

**SAVANNAH RIVER
ECOLOGY LABORATORY**

**ANNUAL TECHNICAL PROGRESS REPORT
OF ECOLOGICAL RESEARCH FOR FY2011**

Supported under Cooperative Agreement
DE-FC09-96SR18546

between
The University of Georgia
and the
U.S. Department of Energy
for the period of
1 October 2010 – 30 September 2011

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SAVANNAH RIVER ECOLOGY LABORATORY FY2011 OVERVIEW

The Savannah River Ecology Laboratory (SREL) is a research unit of The University of Georgia (UGA) that has been conducting ecological research on the Savannah River Site (SRS) near Aiken, South Carolina for 60 years. The overall mission of the Laboratory is to enhance our understanding of the environment by acquiring and communicating knowledge of ecological processes and principles that contribute to sound environmental stewardship. In addition, as directed in the Cooperative Agreement with the U.S. Department of Energy (DOE), SREL will provide the public with an independent evaluation of the ecological effects of SRS operations on the environment. Toward these goals, SREL conducts fundamental and applied ecological research, as well as education and outreach programs.

The Laboratory's research mission during the 2011 fiscal year was fulfilled with the publication of 33 journal articles and book chapters by faculty, technical staff, students, and visiting scientists. One book was also authored by SREL faculty and staff members. Additional journal articles and books have been submitted or are in press. Significantly, SREL outreach activities reached over 33,000 people of all ages. Other noteworthy events took place as faculty members, staff, and graduate students received awards. These are described in the section 'Special Accomplishments of SREL Personnel'.

The vision, structure, and operations of SREL continued to evolve since changes in funding structure were instituted in FY07. Funding from EM-HQ consists of two individual projects and SRS-based project funding reflects the specific needs of EM. The funding mechanism currently in place necessitated a conversion to programs that are entrepreneurial and interdisciplinary, and to funding strategies that are competitive, responsive to sponsors' requirements, and based on a diverse and sustainable foundation. This recognition required restructuring of research and supporting infrastructure.

Although these changes were very challenging, a reduced, but robust SREL presence continued to operate on the SRS in FY11. Currently, SREL's total employment is approximately 60 faculty, technicians, students, and support staff. The number of employees and level of funding is lean but ensures continued progress toward stated objectives and does not compromise safety and security. New partnerships and collaborations with the Athens campus and other agencies continue to be explored and developed in order to maximize the use of SREL assets. Graduate student programs have continued with funding provided by external grants, UGA, or the student's host university.

SREL faculty have responded to the revised structure and have sought financial support from multiple external funding agencies, DOE-EM, DOE-NNSA (National Nuclear Security Administration), and SRNS-ACP (Savannah River Nuclear Solutions-Area Closure Projects), and UGA has provided temporary infrastructure support to SREL through this transitional period. Funding, made available as a result of the ARRA-DOE

program, has facilitated SREL program development. The Cooperative Agreement with DOE allows SREL/UGA access to the SRS through 30 November 2011. Continued funding for SREL has been strongly supported by the local community for its role in research, environmental monitoring, and education/outreach programs for local schools and the general public.

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

Researchers at SREL had funding from 23 external grants during FY11. Sources of grant awards range from private foundations such as the National Fish and Wildlife Foundation to federal agencies such as the U.S. Department of Interior, the National Science Foundation, and the Department of Defense.

SREL faculty members hold positions in varied departments at the University of Georgia. In addition, several SREL faculty members (and emeritus faculty) have adjunct status at other colleges and universities. Faculty, staff, and students also are active in providing outreach and service to the scientific community. Representatives from SREL hold editorial or committee positions in national groups and organizations and also serve on several UGA academic and administrative committees. SREL faculty members continue to make scientific presentations, contribute posters to scientific meetings, and present seminars at colleges and universities.

Participants in the SREL Education Program during FY11 included 10 undergraduate students and 24 graduate students from numerous colleges and universities in the United States.

