Savannah River Ecology Laboratory

Annual Technical Progress Report

2004

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Annual Technical Progress Report of Ecological Research

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Savannah River Ecology Laboratory
··· FY2004 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 over 53 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 2004 fiscal year was fulfilled with the publication of 86 journal articles and book chapters by faculty, technical staff, students, and visiting scientists. An additional 53 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 6.

Notable scientific accomplishments include work conducted on contaminant transport in riparian systems, the role of sulfate-reducing bacteria in contaminant transport, and seasonal wetlands.

- SREL and WSRC jointly published a report (SREL-71-UC-66e, WSRC-TR-2003-00312) based on a workshop that assessed potential research opportunities for studies on contaminant transport in riparian systems. A consensus among workshop participants from around the nation was that DOE-ERSD should expand its research focus to include research on the fate and transport of contaminants in fluvial riparian systems.
- Research by SREL's Andrew Neal and others from Pacific Northwest National Laboratory, Montana State University and Washington State University characterized uranium complexes formed at hematite surfaces colonized by sulfate-reducing bacteria. Among other things their results, published in *Environmental Science & Technology*, suggest higher levels of uranium associated with hematite surfaces colonized by sulfate-reducing bacteria than with bacteria-free surfaces. Such knowledge is important in modeling contaminant transport in the environment as well as for optimizing bioremediation methods for reducible metals and radionuclides.
- Dr. Rebecca Sharitz had an invited review paper about Carolina bays of the Southeast published in the September 2003 issue of *Wetlands*. Carolina bays are isolated depression wetlands that are unique to the southeastern Coastal Plain. Thousands of them exist, including hundreds on the Savannah River Site. They play an important role as critical habitat for plants and animals and in maintaining regional biodiversity.

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

- Dr. Tom Hinton co-authored an article titled "Avoiding Destructive Remediation at DOE Sites" that was published in the March 12, 2004 issue of the prestigious journal *Science* (SREL reprint 2728, see page 72). The article highlighted PAR Pond on the Savannah River Site and advocated that scientifically sound risk assessments, based on realistic land-use scenarios, be used to a guide cleanup decisions.

Remediation of Par Pond was the subject of a paper in Science.

- Dr. Travis Glenn was promoted in 2004 to Associate Research Ecologist. Dr. Glenn, who originally came to SREL in September 1998, has established a molecular ecology program specializing in the application of molecular genetics tools to problems in conservation biology, genotoxicology, population genetics, and natural resources management.
- SREL research scientists Drs. Christopher Romanek, Andrew Neal, Gary Mills, and Chuanlun Zhang, with collaborators from The University of Georgia, Portland State University, the University of Maine, the University of Maryland, and Cornell University, received a million dollar grant from the National Science Foundation's Microbial Observatory program to work in Kamchatka, Russia. The goal of this unique interdisciplinary effort is to increase understanding of geochemical/microbial interactions in hydrothermal systems. The research team plans to travel to Russia several times over the course of the four-year grant. On the heals of this award, Drs. Zhang, Romanek, and Mills received a second NSF grant of over $450K to study the microbiology of hot springs in the basin and range province of Nevada. This three-year grant, which includes Dr. Juergen Wiegel of the Department of Microbiology at UGA, is focused on studying the effects of pH, temperature, and alkalinity on one particular group of microorganisms, the crenarchaeota, in hot spring environments. In a preliminary investigation, soon to be published in *Applied Environmental Microbiology*, the group discovered an archaeal lipid biomarker that was previously only found in marine environments. The group plans on characterizing community structure using various culture and molecular techniques and determining the chemical and stable isotope fingerprint of lipid biomarkers to understand how this unusual group of microorganisms has adapted to life in extreme environments.

During spring 2003 SREL underwent a Program Review by the UGA Program Review and Assessment Committee and the Office of the Vice President for Instruction. The purpose of the review was to provide a systematic means of assuring the continuous improvement of the University's academic, research, and service programs. The assessment team provided a report of their findings in September 2003, concluding that SREL is a "unique and outstanding program under the UGA umbrella that brings the University significant national and international recognition." Major recommendations included developing a strategic plan for SREL within the UGA framework, providing annual refresher training on performance evaluations to supervisors, adding new 0.51 FTE joint academic positions with UGA departments as appropriate, establishing new Eugene Odum Research Fellowships for graduate students with SREL overhead return funds from outside grants, considering the construction of a new SREL laboratory facility outside the SRS security fence, better advertising SREL graduate and undergraduate programs to UGA students, implementing an evaluation vehicle for SREL outreach activities, securing 0.51 FTE staff appointments with UGA Public Service and Outreach (PS&O) for some SREL outreach staff, and improving the linkage of SREL outreach efforts with UGA PS&O programs.

In November, 2003, the Environmental Remediation Sciences Division of the DOE Office of Biological and Environmental Research (OBER) conducted a Programmatic Alignment Review of SREL to determine whether SREL's research activities were consistent with the programmatic activities and overall mission of OBER. Five external reviewers, selected by OBER, and several DOE-SC personnel attended the two-day review, which focused on SREL's research programs as well as the education and outreach programs. As a result of this review, OBER recommended that: (1) SREL focus on core competencies that support the ERSD mission (including systems-scale evaluation of environmental fate and transport of contaminants, radioecology, and hypothesis-driven research that supports the remediation of such contaminants); (2) SREL recruit more postdoctoral fellows and graduate students; (3) SREL extend its research to other DOE sites and take a more formalized team approach to research projects; (4) SREL's long-term databases be made available to a wider audience; (5) the Lab's education and outreach activities develop a means to quantify program impacts; and (6) SREL continue to support SRS research needs.

The research, education, and outreach capabilities of the Laboratory were highlighted at the annual meeting of the

Ecological Society of America in Savannah, GA, in August, 2003. To advertise Lab capabilities to the national ecological community, SREL prepared a large display that summarized new research initiatives as well as the education and outreach programs. Written materials were also available for distribution to meeting participants. One focus of the SREL display was the need for a southeastern National Ecological Observatory Network (NEON). SREL and The University of Georgia were instrumental in initiating regional meetings on the feasibility of a southeastern NEON; the ESA meeting served as an excellent venue from which to recruit interested partners in this endeavor.

Partnering with UGA, with funding provided by the Office of the Vice President for Research, SREL hosted a Southeastern National Ecological Observatory Network (SEEON) Planning Workshop at the Lab's conference center in October, 2003. The meeting theme was "Visualization and Forecasting of Environmental Changes in the Southeastern U.S. (and Beyond)." Workshop organizers included Paul Bertsch from SREL and Alan Covich from the UGA Institute of Ecology. SREL faculty Rebecca Sharitz, Steve Harper, and Christopher Romanek served as workshop co-chairs. More than 50 attendees from across the southeastern U.S. and beyond participated in discussions and break-out sessions. As a result of this initial workshop, a SEEON website was developed (http://www.seeon.org) and a final report of the meeting proceedings was posted there. A follow-up workshop, held in February 2004 at the Kennedy Space Center and hosted by the University of Florida and NASA, focused on the development of novel sensors and instruments, networking of sensor arrays, advanced data management and interpretation, and other information technology issues related to an ecological network. Three SREL faculty and staff attended.

Drs. Tom Hinton and Travis Glenn of SREL, with colleagues from the UGA Aquatic Biotechnology and Environmental Laboratory (ABEL), hosted a workshop on "Exploring Potential Collaborative Research in Human Health and Ecotoxicology Risks Using Medaka as a Model Organism" in March, 2004 at UGA. The workshop was co-funded by SREL and several UGA entities, including the Office of the Vice President for Research, ABEL, and the Warnell School of Forest Resrouces. Approximately 45 attendees, including scientists from Japan, Sweden, and the Ukraine, came together to discuss multidisciplinary research opportunities using the Japanese Medaka fish as a model organism. After the formal workshop in Athens, many of the workshop participants came to the SRS to tour SREL's Low Dose Rate Irradiation Facility (LoDif) at Par Pond.

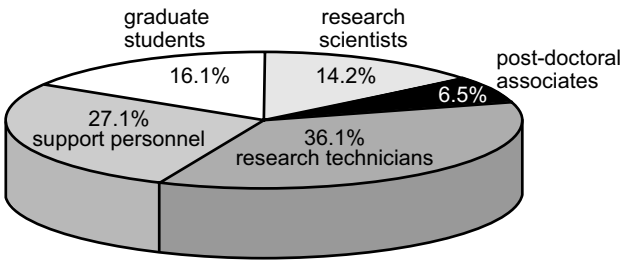
SREL's LoDif facility.

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 covers a five-year period from July 1, 2001 through June 30, 2006. The estimated total cost of this agreement is almost $53 million, with DOE contributing about $50 million and the University of Georgia about $3 million. Additional funding, about $975K in FY04, comes from contracts and grants involving a variety of other organizations. SREL's total operating budgets from DOE and other federal sources in FY01, FY02, FY03, and FY04 were $9.7, $10.2, $10.2, and $9.11 million, respectively. In FY04 SREL received about $7.6M from DOE Office of Science (DOE-SC); the FY05 budget from DOE-SC is projected to be $7.78 million. SREL also receives almost $550,000 per year from The University of Georgia. During FY04 an additional $509K was received from DOE and WSRC for SRS-related tasks.

Researchers at SREL currently have funding totaling about $975K from 27 grants in addition to funds provided by DOE-SR. Sources of grant awards range from private foundations such as the American Honda Foundation to federal agencies such as the U.S. Environmental Protection Agency, the National Science Foundation, and the Department of Defense (DoD). Important grants received this year included an award of $100K from the National

Science Foundation to Dr. Domy Adriano for a pilot study on regulation of metal bioavailability in a floodplain continuum and $69K from the American Honda Foundation to Dr. Whit Gibbons and others for a hands-on "research-to-classroom" outreach science program.

SREL currently has a permanent staff of about 155 people, nearly all of whom are employees of The University of Georgia. The staff includes 22 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 (56), clerical and other support personnel (42), and graduate students (25) comprise the remaining staff categories.



In addition to holding faculty positions in 13 departments at the University of Georgia, various SREL faculty have adjunct status at 14 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.

SREL purchased one major equipment item in FY04. An epifluorescence microscope was purchased for one of the Laboratory's new research scientists, at a cost of about $95,000.

Participants in the SREL Education Program during FY04 came from schools located throughout the United States and included 18 undergraduate students and 44 graduate students. The graduate students came from four different universities in the U.S. and abroad, emphasizing the national and international stature of the SREL program. In the past year 12 graduate students from SREL earned Masters Degrees and 3 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 401 talks, 21 tours, 26 exhibits, and 59 workshops, reaching a total of 85,425 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. Educational products produced during the past year included a metric ruler/bookmark and an emergency services calendar that depicts animals, plants, and habitats of the SRS, produced in cooperation with Westinghouse Savannah River Company. In addition, a website (www.kidsdoscience.org) was developed to provide lesson plans and associated materials to teachers and students in conjunction with a hands-on "research-to-classroom" science education program conducted by SREL Outreach personnel and funded by the American Honda Foundation. These new materials and other Outreach products have been extremely popular and thousands of copies have been distributed during the past year.

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, is offering 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. Three

students are continuing into the second year of coursework for the degree. Four students have completed all required coursework and are working on the research component of the degree and three students have graduated from the program. In an effort to expand their audience, the SREL Outreach Program presented 10 Ecotalks via distance learning to 36 elementary, middle, and high schools in both South Carolina and Georgia. This approach allows Outreach staff to reduce animal handling and transport time and reach multiple schools simultaneously.

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 2,169 people visited the facility.

Representatives of the Laboratory also serve local and statewide communities by organizing a canned goods drive in November, 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. William Hopkins was selected by the Society of Environmental Toxicology and Chemistry (SETAC) World Council to a three-year term on the editorial board of the journal *Environmental Toxicology and Chemistry*.

Dr. Tom Hinton was selected as one of only eight international scientists, and the only one from North America, to participate in a review of two laboratories associated with the French ENVIRHOM research program, which was established to study the effects of chronic low-level irradiation on humans and the environment.

The U.S. Department of Agriculture recognized the efforts of SREL researchers **John Mulhouse** and **Paul Stankus** with awards "In commendation for outstanding emergency response to aid an injured co-worker during joint research activities of the USDA Forest Service Southern Research Station and the Savannah River Ecology Laboratory." John and Paul immediately notified SRS emergency personnel when a student with whom they were working exhibited an extreme allergic reaction after being stung by fire ants.

Erin Casey, an SREL graduate student and laboratory technician, was selected to represent western South Carolina Rotary 7750 during a five-week trip to South Africa. She will be joining three other young professionals from South Carolina who will enjoy the all-expenses-paid trip to Port Elizabeth, South Africa in a Group Study Exchange.

Graduate student **Chris Winne**, a student of **Dr. Whit Gibbons,** received several awards this year for a paper, with Michael Keck, on daily activity patterns in lizards. Awards received included the University of Georgia Institute of Ecology Best Student Publication Award, the Association of Southeastern Biologists Student Research Award, the Eugene P. Odum Award, awarded by the Ecological Society of America, Southeastern Chapter, the Outstanding Student Paper in Herpetology Award from the American Society of Ichthyologists and Herpetologists, Southeastern Division, and a Travel Support Award from the American Society of Ichthyologists and Herpetologists, Southeastern Division.

Dr. Paul Bertsch was appointed to two National Academy of Sciences Committees: the U.S. National Committee for Soil Science and the Committee on Earth Resources. The USNC/SS is the formal representative of the U.S. soil science community to the International Union of Soil Sciences. Its function is to provide advice and input to the union and its activities on behalf of U.S. soil scientists. The Committee on Earth Resources reviews development of new activities relevant to mineral and energy resource affairs for the Board on Earth Sciences and Resources.

Dr. Whit Gibbons received the ASB Senior Research Award from the Association of Southeastern Biologists for the presentation "Terrestrial Habitat: A Vital Component for Herpetofauna of Isolated Wetlands," which also has been published in the journal *Wetlands*.

The **SREL Outreach Program** received "Awards of Distinction" in the 2004 print media competition from The Communicator Awards for two Outreach Products: the PARC poster "From the Mountains to the Coast" and the six-page "American Alligator" flier.

Rosemary Forrest, SREL Public Relations Coordinator, received a Service Merit Award from Partners in Amphibian and Reptile Conservation (PARC) at the Southeast PARC meeting in 2004.

SREL research coordinator **Charles Davis** received a 2004 Affiliate Leaders Scholarship Award from the National Wildlife Federation to attend the NWF annual meeting; only 10 such awards were given nationwide.

Dr. Beverly Collins was elected as Chair of the Vegetation Section of the Ecological Society of America.

Outreach educator **Scottie Moore** was honored at the South Carolina School Boards Association Awards Banquet as a Champion for Public Education.

Susanne Hauswaldt, student of **Dr. Travis Glenn**, received the Gerald and Antonia Wilson Biological Sciences Graduate Student Award from the University of South Carolina Department of Biological Sciences.

Charles Davis was elected in January 2004 as First Vice-President of the South Carolina Wildlife Federation Board of Directors.

Dr. William Hopkins was nominated by two environmental organizations to a National Academy of Sciences/National Research Council Panel on handling coal combustion wastes. Bill also was invited to sit on the Department of Interior Advisory Panel on environmental hazards of minefilling coal combustion wastes.

SREL postdoctoral fellow **Dr. Bonjun Koo** was appointed as an Associate Editor for *Journal of Environmental Monitoring and Restoration*.

Dr. Chuanlun Zhang was selected as a member of the Petroleum Science & Technology Forum of One Hundred Overseas Scholars for 2003.

Dr. Whit Gibbons received a Certificate of Appreciation from Partners in Amphibian and Reptile Conservation (PARC) for serving as PARC National Chair from 1999-2004.

SREL postdoctoral fellow **Dr. Heather Dion** was appointed to a three-year term as an Associate Editor for the Soil Science Society of America Journal.

··· 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 53-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 FY04.

··· 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 FY04 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

Field flow fractionation (FFF) is an aqueous size-based separation technique applicable to the separation of biomolecules, colloids, and bacteria. When interfaced on-line with ICP-Mass Spectrometry detection, elemental data can be collected concurrently. In this study we employed hyperlayer-FFF methodology to separate cells of aerobically grown *Shewanella oneidensis* strain MR-1 from exopolymers present in washed cell suspensions. Cell suspensions were exposed to increasing concentrations of U to establish an adsorption isotherm and with fixed U concentrations at varying pH to establish a pH sorption isotherm. Elution of cells was detected by UV absorbance (254 nm) and the eluant exiting the UV detector was interfaced on-line to ICP-MS to detect U. A linear

sorption isotherm was determined for U solution concentrations from 0.2-6 µM. The pH sorption isotherm showed maximum U sorption to *S. oneidensis* occurs at pH 5. A relatively large molecular compound, presumably a cell exudate, since the cells were determined to remain intact with microscopy, was identified by FFF. This cell exudate complexed U, and at higher pH the exudate appeared to have a greater affinity for U than the cell surface. Thus Fl-FFF interfaced with ICP-MS detection appears to be a powerful analytical technique for metal sorption studies with bacteria. Analysis can be carried out on very small sample volumes and additional speciation information can be gained because soluble organic constituents of the cell suspension also elute from the FFF channel and are resolved from the cells.

After optimization and calibrating the hyperlayer separation conditions and demonstrating that the conditions were applicable to the separation of *S. oneidensis*, washed cell suspensions were amended with increasing concentrations of U to determine an adsorption isotherm. pH conditions were maintained at 5. The amount of U bound by cells increased as a function of initial uranium concentration and the relationship was linear over the 0.2-16 µm concentration range. This result demonstrates the potential of Fl-FFF-ICP-MS to calculate sorption isotherms using small sample volumes and environmentally relevant aqueous metal concentrations. It is also noteworthy that ICP-MS is capable of simultaneous multi-element detection; therefore this technique lends itself to the study of competitive sorption in multiple metal systems.

Uranium sorption as a function of pH was also investigated over the pH range 5-9. Maximum sorption occurs at pH 5 and as pH increases, the formation of hydroxide and carbonato-U solution complexes act to inhibit adsorption to the cell surface. An early eluting U-binding species was identified in the Fl-FFF-ICP-MS fractograms with an elution time of approximately 2 minutes. We hypothesize that this is a bacterially produced extracellular polymeric substance (EPS) that elutes under normal mode separation conditions. Preliminary size exclusion chromatography showed that this compound was approximately 200 kDa, while epifluorescent microscopy of the cell suspensions did not indicate any cell agglomeration. Further investigations of the exopolymer, including purification and characterization studies, are on-going.

Recent discussions with Dr. Alex Beliaev (EMSL) suggest that the high molecular weight exopolymer we are observing with FFF is of a similar mass to that which they are observing by SDS-PAGE under similar growth conditions. This has elicited a collaborative effort between our groups to characterize the exopolymer and its secretion system.

(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

The discharge to an unlined settling basin of M-Area metallurgical process wastes, associated with nuclear weapons production from the mid-1950's until 1985, has led to extensive contamination of groundwater, sediments, and riparian soils within the Tims Branch, Steeds Pond, and Upper Three Runs stream ecosystem. These effluents included substantial quantities of trichloroethylene (TCE), tetrachloroethylene (PCE), nickel, natural and depleted uranium, and other metals including copper, zinc, lead, and chromium. Total U releases to Tims Branch have been estimated at 43,500 kg. Total releases of Ni are unknown, but comparable sediment loadings suggest Ni releases on the same scale as U, if not greater. Most of the metals were retained in wetland and pond sediments along the stream's 5 km impacted corridor. Approximately 144,000 kg of TCE and 820,000 kg PCE were originally released to the M-Area Settling Basin, and the subsurface migration of these solvents has resulted in large-scale vadose zone and aquifer contamination. Outcropping of TCE- and PCE-laded groundwater into the Tims Branch system is a potential concern, and containment measures have

been initiated. Natural attenuation of this plume has been proposed as a cost-effective remediation option, and endogenous microbial populations capable of degrading TCE via aerobic and anaerobic pathways have been identified. Riparian and wetland systems are valued for their natural attenuation capacity resulting from the synergy of high organic matter content, diverse microbial populations, and the wide range of geo-chemical conditions, which lead to the biodegradation of organics and the biotransformation of metals. However, as numerous DOE, DoD, and other sites are contaminated with mixtures of organics and inorganics, including heavy metals and radionuclides at potentially toxic levels, it is critical to understand the effect of these contaminants on microbial populations and the consequent influence on *in situ* biodegradation of organics in support of intrinsic and enhanced biodegradation as well as on the reduction of U(VI).

