004–August 31, 2005
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, 2005
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 ($188,700 in FY05)
Period	2002–2005
PI	Rebecca R. Sharitz
Project Title	Vegetation Establishment Success in Restored Carolina Bay Depressions on the Savannah River Site, South Carolina
Funding Agency	USDA Forest Service
Budget	$43,750
Period	August 6, 2001–May 31, 2004
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, 2005
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	Ramunas Stephanauskas
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 Strojan
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, 2005
PI	Barbara E. Taylor
Project Title	Aquatic Invertebrates in Carolina Bays and Other Wetland Ponds Before and After Restoration Treatments

Funding Agency	US Department of Agriculture
Budget	$10,000
Period	September 1, 2003–September 30, 2005

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 Chemolighoautotrophic Archaea in Nevada Hot Springs
Funding Agency	National Science Foundation
Budget	$147,053
Period	May 1, 2004–July 31, 2005

◆◆◆ Publications ◆◆◆

Books Published in FY05

Gibbons, W. and M. Dorcas. 2005. Snakes of the Southeast. University of Georgia Press, Athens, GA. 253 pages.

Marcy, B.C., Jr., D.E. Fletcher, F.D. Martin, M.H. Paller, and M.J.M. Reichert. 2005. Fishes of the Middle Savannah River Basin With Emphasis on the Savannah River Site. University of Georgia Press, Athens, GA. 462 pages.

NERP Documents Published in FY05

DeBiase, A.E. and B.E. Taylor. 2005. Microcrustaceans (Branchiopoda and Copepoda) of Wetland Ponds and Impoundments on the Savannah River Site, Aiken, South Carolina. SRO-NERP-28. SRS National Environmental Research park Program, Aiken, SC.

Journal Articles Published in FY05

2771 Sever, D.M. and W.A. Hopkins. 2004. Oviductal sperm storage in the ground skink *Scincella laterale* holbrook (Reptilia: Scincidae). Journal of Experimental Zoology 301A:599-611.

2772 Bolan, N., D. Adriano and S. Mahimairaja. 2004. Distribution and bioavailability of trace elements in livestock and poultry manure by-products. Critical Reviews in Environmental Science and Technology 34:291-338.

2773 Jackson, B.P., P.V. Winger and P.J. Lasier. 2004. Atmospheric lead deposition to Okefenokee Swamp, Georgia, USA. Environmental Pollution 130:445-451.

2774 Battaglia, L.L., B.S. Collins and P.B. Weisenhorn. 2004. *Quercus michauxii* regeneration in and around aging canopy gaps. Canadian Journal of Forest Research 34:1359-1364.

2775 Hopkins, W.A., B.P. Staub, J.A. Baionno, B.P. Jackson, J.H. Roe and N.B. Ford. 2004. Trophic and maternal transfer of selenium in brown house snakes (*Lamprophis fuliginosus*). Ecotoxicology and Environmental Safety 58:285-293.

2776 Unrine, J.M. and C.H. Jagoe. 2004. Dietary mercury exposure and bioaccumulation in larvae of the southern leopard frog, *Rana sphenocephala*. RMZ-Materials and Geoenvironment 51:1418-1421.

2777 Unrine, J.M., C.H. Jagoe, W.A. Hopkins and H.A. Brant. 2004. Adverse effects of environmentally relevant dietary mercury exposure in larvae of the southern leopard frog, *Rana sphenocephala*. RMZ-Materials and Geoenvironment 51:1422-1425.

2778 Li, Y., H. Vali, S.K. Sears, J. Yang, B. Deng and C.L. Zhang. 2004. Iron reduction and alteration of nontronite NAu-2 by a sulfate-reducing bacterium. Geochimica et Cosmochimica Acta 68:3251-3260.

2779 Zhang, C.L., B.W. Fouke, G.T. Bonheyo, A.D. Peacock, D.C. White, Y. Huang and C.S. Romanek. 2004. Lipid biomarkers and carbon-isotopes of modern travertine deposits (Yellowstone National Park, USA): implications for biogeochemical dynamics in hot-spring systems. Geochimica et Cosmochimica Acta 68:3157-3169.