The SREL Outreach Program communicates scientific awareness to area schools and the general public, an audience which differs from science professionals. During the past year, SREL presented 263 talks, 65 tours, and 15 exhibits, reaching a total of over 33,000 people. Topics for these presentations included ecological studies of reptiles and amphibians, southeastern plants and habitats, long-term research, safety, biodiversity, local wetlands and watersheds, conservation, and careers in ecology and research. In the past year, SREL has participated in the SRS public tour program (two tours per month of 30-40 citizens). SREL participates by providing presentations on the history and research of the lab as well as a "show and tell" session featuring research animals native to the SRS. In addition, outreach personnel have initiated and promoted a monthly tour for SRS employees.

The SREL Conference Center has continued to be a valuable asset to SREL and other groups on the SRS. SREL used the facility to host numerous meetings and environmental education programs for students, teachers, and other groups this past year. The facility is also used by DOE and the USDA Forest Service when it is available.

During FY11, the UGA Vice President for Research, Dr. David C. Lee, approved the initiation of a search for a new SREL Director. Advertisement appeared in the fall of 2010 with subsequent interviews, selection and negotiations with selected candidate. Dr. O. E. (Gene) Rhodes, Jr. was successfully hired with a start date of January 1, 2012.

SPECIAL ACCOMPLISHMENTS OF SREL PERSONNEL IN FY2011

In FY2011, several SREL faculty members were honored by their scientific peers.

Dr. J. Whitfield Gibbons, an emeritus senior ecologist, was awarded the first National Meritorious Teaching Award in Herpetology, sponsored by the American Society of Ichthyologists and Herpetologists, the Herpetologists' League, and the Society for the Study of Amphibians and Reptiles. He also received the first PARC (Partners in Amphibian and Reptile Conservation) Visionary Leader Award.

Dr. J Vaun McArthur, a senior ecologist, was awarded the outstanding teaching award by the Odum School of Ecology at the University of Georgia. He teaches over 400 students each year in the Introductory Ecology course for non-majors. Dr, McArthur also received a Distinguished Service award from the American Society for Microbiology as a member of the Editorial Board.

Dr. John C. Seaman, Dr. Tracey C. Tuberville, Dr. Stacey Lance, Julian Singer and others received a grant from the Nuclear Regulatory Commission Education Grants Program entitled *GA/SC University Partnership Curriculum for Environmental Radiation Protection*. This is very significant as it begins to teach Maymester courses at SREL regarding environmental protection.

Several SREL publications and research projects generating scientific and public interest:

A publication by **Dr. Tracey D. Tuberville (Tuberville, T.D., T.M., Norton, B.J. Waffa*, C. Hagen, and T.C. Glenn**. 2011. Mating system in a gopher tortoise population established through multiple translocations: Apparent advantage of prior residence. *Biological Conservation* 144:175-183) was publicized in the Science Daily report and the conservation journal *Oryx*.

ACP-funded research on contaminant monitoring in long-lived reptiles by **Dr. Tracey D. Tuberville's** research lab was mentioned in USA Today. It was also featured in an informational video for Savannah River Site employees.

Drs. Kurt A. Buhlmann and Tracey D. Tuberville have been involved with a project to reintroduce Blandings turtles to a national wildlife refuge in Massachusetts, in collaboration with USFWS, a local vocational high school, and regional biologists. The project was featured in a video produced by USFWS and posted on YouTube.

Dr. I. L. Brisbin and a graduate student, Tomas Condon, also contributed to a prime-time documentary entitled "The Taming of the Wild" which was aired by National Geographic Television in 2011 and a companion article with the same

name was published in Volume 219, No. 2 (March, 2011) of the *National Geographic Magazine*. These described the biological, ecological and genetic processes through which certain unique species of wild animals were domesticated by man and how these important domestic animals and the evolution from their increasingly endangered surviving wild counterparts, are still being studied today.