Influence of radionuclide and heavy metal input upon microbial soil populations within a riparian system

Investigators: A.L. Neal, G.L. Mills, C. Bagwell (SRNL), and C.L. Zhang

We investigated correlations between contaminant metals and the molecular composition of sedimentary microbial communities from two locations in the Steed Pond system. Soil metal burdens were assessed with ICP-MS; C-N-S measurements were also made. Environmental DNA was extracted using a laboratory developed protocol to overcome problems associated with the high metal and humic acid contents of the sediments. Phospholipid fatty acids were also extracted using a laboratory designed protocol. Microbial community structure was assessed by 16S rRNA gene based T-RFLP with *Hae*III and *Rsa*I restriction endonucleases and PLFA analysis. Near-full length amplimers were obtained from all core depths; however T-RFLP failed to detect products within the 100-1000 bp range from Steed Pond surface sediments containing highest concentrations of metals (3006 ppm U; 3238 ppm Ni). At depth, with metal burdens as low as 3.4 ppm U and 21 ppm Ni, a complex, though conserved fragment pattern was observed between soil cores. This conservation was also observed with PLFA analysis of extracted lipids. Downstream Pond 25 cores exhibited the reciprocal pattern, community complexity mirrored contaminant burden and decreased with depth.

Microbial community structure appears to be differentially responsive to contaminant burdens in the Steed Pond system, but metal reducing bacteria are present within severely contaminated surface layers. Bacterial strains isolated from contaminated sediments from Pond 25 appear to be more resistant to metals (Ni and Cu) than isolates from uncontaminated sediments from Boggy Gut. We are currently characterizing the isolates with a view to performing comparative studies of metal toxicity and determining the nature of the acquired metal resistance. These results were presented at the 104th General Meeting of the American Society for Microbiology, May 2004.

We also completed preliminary characterization of microbial communities in riparian soils of the Lower Tims Branch stream system, which have different degrees of heavy metal contamination. Samples were collected along a transect from the lower to the upper bank of the creek with each of two samples about 3 feet apart. Preliminary results show the bacterial diversity at Lower Tims Branch was extremely high. A total of 43 different bands were excised from the DGGE gels. Most of the bands gave sequences that are distantly related to identified species. This suggests that microorganisms at the Lower Tims Branch are largely unknown. The dominant bacteria in each soil sample were different, though the same species could be found at all sample locations. These results were presented at the American Society for Microbiology Annual Meeting, May 2004.

Effect of Ni and pH on the growth of Steed Pond bacteria

Investigators: P.M. Bertsch, P. Morris (MUSC), and B.P. Jackson

We have also been investigating the toxicity of Ni to a constitutive TCE degrader, *B. cepacia* $PR1_{301}$ (PR1) at concentrations found in Steed Pond using carefully

selected (from the perspective of chemical speciation) growth media. Growth of PR1 was the same at pH 5, 6, and 7 in the absence of Ni. This pH range was examined because preliminary experiments using two microorganisms (*S. aureofaciens* and *K. cystarginea)* isolated from Steed Pond demonstrated greater growth at pH 5, the native pH of Steed Pond sediments, compared to pH 6 and 7 and because most metal toxicity studies to microorganisms in the literature have been performed at pH 7. In the presence of Ni, growth of PR1 was influenced by the pH of the medium. Growth was inhibited above 17.04 mM Ni at pH 5, 3.41 mM at pH 6, and above 0.85 mM at pH 7, even though calculations of Ni speciation predict decreasing free metal concentrations with increasing pH. Preliminary proteomics investigations have also provided evidence for up-regulation of the stress protein GrowEL at pH 5 under Ni exposure, which is not expressed at higher pH values. In contrast, our studies using *Rolstonia metallodurans* CH34 and 31A found greater inhibition of growth at pH 5 compared to 6. A manuscript based on this work is currently in review in *Environmental Toxicology and Chemistry*. We are also examining the mechanisms of enhanced Ni toxicity with increasing pH and expanding the proteomics studies.

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.

FY04 milestones for this research were to (1) initiate field research along geomorphological gradients to characterize land-use effects on the structure and function of upland forests; (2) quantify the extent to which these factors consequently affect important drivers of biogeochemical processes (e.g., dynamics of redox, carbon, moisture, and nutrients) that influence remediation of contaminants at the wetland-upland interface; and (3) collect biogeochemical field data along wetland-upland gradients under different land-use conditions. 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 (3) burned [2004] upland, thinned [ca. 2000] slope, and interface. In spring, 2004, we initiated field sampling to characterize the biogeochemical effects of land use in each set of sites. Pre- and post-fire sampling of lysimeters, deployed in upland, slope, and upland-wetland interface regions in each set of sites, revealed land use did not affect NO_3-N concentrations, but samples from the slope were marginally higher than those from the upland or the interface. Position on the topographic gradient did not affect NH_4-N, but samples from the set of undisturbed sites were marginally higher than those from the set of sites with recently burned upland. Mass of organic layer samples, collected along the topographic gradient in each set of sites, differed (p=0.008) over the upland-to-wetland gradient, and was less at the upland-wetland interface than at slope or upland positions. Organic layer mass also differed with land use (p=0.0001). Downslope sites leading from burned and cleared uplands had less organic layer mass than those leading from burned or undisturbed uplands. These initial field

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

(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

Coal fly ash was released into a bottomland and swamp forest from 1967 to 1977 as a result of runoff from a filled settling basin. Up to a 2 m depth of fly ash was deposited in some areas and total aerial coverage was approximately 40 hectares. We characterized the extent (length, width, and depth) of fly ash in a 3-dimensional grid at the site. Elemental analyses were performed on samples of the surface litter layer, fly ash, original soil layer, and selected plant, invertebrate, and amphibian species taken from the impacted area and adjacent areas with no ash. Plant community composition in the fly ash area was also determined and contrasted to an adjacent clearcut and a thermally affected stream delta, both of similar age.

Arsenic (As) and selenium (Se) were the primary contaminants-of-concern, with maximum concentrations of 8 ppm and 7 ppm in the forest floor litter layer, 85 ppm and 11 ppm in the fly ash, and 18 ppm and 11 ppm in the original soil, respectively. Heavy metal concentrations of the 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. 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.

Plant species composition on the ash plume area has shifted to an earlier successional stage, but community characteristics such as basal area and density are similar to those of an adjacent non-contaminated early successional cut-over site. The present vegetation is in transition from the former mature forest community to an impacted and/or recovering community. The amphibian and reptile 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, respectively. Since the plant community is adjusting to the deposited ash, the best management for this site might be to leave the ash in place. However, it must be demonstrated that the ash contaminants are not migrating from the area of original ash deposition or 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

To directly determine cellular response of biologically available metals, we have developed transgenic nematodes (*Caenorhabditis elegans)* with a metal-specific promotor, metallothionein-2, linked to a Green Fluorescent Protein reporter (mtl-2::GFP). We

developed several lines of mtl-2::GFP transgenic worms by micro-injecting the mtl-2::GFP construct into the gonads of the *C. elegans*. Resulting transgenic worms were then bombarded with radiation to produce three lines that gave clear and stable patterns of GFP expression in multiple generations of worms. We focused on characterizing the line with the brightest expression of GFP in response to Cd. All assays have focused on aquatic exposure. Assays of GFP expression were initially done using a microscope and digital video camera. Expression in live worms was difficult to assay because the low amount of light given from the GFP required time integration of images for accurate quantitation, but the exposures were blurred from movement of the worms. Worms were subsequently sedated or killed prior to GFP assay. Increased levels of GFP were noted following exposure to 30 µM $CdCl_2$; significantly less than the concentration required to effect behavior, feeding, reproduction, etc. Although this was successful, it reduced a major advantage of the overall approach assay: real-time assays of animal response to heavy metals (both up regulation and return to homeostasis). Additionally, the amount of time and energy associated with quantitation with this method was not ideal. Thus, we determined that methods for high throughput assays of GFP expression would be necessary.

We spent significant resources developing a 96-well plate assay of GFP expression in the mtl-2::GFP *C. elegans*. After determining that the overall approach would work, we purchased a 96-well plate reader that could be dedicated to this research. Initial results with the new plate reader suggest that sensitivity is increased by about an order of magnitude relative to use of a microscope and video capture. The limitation in the number of assays that can now be performed is in the production of stage/age specific worms, not quantification of the GFP.

Manuscripts to describe the mtl-2::GFP worms, as well as another strain to detect environmental mutagens, are in preparation. Publication of these manuscripts must be coordinated with student theses, as well as potential patent applications. Thus, they will not be submitted until FY05.

Collaboration with Dr. Angela Stormberg, Ecological and Cultural Resources Department, Idaho National Environmental and Engineering Laboratory, was initiated. An LDRD proposal was submitted and funded through INEEL. Dr. Stormberg visited the laboratory of Dr. Philip Williams, UGA, to learn laboratory rearing methods and toxicity assays for *C. elegans*. Dr. Stormberg was also introduced to the mtl-2::GFP worms. A graduate student who finished her degree in the spring of 2004 was hired as technician with LDRD funds. Dr. Stormberg received final clearance to establish *C. elegans* facilities at INEEL in June 2004. Transfer of technologies developed at SREL/UGA to INEEL will continue in early FY05.

Trace metal uptake and spatial distributions of metals in *C. elegans* were also investigated. Recently a standardized method for the use of *C. elegans* as a test organism in soil toxicity testing has been adopted by the American Society for Testing and Materials (ASTM 2001). The relatively short duration of the toxicity test, coupled with the ease of recovery of *C. elegans* from the soil media, suggest that this organism may be useful in bioaccumulation studies. To date, however, there have been few studies that have attempted to quantify whole body uptake of *C. elegans* exposed to trace elements or to study qualitative differences in trace element accumulation. Thus, we used synchrotron-based X-ray fluorescence (S-XRF) to probe the spatial variability and localized concentration of Cu and Pb in *C. elegans* exposed to those metals in aqueous solution. *C. elegans* were exposed to solutions of Cu and Pb at the EC_{50} concentration for movement (30 mg L^{-1} and 10 mg L^{-1}, respectively) for 24 hrs. Nematodes were washed and mounted in agar gel on kapton tape for presentation to the X-ray beam line. Mapping of Cu and Pb distribution and XRF spectra of Cu and Pb hot-spots were collected using a 0.010 mm focused monochromatic X ray beam at an energy set just above the Pb Lâ binding energy. Nematodes were mapped in 2D using a 6 by 15 micron step size. Localized hot-spots of Cu and Pb were quantified by collecting XRF spectra at these locations. Calibration of XRF response

was performed using a agar gel spiked with known concentrations of Cu and Pb. Clear qualitative distribution differences are apparent between Cu and Pb, with Cu uptake being relatively homogeneous throughout the nematode whereas Pb uptake was highly localized in the anterior. Quantitative XRF analysis of Cu and Pb hot-spots revealed that Cu concentrations were on the order of 80 µg g^{-1} whereas Pb concentrations exceeded 1000 µg g^{-1}.

(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.

Most mercury released to the environment is in inorganic forms, while methyl mercury is the form that generally accumulates in fish and wildlife. Thus, better understanding of the processes that influence or control *in situ* mercury methylation, speciation, and transport is essential to assessing and mitigating mercury contamination in the environment. One study completed this year examined mercury distribution and speciation in amphibian larvae in wetlands, and their food items. We found that mercury concentrations in larvae from wetlands without obvious local sources of pollution were similar to those that caused adverse effects on growth and development in laboratory experiments. This indicates the importance of atmospherically-transported mercury in these systems. Amphibian larvae from these wetlands also act as prey items for a variety of wildlife, so such mercury concentrations might indicate risks to consumer species. We used the speciation data to calculate bio-accumulation factors for inorganic and methylmercury in a simple food web composed of periphyton to amphibian larvae. Our results indicate that standard EPA models do not accurately predict mercury bioaccumulation in these wetland systems. A manuscript on these findings has been submitted to *Environmental Pollution*.

For eight years we have sampled tissues (blood, down, feathers) and food items from wood storks *(Mycteria americana*), an endangered species that nests in the southeastern U.S. Based on this long-term data set, we observed that mercury exposure and accumulation in nestling wood storks varied with location and annual rainfall patterns. Parent storks forage in wetlands near the nesting colony and return prey (mostly fish) 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. We hypothesize that this is related to higher methylation potential in freshwater systems. 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. A letter describing this relationship was submitted to *Nature*; it is now being expanded into a full manuscript for publication in another journal.

In addition to wood storks, feather samples have been collected from more than 10 other species of nestling wading birds (including egrets, herons, and ibis). These 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. Collections will continue in the coming year. Mercury concentrations and stable isotope ratios (C and N) in these samples are now being measured. Carbon isotope ratios will be used to differentiate between fresh water and estuarine/salt water foraging sites, and nitrogen isotope ratios will provide information on trophic position within local food webs. Since mercury biomagnifies (concentrations increase with trophic position), the stable isotope data and mercury concentrations for multiple species will provide a unique opportunity to evaluate the relative impacts of trophic position and feeding habitat on contaminant bioaccumulation in bird populations. Studies will also begin in FY05 on the kinetics of mercury accumulation and elimination in biota from freshwater wetlands.

(C) Earthworms as biomonitors for uranium and nickel

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

The EPA's Earthworm subchronic toxicity test (EST)

forms part of the Ecological Effects Test Guidelines, allowing the characterization of potential ecological risk from soil contamination by exposing earthworms to various incremental mixtures of the test soil over a period of 28 days, and measuring mortality. Toxicity tests like this form an increasingly important part of how regulatory bodies determine risk by attempting to measure bioavailability. Although it represents an important step forward from using purely chemical methods to determine a complex biological endpoint, the predictive power of these toxicity tests is limited. As part of SREL's continuing attempt to advance the accuracy of bioavailability characterization, tests are being run on soils from the Ni- and U- contaminated Steed Pond-Tims Branch riparian corridor. This is a well characterized ecosystem, which is useful for evaluating biomonitors and bioavailability test systems. In particular, it is thought that the EST may have limited application to U. Chemical tests, such as the frequently used sequential extraction scheme of Tessier et al. (1979, *Analytical Chem.* 51:844-851), have suggested that only Ni is bioavailable in this ecosystem, and U does not present a human or ecological health risk.

Key elements of the EST were varied to investigate for certain artifacts. While numerous soil physical and chemical factors influence bioavailability, the organic matter content of the contaminated soil has a significant role in controlling U bioavailability; therefore, one set of bioassays added organic material in the form of peat supplements to the standard bioassay. There was no earthworm mortality found during the first EST, although there were significant differences in the U concentration within exposed earthworms. As a general rule, the lower the percentage of Tims Branch (TB) soil, the more effective peat addition was at reducing the U concentration of the earthworms. Within each TB treatment level, the more peat added, the lower the U concentration of the exposed earthworms. Addition of 20% peat reduced earthworm U concentration by 44-58% across all TB mixture rates.

Our work has confirmed several problems with the EST. Firstly, the lack of mortality may be erroneously equated to a lack of ecological risk in this case, despite the accumulation of ≈200 mg g^{-1} U within earthworms. Secondly, interaction with components of the artificial soil throws into question the applicability of this test, at least for certain metals. Our experiments suggest an interactive effect, with artifacts from $CaCO_3$ or organic matter addition increasingly influential at lower contaminant burdens.

One of the most important issues in the use of the EST remains that uptake does not consistently equate with toxicity, and it is upon this assumption that the EST is based. Organisms such as earthworms are known to avoid toxic effects by sequestering metals within specialized structures within their bodies. These structures or granules contain calcium pyrophosphate, and are produced by organelles known as chloragocytes, which occur in chloragogenous tissue located on either side of the digestive tract. When contaminated soil moves through the earthworm gut, excess metals can be sequestered by the chloragocytes before the food is assimilated. Hence, the worms can contain elevated concentrations of metals without excessive mortality. The central tenet of biomonitor use is that the organism is affected by the contaminant in a predictable manner, and that it does not posses any adaptive mechanisms which obscure the dose response relationship. We have shown that there are numerous issues inherent in the EST which limits its use for assessing the risk due to the presence of U in the soil.

The application of spatially-resolved metal analysis techniques, such as synchrotron X-ray fluorescence microspectroscopy (SXRF), allows the distribution and concentrations of metals to be imaged within the context of the particular biological structures in which they are contained. Knowing where the metal is primarily located allows inferences to be made about the transport route into and its toxicity to the organism. In this case, worms were collected from soils within the Tims Branch depositional system and preserved in formaldehyde prior to dissection, embedding, and SXRF analysis. The digestive system of several individual earthworms were dissected from the remaining tissue, specifically the upper digestive tract. Calciferous glands or chloragogenous tissue is located in the upper

intestinal tract, between the esophagus and the crop. Once the tract was removed, several samples were prepared by embedding the soft tissues in Nanoplast™ resin, which maintains sample integrity by allowing hydrated samples to be embedded.

The samples were analyzed using synchrotron X-ray fluorescence (SXRF) microspectroscopy at beamline X26A of the National Synchrotron Light Source. Compositional maps were generated for numerous metals, confirming that Ni was not accumulated in significantly elevated amounts within the bodies of earthworms, confirming findings from the earlier EST. However, images of the Ca and U distribution of the gut clearly show a localized and relatively elevated distribution of these elements within the calciferous tissue of the earthworm. Calcium occurs in other regions of the digestive system, but it is only in these tissues that both U and Ca are found.

Work is ongoing to isolate these enriched regions and determine whether the speciation of the U is being distinct from that found within U contaminated soils, using x-ray absorption spectroscopy (XAS) and size exclusion chromatography (SEC).

(D) Whole-lake experiments on the dynamics of cesium

Investigators: T.G. Hinton and B. Taylor, with collaborators from Colorado State University

To document the short-term dynamics of cesium in an aquatic system, we conducted a whole-lake experiment by introducing 4 kg of stable Cs into an 11.4 ha reservoir located on the SRS. We were interested in the dynamics of the stable Cs throughout the reservoir ecosystem, and on its impacts to the residual ^{137}Cs contamination that occurred from releases some 40 years ago. The work is interesting because whole-lake experiments provide a realistic experimental scale, but are rare and difficult to conduct, and because little is known about how the reservoir's large mass of macrophytic plants (approximately 26,000 kg) might influence Cs dynamics. A recently submitted manuscript to *Journal of Environmental Radioactivity* documents stable and radioactive Cs dynamics during the first 260 days of the experiment. The data are important for modeling early Cs dynamics within a contaminated reservoir (as might occur from a terrorist use of a dirty bomb), and in testing if the addition of stable Cs might be an effective countermeasure/remediation strategy for radioactive Cs contamination. We anticipate another two manuscripts from the data in FY05.

(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 organo-selenium 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 analysed 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 analysed 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.

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.

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 on Ni and U. Previous studies have shown that Ni is bioavailable at this site with increased concentrations detected is 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 Steed 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.

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 analyses 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 N, 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*.

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.