2780 Roe, J.H., W.A. Hopkins, J.A. Baionno, B.P. Staub, C.L. Rowe and B.P. Jackson. 2004. Maternal transfer of selenium in *Alligator mississippiensis* nesting downstream from a coal-burning power plant. Environmental Toxicology and Chemistry 23:1969-1972.

2781 Glaudas, X. 2004. Do cottonmouths (*Agkistrodon piscivorus*) habituate to human confrontations? Southeastern Naturalist 3:129-138.

2782 Schable, N.A., B.C. Faircloth, W.E. Palmer, J.P. Carroll, L.W. Burger, L.A. Brennan, C. Hagen and T.C. Glenn. 2004. Tetranucleotide and dinucleotide microsatellite loci from the northern bobwhite (*Colinus virginianus*). Molecular Ecology 4:415-419.

2783 Jørgensen, N.O.G., R. Stepanauskas, A.U. Pedersen, M. Hansen and O. Nybroe. 2003. Occurrence and degradation of peptidoglycan in aquatic environments. FEMS Microbiology Ecology 46:269-280.

2784 Stepanauskas, R., M. Moran, B.A. Bergamaschi and J.T. Hollibaugh. 2003. Covariance of bacterioplankton composition and environmental variables in a temperate delta

system. Aquatic Microbial Ecology 31:85-98.

2785 Kekli, A., A. Aldahan, M. Meili, G. Possnert, N. Buraglio and R. Stepanauskas. 2003. ^{129}I in Swedish rivers: distribution and sources. The Science of Total Environment 309:161-172.

2786 Duncan, L., J. Dilustro and B. Collins. 2004. Avian response to forest management and military training activities at Fort Benning, Georgia. Georgia Journal of Science 62:95-103.

2787 Shao, Q., M.D. Wilson, C.S. Romanek and K.A. Hobson. 2004. Time series analysis of elemental and isotopic data from biomineralized whale tissue. Environmental and Ecological Statistics 11:323-337.

2788 Bertsch, P.M. and C.L. Strojan. 2004. Science in support of assessing ecological risk and implementing novel remediation strategies at the Savannah River Site, SC. Waste Management 2004 Conference, February 29-March 4, 2004, Tucson, AZ.

2789 DeVault, T.L., B.D. Reinhart, I.L. Brisbin, Jr. and O.E. Rhodes, Jr. 2004. Home ranges of sympatric black and turkey vultures in South Carolina. The Condor 106:706-711.

2790 Adriano, D.C., W.W. Wenzel, J. Vangronsveld and N.S. Bolan. 2004. Role of assisted natural remediation in environmental cleanup. Geoderma 122:121-142.

2791 Hinton, T.G., J.S. Bedford, J.D. Congdon and F.W. Whicker. 2004. Effects of radiation on the environment: A need to question old paradigms and enhance collaboration among radiation biologists and radiation ecologists. Radiation Research 162:332-338.

2792 Zhao, D., B.E. Borders and M.D. Wilson. 2003. Individual-tree diameter growth and mortality models for bottomland mixed-species hardwood stands in the lower Mississippi alluvial valley. Forest Ecology and Management 199:307-322.

2793 Pearson, A., Z. Huang, A.E. Ingalls, C.S. Romanek, J. Wiegel, K.H. Freeman, R.H. Smittenberg and C.L. Zhang. 2004. Nonmarine crenarchaeol in Nevada hot springs. Applied and Environmental Microbiology 70:5229-5237.

2794 Rothermel, B.B. 2004. Migratory success of juveniles: a potential constraint on connectivity for pond-breeding amphibians. Ecological Applications 14:1535-1546.

2795 Wilson, M., W.P. McCormick and T.G. Hinton. 2004. The maximally exposed individual—comparison of maximum likelihood estimation of high quantiles to an extreme value estimate. Risk Analysis 24:1143-1151.