Interviews with National Geographic writers and producers and on-location filming of the portion of this production concerned the evolution of the domestic chicken from its wild ancestor, the Red Jungle Fowl. Dr. I. L. Brisbin's studies of the behavior, ecology and conservation biology of the Red Jungle Fowl and its hybrids have been conducted at the SREL for over thirty years and have been associated with a program to develop a free-ranging strain of feral/domestic chickens which have been used to study the uptake and concentration of radionuclides and other environmental contaminants in naturally-contaminated field habitats of the Savannah River Site.

The behavior and ecology of these free-ranging birds are now contributing to a better understanding of how free-ranging "backyard" poultry could potentially be managed as a source of edible food protein within radioactively contaminated suburban and rural areas such as those surrounding the sites of the Chernobyl and Fukushima nuclear accidents.

SREL faculty published one book in FY2011.

FROGS: THE ANIMAL ANSWER GUIDE. 2011. (Mike Dorcas and Whit Gibbons).
Johns Hopkins University Press.

SREL faculty and students also served on numerous editorial boards of scientific journals, including:

Dr. Kurt A. Buhlmann –

Chelonian Conservation and Biology
Northwest PARC Habitat Management Guidelines
Habitat Management Guidelines for Amphibians and Reptiles of the Midwestern
United States
PARC Inventory and Monitoring Techniques Manual
Conservation Biology of Tortoises and Freshwater Turtles

Dr. J Vaun McArthur –

Applied and Environmental Microbiology
ISRN Ecology (International Scholarly Research Network)
Herpetological Review – Natural History Notes

SREL faculty and students were also societal officers and provided service to other scientific societies, including:

Dr. John C. Seaman - Will be the host of the International Conference on the Biogeochemistry of Trace Elements (ICOBTE) meeting in 2013, active planning is already underway; served on the Technical Planning Committee for the 2011 Georgia Water Resources Conference; served as Division Chair for Soil Chemistry of the Soil Science Society of America.

Dr. Stacey Lance conducted a NSF-OISE funded US-Mexico workshop on evolutionary genomics of non-model species: next-gen sequences, data management and hypothesis testing.

Dr. Rebecca R. Sharitz – Member of the Executive Board of Audubon South Carolina and the Executive Committee of UGA Plant Biology Department

Dr. Tracey D. Tuberville – Member of the Florida Fish and Wildlife Conservation Commission; Co-Founder and Co-Chair of the Southeast Partners in Amphibian and Reptile Conservation Reintroduction Working Group; South Carolina State Representative to the Gopher Tortoise Council; Instructor at the Envirovet Training Program, St. Catherines Island / White Oak Plantation; Biological expert for the Indigo Snake Captive Propagation and Repatriation Meeting; Biological expert on gopher tortoise population biology and reintroduction technologies for the South Carolina Department of Natural Resources, including contributor to state conservation plan for gopher tortoises (currently under review by agency); Served as biological expert on population viability and reintroduction of Blanding's turtles for the U.S. Fish and Wildlife Service, Great Meadows National Wildlife Refuge complex.

SREL faculty and staff also provide numerous services to the Savannah River Site and the overall CSRA public.

Dean Fletcher is producing numerous GIS layers and maps that will be widely used by researchers and managers working on SRS streams. GIS layers and maps delineate perennial, intermittent and ephemeral streams, flow impediments including both active and abandoned dams, levees and crossings, rip rap, and check dams. Areas of severe erosion, incision, or sedimentation are being recorded. Stream reaches, modified by ditching or channelization, are being mapped. Runoff patterns from industrial areas and outfall routes are being drawn on GIS layers based on field data.

Drs. Rebecca Sharitz and Kurt Buhlmann serve on the Craig's Pond Advisory Committee to define an active management plan for this Carolina bay, including discussions about fire management and gopher frog habitat use.