(F) Use of Monitored Natural Attenuation for chlorinated solvent remediation

Investigators: L.A. Newman and faculty at University of South Carolina

Monitored Natural Attenuation (MNA) is a concept that is gathering interest, but in the absence of a scientific basis it may be viewed skeptically by the regulatory community and the general public. Natural remediation does take place; what is less well understood is how to predict the suitability of a given site for MNA. In FY 04, we planned to complete a study of a system where a chlorinated solvent plume is entering a wetland area and potentially impacting a stream system to determine

if natural remediation is taking place. This included examination of soil and near-surface water for contaminants; sampling of tree cores for evidence of contaminant uptake by the trees; examination of soil physical conditions such as percent saturation, dissolved oxygen levels in soil and near surface water and redox potential; and determination of how these conditions affect the concentrations and metabolism of the contaminants.

To date, we have completed several samplings along the Pen Branch creek on the SRS, where trichloroethylene and perchloroethylene are seeping from the Chemical, Metal, and Pesticide disposal area. Soil, groundwater, and tree core samples were analyzed for the presence of the parent compounds, as well as the degradation product dichloroethylene. To date, we have found only one section along the creek where PCE is being detected, but no degradation products have been seen. At each sampling location, we also recorded soil moisture levels, oxygen levels, and redox conditions. Additional on-going analyses include examining the state and concentration of potential electron donors. In summer 2004, we will start looking at the microbial communities to determine if potential degrading organisms are present at the site. The student working on this project has just successfully defended her thesis proposal to her graduate committee, and will be spending the summer and fall doing sampling and analyses. In FY05, we plan to continue sampling for the calendar year and write a paper on our findings at this sampling site. We also hope to be able to work with WSRC personnel to help them determine if MNA is a viable option for this site. (This project is not funded by SREL core dollars.)

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. 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.

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. Taylor et al. presented a paper on these results at the annual meeting of the American Society of Limnology and Oceanography. 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. Further analysis and modeling will focus on this issue, and preparation of manuscripts from this project will be completed in FY05.

(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 recent years in using indigenous organisms as indicators of environmental contamination, it has been suggested that turtles could be good indicators of metal contamination because they are extremely long-lived, have 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 (keritin) coating, within which periodic growth deposition bands are discernible, providing 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 a bioindicators. To further explore this idea, in June 2003 five hundred hatchling red-eared sliders (*Trachemys scripta elegans*) were divided evenly into twelve tanks and each tank was assigned one of four treatment groups (control, 25 mgL^{-1}, 100 mgL^{-1}, and 400 mgL^{-1}). With the exception of January and February, turtles have been dosed with nickel citrate ($Ni_3(C_6H_5O_7)_2 \cdot XH_2O$) on a bi-monthly gavaging regime. The nickel is administered orally using a transfer pipette, and regurgitation events are recorded. Measurements of individuals were taken upon initial receipt of turtles and prior to all gavaging events. To date 250 individuals have died. However, there is no dose-related mortality trend, nor has a correlation been found between number of deaths and position of tank. The deaths are most likely due to a naturally high mortality in hatchlings in addition to the initial stresses on hatchlings due to being in captivity.

All measurements indicate notable growth of surviving turtles regardless of treatment group, with as much as a 3-4 fold increase in mass for some individuals. Growth rates do not differ significantly among treatment groups or among tanks at this time. A significant treatment effect on shape was detected, with shape being a function of plastron length divided by plastron width, with the 100 mgL^{-1} group being significantly different than control and 25 mgL^{-1} groups. However, additional analyses are needed for all growth data. Marginal bone fragments have been sectioned from individuals in high dose and control tanks, and analyzed using synchrotron-based X-ray microanalysis at beamline X26-A at the National Synchrotron Light Source, Brookhaven National Laboratory. Preliminary results indicate spatially variable higher nickel concentrations in 400 mgL^{-1} group turtles in comparison to control group. In addition, preliminary ICP-MS analyses have been conducted using marginal scute sections.

Additional trials of all analytical techniques, including synchrotron-based microanalysis, ICP-MS (including laser ablation), are necessary for more conclusive results. The bi-monthly gavaging regime is planned for another growth season, 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 at the conclusion of dosing rounds. 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, June, 2004.

(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

The use of the chemical composition of tree rings, or dendroanalysis, to inform about past and present environmental quality has been successfully used for climate change (e.g. atmospheric CO_2 or SO_x concentrations) and to assess groundwater quality, but its application to heavy metals in the soil has been more controversial. Researchers have made connections between contaminant history and the concentrations of metals within annual rings, but in some cases post-growth metal translocation between annual rings has confounded dendroanalysis. Of particular interest to researchers at SREL are the processes that occur within woody plants that successfully occupy extremely metal-contaminated areas. Comparative research has been conducted on the transport, distribution and speciation of metals within hyperaccumulator plants. However, the ultimate aim of this work is to inform on risk assessment and remedial issues for the clean up of DOE lands, and therefore the focus remains on the large body of plants that survive on contaminated sites, how they can be used in biomonitoring, and the contribution they make to trophic transfer.

The Steed Pond former settling basin is a well-studied contaminated wetland ecosystem used to investigate processes central to the use of trees as biomonitors. Interpreting the metal composition of annual rings requires an understanding of the transport and reactions of metals within the vascular system of the tree. Spatial metal analysis techniques such as SXRF and CMT are critical tools, as they allow us to image the distribution on a mm scale, measure metal abundance, and increasingly, determine the binding environment of metals.

Cores were collected from a metal-impacted black willow (*Salix nigra* L.) growing in an area where total soil Ni concentrations were 266 (±65) mg kg^{-1}. Previous studies had shown that this tree had taken up above normal concentrations of Ni. Multiple annual rings were analyzed using SXRF and also by subjecting the data to cluster recognition analysis to determine whether metal abundances were statistically significantly different between annual rings. A second study focused on the Ni-enriched inclusions observed in earlier studies, which were thought to be the transported form of Ni present within the lumen of the xylem vessels. Further studies also used X-ray absorption spectroscopy (XAS) to determine the nearest neighbours and bond lengths of Ni to determine speciation. Cluster recognition analysis allowed the data to be separated into Ni-rich and Ni-poor areas, and these clusters were applied to the remaining elements for comparison of metal co-association between the metal enriched and non-enriched annual rings. Statistical analysis confirmed that the Ni, Cu, and Mn concentrations in 1996 were significantly different from previous years (in all cases, $P<0.0001$). Most importantly, there does not appear to be any indication of post-growth translocation between annual rings shown here, some eight years after enrichment with Ni, because the transition in metal abundance is sharp, rather than gradual. Further, statistical analysis showed that there were strong correlations between the distribution and concentration of Ni and Mn. Over the entire core sample this correlation was $r^2=0.7792$. Cluster recognition analysis showed that the correlation differed between annual rings, with an $r^2=0.42$ in the

Ni-rich ring, and r^2=0.12 in non-enriched annual rings. Smaller, Ni-enriched inclusions also correlate strongly with Mn, and it is believed that these elements may be bound to similar ligands within plant tissues.

Computed microtomography was conducted on a sample of woody tissue excavated from a Ni-enriched annual ring. The sample was analyzed via SXRF and three-dimensional images were constructed from individual sinograms or 'slices' of the sample. Highly concentrated inclusions, where the concentration of Ni can be almost 1000 mg kg^{-1}, were identified as Ni within sap moving through the xylem vessels, and were one of the first such images to be produced using this technique. Ongoing studies are using X-ray absorption spectroscopy and Fourier Transformed Mass Spectroscopy to identify the ligands to which the Ni is attached in this transported form.

(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

As the DOE moves toward a completion of site cleanup and restoration activities at the SRS, it becomes increasingly important to understand whether past nuclear industrial activities at the site have had persistent effects on the levels and distributions of various nuclear industrial contaminants in site biota. As one of the most abundant and widely-distributed predator species in the SRS' terrestrial food webs, the gray fox is particularly appropriate to study as an indicator of site-wide contaminant distribution. At the same time, the broadly omnivorous diet of this species ensures that contaminant distribution data will be relevant to a variety of trophic relationships within the site's food webs.

Muscle samples from 72 gray foxes (*Urocyon cinereoargenteus*), collected from locations on (n=42) and from off (n=30) the SRS, have been analyzed for radiocesium, heavy metals, and stable isotopes. Preliminary statistical analyses of these data indicated significant differences between radiocesium levels, trophic relationships (as revealed by stable isotope analyses), and levels of mercury and selenium for foxes collected on vs. off the SRS. The implications of these differences for both present and future SRS site management are now being considered as writing begins on a manuscript summarizing these results. The fact that preliminary analyses have shown significant differences in contaminant burdens and trophic relationships between on- and off-site foxes will provide a unique opportunity to evaluate the ways that closure of the SRS to public access and resulting habitat alterations have altered gray fox trophic relationships. An evaluation can then be made of how altered trophic relationships may or may not have contributed to the observed differences in contaminant burdens.

(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

American alligators (*Alligator mississippiensis*) are the top-level predators in many southeastern aquatic ecosystems. Because of their trophic position and long life span, they can accumulate considerable contaminant burdens, particularly of persistent pollutants like methyl mercury and pesticides. Because they are mobile, and many states now allow hunting and harvesting of wild alligators, they may also serve as vectors of contaminant movement into humans who consume alligator meat. A graduate thesis was completed describing mercury contamination in wild alligators in four southeastern states: Alabama (n=10), Georgia (n=16), South Carolina (other than the SRS; n=3), and Louisiana (n=27). The 27 Louisiana alligators were also used to examine distribution of mercury and other elements among tissues (including muscle, liver, kidney, gonad, etc.). This work is the basis of two papers that are currently undergoing internal review prior to submission. The first paper showed all alligators sampled had detectable mercury concentrations in both liver and tail muscle samples. The highest liver mercury levels were found in Glynn and Camden Counties in Georgia; South Carolina and

Alabama alligators had intermediate liver mercury levels and Louisiana alligators had the lowest mercury concentrations of both liver and tail muscle.

Stable isotopes of carbon and nitrogen were measured in alligator tissues to estimate trophic position and dietary carbon sources. Nitrogen stable isotope ratios were also the highest in the Glynn and Camden Co., Georgia alligators, indicating that they were feeding at higher trophic levels than those from the other locations. There were no clear differences in carbon sources for the alligator populations sampled. The stable nitrogen ratios confirmed that alligators feeding at higher levels within food webs tend to have the highest levels of mercury contamination.

This information, along with the relationships observed among mercury concentrations in various organ/tissue compartments of Louisiana alligators, provides useful insight into the factors that influence mercury levels in alligators on the SRS. One of the largest alligators ever captured in South Carolina was found dead in Par Pond on the SRS several years ago. Tissue mercury concentrations were high enough to suggest mercury toxicosis as a contributor to the death of this alligator. Thus, an understanding of the processes and parameters that influence mercury uptake in alligators is extremely important, particularly with regard to the need to predict the outcome of various reservoir management strategies that may be employed as DOE completes environmental clean-up and restoration activities at this site.

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) Mapping distributions of radiocesium in SRS reservoirs using hydroacoustic surveys

Investigators: S.J. Harper, T.G. Hinton, and M.D. Wilson

To characterize and predict the distribution and dynamics of contaminants, a generalizable, spatially-explicit modeling approach is needed. In former cooling reservoirs at the SRS, surveys indicate that the vast majority of ^{137}Cs contamination is confined to sediments, though tremendous variability in concentration has been observed among sample locations. Much of this variation is likely attributable to underlying spatial heterogeneity in sediment characteristics. Given this variability, 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 and document subsequent declines given spatial heterogeneity. Advances in the field of hydroacoustics afford a technological solution to this problem; statistical power can be enhanced by accounting for spatial variability in factors known to influence radionuclides, including bathymetric features (depth, slope) and sediment characteristics (soil texture, water content, organic matter). Using GIS to evaluate energy return profiles obtained from extensive hydroacoustic surveys, the spatial distributions of these important variables were mapped at fine spatial scales over broad areas for Pond B. Dredge samples were taken and laboratory analyses were conducted to quantify ^{137}Cs concentrations, moisture content, organic content, and texture (percent sand, silt, and clay) of sediments. A statistical model was developed to relate ^{137}Cs with hydroacoustically-

derived variables; significant main and interactive effects were found, and the model explained 73% of observed variation. Geostatistical techniques allowed a detailed, full-coverage map of ^{137}Cs in surface sediments of Pond B to be generated from results of hydroacoustic surveys. Greater understanding of the distributions of radionuclides within reservoirs will enhance scientific understanding of important reservoir processes (e.g., sediment focusing), which in turn will allow better resource management. For example, such information could be used to predict how contaminants would respond to hydrologic or environmental changes (e.g., reservoir draw-down), and could also be used to provide enhanced risk estimates for fish and wildlife species that utilize reservoir ecosystems.

This research supported the training of one IAEA Postdoctoral Fellow and two NSF REU students. Results were presented at annual meetings of the American Water Resources Association (AWRA) and the American Society of Limnology and Oceanography (ASLO). A peer-reviewed manuscript detailing this research is in progress. Future work will include model validation and the development of spatially-explicit simulation models of ^{137}Cs dynamics that incorporate the spatial and temporal dynamics of sediment deposition and resuspension as influenced by the effects of wind and other extrinsic factors.

(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. Such parameteric approaches are powerful and well developed, but assumptions of normality are frequently not met in environmental sampling. In fact, frequency distributions of contaminants in organisms are often non-normal, and may be highly skewed. Knowledge of underlying distributions and their properties are critical to selection of appropriate data analysis techniques. The frequency distribution of contaminants also highly influences probability-based risk assessment models. 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). We are presently extending this analysis to other contaminants, particularly heavy metals such as mercury. In the past year existing data sets have been identified. These include concentration data on Hg and other heavy metals in fish, amphibians, and birds. Most of these data come from sites on the SRS, but we are including other locations as appropriate and available. Additional data sets on radionuclide concentrations from highly contaminated areas near Chornobyl, Ukraine were also obtained this year. We have begun analyzing these data sets to determine the best-fitting frequency distributions. We will continue these goodness of fit analyses in FY05, and incorporate additional data from existing literature as needed. It is anticipated that these studies will yield one or two manuscripts for submission in the coming year.

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, T.C. Glenn, and C.S. Romanek

The concept of sentinel species suggests an efficient and cost effective method to monitor pollutant uptake and effects at contaminated sites. In this approach, a population of a native or closely related surrogate animal species is established at a site of interest. The animals are allowed to range freely and feed, and are recaptured at intervals for non-lethal sampling. They are then returned to the site for continued exposure. In this way, time series data about contaminant

exposure, accumulation, and effects can be obtained. Past studies at terrestrial and aquatic habitats have employed this methodology to determine radiocesium accumulation kinetics and to explore potential effects of long term, chronic radionuclide exposure. We are presently maintaining experimental populations of two candidate species, the eastern box turtle (*Terrapene carolina*), and a specially developed strain of feral bantam chicken (*Gallus gallus*). These will allow studies in either terrestrial or aquatic habitats, and so can be employed at various sites depending on future research and management needs. Both of these species have been successfully used in past release-recapture studies at SREL and both have proven to be logistically and ecologically suited to serve as sentinel species in a variety of contaminated terrestrial waste sites on the SRS. If an aquatic system should be chosen for future research focus, tamed game-farm mallard ducks (*Anas platyrhynchos*) would be the species chosen for release-recapture studies, with stock being ordered from a commercial supplier as needed.

In the past year, we initiated studies of barn swallows (*Hirundo rustica*) as a sentinel species in both contaminated and uncontaminated habitats on the SRS. This study, which is partially supported by the SRS task funding**,** has provided preliminary data that will help characterize these birds as a sentinel species for both aquatic and terrestrial systems on the site. Barn swallows are opportunistic insectivores that forage over both aquatic and terrestrial habitats. Their prey could thus reflect food-web contamination in either kind of area. Moreover, barn swallows build their nests from mud that may be gathered from areas with past contaminant inputs from SRS site-related activities. The presence of gamma-emitting contaminants in the birds' nesting materials would expose their eggs and growing nestlings to an external radiation dose in addition to any burden accumulated from the consumption of contaminated prey. We are addressing this concern by collecting tissues from nestlings to measure internal concentrations of radionuclides and heavy metals, and by placing thermoluminescent dosimeters (TLDs) in nests to evaluate external gamma exposure. This study builds on previous work with swallows at Chornobyl and at the Idaho National Engineering and Environmental Laboratory.

During this past breeding season, we began monitoring about 120 barn swallow nests for breeding success. Mud samples have also been collected from many of these nests and preliminary analyses of radiocesium have been completed. We have also banded over 30 adult swallows and 50 nestlings associated with these nests. Later returns/recaptures of such banded birds will allow us to estimate survival rates and changes in contaminant body burdens over time. We are coordinating our sampling with researchers studying contaminant dynamics and population genetics of barn swallows in the vicinity of the Chornobyl. Dr. T. Mousseau of the University of South Carolina has visited our field research sites and we have made plans to compare the data we are collecting with similar information gathered for this species in the Ukraine.

(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

The Tims Branch watershed on the SRS was contaminated by effluents containing Ni, U, and other heavy metals. We collected small mammals from the Tims Branch watershed and a nearby reference site and analyzed their tissues for pollutant metals and radionuclides. This study included examination of spatial and temporal variation in body concentrations in small mammals foraging in the area. Contaminant analyses have been completed, all data have been analyzed, and a Master's thesis has been written and defended describing the distribution of radionuclide and heavy metal contaminants in small mammals collected over a two-year period. Stable isotope analyses also allowed a simultaneous characterization of the trophic structure of this small mammal community. In the six species studied, there was no detectable relationship between trophic position and tissue metal concentrations. This suggests that there is little or no biomagnification of these contaminants with

higher trophic levels within the small mammal community. Season was the only factor significantly related to the level of metal contamination, with animals captured in the fall having higher contaminant burdens. Manuscripts detailing these findings are currently in preparation.

(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.

We have completed radiocesium analyses on 259 pork muscle samples from feral swine collected during all seasons of the year and from a variety of locations on the SRS and from 74 swine collected from an off-site location on the Lower Coastal Plain (Ossabaw Island, Chatham Co., GA). Studies over the past 25 years have confirmed the presence of elevated and easily quantifiable levels of radiocesium in the swine from Ossabaw Island, resulting from natural global atmospheric processes. Swine from Ossabaw are thus well suited to serve as a control population to compare to those from the SRS, where these same natural fallout processes are augmented by access to a number of wetland habitats that have received past anthropogenic releases of radiocesium and other site-related contaminants.

Heavy metal and stable isotope analyses have not yet been completed on all samples analyzed for radiocesium. However, the samples have been processed and are now ready for stable isotope analyses. When completed, stable isotope analyses will also contribute to the research goals of Milestone 7(C) as listed under the Risk section of this report.

In addition to the contaminant analyses described above, a draft manuscript has being completed analyzing whole body weights of 10,530 feral swine collected on the SRS over the past 30+ years. When combined with weight-specific contaminant burdens, the body weight data will allow us to estimate the total amount of meat of a given contaminant level that a hunter would be able to take home and consume as food. This information, will, in turn allow us to parameterize population-level risk assessment models for this potential pathway for exposure of the public to SRS-generated environmental contaminants, particularly radiocesium and heavy metals. Stable isotope data will provide further insight into the role of factors such as specific trophic levels used by individuals of this broadly omnivorous species, in determining the contaminant burdens observed.

(D) Herpetofaunal diversity at an SRS seasonal wetland

Investigators: J.W. Gibbons and collaborators

Isolated wetlands in the southeastern United States, documented as having high species diversities and being essential to many groups of organisms, have become increasingly threatened. The steady decline in numbers of these critical wetlands from losses to agriculture and commercial development since the beginning of the 20th century is well documented. Recent regulatory changes have further exacerbated the situation. Despite the perceived value of isolated wetlands to regional biodiversity, few studies have demonstrated quantitatively the complete productivity achievable within single isolated wetlands over measurable time frames. Our purpose is to use our long-term experience and resultant data from the herpetofaunal community on the SRS to provide documentation of what is potentially lost by the elimination of a single isolated wetland in the Coastal Plain of the southeastern United States, where the highest species densities of U.S. herpetofauna are found.