2796 Vali, H., B. Weiss, Y. Li, S.K. Sears, S.S. Kim, J.L. Kirschvink and C.L. Zhang. 2004. Formation of tabular single-domain magnetite induced by *Geobacter metallireducens* GS-15. Proceedings of the National Academy of Sciences 101:16121-16126.

2797 Hinton, T.G., D.P. Coughlin, Yi Yi, and L.C. Marsh. 2004. Low dose rate irradiation facility: initial study on chronic exposures to medaka. Journal of Environmental Radioactivity 74:43-55.

2798 Punshon, T., A.L. Neal and B.P. Jackson. 2004. Cadmium. pp. 171-208. *In:* Trace and Ultratrace Elements in Plants and Soil. I. Shtangeeva (ed.). WIT Press.

2799 Paller, M.H., C.H. Jagoe, H. Bennett, H.A. Brant and J.A. Bowers. 2004. Influence of methylmercury from tributary streams on mercury levels in Savannah River Asiatic clams. Science of the Total Environment 325:209-219.

2800 Collins, B.S., JV. McArthur and R.R. Sharitz. 2004. Plant effects on microbial assemblages and remediation of acidic coal pile runoff in mesocosm treatment wetlands. Ecological Engineering 23:107-115.

2801 Hopkins, W.A., C.T. Winne and S.E. DuRant. 2004. Differential swimming performance of two natricine snakes exposed to a cholinesterase-inhibiting pesticide. Environmental Pollution 133:531-540.

2802 Wickliffe, J.K., A.M. Bickham, B.E. Rodgers, R.K. Chesser, C.J. Phillips, S.P. Gaschak, J.A. Goryanaya, I. Chizhevsky and R.J. Baker. 2003. Exposure to chronic, low-dose rate ã-Radiation at Chornobyl does not induce point mutations in Big Blue Mice. Environmental and Molecular Mutagenesis 42:11-18.

2803 Roe, J.H., W.H. Hopkins, J.W. Snodgrass and J.D. Congdon. 2004. The influence of circadian rhythms on pre- and post-prandial metabolism in the snake *Lamprophis fuliginosus*. Comparative Biochemistry and Physiology Part A 139:159-168.

2804 Unrine, J.M. and C.H. Jagoe. 2004. Dietary mercury exposure and bioaccumulation in southern leopard frog (*Rana sphenocephala*) larvae. Environmental Toxicology and Chemistry 23:2956-2963.

2805 Unrine, J.M., C.H. Jagoe, W.A. Hopkins and H.A. Brant. 2004. Adverse effects of ecologically relevant dietary mercury exposure in southern leopard frog (*Rana sphenocephala*) larvae. Environmental Toxicology and Chemistry 23:2964-2970.

2806 Dilustro, J., B. Collins, L. Duncan and C. Crawford. 2005.

Moisture and soil texture effects on soil $C0_2$ efflux components in southeastern mixed pine forests. Forest Ecology and Management 204:85-95.

2807 Newman, L.A. and C.M. Reynolds. 2004. Phytodegradation of organic compounds. Current Opinion in Biotechnology 15:225-230.

2808 Magnuson, T.S., A.L. Neal and G.G. Geesey. 2004. Combining *in situ* reverse transcriptase polymerase chain reaction, optical microscopy, and x-ray photoelectron spectroscopy to investigate mineral surface-associated microbial activities. Microbial Ecology 48:578-588.

2809 Hopkins, W.A., J.W. Snodgrass, J.A. Baionno, J.H. Roe, B.P. Staub and B.P. Jackson. 2005. Functional relationships among selenium concentrations in the diet, target tissues, and nondestructive tissue samples of two species of snakes. Environmental Toxicology and Chemistry 24:344-351.

2810 Collins, B.S., R.R. Sharitz and D.P. Coughlin. 2005. Elemental composition of native wetland plants in constructed mesocosm treatment wetlands. Bioresource Technology 96:937-948.

2811 Hopkins, W.A., B.P. Staub, J.A. Baionno, B.P. Jackson and L.G. Talent. 2005. Transfer of selenium from prey to predators in a simulated terrestrial food chain. Environmental Pollution 134:447-456.