David Scott, Sean Poppy and others continue to provide Public Outreach Activities, such as Earth Day talks, exhibits, watershed tours and special activities at

Hidden Bay in Aiken including talks, education/service projects, and coordinated public workdays for invasive species removal.

SREL faculty also contributed to other SRS, regional, national, and international scientific efforts

During the last week of July, 2011, **Dr. I.L. Brisbin**, a Senior Research Scientist Emeritus, was invited by the American Ornithologists' Union (AOU) to organize and chair a special workshop on the issue of possible impacts upon global populations of birds, as a result of Japan's Fukushima nuclear accident in March of this year. The AOU is North America's oldest and largest professional organization of scientists who study birds, and the Fukushima workshop which Dr. Brisbin held this year as part of the AOU's annual meeting in Jacksonville, Florida, was a follow-up to a workshop which he was similarly asked to organize and chair at an earlier AOU annual meeting, concerning the global impacts upon birds of the radioactive releases resulting from the Chernobyl nuclear accident in the former Soviet Union almost exactly 25 years ago. In particular this workshop pointed to the need for data on regional and global transport of nuclear contaminants by waterfowl and other game birds which may be consumed by the hunting public as food. Other presenters at this year's workshop included Dr. Tim Mousseau of the University of South Carolina who has maintained a long-time study of the radioecology of birds in the exclusion zone of the Chernobyl site, and also faculty of Georgia Southern University of Statesboro, Georgia, whose campus is located close to the presently expanding Plant Vogtle Nuclear Power Plant.

Dr. Cary Tuckfield worked with Dr. John Kilgo of the US Forest Service, Savannah River Station on a project which estimated the bird density within restored Carolina Bay wetlands. This project was an extension of The Carolina Bay Restoration Project for which a final report was recently generated (Barton et al. 2007). Prior to the establishment of the Savannah River Site (SRS) in 1950 many of the endemic Carolina Bay wetlands on this site were drained, ditched and/or disturbed. The SRS Wetlands Mitigation Bank was established in 1997 as part of a concerted effort within DOE to restore degraded Carolina bays. A collaborative research project was conducted from 2000-2006 to demonstrate, if possible, the effectiveness of various treatment combinations to restore selected Carolina bays to a more typical undisturbed ecological status. By experimental design some bays were randomly selected for restoration using 4 method combinations (2 bay x 2 margin treatments). Subsequently, bird species numbers were measured by line transect methods to estimate and contrast species density in restored vs. non-restored bays.

In the end, this study provided weak evidence for a broad effect of Carolina bay wetland restoration methods on increasing bird populations. In 2 of 12 species (Carolina chickadee and Eastern Rufous-sided towhee), with observation counts

≥ 300 , there was a statistically significant ($p < .05$) difference in bird density between the two margin treatment methods, viz., mixed vs. pine. Both differences (i.e., mixed – pine) were negative indicating a greater density of birds in the pine margin treatment than the mixed margin treatment.

Dr. Tuckfield also was a collaborator in several other projects off of the SRS. He collaborated with US Forest Service biologists from the Luquillo Experimental Forest Station, Puerto Rico, *Evaluation of Tropical Agricultural Practices* to examine relationships between coffee plantations on the Pacific slopes in Nicaragua with bird species diversity and richness. Another project, *Statistical Stream Reach Analysis of an Endangered Fish Species* examined physical stream reach metrics and the occurrence of leatherside chubs, an endangered fish species endemic the Great Basin of the West. This work was conducted in collaboration with the Wyoming Game and Fish and Brigham Young University.

OVERVIEW OF RESEARCH THEMES

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

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

The FY11 SREL research plan can be divided into three critical research areas:

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

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

ENVIRONMENTAL CHARACTERIZATION

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

Environmental Professional Training

Ken McLeod

SREL, in conjunction with ACP Engineering, planned and held a series of short courses during 2010 and 2011. The major course objectives were to broaden the environmental restoration knowledge of individuals who may not have formal training in specific areas and to provide refresher training for those with experience in this field. The series focused on technical topics appropriate for environmental professionals working on SRS projects.