Ellenton Bay, a Carolina bay, is an isolated freshwater wetland located on the SRS, where wetlands have been protected since 1951 from most of the environmental perturbations typically resulting from agricultural, urban, and industrial alterations in the region. Studies at Ellenton Bay from 1968 through 2004 suggest that the herpetofaunal species diversity naturally characteristic of the region was existent within 25 years of establishment of the SRS and the shift toward more natural (i.e. non-agricultural) conditions; herpetofaunal species diversity remains high at this site today.

- Based on determinations of the herpetofaunal species diversity and biomass production of Ellenton Bay and its peripheral terrestrial habitat during a single year (February 2003-February 2004) that immediately following a severe, three-year drought, we concluded that wetlands can recover from natural droughts if the natural landscape, metapopulation framework, and habitat connectivity have remained intact, as was the apparent situation for the SRS populations.
- During one year of sampling at Ellenton Bay a total of 409,048 reptiles and amphibians were captured, indicating a high level of productivity, herpetofaunal abundance, and diversity. Of those captures, 24 species were amphibians and 36 were reptiles.
- The biomass productivity for amphibians during the single year of sampling at Ellenton Bay, based on captures of metamorphs of the four most abundant species (two frogs, two salamanders), was more than one and a half metric tons (1,541kg).

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

Analysis of lipid biomarkers, including phospholipid fatty acids (PLFA), hopanoic acids, and ether lipids have been shown to be a useful culture-independent technique for determining the identity and biomass of microorganisms in soils and sediments. Our preliminary study was to determine the community structures of bacteria from different soil environments at the SRS. The samples included the lower Tims Branch (soil), Beaver Dam Creek (sediment), and the sand and mud from a creek running through the lower Tims Branch. The number of fatty acids identified ranges from 42 to 69, suggesting that extremely diverse groups of organisms exist in the soil and sediment environments. Total biomass in these samples ranges from 1441 pmol/g to 8923 pmol/g. However, this variation does not correspond to the number of fatty acids in each sample, suggesting that biomass and diversity do not co-vary. Fatty acids in these samples are dominated by saturated compounds (27.5-32.6%), monounsaturated compounds (27.4-42.4%), and branched compounds (15.0-36.4%). This distribution is consistent with dominance of bacterial fatty acids in the biomass. On the other hand, polyunsaturated fatty acids that are indicative of eukaryotes are low (1.3-8.1%), suggesting low biomass from eukaryotic organisms. These results provide valuable information on microbial community structure in the soil and sediment environments. They also lay the foundation for our planned ^{13}C-labeling experiments to identify extant microbial populations contributing to the bacteria biomass in the natural environment.

(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 that allow the screening of the 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. In short, environmental samples are amended with the adverse chemical of interest (e.g. an antibiotic or a heavy metal), then resistant and sensitive cells are marked using viability stains, sorted with a flow-cytometer, and subsequently analyzed with a suite of molecular biology techniques. We have analyzed a number of freshwater samples using direct viable counts and propidium iodine techniques to mark viable versus dead or dormant cells. Sorting of bacteria with a MoFlo (Cytomation) cell sorter resulted in a significant separation of viable and non-viable cells during the first sorting cycle. Using water from the Savannah River and D-area ash basins, we screened the entire microbial community to determine the effect of elevated heavy metals in the water column of the ash basins relative to the source water (Savannah River). In addition, we obtained source and outfall water from two additional ash basins at coal fired plants in South Carolina and Wisconsin. Our results demonstrate that exposure to ash basin water increases resistance/tolerance of bacteria to eleven different antibiotics, Hg, Cd, Ni, and Pb.

We have also begun a series of selection experiments. These results indicate that exposures to ash basin water or controlled experiments adding either Cd or Ni do select for increased levels of antibiotic resistance and increased levels of multiple antibiotic resistance. In addition, these results indicate that exposure to antibiotics increased the level of metal resistance and tolerance. Results were presented at the Annual Meeting of the American Society of Microbiology, May 2004.

(C) Evaluating the efficacy of standard testing methods for *E. coli* in the environment

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

Preliminary results indicate that many bacteria are misidentified the U.S. EPA's standard test kit method to identify *Escherichia coli* in the environment. Such misidentifications may have serious implications for the interpretation of microbial contamination data routinely collected by federal, state, and local agencies. Using commercially prepared test kits, we analyzed environmental water samples collected from Four Mile Creek on the SRS for the prevalence of *E. coli* in sediments and water. A large waste-water treatment facility discharges treated effluent into the Four Mile Creek system. We successfully isolated numerous strains of *E. coli* from these samples. We then sequenced 100 of the strains and none of them were positive for *E. coli*. This result was unexpected. We have obtained samples of *E. coli* from colleagues at the Ft. Johnson NOAA laboratory in Charleston, SC. These samples have been sequenced and all are either *E. coli* or closely related organisms. We are collecting additional samples from Four Mile Creek to determine whether the test kits were faulty or whether some other process is causing the negative results.

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.

Soils and sediments in many natural and man-made systems are subject to periodic flooding and drying. Such flooding and drying can be frequent or infrequent, and 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 can influence metal speciation. Our previous work, as well as that of others, has suggested that mercury bioavailability and accumulation, in particular, is related to hydroperiod and flooding of soils and sediments. We performed preliminary experiments in laboratory mesocosms to investigate metal bioavailability to crayfish (*Procambarus clarkii*) under different flooding and drying regimes. Repeated flooding increased bioavailability of some metals. These results were presented at a national meeting (Society for Environmental Toxicology and Chemistry). We

continued collection of samples of biota from Carolina bays, seasonal wetlands in the southeastern Coastal Plain that typically flood and dry seasonally. Our previous studies had demonstrated a relationship between bay hydroperiod (relative amount of time that the bay contains water) and fish mercury concentrations. Surveys of amphibian larvae did not find a similar relationship. However, amphibian larvae accumulate both inorganic and organic mercury species, whereas most mercury in fish is methyl mercury. Differences in accumulation patterns among fish and amphibians may reflect differences in the form of mercury accumulated. This again reflects the importance of metal speciation to bioavailability. In the coming year, we plan to place crayfish in bays with different hydroperiods, and sample them to measure metal uptake over time. We hypothesize that uptake will be higher in bays with shorter hydroperiods. We will also continue sampling native biota from permanent and temporary aquatic habitats to examine differences among species and wetlands. Results of these studies will be valuable in evaluating potential impacts of wetland remediation projects, particularly those where water levels will 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.

For amphibians that breed in seasonal ponds, a great component of variation in reproductive success is caused by year-to-year differences in the times that the ponds fill and dry. At Rainbow Bay on the SRS, for example, long-term census data for the marbled salamander (*Ambystoma opacum*) showed that the pond dried before the larvae had completed development in 6 of 22 years, resulting in complete loss of the year's cohort. Using a computer simulation model written by Taylor, we studied demographic responses of *A. opacum* to conditions during aquatic and terrestrial stages of the cycle and to frequencies of randomly imposed reproductive failure. The model was formulated and parameterized using SREL's extensive field and experimental data. Larval growth and survival are density-dependent, and females are tracked individually after metamorphosis. Two important results are: (1) extreme sensitivity of populations to survival rates in the terrestrial stage of the life cycle; and (2) nontrivial probabilities of local extinction due to natural variation in filling and drying time of the pond. The first result expands the scope of prudent management from the breeding pond to the adjacent terrestrial habitat. The second result implies that occasional colonists may be required to maintain the local population and thus expands the scope of prudent management from a single population to multiple populations in a landscape that permits exchange of migrants. A manuscript by Taylor, Scott, and Gibbons describing this work has been accepted by *Conservation Biology*.

··· Ecological Risks and Effects ···

Investigators in the Ecological Risks and Effects Research Theme at SREL conducted a wide variety of research during FY04 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 FY04 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 FY04 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

Little is known about effects of environmentally realistic concentrations and chemical species of dietary mercury on developing amphibians. Amphibians feeding in wetlands can accumulate mercury from their diet, but it is unclear whether mercury negatively affects growth and metamorphosis, and whether current exposure levels will affect amphibian populations. In FY04, we completed a study on the trophic transfer of mercury from *aufwuchs* (periphyton, containing a quantified mixture of inorganic and organic Hg) to amphibians, and examined effects on larval development and life history traits important to population dynamics. Larvae of the southern leopard frog (*Rana sphenocephala*) were exposed to experimental diets intended to mimic mercury concentrations and speciation in *aufwuchs* observed from aquatic systems contaminated by atmospheric deposition. Observations on rates of mortality, malformation, and larval growth and

development were made for 254 days. Increased incidence of mortality, malformation, and changes in growth and development were observed at concentrations that reflect the highest concentrations expected in the amphibian diet from atmospheric deposition (1,500-3,300 ng Hg/g DW). The results of this study are probably more ecologically realistic than results obtained from previous studies of aqueous mercury toxicity and suggest that dietary mercury exposure in habitats primarily contaminated by atmospheric deposition has the potential to cause adverse effects in amphibian larvae. This research was presented at the annual meeting of the Society of Environmental Toxicology and Chemistry, two University of Georgia sponsored platform presentations, at the 7th International conference on mercury as a global pollutant (Ljubljana, Slovenia), and is in press within *Environmental Toxicology and Chemistry.*

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 made significant strides toward developing 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. A simulated food chain approach allowed manipulation of the chemical species of contaminants of interest, and therefore proved to be a powerful tool for assessing the relationship between speciation and bioavailability. In our first experiment, we fed commercial feed laden with seleno-D,L-methionine (30 ì g/g dry mass) to crickets (*Acheta domestica*). Crickets fed Se-contaminated food accumulated ~15 ì g/g Se (dry mass) in their tissues after 5-7 days. Se-enriched crickets were then fed to juvenile male and female lizards (*Sceloporus occidentalis*) for 98 days while conspecifics were simultaneously fed un-contaminated (~0.05 ì g/g Se) crickets. We compared Se accumulation, survival, food consumption, growth, and body condition of lizards between sexes and dietary treatments. At the end of the study, lizards fed Se-contaminated crickets had mean tissue Se concentrations ranging from 9.3 -14.1 ì g/g dry mass compared to Se concentrations of <1.3 ì g/g in tissues of control lizards. Distribution of Se among tissues differed between sexes; males retained more Se in their carcass whereas females partitioned more Se into their gonads. Although maternal transfer was not examined in this study, female gonad concentrations (14 ì g/g Se) approached the highest of thresholds (16 ì g/g) for reproductive toxicity in oviparous vertebrates, suggesting that dietary exposure of ~15 ì g/g Se could pose significant risks to reproductive success in lizards. Although there were significant differences in growth and body condition between sexes (males grew larger but females had higher body condition), there was no consistent effect of dietary treatment on these sublethal parameters, food consumption, or survival (100% in all treatments). The simplified food chain approach proved to be an ecologically relevant method (unlike traditional gavage methods) of exposing small vertebrates to dietary contaminants, and forms the foundation for future studies on maternal transfer, teratogenicity, chemical speciation, and bioavailability in terrestrial predators. This research was presented at the annual meeting of the Society of Environmental Toxicology and Chemistry and a manuscript has been submitted to *Environmental Pollution* for consideration.

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

Since the mid-1970's, huge advances have been made in the technology associated with molecular and cellular biology. Increased sophistication and automation of molecular methods have greatly increased the identification of molecular damage. These advances have led to the use of molecular or chromosomal damage as a plausible indicator or early warning of ecological risks to exposed biota, with the tacit assumption that such damage endpoints are indicative of damage to the individual's health. Unfortunately, the relationship between the level of molecular or chromosomal damage and the level of effect necessary to impact an individual organism or, more importantly, a population of organisms, has yet to be determined. As a result, a disjunction exists between cellular or molecular damage as an effect endpoint for biota and concomitant impacts at the individual and population levels of biological organization.

We are conducting several research projects to understand how effects are integrated across different levels of biological organization. Such research will give population-level relevance to molecular-level markers of contaminant effects. In FY04 we published on a promising molecular technique that uses the frequency of radiation-induced chromosome aberrations as a population-relevant biomarker, and that might allow us to couple effects at different levels of biological organization (SREL reprint 2701). The chromosome aberrations serve as a lifetime biological dosimeter. Thus, no assumptions are needed relative to external exposure rates or the movement of organisms into and out of contaminated areas. A biological dosimeter that integrates dose over the lifetime of the organism could greatly improve accuracy and reduce uncertainties associated with ecological risk assessments.

The individual and population-level importance of molecular damage has direct implications to DOE's method of determining risk to biota exposed to ionizing radiation. Currently the DOE guidance for ecological risk assessments for biota exposed to radiation is based on dose rate limits set by the International Atomic Energy Agency. The IAEA recommendations are for the maximally exposed individuals in the population. DOE recognized the difficulty in determining dose rates to maximally exposed individuals and changed the IAEA paradigm by assuming that the same dose rate limits could apply to representative animals in the population rather than the maximally exposed ones. However, if the dose of a representative individual is estimated using the sample mean, this change in dose limit interpretation could result in 50% or more of the population receiving dose rates greater than the suggested limits, rather than only maximally exposed individuals, as the original IAEA guidelines suggested. In FY04, we published on the implications of DOE altering the IAEA paradigm, and suggested viable alternatives that meet both the IAEA expectations and DOE's risk assessment needs (SREL reprint 2706; and in press, *J. Risk Analysis*). We suggest shifting the regulatory criterion appropriately to argue that if the top 1% (as opposed to the maximum) of the population has a dose rate less than or equal to the regulatory limit, then the population is adequately protected; the Maximum Likelihood Estimate of the 99th percentile can then be used to estimate the true population percentile.

(B) Development of biomarkers to measure transgenerational risks from contaminant exposure
Investigators: T.C. Glenn, T.G. Hinton, C.H. Jagoe, and I.L. Brisbin, Jr.

Knowledge of mutation rates in biota can help determine the need to undertake complete site cleanup vs. site remediation vs. monitored natural attenuation

in the management of CERCLA sites. DNA markers are one way of determining mutation rates. Mutation rates in microsatellite DNA loci (repeating sections of DNA) are being used as a biomarker of contaminant exposure and transgenerational risk (i.e., mutations passed from parents to offspring).

During FY04, microsatellite DNA markers were developed and manuscripts were published for the southern dusky salamander, red-spotted newt, southern flying squirrel, wood stork, and cattail. DNA markers have also been identified and manuscripts are in press from three additional species, including the northern bobwhite quail, northern boreal owl, and gray fox. Seven of these species inhabit the SRS and may be receptors of contaminants or otherwise impacted by site activities. Cattails are part of an on-going study to investigate the genetic effects of the Chornobyl accident in Ukraine. Most genetic parameters investigated in the cattails were correlated with radionuclide contaminant levels or distance from the Chornobyl power plant. The DNA markers developed in the other species will serve as tools for future studies.

A manuscript comparing mutation rates among populations of American alligators is being revised. Data analysis for a similar study was recently completed by collaborators at the National Environmental and Engineering Laboratory using *Peromyscus* as the model. Neither study found support for increased mutation rates. Both of these studies indicate that contaminant levels at the DOE sites studied are below the threshold which would increase mutation rates at microsatellite DNA loci. It was determined that the manuscripts and resulting publications from these two studies would benefit from coordination. Thus, submission of the alligator manuscript will be delayed until FY 05.

We also began collecting samples for a molecular genetic assessment of the mutation rates between nesting female wood ducks (*Aix sponsa)* and their eggs/ducklings on the contaminated Par Pond CERCLA site of the SRS. A previous study showed evidence of either transgenerational mutational effects or the effects of nest parasitism in wood ducks nesting at the Par Pond CERCLA site, but these two possibilities could not be distinguished from one another without further analyses. To date, DNA samples have been collected from nesting female ducks, ducklings, membranes from additional hatched eggs (from which ducklings could not be obtained), and unhatched eggs. This material is being archived for later DNA extraction and amplification to estimate the *in situ* mutation rate in birds nesting in this contaminated habitat. The mating system of wood ducks on Par Pond will be investigated in FY05, prior to larger scale studies comparing mutation rates of multiple populations, because the mating system must be known before mutation rates can be estimated.

Although field surveys provide an important source of information about specific sites, basic research using manipulative studies provides powerful base-line information to better determine which field sites are likely to need further investigation. Samples are now in hand for a direct assessment of microsatellite mutation rates among offspring of medaka fish whose parents were exposed to varying levels of ^{137}Cs, to determine if transgenerational effects exist. All exposures and breeding took place at the Low Dose Rate Irradiation Facility and Par Pond research labs. Personnel have been recruited and are now being trained to assess microsatellite DNA loci for these samples. A highly skilled post-doctoral researcher has been hired to lead this project beginning in the second quarter of FY 05.

(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

Model organisms are often used in research to facilitate understanding. Fish are recognized as important comparative animal models for addressing questions related to a variety of important processes and diseases, including cancer, DNA repair, mutations, infectious diseases, developmental biology, endocrine disruption,

and genetics. One of the best-developed laboratory models for comparative carcinogenesis studies is the Japanese rice fish, medaka (*Oryzias latipes)*. In late March, 2004, SREL organized and hosted an international workshop that centered on using medaka as a model for coupling effects across levels of biological organization, human disease, ecological risk analyses, and transgenerational studies. The goal of the workshop was to form a consortium of scientists interested in multi-disciplinary, multi-institutional research relative to human and ecological risks using medaka as a model organism. The rationale is that by sharing a variety of resources, including new tools, procedures, strains, facilities, or samples from collaborative projects, as well as, examining multiple endpoints simultaneously, we will maximize efficiency and efficacy of hypothesis testing.

The workshop was held on The University of Georgia campus (24-27 March 2004) and was divided into the following sessions:

- Session 1 *Medaka Biology, Genomics, Mutants and Transgenics*
- Session 2 *Introduction to Approaches, Techniques and Tools*
- Session 3 *Examining Multiple Endpoints and Integrating Effects Across Levels of Biological Organizations*
- Session 4 *Medaka as a Model For Human Disease*
- Session 5 *Approaches and Challenges of Trans-generational Analyses*
- Session 6 *Path Forward*

An international consortium has formed among the workshop participants that will exchange ideas, facilitate collaboration, and prepare research proposals. A workshop report is being prepared.

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

Migratory species that frequent contaminated sites can be vectors of contaminant dispersal. Quantifying contaminant dispersal is important in assessing risks. A manuscript has been written describing the long-term/long-distance movements of 5,672 ring-necked ducks (*Aythya collaris*) that were captured and banded during their winter stay on the SRS's contaminated, Par Pond reservoir between 1985-1995 (in press, *Oriole*). This paper describes the recoveries of 592 of those banded ducks, most of which (>95%) were reported by hunters from throughout North America and Cuba. The majority of band recoveries were from areas near the SRS in South Carolina (37.8%) and Georgia (15.0%). Direct recoveries (i.e. those occurring within the same winter as the banding) comprised 17.6% of all band returns and represented those birds which would be the most likely to serve as potential vectors of SRS on-site contaminants to the food chain of the hunting public. Most of these direct recoveries were from South Carolina (79.8%) and Georgia (13.4%), with others coming from Florida (5.8%) and Alabama (1.0%).

Data on the long-term declines of radiocesium and other contaminants in these birds are needed to assess the degree to which such mobile migratory birds may pose a risk of conveying site contaminants to the food chain of the hunting public. The magnitude of such long-term declines in contaminant levels has been shown to be related to site management activities, and long-term baseline data are needed to evaluate such changes. Long-term efforts have been under way to collect periodic year-long samples of migratory waterfowl, particularly American coots (*Fulica Americana*), wintering on abandoned reactor cooling reservoirs on the SRS. Plans to collect an additional year's annual cycle of coots could not be undertaken this year because wintering coots failed to appear on SRS reservoirs in sufficient numbers to allow the collection of meaningful sample sizes.