2812 Jackson, B.P., J.F. Ranville and A.L. Neal. 2005. Application of flow field flow fractionation-ICPMS for the study of uranium binding in bacterial cell suspensions. Analytical Chemistry 77:1393-1397.

2813 Sawyer, R.H., L. Rogers, L. Washington, T.C. Glenn and L.W. Knapp. 2005. Evolutionary origin of the feather epidermis. Developmental Dynamics 232:256-267.

2814 Mou, X., M.A. Moran, R. Stepanauskas, J.M. Gonzalez and R.E. Hodson. 2005. Flow-cytometric cell sorting and subsequent molecular analyses for culture-independent identification of bacterioplankton involved in dimethylsufoniopropionate transformations. Applied and Environmental Microbiology 71:1405-1416.

2815 Metts, B.S., W.A. Hopkins and J.P. Nestor. 2005. Interaction of an insecticide with larval density in pond-breeding salamanders (*Ambystoma*). Freshwater Biology 50:685-696.

2816 Glaudas, X., T.M. Farrell and P.G. May. 2005. Defensive behavior of free-ranging pygmy rattlesnakes. Copeia 1:196-200.

2817 Winne, C.T. and M.B. Keck. 2005. Intraspecific differences in thermal tolerance of the diamondback watersnake (*Nerodia rhombifer*): effects of ontogeny, latitude, and sex. Comparative Biochemistry and Physiology, Part A 140:141-149.

2818 Pinder, J.E., III, T.G. Hinton and F.W. Whicker. 2005. The influence of a whole-lake addition of stable cesium on the remobilization of aged ^{137}Cs in a contaminated reservoir. Journal of Environmental Radioactivity 80:225-243.

2819 Unrine, J.M., C.H. Jagoe, A.C. Brinton, H.A. Brant and N.T. Garvin. 2005. Dietary mercury exposure and bioaccumulation in amphibian larvae inhabiting Carolina bay wetlands. Environmental Pollution 135:245-253.

2820 Hauswaldt, J.S. and T.C. Glenn. 2005. Population genetics of the diamondback terrapin (*Malaclemys terrapin*). Molecular Ecology 14:723-732.

2821 Smith, R.B., T.D. Tuberville, A.L. Chambers, K.M. Herpich and J.E. Berish. 2005. Gopher tortoise burrow surveys: External characteristics, burrow cameras, and truth. Applied Herpetology 2:161-170.

2822 Taylor, B.E. and A.E. DeBiase. 2005. Are microcrustaceans useful for assessing success of wetland pond restoration? (South Carolina). Ecological Restoration 23:56-57.

2823 Croshaw, D.A. 2005. Cryptic behavior is independent of dorsal color polymorphism in juvenile northern leopard frogs (*Rana pipiens*). Journal of Herpetology 39:125-129.

2824 Zhao, D., M. Wilson and B.E. Borders. 2005. Modeling response curves and testing treatment effects in repeated measures experiments: a multilevel nonlinear mixed-effects model approach. Canadian Journal for Research 35:122-132.

2825 Zhang, C.L., Z. Huang, J. Cantu, R.D. Pancost, R.L. Brigmon, T.W. Lyons and R. Sassen. 2005. Lipid biomarkers and carbon isotope signatures of a microbial (*Beggiatoa*) mat associated with gas hydrates in the Gulf of Mexico. Applied and Environmental Microbiology 71:2106-2112.

2826 Cooper, D.C., A L. Neal, R.K. Kukkadapu, D. Brewe, A. Coby and F.W. Picardal. 2005. Effects of sediment iron mineral composition on microbially mediated changes in divalent metal speciation: Importance of ferrihydrite. Geochimica et Cosmochimica Acta 69:1739-1754.

2827 Jackson, B.P., J.F. Ranville, P.M. Bertsch and A.G. Sowder. 2005. Characterization of colloidal and humic-bound Ni and U in the "dissolved" fraction of contaminated sediment extracts. Environmental Science & Technology 39:2478-2485.