18 topics were suggested at the beginning of 2010 and by year end 2011, 13 classes were held. Below is a breakdown of the details of each:

The first course, *Target Compound List/Target Analyte List at the SRS*, was a 2 day class taught by Walt Kubilius, SRNS. Each student earned 0.9 Continuing Education Units (CEUs). There were 22 in attendance.

The second course, *Steed Pond*, was taught by Karen Gaines, Eastern Illinois University and John Seaman, SREL. There were 18 in attendance, each earning 0.55 CEUs.

The third course, *Bioavailability and Toxicity of Metals*, was taught by Gary Mills, SREL. Each of the 26 attendees earned 0.4 CEUs. Because of the large number of participants, this class was offered a second time.

The fourth course, *Ecological/Biota Studies at SRS*, was taught by Larry Bryan, SREL. Eleven individuals attended the course and each earned 0.4 CEUs.

The fifth course, *LIDAR and Imagery*, was taught by Siobhan Scott, SRNS. Each attendee earned 0.3 CEUs, with 12 in attendance.

The sixth course, *Bioremediation and Environmental Microbiology*, was taught by J. Vaun McArthur, SREL. There were 12 in attendance, each earning 0.4 CEUs.

The seventh course, *Bioavailability and Toxicity of Metals*, was taught (for the 2nd time) by Gary Mills, SREL. Each attendee earned 0.3 CEUs and 11 were in attendance.

The eighth course, *PCBs*, was taught by Nancy Lowry, SRNS. Six people attended this course and earned 0.65 CEUs each.

The ninth course, *Geology of SRS/CSRA*, was taught by Laura Bagwell, SRNL. Each of the five attendees earned 0.5 CEUs.

The tenth course, *Southeastern Climate Change and Implications*, taught by David Stooksbury, UGA. Twenty attended the course and each earned 0.3 CEUs.

The eleventh course, *Archeology*, was taught by George Wingard, USC. Each attendee earned 0.3 CEUs and there were 18 in attendance.

The twelfth course, *Toxicity Reduction in Outfalls at the SRS*, was taught by Nancy Halverson, SRNL. There were 19 in attendance, each earning 0.2 CEUs.

The final course, *VZ Comml*, was taught by Greg Rucker, SRNS. Each of the 18 attendees earned 0.4 CEUs.

A student attending all 13 courses earned 5.6 CEUs over the 2 year period. A total of 198 student/session were provided and 85.0 CEUs were earned. Many of the students attended multiple courses. Most classes were held at the SREL Distance Learning Facility, however two were held at the 766-H training facility and one was held in 730-4B.

Developing an Automated Remote Stream Monitoring Network within the Tims Branch/Steed Pond System

John Seaman, Julian Singer, Shea Buettner¹, and Aaron Thompson²

¹UGA Graduate Student, ²UGA Assistant Professor, Crop and Soil Science Department

Conventional approaches to stream monitoring for contaminants generally consist of discharge estimates based on stream depth calibrated with manual stream profile data, and water samples collected at arbitrary intervals for chemical analysis. However, previous studies conducted by SREL in the early 1990s demonstrated that erosion and sediment transport was a significant vector for contaminant migration within the Tims Branch/Steed Pond (TBSP) system, while routine monitoring efforts observed little contaminant movement associated with base flow. Stream sampling to account for such discrepancies, however, can be logistically and financially prohibitive. Monitoring efforts are further complicated by the characteristic lag between precipitation, maximum stream discharge, and particulate mobilization that depend on the precipitation rate, stream location, watershed moisture status and other complex, interrelated factors which hinder the development of a standardized monitoring protocol that is applicable to all stream sites, even those within the same watershed. Therefore, a responsive monitoring system designed to collect discrete stream-water samples when triggered by any number of user-defined environmental parameters reflects a significant improvement

over conventional methods. To address such limitations, SREL is developing an automated stream monitoring system that responds to transient flow conditions in a manner designed to evaluate the impact of episodic precipitation events on the export of contaminants within TBSP. Furthermore, the proposed system makes use of SREL's FCC dedicated transmission frequency to remotely monitor and control system performance, providing real-time data acquisition capability.