(B) Risk associated with remediation

Investigator: L.A. Newman

We conducted a study to determine if phytoirrigation of trichloroethylene (TCE) would have a negative impact on two soil organisms, earthworms and the nematode *Caenorhabditis elegans*. We have completed this study and are in the process of internally reviewing a manuscript on the findings. We found that the levels of TCE typically applied to a phytoirrigation site did not adversely affect earthworm growth or outward physical form, and that there were no detectable levels of metabolites in the worms' bodies. For the nematode study, we used motility as the most sensitive indicator of toxicity to the TCE, and also looked at mortality. There was no change in motility at levels that would be seen at a field site; the EC_{50} for motility was not reached until pore water concentration of TCE reached 9.7 ppm, and differences in motility were not significant until concentrations reached 10.7 ppm. Even though the *C. elegans* were exposed to TCE concentrations of up to 10 ppm, no mortality was seen after continuous exposures of 24 hours. This project is funded by the National Institute for Environmental Health Sciences (grant P42 ES04696).

(C) Determining the most sensitive species to organic contaminants

Investigators: W. Hopkins and scientists from USGS-Patuxent Wildlife Center

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, we completed a study in FY04 that characterized aspects of the cytochrome P450 system in two species of oviparous snakes (family Colubridae) common to North America (*Elaphe gutatta*) and Africa (*Lamprophis fuliginosus*). We injected snakes with 45 mg/kg 3-methylcholanthrene (3-MC) and performed four resorufin generating *O*-dealkylase assays on hepatic microsomes; CYP1A induction was determined by protein separation and chromatography. Our data suggest that reptiles have many of the key components of the mammalian mixed function oxygenase (MFO) system, but that the quantity and activities of these components are reduced in reptiles compared to other vertebrates (e.g., mammals). However, our findings also suggest that detoxification capacity differs widely among reptiles with different evolutionary histories, indicating that future work on additional reptile species is needed before broad conclusions can be drawn about reptilian sensitivity to organic contaminants. We have completed a draft of the manuscript and it is currently undergoing internal peer review. We anticipate submission of the manuscript to a peer-reviewed journal in early FY05.

(D) Fish as vectors of contaminant dispersal

Investigators: D. Fletcher and collaborators from Clemson University, Savannah River National Laboratory, and WSRC-SGCP

Consumption of contaminated fish by humans or other animals can represent an important biotic pathway for contaminant exposure. Stream fish can accumulate contaminants such as ^{137}Cs from their local environment and disperse them by migrating into new areas. This has the potential of being particularly problematic when fish migrate from a restricted access area, such as the SRS, into publicly accessible waters. Though state and federal regulatory agencies (SCDHEC, EPA) have voiced concerns about the potential of contaminant dispersal from the SRS by migrating fish, little information is available about fish migrations on or around the SRS. To address these concerns, a radiotelemetry study of largemouth bass movements was conducted in collaboration with investigators from Clemson University, Savannah River National Laboratory, and WSRC-SGCP. In FY04 data collection for this 3.5-year study was completed and the data were analyzed. A total of 51 bass tagged in the Steel Creek corridor and 98 tagged in the lower reaches of Steel Creek and the Savannah River were relocated 834 and 1460 times,

respectively.

We examined the effects of season, dissolved oxygen, water temperature, and river discharge on migratory patterns. In general, the likelihood of anglers catching contaminated largemouth bass originating from Steel Creek was much higher near (within 10 km of) Steel Creek, and the likelihood of catching contaminated largemouth bass at greater distances from Steel Creek was greatest during the March through May largemouth bass spawning period.

Escape of fish from Par Pond and L Lake was examined to determine the risk of fish from contaminated SRS reservoirs reaching public waters. Spring counts were higher, with as many as 100 fish per week passing through the Par Pond dam in May 2003. The numbers and species composition of fish that escaped from the two lakes differed. Preliminary analysis suggests that escape from Par Pond is strongly influenced by the location and arrangement of the outlet structure. It appears that fish passage is most prevalent when water is passing over the overflow outlet so that littoral species are washed over the top of the outlet duct rather being sucked through the deep-water gate of the outlet structure. This finding is critical, should development of mitigation strategies be required. A manuscript on bass as vectors of ^{137}Cs dispersal has been submitted to the *Journal of Environmental Radioactivity*.

(E) Endangered species using contaminated sites

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

Contaminated sites on federal lands may pose risks to wildlife inhabiting or foraging in these areas. Wildlife may include endangered species, such as bald eagles. Eagles have nested on the SRS since 1959 and they forage in SRS reservoirs for items to feed their young. These activities raise concerns about exposure to legacy contaminants, including heavy metals and radionuclides. We observed foraging behavior of nesting eagles and collected data on the composition of their diet. We also sampled potential dietary items (fish and waterfowl) from SRS reservoirs, and have completed analyses for a suite of contaminants including radiocesium, Hg, Pb, Cd, As, and Se. Our results show that eagles nesting on the SRS feed primarily on fish, although waterfowl can be a significant dietary component (about 25% of total diet) in some years, depending on availability. Mercury is the major contaminant of concern in SRS reservoirs, and mercury concentrations in prey-size fish usually exceed U.S. Fish and Wildlife Service guidelines for sensitive avian species. Mercury concentrations in waterfowl are typically much lower than those in fish. Consequently, exposure and risks to young eagles due to dietary mercury are lower in years when waterfowl are available as a prey item, and can partially replace fish in the diet. However, predation on waterfowl may have other undesirable effects, including increased risk of Avian Vacuolar Myelinopathy (AVM), a disease that may be associated with consumption of waterfowl by eagles. A manuscript about eagle foraging behavior and prey selection on the SRS has been submitted to *The Southeastern Naturalist*. We have also developed a statistical model based on a nonparametric bootstrap technique, and will now apply this to estimate likely contaminant exposure based on our diet observations and measurements of prey contaminant level.

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 and scientists from USGS-Columbia Environmental Research Center

Because organisms are exposed to contaminants under a wide array of conditions, and the outcome of complex interactions between biotic and abiotic variables is sometimes unpredictable, studies examining such interactions will help to remove some uncertainties from the risk assessment process.

In FY04 we completed two studies examining the interactive effects of biotic and abiotic factors. In the first study, we examined the interactions between stress and infectious disease in amphibians. Two experiments examined the effects of environmental stressors on amphibians and the pathogenicity of a naturally-occurring fungus (*Saprolegnia ferax*). In the laboratory, spotted salamander (*Ambystoma maculatum*) larvae were exposed to the pesticide carbaryl, UV-B radiation, and the pathogenic fungus *Saprolegnia* and the time to mortality was recorded over seven days. In outdoor mesocosms, we exposed southern leopard frog (*Rana sphenocephala*) tadpoles and spotted salamander larvae to carbaryl and fungus and recorded survival, mass at metamorphosis, length of the larval period and, for metamorphosed frogs, percent lipid content. In the laboratory, salamander larvae exposed to all three stressors died more quickly than larvae in other treatments. In the mesocosms, frogs and salamanders responded differently to the stressors. Carbaryl and fungus independently reduced the survival of both amphibian species, but tadpoles exposed to both stressors had lower survival than those exposed to each stressor individually. Exposure to carbaryl alone increased tadpole mass at metamorphosis, but the interaction between carbaryl and fungus decreased mass. This study suggests that exposure to multiple stressors may make amphibian larvae more susceptible to disease, and illustrates the importance of examining multiple factors (both natural and anthropogenic) simultaneously when attempting to determine causes for amphibian declines. We have completed a manuscript that is under internal review and will be submitted to *Conservation Biology* in early FY05.

In a second study, we examined the effects of an insecticide (carbaryl) on two species of *Ambystoma* salamanders experiencing the natural stress of competition. In outdoor mesocosms we manipulated larval density (12 or 36 larvae/mesocosm) and chemical exposure (0, 3.5, or 7.0 mg/L carbaryl). Both species were included in each mesocosm. We determined the effect of treatments on snout-vent length (SVL) of metamorphs, growth rate, lipid reserves, time to metamorphosis, percent survival, and percent metamorphosis. Carbaryl negatively affected all response variables significantly, and the intensity of this effect on SVL, lipid reserves, and growth rate was influenced by density. Additionally, *A. maculatum* and *A. opacum* responded differently to the treatments, with *A. opacum* generally being more sensitive. The effects of carbaryl and increased density on percent metamorphosis were nearly additive, but were generally less than additive on the other variables. Because of the short half-life of carbaryl, 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 study demonstrates the importance of the interactive effects that chemical contamination and natural environmental factors have on salamander assemblages. We have submitted a manuscript for review to *Freshwater Biology.*

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

Uncertainties associated with the effects from chronic low-level exposures to radiation prompted us to construct a Low Dose Rate Irradiation Facility (LoDIF). This unique facility was designed specifically to test the appropriateness of the 10 mGy d^{-1} guideline often espoused as acceptable for protection of aquatic biota from ionizing radiation. We know of no other facility comparable to the LoDIF for conducting studies on chronic exposures to low dose rates. Scientists at the facility use 40 outdoor mesocosms and ^{137}Cs irradiators of three different source strengths to research the effects of chronic low-level irradiation at different levels of biological organization. A manuscript was recently published in *Journal of Environmental Radiation* that describes the facility and the results of a pilot study in which Japanese medaka were chronically irradiated at

the highest dose rate possible within the facility (350 ± 150 mGy d^{-1}). Irradiated fish produced fewer eggs per day (p=0.03), had a lower percentage of viable eggs (p=0.04), and produced a lower percentage of hatchlings (p=0.05). Although these data are not surprising based on the relatively high dose rates, they are important to future work at the LoDIF because they confirm the utility of our chosen model organism for detecting population-level responses, and they illustrate the statistical power achieved from using replicated mesocosms.

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 environmental pollutants causes stress in animals. This stress is manifested in increased metabolic costs, because energy is used to metabolize, sequester, or excrete contaminants, to repair contaminant-induced damage, and to maintain homeostasis. Since more energy is used for these maintenance functions, less is available for growth and reproduction. This can lead to declines in populations in contaminated areas. Conventional biomarkers like induction of metallothionein or changes in glutathione levels indicate exposure to various pollutants, but better measures of increased energetic costs of pollutant exposure are needed. Our preliminary studies have shown changes in ratios of stable nitrogen isotopes in tissues of fish and birds exposed to toxic metals (e.g. Hg). These changes in nitrogen isotope ratios indicate that rates of protein turnover increase with exposure to these pollutants. The increased protein turnover is probably due to processes like increased synthesis of defensive proteins such as metallothionein, and increased repair of damage at the cellular level. An absence of significant mercury-associated changes in metallothionein and glutathione suggests that ä^{15}N shifts may be an initial response to sub-lethal mercury exposure that escapes detection by conventional biochemical assays. However, the exact causes and implications of changes in protein synthesis and degradation with pollutant stress are still unclear.

In the past year, we published a manuscript describing a new method to measure metallothionein in tissues in (SREL reprint 2696). We also submitted a manuscript on changes in nitrogen isotope ratio with mercury exposure to *Environmental Toxicology and Chemistry*.

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 FY04, we began to examine maternal transfer of trace elements (As, Cd, Cu, Cr, Hg, Ni, Se, V, Sr, or Zn) in narrow mouth toads (*Gastrophryne carolinensis*). After collecting adults near a coal burning power plant, toads were bred under captive conditions and eggs reared for 96 hours in uncontaminated water. In the 58 egg clutches examined to date, hatching success was 82 vs. 93% in the contaminated and reference site, respectively. Of the

larvae that hatched, the frequency of developmental abnormalities was 17 vs. 11% and abnormal swimming frequency was 19 vs. 12% in the contaminated and reference site, respectively. Axial malformations (shortening, scoliosis, lordosis) were the most prevalent abnormalities observed. Toads (and their eggs) utilizing the industrial area are currently being analyzed to determine whether they transfer significant quantities of contaminants to their eggs. Our study will help to determine if maternal transfer is an important route of contaminant exposure in amphibians and whether it warrants future study.

In FY04 we were successful at acquiring additional funding to support this work; grants were received from The University of Georgia (Young Faculty Award for WH) and the Eppley Foundation for Scientific Research. Abstracts have been accepted and results of this study will be presented at the upcoming annual meeting of the Ecological Society of America and the World Congress of the Society of Environmental Toxicology and Chemistry. We intend to write and submit a manuscript to a peer-reviewed journal in FY05.

(B) Indirect effects of contaminants

Investigators: W. Hopkins, D. Scott, S. Harper, and B. Jackson

As scheduled, research will commence in FY05.

(C) Use of stable isotopes to elucidate exposure pathways

Investigators: C. Romanek, C.H. Jagoe, I.L. Brisbin, Jr., W. Hopkins, and B. Jackson

Biomagnification of selenium (Se) in aquatic ecosystems could potentially increase the risk of Se toxicity in higher trophic level organisms relative to organisms occupying lower trophic positions; however, studies addressing the question of whether Se biomagnifies have produced conflicting results. In many previous studies trophic structure has been poorly defined, potentially confounding assessments of biomagnification. Furthermore, differences in carbon source for primary consumers (e.g. algal vs. bacterial, terrestrial vs. aquatic) are rarely considered, but could influence Se bioaccumulation patterns. In FY04, nine species of aquatic organisms (including snails, clams, odonate larvae, fish, and amphibian larvae) were collected from a swamp contaminated by trace elements from coal combustion waste. Stable isotopes of nitrogen ($ä^{15}N$) and carbon ($ä^{13}C$) were used to characterize trophic positions and carbon sources within the community. Significant differences in $ä^{15}N$, $ä^{13}C$, and total Se were found among species. Based upon an increase in $ä^{15}N$ of approximately 2-3‰ through each trophic level, our collection of species represented 3-4 trophic levels. Mean $ä^{13}C$ ranged from -27.5 to -20.0 among species, suggesting that primary consumers have multiple carbon sources in this contaminated swamp. Mean Se concentrations ranged from 6.60 to 24.50 mg/g dry mass among species. Through multiple regression analysis we found no relationship between Se body concentration and $ä^{15}N$; however, there was a statistically significant negative relationship between $ä^{13}C$ and Se concentration. To our knowledge, this is the first study examining Se biomagnification using the stable isotope approach and suggests that while trophic level does not influence Se concentrations, Se accumulation may be related to differences in carbon source. Results of this study will be presented at the upcoming World Congress of the Society of Environmental Toxicology and Chemistry. We intend to submit a manuscript to a peer reviewed journal in FY05.

In addition to the D-Area swamp work with Se, stable isotope analyses have been conducted on the SRS at Tims Branch (U, Ni), and Pond B (^{137}Cs) to better understand the flow of contaminants through these impacted ecosystems. In Tims Branch, a variety of organisms have been analyzed for metals and stable carbon and nitrogen isotopes, including: (1) mammals (various rodents, raccoons, foxes, feral hogs), (2) amphibians (various frogs and salamanders), (3) various plants, (4) snakes, (5) arthropods (various insects, crayfish), (6) clams, (7) snails, (8) fish, and (9) annelids. In Pond B, the following organisms have been analyzed for ^{137}Cs and stable carbon and nitrogen isotopes: (1) frogs, (2) various aquatic plants, and (3)

fish (bass, mosquito fish, sunfish). The trophic structure and food webs of these ecosystems will be determined from the stable isotope composition of these various ecosystem components when sample collection and analysis is complete. The resulting structure will be compared to contaminant distribution patterns to understand how various pollutants move through these ecosystems. A model will be developed that demonstrates the pathways and linkages between various ecosystem compartments. These relationships will then be used to construct a model that predicts contaminant transport through ecosystems in more general terms.

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

It has been established that metal exposure can indirectly select for antibiotic-resistant bacteria through common genetic linkages between resistances to metals and antibiotics. We seek to determine how common these linkages are and if common activities at DOE sites promote generating, maintaining, or spreading antibiotic resistance within and among bacterial communities. Major accomplishments of FY04 include:

- Although field studies show strong correlations, they do not provide sufficient evidence to show that elevated heavy metal concentrations cause increases in antibiotic resistance. Thus, manipulative laboratory studies of river waters were initiated to determine if heavy metals could cause increases in antibiotic resistance of bacterio-plankton. Savannah River water microcosms were amended with various types and concentrations of individual metals (Cd and Ni at 0.1 and 1 ì mol L^{-1}) and antibiotics (ampicillin and tetracycline). During a seven-day incubation, metal and antibiotic amendments selected for bacteria resistant to multiple, unrelated toxicants, providing direct evidence that metal and antibiotic resistances can be co-selected, and that metal exposure can indirectly select for bacteria resistant to multiple antibiotics. Specifically, Cd exposure resulted in nearly 100% of the isolates being resistant to metals and/or antibiotics. Bacteria exposed to Cd had high levels of gentamycin and ampicillin resistance and multiple metal resistances. Similar patterns were observed in microcosms with Ni additions. These data are the strongest evidence ever provided to show that heavy metal contamination can increase antibiotic resistance in native stream bacteria.
- A study of antibiotic and heavy metal tolerance of bacterioplankton in river waters was undertaken. Tolerance of the entire bacterioplankton community was assessed using flow cytometry. We found that the frequency of antibiotic-tolerant bacterio-plankton increased during river water passage through ash settling basins of coal-fired power plants, which were contaminated with toxic metals but not antibiotics. Anthropogenic metal emissions greatly exceed emissions of antibiotics and may, therefore, contribute to the maintenance and spread of antibiotic resistance among environmental bacteria, including human pathogens. This research was presented at the 104th general meeting of the American Society for Microbiology. A manuscript is in preparation.

(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 FY04 include:

- We have conducted a series of preliminary experiments to increase our expertise in bacterial matings. We have successfully isolated bacterial

plasmids from both environmental DNA and from DNA extracted from *Aeromonas* isolates collected from five fish species in three stream systems on the SRS. *Aeromonas* are opportunistic human pathogens. We have begun experiments to determine if antibiotic resistance genes from stream bacteria can be laterally transferred to *Aeromonas* isolates. From anecdotal observations by doctors at the Medical College of Georgia infectious disease unit, it appears that there are a number of incidents of fisherman that have become infected with multiple antibiotic resistant *Aeromonas* while handling fish taken from the Savannah River. Our data will confirm whether the genes conferring resistance to the environmental bacteria are the same as those found in the infectious disease unit.

- 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 character-ization 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, C.H. Jagoe, and M.H. Smith

When attempting to draw inferences about the average dose or exposure in a population of non-human biota, investigators often use traditional statistical methods that require the assumption that the data are normally distributed. However, traditional statistical distributions are often inadequate in describing the distributional behavior of dose estimates, exposure, or body burdens in natural populations. Non-traditional, nonparametric statistical techniques can provide higher confidence and power when conducting hypothesis testing, attempting to quantify uncertainty based on experimental results, and estimating total dose or exposure to human and non-human individuals. Bootstrap methods rely on resampling techniques to use the information in the data to estimate the mean, variance, and the quantiles of the underlying distribution. Hence, the bootstrap is an excellent candidate for increasing power and confidence when conducting hypothesis testing on data where distributional assumptions are questionable. Thus, we have been developing the necessary computational techniques for conducting bootstrap hypothesis testing and power analysis about the mean total dose, exposure, or body burden estimates of data sets found at the SRS and other sites. More specifically:

- We have written code for hypothesis testing about the mean dose and power analysis of the hypothesis test in *Splus*. We tested the *Splus* code for both hypothesis testing and power analysis on a data set from communities near Sellafield Nuclear Power Plant in the UK, and submitted manuscripts to *Science of the Total Environment* and *Journal of Radiological Protection*. The papers illustrate the use of the bootstrap hypothesis test and power analysis methods on an otherwise difficult data set and reveal how traditional methods can greatly under-estimate the variance, resulting in false inference. Bootstrap allows estimation of the total variance and hence better inference.
- We obtained data sets of radiocesium body burdens for deer, raccoon, bass, and blue gill from the SRS, and for soil, grass, ants, and toads from the Chornobyl Exclusion Zone. Preliminary analysis of the SRS and Chornobyl data sets has begun.
- Data sets of heavy metals from the SRS have been obtained and exploratory data analysis has begun.