2828 Winne, C.T., M.D. Dorcas and S.M. Poppy. 2005. Population structure, body size, and seasonal activity of black swamp snakes (*Seminatrix pygaea*). Southeastern Naturalist 4:1-14.

2829 Hinton, T.G., A.S. Knox, D.I. Kaplan and R. Sharitz. 2005. Phytoextraction of uranium and thorium by native trees in a contaminated wetland. Journal of Radioanalytical and Nuclear Chemistry 264:417-422.

2830 Kaplan, D.I., T.G. Hinton and A.S. Knox. 2005. Cesium-137 partitioning to wetland sediments and uptake by plants. Journal of Radioanalytical and Nuclear Chemistry 264:393-399.

2831 Adriano, D.C., N.S. Bolan, J. Vangronsveld and W.W. Wenzel. 2005. Heavy Metals. pp. 175-182. *In*: Encyclopedia of Soils in the Environment, edited by D. Hillel. Elsevier Academic Press. Amsterdam, The Netherlands.

2832 Bolan, N.S., D. Curtin and D.C. Adriano. 2005. Acidity. pp. 11-17. *In:* Encyclopedia of Soils in the Environment, edited by D. Hillel. Elsevier Academic Press. Amsterdam, The Netherlands.

2833 Koo, B-J, D.C. Adriano, N.S. Bolan and C.D. Barton. 2005. Root exudates and microorganisms. pp. 421-428. *In:* Encyclopedia of Soils in the Environment, edited by D. Hillel. Elsevier Academic Press. Amsterdam, The Netherlands.

2834 Stepanauskas, R., T.C. Glenn, C.H. Jagoe, R.C. Tuckfield, A.H. Lindell and J McArthur. 2005. Elevated microbial tolerance to metals and antibiotics in metal-contaminated industrial environments. Environmental Science & Technology 39:3671-3678.

2835 Zhao, D., B.E. Borders and M.D. Wilson. 2005. A density-dependent matrix model for bottomland hardwood stands in the lower Mississippi Alluvial Valley. Ecological Modelling 184:381-395.

2836 Jiménez-López, C., C.S. Romanek, F.J. Huertas, H. Ohmoto and E. Caballero. 2004. Oxygen isotope fractionation in synthetic magnesian calcite. Geochimica et Cosmochimica Acta 68:3367-3377.

2837 Zhang, C. 2005. Genome-empowered stable-isotope biogeochemistry: integrating genomics, proteomics and metabolomics. Logical Biology 5:79-80.

2838 Weston, J.L., N.A. Schable and T.C. Glenn. 2004. Characterization of six microsatellite primers for the grey fox (*Urocyon cinereoargenteus*). Molecular Ecology Notes 4:503-505.

2839 Koopman, M.E., N.A. Schable and T.C. Glenn. 2004. Development and optimization of microsatellite DNA primers for boreal owls (*Aegolius funereus*). Molecular Ecology Notes 4:376-378.

2840 Otero-Arnaiz, A., A. Schnabel, T.C. Glenn, N.A. Schable, C. Hagen and L. Ndong. 2005. Isolation and characterization of microsatellite markers in the East African tree, *Acacia brevispica* (Fabaceae: Mimosoideae). Molecular Ecology Notes 5:366-368.

2841 Faircloth, B.C., A. Reid, T. Valentine, S.H. Eo, T.M. Terhune, T.C. Glenn, W.E. Palmer, C.J. Nairn and J.P. Carroll. 2005. Tetranucleotide, trinucleotide, and dinucleotide loci from the bobcat (*Lynx rufus*). Molecular Ecology Notes 5:387-389.

2842 Dion, H.M., C.S. Romanek, T.G. Hinton and P.M. Bertsch. 2005. Cesium-137 in floodplain sediments of the Lower Three Runs Creek on the DOE Savannah River Site. Journal of Radioanalytical and Nuclear Chemistry 264:481-488.