(B) Bayesian methods

Investigators: M.D. Wilson, T.G. Hinton, C.H. Jagoe, and M.H. Smith

Bayesian methods are another non-traditional statistical approach with desirable characteristics for dose and exposure assessments. Bayesian techniques allow for the inclusion of expert knowledge into the analysis of data. The investigator can specify a prior distribution of the parameter of interest, and combine this 'expert knowledge' into the analysis. These techniques often require intensive computational effort, but are well developed in the statistical community; although not well known in other disciplines. Therefore, we are developing the necessary computational techniques for carrying out Bayesian hypothesis testing about the mean total dose or exposure estimates of data sets found at the SRS and other sites. Our efforts will help provide code that will expedite the use of these methods by non-statisticians. The software package *WinBUGS* has been downloaded, installed, and tested. Exploratory analyses using the data set described in Q9A has begun.

(C) Mixture models

Investigators: M.D. Wilson, T.G. Hinton, C.H. Jagoe, and M.H. Smith

Mixture models provide a third approach for assessing dose and exposure. Mixture models are a powerful tool because they allow the identification of a distribution for the data that is a mixture of distributions. If the distribution of the data can be correctly identified, then increased power is achieved over non-parametric approaches. For example, body burdens of a toxic substance are thought to be mostly lognormal at low trophic levels, but tend to become more and more normal at higher trophic levels. For this reason, mixture models at intermediate trophic levels could provide the best model for the distribution of body burdens. Therefore, we are developing the necessary computational techniques for identifying a mixture model to describe distributions of dose and exposure samples, to test the hypothesis that heretofore unidentifiable distributions may be the result of mixtures of distributions, e.g., normal and lognormal. Exploratory analyses using the data set described in Q9A have begun using *Splus* and *Matlab*.

··· 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 FY04.

Report on FY04 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

SREL researchers collaborated with Clark Atlanta and Cornell Universities, and the USFS-Savannah River in the development and refinement of one- and two-dimensional vadose zone solute transport/hydrological cycling models using tritium migration data collected from within the SRS-Mixed Waste Management Facility (MWMF) irrigation site. Tritium movement was monitored through the soil, the vegetation canopy, and the indigenous wildlife (i.e., rodents) in response to various irrigation management scenarios and environmental conditions. The transport model was then used in predicting the efficiency of ongoing remediation efforts under a range of long-term management scenarios that could not be directly evaluated in the field. These efforts have resulted in the recent submission of six manuscripts to peer reviewed journals and/or proceedings, two of which have already been published, and have assisted DOE and the SRS site contractors in their annual reporting to state and federal regulatory agencies. In FY04, SREL drafted two reports to WSRC summarizing an independent evaluation of the efficacy of the current tritium remediation efforts.

In a companion laboratory study, results of which were published in FY04, unsaturated flow apparatus (UFA)-based solute transport experiments using tritium as a conservative tracer and Cr(VI) as a model contaminant indicated that hydrodynamic dispersion, the physical spreading of the plume as it migrates through the soil, increased with decreasing soil water content. Additional laboratory studies utilizing the UFA system to conduct solute transport conditions under variably saturated conditions are ongoing.

Rodent trapping data from the vadose tracer site confirmed that the animals pick up detectable levels of tritium, on average about 1,100 pCi/mL body fluid, still orders of magnitude less than reported toxic concentrations, with concentrations varying in response to the tritium application rate and recent precipitation events. In a companion experiment lab mice were exposed to 8,000 pCi/mL for two weeks before the source was removed. The animals were then sacrificed at several time intervals to estimate the biological half-life of tritium. The tritium half life was found to be about 2.2 days, indicating that body fluid levels would decrease by one half in the first two days after removal

of the source, regardless of the initial source concentration. The half life is considerably longer than previous rodent studies that focused on exposure routes other than oral uptake. Oxidative stress enzyme assays were used to evaluate the effects of tritium exposure at the cellular level, with no statistical difference observed between the exposed and non-exposed (control) mice, even though the lab dose was substantially higher than that of the captured mice from the tritium irrigation site. The results were summarized in two SREL reports to WSRC and the MS thesis of an SREL graduate student

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.

Testing and refinement of the current Automated Vadose Monitoring (AVM) system developed through a collaboration between SREL, Clark Atlanta University, and Dynamax Inc. continued throughout FY04 as part of the ongoing vadose tracer experiments being conducted at the SRS-Mixed Waste Management Facility (MWMF). Difficulties in maintaining the tension necessary for pore-water sampling plagued the prototype system. The formal application for patent protection for the AVM system was withheld pending further design changes in the current pumping scheme that should improve pore water sampling efficiency and reduce the unit cost. Such modifications will be completed during FY05. Additional soil moisture and matrix potential sensors were installed to evaluate their compatibility with the current system. Installation of the radiotelemetry control system was delayed pending FCC approval of a dedicated frequency for instrument control and data transfer on the SRS. SREL's Information Management Specialist was instrumental in receiving FCC approval, which was finally awarded at the end of May 04. Delivery of the remote control and data acquisition equipment is pending. As part of this ongoing effort, the automated monitoring system was modified for use in stream monitoring on Tims Branch and Fourmile Branch to improve our understanding of contaminant levels and sediment loading in riparian systems, which is critical in defining contaminant inventories and export rates. Development of the prototype stream monitoring system is ongoing.

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

In situ redox manipulation (ISRM) studies have been expanded in collaboration with A. Neal to include bioremediation. Current experiments are evaluating the impact of biostimulation and bioaugmentation efforts to induce contaminant metal reduction and/or reductive solvent dechlorination on formation integrity using dynamic column techniques that are more analogous to field conditions experienced during active remediation than more widely used laboratory experimental techniques. At present, two peer-reviewed manuscripts pertaining to *in situ* reduction using dithionite were submitted for publication, with a third to follow shortly in FY05. A proposal entitled 'Stimulating Contaminant Metal Reduction in the Presence of Chlorinated Solvents' by Seaman, Neal, Brigmon (SRTC), Mills, Wilson, and Hesterberg (NC State) was submitted to the Biogeochemistry Program Element of NABIR in response to DOE Office of Science Notice DE-FG01-04ER04-06 to further expand these research efforts. A second proposal entitled 'Enhancing Passive Remediation of Chlorinated Solvents: Transport of Dehalococcoides in SRS Aquifer Systems' was submitted by Seaman, Neal, Mills, and Brigmon (SRTC) in response to the SRS request for Innovative Technologies to Support Passive and Natural Remediation (Need/Opportunity # SR02-3028).

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 and collaborators at the Savannah River National Laboratory

Radionuclide releases into the aquatic systems of the SRS occurred during the early years of site operation. The releases contaminated numerous aquatic systems on the SRS, such as Par Pond, Ponds A, 2, 4, 5, and B, several creeks (e.g. Steel, Lower Three Runs, and Pen Branch), as well as many kilometers of man-made canals that connected the reactors to their cooling ponds. Combined, these aquatic systems represent over 3,000 acres of wetlands that became contaminated with over 500 Ci of ^{137}Cs.

The problems of contaminated aquatic systems and their associated risks are heightened on the SRS because ^{137}Cs is more biologically available here than at any other DOC facility. The enhanced bioavailability of Cs on the SRS is due, in part, to its low adsorptive capability by our sandy soils. We hypothesized that adding naturally occurring clay minerals, with a greater Cs binding capacity, to the contaminated wetlands would sequester ^{137}Cs and reduce its bioavailability. In application, the mineral is broadcast over the surface of the water, where it quickly scavenges ^{137}Cs from the water column as it settles to the bottom sediments. The clay amendments then continue to intercept and adsorb ^{137}Cs from the contaminated sediments, effectively sequestering the contaminant and drastically reducing biological uptake while radioactive decay slowly reduces contaminant concentrations.

In FY04, we published a report to WSRC documenting the *in situ* technique as a promising remediation tool, and a preferred alternative to the extremely destructive and expensive traditional method of "muck and truck." A draft manuscript is being reviewed in-house for submission to *Environmental Science and Technology*. We also have two other manuscripts in press in *Journal of Radioanalytical and Nuclear Chemistry* that address radioactive Cs contamination on the SRS. We also had manuscripts accepted relative to: (1) the phytoremediation of U and Th contaminated wetlands by native trees, (2) the use of sequestering agents to immobilize metals and radionuclides, and (3) the use of mulch to reduce crop contamination caused from the resuspension of contaminated soil. In the phytoremediation study, trees were not effective at removing U and Th within a reasonable period, largely because the contaminants have a low propensity to be taken up by plants. Finally, in FY04, our collective radioecology and remediation experiences, gained from many years of research on the SRS, resulted in a paper in *Science* (SREL reprint 2728), concerned with avoiding destructive remediation at DOE sites.

(B) Testing immobilization capacity of soil amendments for use in phytoextraction

Investigators: D.C. Adriano and collaborators from Universitaat fur Bodenkultur, Vienna, Austria

A companion proof-of-concept study resulted in the issuance of an Austrian patent A1525/2000 by the Austrian Patent and Trademark Office in 2002 and US patent number 6,719,822 issued by the US Patent and Trademark Office in 2004. The invention deals with the integration of two cleanup methods: phytoextraction and chemical immobilization of hazardous chemicals, primarily metals, in contaminated media (soils, sediments, biosolids, etc). Phytoextraction will accumulate the contaminants in plant tissues such as stem and leaf after root uptake; the fallen leaves and twigs on the ground will decompose, releasing the contaminants that can be leached back to the contaminated media. Selected soil amendments will then adsorb and immobilize the contaminants *in situ*. This process can last over several growth cycles of the phytoextracting plant until the immobilization capacity of the soil amendment is exhausted. The process eliminates the need to harvest the plant in conventional phytoextraction and the issue of disposing the large contaminated biomass. The present study evaluated the

immobilization capacity of several soil amendments including clay minerals, alkaline by-products, etc.

(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

Oxidation of pyrites in coal reject and fly ash materials can elevate the acidity concomitant with the generation of high dissolved solids (TDS) such as the sulfates, chlorides, metals, etc. This creates an unfavorable condition in the disposal medium such as a landfill or mining spoil, rendering it inhospitable to grow plants. Certain soil amendments can ameliorate this unfavorable growth environment by providing buffering capacity and minimizing TDS generation. An article on using soil amendments to promote vegetation establishment and control acidity on coal combustion wastes was published as part of a proceedings on "Chemistry of trace elements in fly ash" (SREL reprint 2750).

(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 that Ni is toxic to a constitutive TCE degrader, *B. cepacia* $PR1_{301}$ at concentrations found in Steed Pond, a U and Ni contaminated wetland on the SRS, where TCE contaminated groundwater is outcropping. We have also been investigating the ability of hydroxylapatite (HA) to reduce the solubility of Ni and U in soil pore waters and hypothesize that this will manifest in decreased toxicity. The initial experiments with HA added at 0.01 g mL^{-1} demonstrate that HA is able to ameliorate the growth inhibitory effects of Ni and U to PR1 in liquid culture. PR1 growth was inhibited above 17 mM Ni in the absence of HA and above 34 mM Ni with HA at pH 5, whereas at pH 7, growth was inhibited above 0.85 mM Ni without HA and above 1.7 mM Ni with HA. PR1 growth was inhibited above 4.26 mM U at pH 5 and 7 without HA and above 17 mM at pH 5 and 7 with HA. Aqueous phase dissolved Ni and U concentrations were operationally defined as the amount able to pass through a 0.2 mm filter. Aqueous phase Ni concentrations decreased to 2.1mM Ni with HA (a decrease of 21.1% as compared to samples in the absence of HA) and 1.78 mM Ni (32.3% decrease) at pH 5 and 7, respectively. Aqueous phase U concentrations decreased over time in the presence of PR1 while remaining stable in the absence of PR1, suggesting U sorption to PR1 cells. Aqueous phase U concentrations without HA averaged 0.69 and 0.024 mM U (65.6 and 2.3% of the U initially added) at pH 5 and 7, respectively. After 12 h of growth, U concentrations decreased to 0.076 and <0.0001 mM U (11 and 0% of the starting U concentration) at pH 5 and 7, respectively.

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

We have inserted cDNA libraries from both tobacco and *Arabidopsis* into *E. coli* expression systems, and are screening them for activity against ethylene dibromide (EDB). This compound is dehalogenated and processed in mammals by the same enzyme as trichloroethylene (TCE), with the added advantage that we can screen for increases in bromide ion concentration in the reaction mix. We have developed a new protocol for screening the cells that decreases the response time, as well as increasing sensitivity. We have also done a BLAST search of the DNA sequence for *Arabidopsis* against the substrate-binding domain for the human enzyme involved in TCE degradation, and have found several enzymes with similar domains. We are working to find more information about these enzymes. We are in the process of inserting both of the libraries into yeast expression systems, and will be using that as a supplement to the *E. coli* systems. This project

is funded by the National Institute for Environmental Health Sciences (grant P42 ES04696).

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. In FY04, we planned to characterize 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. However, the root zone community is different spatially at different locations. These results suggest that microbial communities in the rhizosphere and the bulk soil are similarly controlled by spatial heterogeneity that may be related to soil texture or hydrology. Results were presented at the American Society for Microbiology Annual Meeting, May 2004. Experiments are being conducted to relate metal concentration and soil mineralogy to microbial diversity at each sample location.

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

(A) Use of native plant species in the phytoremediation of arsenic and selenium
Investigator: K.W. McLeod

Plants naturally inhabit stressful environments. Traits that allow them to prosper in these habitats might also permit them to survive and potentially sequester contaminants in anthropogenically contaminated habitats. For example, baldcypress is very successful in persisting and even flourishing in some very stressful habitats. Physical stresses include flooding, while success in chemically stressful habitats includes fly ash basins and contaminated seeplines. Low contaminant uptake rates may be at least partially responsible for the success of baldcypress, as water tupelo takes up much higher concentrations of heavy metals when growing in the same environments. Thus, while one species is successful in stressful habitats, it is due to a characteristic that makes it less suitable for phytoremediation. Contaminant uptake rate is one of the key factors to consider when potentially choosing species for phytoremediation.

In D-Area, fly ash has extensively contaminated a bottomland forest. Five tree and four herbaceous species are growing in up to 2 meters of pure fly ash. Regardless, none of these plant species accumulated large concentrations of As or Se, the primary contaminants associated with fly ash. Herbaceous species included murdannia (*Aneilema keisak*), sensitive fern (*Onoclea sensibilis*), clearweed (*Pilea pumila*), and lizard's tail (*Saururus cernuus*). Tree species included red maple (*Acer rubrum*), ash (*Fraxinus* sp.), sycamore (*Platanus occidentalis*),

sweetgum (*Liquidambar styraciflua*), and baldcypress (*Taxodium distichum*). In this particular instance, presence in a stressful habitat has not translated into usefulness for phytoremediation.

(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 studies looking at a 'native' poplar, along with willow, sweet gum, sycamore, and commercial tobacco and compared the uptake and degradation rates of trichloroethylene (TCE) by these species with rates characteristic of hybrid poplar. We compared physical parameters between control and exposed native plant species, looking at height, plant mass, and water uptake rates, and then compared these results with hybrid poplar. For metabolism, we looked at TCE accumulation within the plant, the formation of metabolites such as trichloroethanol and di- and trichloroacetic acid, and transpiration of TCE from the leaves. We have found that native plants are comparable in efficiency with hybrid poplars in the Southeast, with similar levels of water uptake and metabolism of TCE. We are currently conducting a study of evergreen plants, including two varieties of loblolly pine, long-leaf pine, and Leyland cypress, comparing these plants to a hybrid poplar grown under the same conditions. This project is funded by the National Institute for Environmental Health Sciences (grant P42 ES04696).

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

We have completed a study examining the tolerance of varying cultivars and clonal lines of poplar to determine which lines have the best potential for phytoremediation sites in the southeastern U.S. Growth and viability parameters of 31 clonal lines of poplar grown on 8 plots with two distinct soil types were compared. Fertilization and irrigation regimes were altered to give information on susceptibility to drought. Physical parameters examined included mortality, height, and diameter; for hardiness, we examined susceptibility to various fungal diseases and insect pests. We found three clones that stood out for growth, which were different from the clones that had the best survival rates. We are currently writing a manuscript that summarizes these results and makes recommendations on planting regimes that can give the best results overall, as well as offering various planting strategies that would allow use of the high biomass plants, even if they may not have the best survival rates. (This project is not funded by SREL core dollars).

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. In spring, 2004, we initiated a small-scale experiment to compare vegetation establishment treatments on new or unplanted closure caps. "Cap communities" were initiated by planting seeds of a common native grass (*Andropogon virginicus)*, a naturalized legume (*Lespedeza cuneata)*, and a turf grass (centipede) in a split-plot design in mesocosms constructed to mimic closure caps. We will compare plant community responses to different treatments that might affect establishment success (e.g., with or without mulch, fertilizer, and additional watering). In June, 2004, we began a field survey of vegetation on existing closure caps. We also located sites for a field experiment to compare invasion of undesirable woody species between native vegetation and closure cap communities under different mowing treatments.

(B) Succession in disturbed floodplain wetlands and sandhills

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

From 1990 to 1995, a dozen species planting experiments were initiated in the Fourmile Branch delta to investigate the best ways to reestablish vegetation in this post-thermally disturbed habitat. Many of these plantings had not been recently examined. During the past two years, all of the surviving trees have been relocated and measured, some with astounding results. In many cases, survivorship has changed very little over the past decade, but the trees have grown very rapidly, potentially affecting the future direction of forest succession.

Baldcypress has continued to flourish with little change in survivorship over the past 8-10 years, regardless of whether the trees were planted as bareroot seedlings (with or without root pruning) or larger balled-and-burlapped saplings. In many instances, baldcypress saplings are now over 8 meters tall. With abundant seed production, this species is poised to revegetate the rest of the Fourmile delta. Other successful species, including green ash, water tupelo, and overcup oak, have had very good survival and height growth in excess of 5 meters. These surviving and prospering individuals are now affecting the abiotic environment through shading. Subsequent forest succession will depend on establishment of seedlings from these individuals as well as additional species that successfully invade the altered delta habitat. Summary results have been presented at the annual meeting of the Society of Ecological Restoration International.

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. In FY04, 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. Additional surveys of TES populations, currently underway, will lead to development of recommendations for restoration and adaptive management of these sensitive habitats. During FY04, results were presented at the annual meetings of the International Association of Landscape Ecologists, the Association of Southeastern Biologists, the Natural Areas Association, and the Ecological Society of America. We intend to submit a manuscript to a peer-reviewed journal in FY05.

(C) Survey of plants colonizing coal ash basins

Investigators: L.A. Newman and faculty from the University of Kentucky

We have completed the field survey of the 488-D coal ash basin on the SRS. The survey included listing the plants that have colonized the basin, as well as their location and relative abundance. Metal concentrations in the basin and in the plant tissues have been analyzed. We are currently analyzing the data and preparing a manuscript that will look at these parameters and discuss ways that these findings can be used when planning to install a vegetative cap on sites like this.

(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 the Department of Energy-SRS 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. Annual monitoring of the vegetation and invertebrates is expected to continue for five years post-restoration as required to evaluate restoration success and for crediting the restored acreage to the mitigation bank.