2843 Winne, C.T. and M.B. Keck. 2004. Daily activity patterns of Whiptail Lizards (Squamata: Teiidae: *Aspidoscelis*): a proximate response to environmental conditions or an endogenous rhythm? Functional Ecology 18:314-321.

2844 Li, Y., Y. Zheng and B. Fu. 2005. Mössbauer spectroscopy of omphacite and garnet pairs from eclogites: Application to geothermobarometry. American Mineralogist 90:90-100.

2845 Kevbrin, V. V. 2004. Alkalithermophiles: A double challenge from extreme environments. pp. 1-16. *In:* Origins: Genesis, Evolution and Diversity of Life, edited by J. Seckbach. Kluwer Academic Publishers, Dordrecht, NL.

2846 Bolan, N.S., L. Wong and D.C. Adriano. 2004. Nutrient removal from farm effluents. Bioresource Technology 94:251-260.

2847 Ye, Q., Y. Roh, S.L. Carroll, B. Blair, J. Zhou, C.L. Zhang and M.W. Fields. 2004. Alkaline anaerobic respiration: isolation and characterization of a novel alkaliphilic and metal-reducing bacterium. Applied and Environmental Microbiology 70:5595-5602.

2848 Harmon, S.M., J.K. King, J.B. Gladden, G.T. Chandler and L.A. Newman. 2005. Mercury body burdens in *Gambusia holbrooki* and *Erimyzon sucetta* in a wetland mesocosm amended with sulfate. Chemosphere 59:227-233.

2849 Zhang, C.L. and B. Lanoil. 2004. Geomicrobiology and biogeochemistry of gas hydrates and cold seeps. Chemical Geology 205:187-194.

2850 Sassen, R., H.H. Roberts, R. Carney, A.V. Milkov, D.A. DeFreitas, B. Lanoil and C. Zhang. 2004. Free hydrocarbon gas, gas hydrate, and authigenic minerals in chemosynthetic communities of the northern Gulf of Mexico continental slope: relation to microbial processes. Chemical Geology 205:195-217.

2851 Formolo, M.J., T.W. Lyons, C. Zhang, C. Kelley, R. Sassen, J. Horita and D.R. Cole. 2004. Quantifying carbon sources in the formation of authigenic carbonates at gas hydrate sites in the Gulf of Mexico. Chemical Geology 205:253-264.

2852 Sassen, R., S.T. Sweet, D.A. DeFreitas, N.L. Eaker, H.H. Roberts and C. Zhang. 2004. Brine vents on the Gulf of Mexico slope: hydrocarbons, carbonate-barite-uranium mineralization, red beds, and life in an extreme environment. 2004 Gulf Coast Section Society of Economic Paleontologists and Mineralogists 444-463.

2853 Batzer, D.P., S.E. Dietz-Brantley, B.E. Taylor and A.E. DeBiase. 2005. Evaluating regional differences in macroinvertebrate communities from forested depressional wetlands across eastern and central North America. Journal of North America Benthological Society 24:403-414.

2854 Roe, J.H., W.A. Hopkins and B.P. Jackson. 2005. Species- and stage-specific differences in trace element tissue concentrations in amphibians: implications for the disposal of coal-combustion wastes. Environmental Pollution 136:353-363.

2855 Kelsey-Wall, A., J.C. Seaman, C.H. Jagoe, C.E. Dallas and K.F. Gaines. 2005. Rodents as receptor species at a tritium disposal site. Journal of Environmental Radioactivity 82:95-104.

2856 Punshon, T., B.P. Jackson, A. Lanzirotti, W.A. Hopkins, P.M. Bertsch and J. Burger. 2005. Application of synchrotron x-ray microbeam spectroscopy to the determination of metal distribution and speciation of biological tissues. Spectroscopy Letters 38:343-363.

2857 DeVault, T.L., B.D. Reinhart, I.L. Brisbin, Jr. and O.E. Rhodes, Jr. 2005. Flight behavior of black and turkey vultures: implications for reducing bird-aircraft collisions. Journal of Wildlife Management 69:592-599.

2858 Paller, M.H., D.E. Fletcher, T. Jones, S.A. Dyer, J.J. Isely and J.W. Littrell. 2005. Potential of largemouth bass as vectors of ^{137}Cs dispersal. Journal of Environmental Radioactivity 80:27-43.

2859 Snodgrass, J.W., W.A. Hopkins, B.P. Jackson, J.A. Baionno and J. Broughton. 2005. Influence of larval period on responses of overwintering green frog (*Rana Clamitans*) larvae exposed to contaminated sediments. Environmental Toxicology and Chemistry 24:1508-1514.

2860 Mahimairaja, S., N.S. Bolan, D.C. Adriano and B. Robinson. 2005. Arsenic contamination and its risk management in complex environmental settings. pp. 1-82. *In:* Advances in Agronomy, edited by D. L. Sparks. Elsevier/Academic Press. San Diego, CA.

2861 Tague, R.T. and S.A. Foré. 2005. Analysis of the spatial genetic structure of *Passiflora incarnata* in recently disturbed sites. Canadian Journal of Botany 83:420-426.

2862 Harper, S.J. and R. Sharitz. 2005. Delineating sandhill communities: the use of advanced techniques to extract features from satellite imagery. pp. 123-136. *In:* Proceedings of the 4th Southern Forestry and Natural Resources GIS Conference. Warnell School of Forest Resources, University of Georgia, Athens, GA.

2863 Moen, D.S., C.T. Winne and R.N. Reed. 2005. Habitat-mediated shifts and plasticity in the evaporative water loss rates of two congeneric pit vipers (Squamata, Viperidae, *Agkistrodon*). Evolutionary Ecology Research 7:759-766.

2864 Hitchcock, D.R., C.D. Barton, K.T. Rebel, J. Singer, J.C. Seaman, J.D. Strawbridge, S.J. Riha and J.I. Blake. 2005. A containment and disposition strategy for tritium-contaminated groundwater at the Savannah River Site, South Carolina, United States. Environmental Geosciences 12:17-28.

2865 Barton, C.D., L.S. Paddock, C.S. Romanek, S. Maharaj and J. Seaman. 2005. Metal attenuation processes in a landfill containing coal combustion waste: implications for remediation. Environmental Geosciences 12:45-55.

2866 Punshon, T., A. Lanzirotti, S. Harper, P.M. Bertsch and J. Burger. 2005. Distribution and speciation of metals in annual rings of black willow. Journal of Environmental Quality 34:1165-1173.

2867 McCay, T.S. and M.J. Komoroski. 2004. Demographic responses of shrews to removal of coarse woody debris in a managed pine forest. Forest Ecology and Management 189:387-395.

2868 Willson, J.D., C.T. Winne and L.A. Fedewa. 2005. Unveiling escape and capture rates of aquatic snakes and salamanders (*Siren spp.* and *Amphiuma means*) in commercial funnel traps. Journal of Freshwater Ecology 20:397-404.