In FY04 we were successful in acquiring additional funding to continue the vegetation research. A grant was received from the USDA Forest Service for phase two of this restoration project. Results were presented at the annual meetings of the Society of Wetland Scientists and the Geological Society of America. We published a manuscript in *Wetlands* describing potential losses of Carolina bays under current interpretation of the Clean Water Act and the need for protection and restoration actions. In addition, we submitted two manuscripts characterizing plant communities of reference depression wetlands to peer-reviewed journals (*Wetlands* and *Journal of the Torrey Botanical Society*). We intend to submit a manuscript on vegetation response to restoration treatments to a peer-reviewed journal in FY05.

In FY04 we were also successful in acquiring additional funding for studies on aquatic invertebrates. A grant was received from the USDA forest service to continue monitoring and to initiate studies of trophic structure using stable isotopes of C and N. Results were presented at the annual meeting of the North American Benthological Society. We published a study on fairy and clam shrimps (large zooplankton narrowly specialized to ephemeral habitats) in *Southeastern Naturalist*, a new regional journal and we revised a manuscript (*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 were invited by the editor of *Ecological Restoration* to submit a research report on responses of microcrustaceans to wetland pond restoration. We also published a paper that provides historical data on natural fluctuations of environmental conditions in these habitats.

··· 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 (SREL) continues to operate successfully under the work-smart safety and environmental standards that resulted from SREL's participation in U.S. Department of Energy's (DOE) Necessary and Sufficient process. These standards continue to address the hazards associated with SREL operations by permitting a focused effort on the health and safety issues most pertinent to SREL operations.

SREL maintains a commitment of 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 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 50 individuals. 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 groups in 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. SCDHEC conducted an inspection of SREL hazardous waste management areas in October, 2003 with no deficiencies identified. 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's Environmental Management System was assessed by DOE-SR in May 2004 with no deficiencies identified. 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 338 pounds of excess hazardous laboratory chemicals and laboratory hazardous wastes to a hazardous waste disposal firm (May 2004).

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. During the past year, the SREL Distance Learning Program continued to focus its efforts on programming related to SREL core programs in ecology and environmental science. The primary 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. Three students are continuing into the second year of coursework for the degree, four students have completed all required coursework and are working on the research component of the degree, and three students have graduated from the program.

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 10 lectures on various ecological topics to students in 36 South Carolina and Georgia K-12 schools.

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 FY04 and the Central Archive Data Repository now has information covering over 496 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 FY04. 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

J Vaun McArthur

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 FY04 are listed in Table 1 (pages 55-56).

The SREL Education Program has averaged 20 undergraduate students per year since 1968. These students, from over 265 different colleges and universities, have been co-authors on 150+ peer reviewed research publications; more than 175 of these students have gone on to pursue careers in science. The Undergraduate-Research Experience for Undergraduates, funded by the National Science Foundation, sponsored 13 students this year. In addition, we sponsored one student funded by NOAA, two students funded by SREL, and two funded by DOE through South Carolina State University.

Since 1967, an average of six students a year have completed graduate studies at SREL and over 310 dissertations and theses have been written. During FY04 fifteen students completed their degree requirements (twelve M.S. and three Ph.D.). Since 1985, our graduate students have won over 190 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 6).

SREL Graduate Students Completing Degree Requirements:

Kimberly Andrews M.S. University of Georgia 2004
J. Whitfield Gibbons
Laura Burbage M.S. University of Georgia 2004
Rebecca R. Sharitz
Elizabeth Burgess M.S. University of Georgia 2003
J Vaun McArthur
Erin Clark M.S. University of Georgia 2003
J. Whitfield Gibbons
James Cumbee M.S. University of Georgia 2003
I. Lehr Brisbin
Luke Fedewa M.S. University of Georgia 2003
J. Whitfield Gibbons
Xavier Glaudas M.S. University of Georgia 2003
J. Whitfield Gibbons
Virginia Jin Ph.D. University of Georgia 2004
Rebecca R. Sharitz
Audrey Majeske M.S. University of Georgia 2003
Charles H, Jagoe
Liberty Moore M.S. University of Georgia 2004
I. Lehr Brisbin
John Mulhouse M.S. University of Georgia 2004
Rebecca R. Sharitz
James Novak Ph.D. University of Georgia 2003
Michael H. Smith
Elizabeth Richardson M.S. University of Georgia 2004 J Vaun McArthur
Jason Unrine Ph.D. University of Georgia 2004
Charles H. Jagoe
Lucas Wilkinson M.S. University of Georgia 2003
J. Whitfield Gibbons

Table 1A. SREL Undergraduate Student Program Participants

Student	Academic Institution	Faculty Advisor
Keith Thomas Beckman	Pensacola Christian College, FL	Beverly Collins
Lauren Melissa Booth	University of South Carolina, Aiken	Andrew Neal
Christy Amber Bowersox	Lycoming College, PA	Steven Harper
Robert Rashad Brightharp	Clemson University, SC	John Seaman
Bonnie Louise Coggins	University of South Carolina, Columbia	Machelle Wilson
Kathryn Anne Copenhaver	Coastal Carolina University, SC	Travis Glenn
Aaliyah Desirae Green	Rutgers University, NJ	J. Whitfield Gibbons
Booker Talzaferro Harrison	Savannah State University, GA	Charles Jagoe
Kate Louise Hertweck	Western Kentucky University	Travis Glenn
Rhesa Nichole Ledbetter	Idaho State University	Andrew Neal
Thomas Marshall Luhring	University of Georgia	J. Whitfield Gibbons
Giovanna McClenachan	Dickinson College, PA	Steven Harper
Abel Munoz	East Texas Baptist University	I Lehr Brisbin
Teri Elizabeth Taylor	College of Charleston, SC	Kenneth McLeod
Mary Scarlett Tudor	Ohio University	William Hopkins
Justin Mychael Nelson	South Carolina State University	Gary Mills
Coby Rashard Williams	South Carolina State University	Gary Mills
Angel Marcella Washington	South Carolina State University	Charles Jagoe

Table 1B. SREL Graduate Student Program Participants

Student	Degree	Institution	Faculty Advisor
Kimberly Andrews	M.S./Ph.D.	University of Georgia, Athens	J. Whitfield Gibbons
*Matthew Atkinson	M.S.	University of Georgia, Athens	Machelle Wilson
Laura Burbage	M.S.	University of Georgia, Athens	Rebecca Sharitz
Elizabeth Burgess	M.S./Ph.D.	University of Georgia, Athens	J Vaun McArthur/Andrew Neal
Monica Carroll	Ph.D.	University of Georgia, Athens	Christopher Romanek
Mara Cea	Ph.D.	La Frontera University, Chile	Gary Mills
Erin Clark	M.S.	University of Georgia, Athens	J. Whitfield Gibbons
Dana Cook	Ph.D.	University of Georgia, Athens	Andrew Neal
James Cumbee	M.S.	University of Georgia, Athens	I Lehr Brisbin
Sara Drake	M.S.	University of Georgia, Athens	Beverly Collins
William Duval	Ph.D.	University of Georgia, Athens	Rebecca Sharitz
Luke Fedewa	M.S.	University of Georgia, Athens	J. Whitfield Gibbons
*Bobbi Fokidis	M.S.	Arkansas State University, Jonesboro	Travis Glenn
Xavier Glaudas	M.S.	University of Georgia, Athens	J. Whitfield Gibbons
Gabrielle Graeter	M.S.	University of Georgia, Athens	J. Whitfield Gibbons
Ellen Hepfer	M.S.	University of Georgia, Athens	Machelle Wilson
Ryan Holem	M.S.	University of Georgia, Athens	William Hopkins
Virginia Jin	Ph.D.	University of Georgia, Athens	Rebecca Sharitz
Glenn Kirkland	M.S.	University of Georgia, Athens	Travis Glenn/I Lehr Brisbin
Yong Jin Lee	Ph.D.	University of Georgia, Athens	Christopher Romanek
Audrey Majeske	M.S.	University of Georgia, Athens	Charles Jagoe
Frantisek Majs	Ph.D.	University of Georgia, Athens	John Seaman
Liberty Moore	M.S.	University of Georgia, Athens	I. Lehr Brisbin
John Mulhouse	M.S.	University of Georgia, Athens	Rebecca Sharitz
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
Elizabeth Richardson	M.S.	University of Georgia, Athens	J Vaun McArthur
Steven Schaff	Ph.D.	University of Georgia, Athens	Kenneth McLeod
Julian Singer	M.S.	University of Georgia, Athens	John Seaman
Amy Squire	M.S.	University of Georgia, Athens	Rebecca Sharitz
Brian Todd	Ph.D.	University of Georgia, Athens	J. Whitfield Gibbons
Olga Tsyusko	Ph.D.	University of Georgia, Athens	Michael Smith
Susan Turner	Ph.D.	University of Georgia, Athens	Rebecca Sharitz
Jason Unrine	Ph.D.	University of Georgia, Athens	Charles Jagoe
*Arlena Wartell	Ph.D.	University of Georgia, Athens	Travis Glenn
Lucas Wilkinson	M.S.	University of Georgia, Athens	J. Whitfield Gibbons
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
Cameron Young	Ph.D.	University of Georgia, Athens	J. Whitfield Gibbons
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

The Savannah River Ecology Laboratory's missions of research, outreach, and education have positioned the lab as a respected resource in the greater Aiken-Augusta area. The intellectual independence of academic research assures the local community of objective research on the impacts of site operations on ecosystems of the SRS and the region. In addition, the Environmental Outreach Program's 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* – undergirds SREL's outreach efforts.

SREL uses information from its own research efforts to educate the public locally, regionally, and nationally. The environmental commitments of SREL extend beyond the lab, the SRS, and even the larger local area. Issues as diverse as amphibian and reptile population declines, potential responses of organisms to local contamination, the distribution and abundance of sensitive species, monitored natural attenuation programs, and the 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. Some of the ways this is accomplished include the following:

- During the past year, SREL scheduled 401 talks, 21 tours, 26 exhibits, and 59 workshops, reaching a total of 85,425 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, and careers in ecology and research.
- Over 350 4th and 5th grade students and 17 teachers at two Aiken 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.
- Teachers at East Aiken Elementary School were guided through 8-week long earthworm experiments that they conducted with their classes.
- School groups enjoy field trips to the Laboratory's Conference Center and speakers from SREL go to schools, presenting programs on conservation, the process of science, and environmental stewardship.
- Teachers are trained in methods of teaching ecology during workshops and leave with materials produced by the Outreach staff.
- Increased demand for Outreach speakers has required greater use of the Distance Learning Facility, reaching a total of 928 additional students in K-12.
- 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 continued its new "research-to-classroom" hands-on science program for elementary school students. With funding from the American Honda Foundation (AHF; $70,000) and partial funding from The Christensen Fund ($25,000), SREL presented five workshops to every 4th and 5th grade class at East Aiken and Greendale Elementary schools, two Title I schools in Aiken County, SC. 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. SREL is seeking additional funding to continue this program in future years.

Fifth grade students learn how to do serial dilutions as part of an outreach unit on ecotoxicology.

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.

One noteworthy event in which SREL participated was the regional EcoMeet, where 28 teams from regional middle schools participated in an ecological science-bowl; SREL's booth required students to understand and use the scientific method. SREL is a founding member of the Central Savannah River Area Environmental Science Education Cooperative (CSRA ESEC), which sponsors the annual EcoMeet as well as other science-related programs throughout the year. 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 aided the Southern Environmental Law Center in the production of a brochure titled "At Risk: South Carolina's 'Isolated' Wetlands."

Associated with the AHF funding, SREL created a new 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. Next year we will continue to add resource materials, as well as develop a "student products" section of the site that will serve as a student site for posting writing, photos, and other materials that students create over the course of the project. A new print product produced this year was a *Methods of Science* bookmark.

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* 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.

Two of last year's products, the *American Alligator* brochure and a poster on amphibians and reptiles, received "Awards of Distinction" in the 2004 print media competition from the Communicator Awards, an international competition honoring excellence in communication. The Award of Distinction is awarded for projects that exceed industry standards in communicating the message or idea.

The Outreach Program also continues to distribute previously published materials including fliers on *Carnivorous Plants and Their Habitats* and *An Amphibian's Eye View of Wetlands*; a children's comic book entitled *Stepping into Ecology: the Ecological Adventures of Mud E. Boot*; a flier on *Is it a Water Moccasin?*; a booklet on *The Snakes of Georgia and South Carolina*, a *Chemistry – it's all about the nature of things* sticker 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; the *Amphibian's Eye View* and the *Water Moccasin* products had their 3rd printing this 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 2004 SREL researchers provided information to such diverse media outlets as *The Philadelphia Inquirer, The Washington Post, Nick Jr., Animal Planet, MSNBC, National Geographic, Mother Jones News, The Christian Science Monitor,* the *Chronicle of Higher Education,* the *Associated Press, Martha Stewart Living, Golf Week, Readers Digest, Wildlife Magazine, Utemagasinet* (Sweden), *National Geographic TV, Smithsonian* and many local news outlets in the Southeast such as *The Augusta Chronicle, The Charleston Post and Courier, The State, The Aiken Standard, Savannah Morning News* and the *Asheville Citizen News.* Topics in the news ranged from animal behavior to environmental impacts to SREL research in Kamchatka, Russia. In addition, Turner Broadcasting produced a half-hour special on SREL for The Natural South feature series.

The Public Relations office screens most inquiries from the press, directing reporters to the most helpful 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.

Also notable this year was the creation of an individual Strategic Plan for the Environmental Outreach Program. This complements the overall SREL Strategic Plan and guides our activities along predetermined pathways.

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 are anticipated. SREL and the USFS-SR also verify/update Set-Aside boundary lines that are concordant with prescribed forest stand treatments. To date, boundaries for 10 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 anticipates a release of the latest version to the SRS 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 5 management plans, completing plans for the SAF Natural Areas (Set-Asides No. 18 and 29) and the Loblolly Pine Forest (Area No.4). Plans for the Rainbow Bay Amphibian Reserve (Area No.16) and the Craig Pond Set-Aside (Area No. 17) were also worked on. Approval was received from the Set-Aside Task Group to conduct a first pine thinning treatment in the Rainbow Bay Set-Aside; approximately 17 acres (6.9 ha) of pine was marked for removal, pending final approval of the final management plan. A 30-acre (12.2 ha) pine thinning treatment in the Flamingo Bay Set-Aside (Area No. 21) was delayed until early FY05 for research and USFS-SR logistical reasons. 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. However, power line ROW vegetation in Ellenton Bay and Risher Pond was cleared by SCE&G.

Research in Set-Aside Areas

Set-Asides continued to be well used by researchers both as research study sites and as reference sites for collections of uncontaminated plants, animals, soils, or water. Publications resulting from research in Set-Asides are listed below. Groups other than SREL also use Set-Aside Areas. SRNL continued to use organisms collected in Set-Asides as "controls" 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 continued to conduct archaeological investigations around Set-Aside Carolina bays. In cooperative efforts, SREL and USFS-SR researchers continue 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.

FY04 SREL documents, publications, theses, and dissertations that used DOE Set-Aside Areas

Thirty-six documents were published this year reporting research from the Set-Aside Areas. Recent SREL documents, scientific publications, theses, and dissertations that used or referenced DOE Set-Aside Areas during FY04 include:

Anonymous. 2003. At Risk: South Carolina's "Isolated" Wetlands. The Southern Environmental Law Center, Chapel Hill, NC.

Boring, C.S. 2003. Home range, habitat use, and radiocesium burdens of raccoons (*Procyon lotor*) inhabiting the U.S. Department of Energy's Savannah River Site. M.S. Rutgers University, New Brunswick, NJ.

Bridges, C.M., C.L. Rowe, and W.A. Hopkins. 2003. Amphibian conservation genetics. p. 59-71. *In* Amphibian Decline: An

Integrated Analysis of Multiple Stressor Effects, edited by G. Linder, S. Krest, and D. Sparling. Society of Environmental Toxicology and Chemistry.

Burgess, E. 2003. An analysis of mercury resistance in stream sediment bacteria, using teminal retriction fragment length polymorphism (TRFLP). M.S. University of Georgia, Athens, GA.

Croshaw, D.A. and T.C. Glenn. 2003. Polymorphic tetranucleotide microsatellite DNA loci from the southern dusky salamander (*Desmognathus auriculatus*). Molecular Ecology Notes 3:623-625.

Davis, C.E. 2004. Stewardship management plans for the Boiling Springs and Scrub Oak Natural Areas on the Savannah River Site Site. Savannah River Ecology Laboratory, University of Georgia, Aiken, SC.

Davis, C.E. 2004. Stewardship management plan for the Loblolly Pine Forest Set-Aside Area on the Savannah River Site. Savannah River Ecology Laboratory, University of Georgia, Aiken, SC.

DeBiase, A. E. and B. E. Taylor. 2003. New reports of fairy shrimps (Crustacea: Anostraca and clam shrimps (Crustacea: Laevicaudata and Spinicaudata) from South Carolina. Southeastern Naturalist 2:207-216.

DeVault, T.L., I.L. Brisbin, Jr., and O.E. Rhodes, Jr. 2004. Factors influencing the acquisition of rodent carrion by vertebrate scavengers and decomposers. Canadian Journal of Zoology 82:502-509.

Fedewa, L.A. 2003. Microbial ecology of developing southern toads, spring peepers, and narrow-mouthed toads. M.S. University of Georgia, Athens, GA.

Fletcher, D.E., E.E. Dakin, B.A. Porter, and J.C. Avise. 2004. Spawning behavior and genetic parentage in the pirate perch (*Aphredoderus sayanus*), a fish with an enigmatic reproductive morphology. Copeia 1:1-10.

Gaines, K.F. 2003. Spatial Modeling of Receptor Species for Ecological Risk Assessment Activities on the Department of Energy's Savannah River Site. PhD Dissertation, University of South Carolina, Columbia, SC.

Gibbons, J.W. and K.M. Andrews. 2004. PIT tagging: Simple technology at its best. BioScience 54:447-454.

Gibbons, J.W. 2003. Terrestrial habitat: A vital component for herpetofauna of isolated wetlands. Wetlands 23:630-635.

Gibbons, J.W. 2003. Societal values and attitudes: history and sociological impact on amphibian conservation problems. p. 214-227. *In* Amphibian Conservation, edited by R.D. Semlitsch. Smithsonian Institution Press.

Gibbons, J.W. and K.L. McGlothlin. 2003. A Changing Balance: An Ecological Perspective on the Loss of Biodiversity. p. 29-54. *In* Loss of Biodiversity: Exploring Environmental Challenges–A Multidisciplinary Approach, edited by S. L. Spray and K.L. McGlothlin. Rowman & Littlefield Publishers, Inc.

Glaudas, X. 2003. Patterns of Defensive Behavior in Pit Vipers of the Southeastern United States. M.S. University of Georgia, Athens, Georgia.

Hopkins, W.A., J.H. Roe, T. Philippi, and J.D. Congdon. 2004. Standard and digestive metabolism in the banded water snake, *Nerodia fasciata fasciata*. Comparative Biochemistry and Physiology Part A 137:141-149.

Hopkins, W.A., B.P. Staub, J.W. Snodgrass, B.E. Taylor, A.E. DeBiase, J.H. Roe, B.P. Jackson, and J. D. Congdon. 2004. Responses of benthic fish exposed to contaminants in outdoor microcosms–examining the ecological relevance of previous laboratory toxicity tests. Aquatic Toxicology 68:1-12.

Hopkins, W.A., J.W. Snodgrass, B.P. Staub, B.P. Jackson, and J.D. Congdon. 2003. Altered swimming performance of a benthic fish (*Erimyzon sucetta*) exposed to contaminated sediments. Archives of Environmental Contamination and Toxicology 44:383-389.

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Staub, B.P., W.A. Hopkins, J. Novak, and J.D. Congdon. 2004. Respiratory and reproductive characteristics of eastern mosquitofish (*Gambusia holbrooki*) inhabiting a coal ash settling basin. Archives of Environmental Contamination and Toxicology 46:96-101.