2869 Sawyer, R.H., T.C. Glenn, J.O. French and L.W. Knapp. 2005. Developing antibodies to synthetic peptides based on

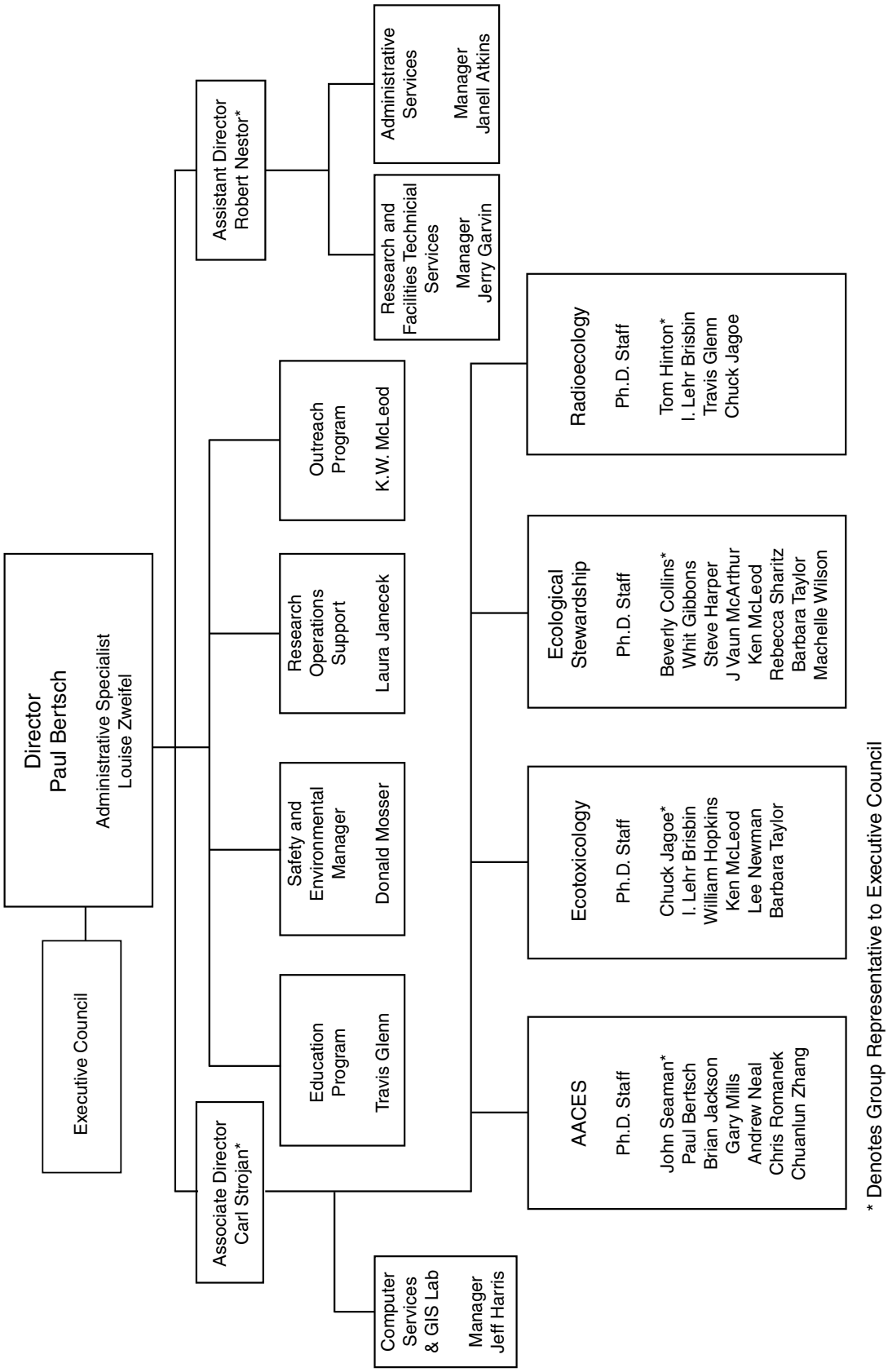
comparative DNA sequencing of multigene families. Methods in Enzymology 395:636-652.

2870 Glenn, T.C. and N.A. Schable. 2005. Isolating microsatellite DNA loci. Methods in Enzymology 395:202-222.

2871 Wenzel, W.W., E. Lombi and D.C. Adriano. 2004. Biogeochemical processes in the rhizosphere: role in phytoremediation of metal-polluted soils. pp. 273-303. *In*: Heavy Metal Stress in Plants: From Biomolecules to Ecosystems, edited by M. Prasad and J. Hagemeyer. Springer Verlag Berlin Heidelberg New York.

2872 Wilson, M. and D. Jackson. 2005. Uncertainty and power at low levels of incurred radiation dose. Journal of Radiological Protection 25:51-66.

◆◆◆ SREL Organizational Chart ◆◆◆



* Denotes Group Representative to Executive Council

The University of Georgia

Savannah River Ecology Laboratory

http://www.uga.edu/srel/