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Weston, J.L. and I.L. Brisbin, Jr. 2003. Demographics of a protected population of gray foxes (*Urocyon cinereoargenteus*) in South Carolina. Journal of Mammalogy 84:996-1005.

White, D.L. 2004. Deerskins and Cotton. Ecological impacts of historical land use in the Central Savannah River Area of the Southeastern US before 1950. Final Report. USDA Forest Service, Savannah River, Aiken, SC.

Wilkinson, L. 2003. Patterns of reproductive allocation: clutch and egg size variation in three freshwater turtles. M.S. University of Georgia, Athens, GA.

··· 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	U.S. Department of Energy/Office of Science EMSP
Budget	$478,000 total (no additional funding in FY04)
Period	September 1, 2002–September 30, 2005

PI	Paul M. Bertsch
Project Title	The Environmental Fate of Arsenic from Poultry Litter
Funding Agency	U.S. Department of Agriculture
Budget	$164,500 total (no additional funding in FY04)
Period	December 1, 2000–November 30, 2003

PI	Paul M. Bertsch
Project Title	Examination of Coastal Aquaculture Effluent and Receiving Water Quality Throughout the Tidal Cycle
Funding Agency	U.S. Department of Commerce
Budget	$94,094
Period	July 1, 2003–June 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	$67,255
Period	July 1, 2003–June 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	$15,335
Period	April 1, 2003–September 30, 2003

PI	I. Lehr Brisbin, Jr.
Project Title	Wood Stork Foraging Behavior in the Coastal Environment
Funding Agency	USDI–U.S. Fish and Wildlife Service
Budget	$28,280
Period	September 1, 2000–December 31, 2003

PI	I. Lehr Brisbin, Jr.
Project Title	Ossabaw Research on Raccoons and Feral Hogs
Funding Agency	The Ecology Committee of Savannah Presbytery
Budget	$9,100
Period	July 1, 2002–November 15, 2003

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	$73,327
Period	October 1, 2003–September 30, 2004

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, 2004

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–November 15, 2004

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

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 ($275,000in FY04)
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	$10,000
Period	July 1, 2003–June 30, 2004

PI	J. Whitfield Gibbons
Project Title	PARC Program
Funding Agency	USDA Forest Service
Budget	$100,000
Period	July 2003–June 2004

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 U.S. 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 U.S. 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 $250,032
Period	April 1, 2001–April 24, 2006

PI	J. Whitfield Gibbons and William Hopkins
Project Title	Sublethal Effects of Pesticide Exposure
Funding Agency	U.S. Golf Association
Budget	$80,175 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	PARC-ARMI Database: Inventory, Monitoring, and Research Database for Amphibians and Reptiles on Federal Land
Funding Agency	U.S. Geological Survey
Budget	$20,000
Period	April 5, 2002–August 31, 2004

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, 2004

PI	J. Whitfield Gibbons
Project Title	Amphibian and Reptile Inventory of Sumter National Forest
Funding Agency	USDA Forest Service
Budget	$20,000
Period	September 18, 2001–September 30, 2004

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	$59,513
Period	May 15, 2003–April 30, 2004

PI	J. Whitfield Gibbons
Project Title	Operation of the NARCAM Website
Funding Agency	U.S. Department of Interior
Budget	$25,000
Period	August 1, 2003–August 31, 2004

PI	J. Whitfield Gibbons
Project Title	Turtle Management on National Wildlife Refuges
Funding Agency	U.S. Department of Interior
Budget	$60,000
Period	March 1, 2004–February 28, 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	Double Enrichment of Isolated DNA of *Protea punctata*
Funding Agency	University of Connecticut
Budget	$5,500
Period	June 1, 2003–June 30, 2004

PI	Travis Glenn
Project Title	Support for Turtle Conservation
Funding Agency	National Fish and Wildlife Foundation
Budget	$7,013
Period	July 1, 2003–June 30, 2004

PI	Travis Glenn and Charles H. Jagoe
Project Title	Development and Use of Transgenic *Caenorhabditis elegans* to Measure Bioavailability of Metals and Mutagenicity in Contaminated Media
Funding Agency	Bechtel BWXT Idaho, Ltc.
Budget	$22,100
Period	March 15, 2004–September 30, 2004

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	U.S. 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	$129,200
Period	May 1, 2002–April 30, 2004
PI	Kenneth W. McLeod
Project Title	Assessment of Harvesting Bottomland Hardwood Sites on Plant Composition and Ecosystem Processes
Funding Agency	USDA Forest Service
Budget	$114,930
Period	February 1, 2002–January 31, 2004
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, 2004
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	$11,600
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, 2004
PI	John C. Seaman
Project Title	Tritium Distribution, Mixing, and Transport at the Tritiated Water Management Facility
Funding Agency	USDA Forest Service

Budget	$31,145
Period	April 15, 2003–December 31, 2004

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 ($229,100 in FY04)
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	Barbara E. Taylor
Project Title	Characterization of Invertebrate Assemblages in Carolina Bays and other Wetland Ponds Before and After Restoration
Funding Agency	USDA Forest Service
Budget	$5,000
Period	August 29, 2002–September 30, 2003

PI	Barbara E. Taylor
Project Title	Aquatic Invertebrates in Carolina Bays and Other Wetland Ponds Before and After Restoration Treatments
Funding Agency	U.S. 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, 2004

PI	Chuanlun Zhang
Project Title	Using Carbon Isotopes to Evaluate the Effectiveness of Carbon Sequestration in Coal Fly Ash
Funding Agency	UT-Battelle, LLC
Budget	$14,240
Period	April 1, 2003–March 31, 2004

PI	Chuanlun Zhang
Project Title	Biogeochemical Processes and Community Dynamics in Gas Hydrate Systems in the Gulf of Mexico
Funding Agency	National Science Foundation
Budget	$258,592 total award
Period	September 1, 2002–December 31, 2003

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	$8,880
Period	May 1, 2004–July 31, 2004

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2685 DeVault, T.L., O.E. Rhodes, Jr., and J.A. Shivik. 2003. Scavenging by vertebrates: behavioral, ecological, and evolutionary perspectives on an important energy transfer pathway in terrestrial ecosystems. Oikos 102:225-234.

2686 Bolan, N.S., M.A. Khan, J. Donaldson, D.C. Adriano, and C. Matthew. 2003. Distribution and bioavailability of copper in farm effluent. The Science of the Total Environment 309:225-236.

2687 Gibbons, J.W. 2003. Societal values and attitudes: their history and sociological impact on amphibian conservation problems. p. 214-227. *In* Amphibian Conservation, edited by R. D. Semlitsch. Smithsonian Institution Press.

2688 DeBiase, A.E. and B.E. Taylor. 2003. New reports of fairy shrimps (Crustacea: Anostraca and clam shrimps (Crustacea: Laevicaudata and Spinicaudata) from South Carolina. Southeastern Naturalist 2:207-216.

2689 Hopkins, W.A., J.W. Snodgrass, B.P. Staub, B.P. Jackson, and J.D. Congdon. 2003. Altered swimming performance of a benthic fish (*Erimyzon sucetta*) exposed to contaminated sediments. Archives of Environmental Contamination and Toxicology 44:383-389.

2690 Hauswaldt, J.S. and T.C. Glenn. 2003. Microsatellite DNA loci from the diamondback terrapin (*Malaclemys terrapin*). Molecular Ecology Notes 3:174-176.

2691 Kumar, K.S., W.W. Bowerman, T.L. DeVault, T. Takasuga, O.E. Rhodes, Jr., I.L. Brisbin, Jr., and S. Masunaga. 2003. Chlorinated hydrocarbon contaminants in blood of black and turkey vultures from Savannah River Site, South Carolina, USA. Chemosphere 53:173-182.

2692 DeVault, T.L., W.L. Stephens, B.D. Reinhart, O.E. Rhodes, Jr., and I.L. Brisbin, Jr. 2003. Aerial telemetry accuracy in a forested landscape. Journal of Raptor Research 37:147-151.

2693 Jackson, B.P., P.L. Shaw-Allen, W.A. Hopkins, and P.M. Bertsch. 2002. Trace element speciation in largemouth bass (*Micropterus salmoides*) from a fly ash settling basin by liquid chromatography-ICP-MS. Analytical Bioanalytical Chemistry 374:203-211.

2694 Congdon, J.D., R.D. Nagle, O.M. Kinney, R.C. van Loben Sels, T. Quinter, and D.W. Tinkle. 2003. Testing hypotheses of aging in long-lived painted turtles (*Chrysemys picta*). Experimental Gerontology 38:765-772.

2695 Dorcas, M.E., S.M. Poppy, C.H. Ernst, and J.W. Gibbons. 2003. *Regina alleni* (Striped crayfish snake). Catalogue of American Amphibians and Reptiles 778:1-778.4.

2696 Shaw-Allen, P., M. Elliott, and C. H. Jagoe. 2003. A microscaled mercury saturation assay for metallothionein in fish. Environmental Toxicology & Chemistry 22:2005-2012.

2697 Bryan, A.L., Jr., W.A. Hopkins, J.A. Baionno, and B.P. Jackson. 2003. Maternal transfer of contaminants to eggs in common grackles (*Quiscalus quiscala*) nesting on coal fly ash basins. Archives of Environmental Contamination and Toxicology 45:273-277.

2698 Weston, J.L. and I.L. Brisbin, Jr. 2003. Demographics of a protected population of gray foxes (*Urocyon cinereoargenteus*) in South Carolina. Journal of Mammalogy 84:996-1005.

2699 Seaman, J.C., S.A. Aburime, J.M. Hutchison, and J.H. Singer. 2003. Evaluating vadose transport processes using centrifugation methods. p. 233-242. *In* Proceedings of the 2002 National Conference on Environmental Science and Technology, edited by G. Uzochukwu, K. Schimmel, G. Reddy, S. Chang and V. Kabadi. September 8-10, 2002, Greensboro, NC. Battelle Press.

2700 Howe, J.A., R.H. Loeppert, V.J. Derose, D.B. Hunter, and P.M. Bertsch. 2003. Localization and speciation of chromium in subterranean clover using XRF, XANES, and EPR spectroscopy. Environmental Science & Technology 37:4091-4097.

2701 Ulsh, B., T.G. Hinton, J.D. Congdon, L.C. Dugan, F.W. Whicker, and J.S. Bedford. 2003. Environmental biodosimetry: a biologically relevant tool for ecological risk assessment and biomonitoring. Journal of Environmental Radioactivity 66:121-139.

2702 Aburime, S.A., J.C. Seaman, J.H. Singer, and T.S. Steenhuis. 2003. Reliability of contaminant transport modeling on vadose zone sampling methods in structured soils. p. 137-151. *In* Proceedings of the 2002 National Conference on Environmental Science and Technology, edited by Uzochukwu, K. Schimmel, G. Reddy, S. Chang, and V. Kabadi. September 8-10, 2002, Greensboro, NC. Battelle Press.

2703 Collins, B. 2003. Ground layer competition and herbivory effects on cherrybark oak (*Quercus pagoda* Raf.) regeneration in experimental canopy gaps. Journal of the Torrey Botanical Society 130:147-157.

2704 Sharitz, R.R. 2003. Carolina Bay wetlands: Unique habitats of the southeastern United States. Wetlands 23:550-562.

2705 Gibbons, J.W. 2003. Terrestrial habitat: A vital component for herpetofauna of isolated wetlands. Wetlands 23:630-635.

2706 Wilson, M.D. and T.G. Hinton. 2003. Comparative bias associated with various estimates of dose to maximally exposed individuals. Health Physics 85:585-593.

2707 Beck, C.W. and J.D. Congdon. 2003. Energetics of metamorphic climax in the southern toad (*Bufo terrestris*). Oecologia 137:344-351.

2708 Smith, M.H., T.K. Oleksyk, and O. Tsyusko-Omeltchenko. 2003. Effect of trophic level on the radiocesium frequency distribution in aquatic and terrestrial ecosystems at Chornobyl and nuclear sites in the United States. p. 37-48. *In* Proceeding of the International Symposium: Transfer of radionuclides in biosphere - Prediction and Assessment, edited by H. Amano and S. Uchida, Japan Atomic Energy Research Institute Conference 2003-010.

2709 Hutchison, J.M., J.C. Seaman, S.A. Aburime, and D.E. Radcliffe. 2003. Chromate transport and retention in variably saturated soil columns. Vadose Zone Journal 2:702-714.

2710 Tomasulo-Seccomandi, A.M., N.A. Schable, A.L. Bryan, Jr., I.L. Brisbin, Jr., S.N. Del Lama, and T.C. Glenn. 2003. Development of microsatellite DNA loci from the wood stork (Aves, Ciconiidae, *Mycteria americana*). Molecular Ecology Notes 3:563-566.

2711 Croshaw, D.A. and T.C. Glenn. 2003. Seven polymorphic microsatellite DNA loci from the red-spotted newt (*Notophthalmus viridescens*). Molecular Ecology Notes 3:514-516.

2712 Croshaw, D.A. and T.C. Glenn. 2003. Polymorphic tetranucleotide microsatellite DNA loci from the southern dusky salamander (*Desmognathus auriculatus*). Molecular Ecology Notes 3:623-625.

2713 McLeod, K.W. and T.G. Ciravolo. 2003. Sensitivity of water tupelo (*Nyssa aquatica*) and bald cypress (*Taxodium distichum*) seedlings to manganese enrichment under water-saturated conditions. Environmental Toxicology and Chemistry 22:2948-2951.

2714 Neal, A.L., K.M. Rosso, G.G. Geesey, Y.A. Gorby, and B.J. Little. 2003. Surface structure effects on direct reduction of iron oxides by *Shewanella oneidensis*. Geochimica et Cosmochimica Acta 67:4489-4503.

2715 Bolan, N.S., D.C. Adriano, P. Duraisamy, and A. Mani. 2003. Immobilization and phytoavailability of cadmium in variable charge soils. III. Effect of biosolid compost addition. Plant and Soil 256:231-241.

2716 Litzgus, J.D. and W.A. Hopkins. 2003. Effect of temperature on metabolic rate of the mud turtle (*Kinosternon subrubrum*). Journal of Thermal Biology 28:595-600.

2717 Gibbons, J.W. and K.L. McGlothlin. 2003. A Changing Balance: An Ecological Perspective on the Loss of Biodiversity. p. 29-54. *In* Loss of Biodiversity: Exploring Environmental Challenges - A Multidisciplinary Approach, edited by S. L. Spray and K. L. McGlothlin. Rowman & Littlefield Publishers, Inc.: 179

2718 Staton, J.L., B.E. Taylor, N.V. Schizas, R. Wetzer, T.C. Glenn, and B.C. Coull. 2003. Mitochondrial gene diversity of *Skistodiaptomus mississippiensis* in impoundments of the upper coastal plain near Aiken, South Carolina, USA. Arch. Hydrobiol. 158:215-231.

2719 DeVault, T.L., O.E. Rhodes, Jr., and L.M. Smith. 2003. Condition indices for wintering American wigeon. Wildlife Society Bulletin 31:1132-1137.

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2721 Hopkins, W.A., J.H. Roe, T. Philippi, and J.D. Congdon. 2004. Standard and digestive metabolism in the banded water snake, *Nerodia fasciata fasciata*. Comparative Biochemistry and Physiology Part A 137:141-149.

2722 Dorcas, M.E., W.A. Hopkins, and J.H. Roe. 2004. Effects of body mass and temperature on standard metabolic rate in the eastern diamondback rattlesnake (*Crotalus adamanteus*). Copeia 1:145-151.

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2724 Staub, B.P., W.A. Hopkins, J. Novak, and J.D. Congdon. 2004. Respiratory and reproductive characteristics of eastern mosquitofish (*Gambusia holbrooki*) inhabiting a coal ash settling basin. Archives of Environmental Contamination and Toxicology 46:96-101.

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leaved cattail *Typha angustifolia*. Molecular Ecology Notes 3:535-538.

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2728 Whicker, F.W., T.G. Hinton, M.M. MacDonell, J.E. Pinder, III, and L.J. Habegger. 2004. Avoiding destructive remediation at DOE sites. Science 303:1615-1616.

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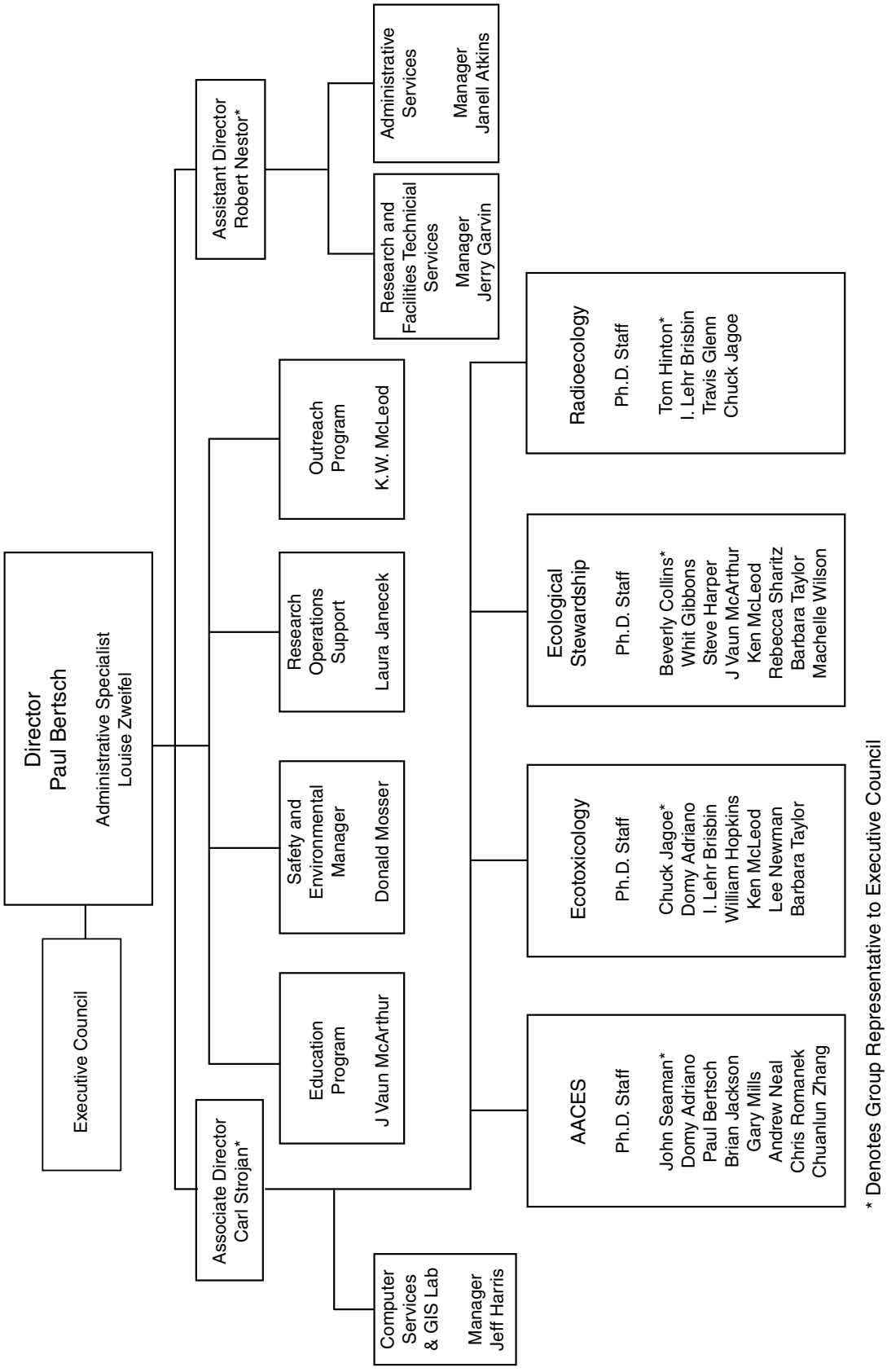
··· SREL Organizational Chart ···



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