Throughout 2011, SREL continued to conduct periodic stream discharge sampling for evaluating the impact of mobilized particulates on U and Ni export. In April 2011, SREL received final approval of a modified Site Clearance permit for installing the requisite equipment. SREL also worked in coordination with Radcon to redefine the soil contamination area adjacent to Steed Pond in an effort to simplify equipment installation and routine maintenance of the prototype monitoring system. Because of such logistical delays, the project timeline was extended without any increase in project budget. After Radcon approval, the prototype system, including a separate data transmission tower, was installed in June. During the first months of operation, the dedicated YSI water quality sonde malfunctioned and was returned to the manufacturer for repair. Even with the signal tower installation, data transfer was unreliable, and the transmission system was eventually returned to Campbell Scientific, Inc to repair moisture damage from an unseen leak. Of the chemical parameters being monitored (e.g., pH, turbidity, electrical conductivity, etc.), the oxidation reduction potential (ORP) appeared to be the least reliable, failing to maintain calibration over limited duration, i.e., 7 to 10 days.

With everything functioning properly, automated sampling data collected towards the end of 2011 indicated that the level of colloidal particulates mobilized during precipitation events no longer directly correlated with increasing TB stream flow. Initially, this lack of stream response was attributed to the re-vegetation of Steed Pond, which presumably limits the continued erosion of contaminated sediments. However, a closer look at stream profile data indicated that the base stream water depth had increased dramatically when compared to earlier in the year. This anomaly was recently attributed to the construction of a beaver dam about 100 meters downstream from the TBSP sampling location. Both manual and automated sampling will resume once the obstruction has been removed.

In addition to field efforts, a series of laboratory batch equilibration experiments were initiated using flood plain sediments from Tims Branch to evaluate the impact of changes in redox status on solid-phase U partitioning and the dispersion and mobilization of colloidal soil materials. Although U solubility generally decreases under reducing conditions, the resulting increase in pH and dissolution of soil aggregating agents, particularly Fe oxides, may potentially increase the mobility of colloid-bound U. For the initial batch experiments, soils were incubated in an anoxic environment to induce reduction. At predetermined time intervals, the soils were removed from the anoxic chamber and suspended colloidal materials were fractionated by centrifugation into a <100 nm and 100-1000 nm size separates that were then analyzed by inductively coupled plasma-optical emission spectrometry (ICP-OES). Although it appears that some Fe reduction has occurred, as indicated by the increase in the < 100 nm Fe

fraction, more particulate Fe and U were suspended with continued incubation. In contrast, incubation does very little to the level of soluble Ni or other common soluble cations (Ca, Mg, Na, etc.) present in either size separate. These results indicate that shifting redox conditions influence the mobilization/stabilization of colloidal materials in a manner that may impact contaminant fate and transport.

As part of the current project, SREL also worked in collaboration with SRNL (Dr. B. Looney) to refine sample digestion methods for determining the tin (Sn) concentration in water samples collected from Tims Branch. Water samples were collected at the air stripper feeding the A14 outfall, the location where Sn is added to the treated water to mitigate mercury (Hg) releases. The water samples were then acidified using a range of treatments (1-2% HNO₃ or HNO₃ combined with HCl) to determine total metal recovery by ICP-MS. Unfortunately, tin levels in all samples were considerably less than expected, i.e., < 10 pp, reflecting the poor solubility of Sn, even under extremely acidic conditions. SREL is currently working with SRNL to develop alternative sample digestion/stabilization methods to facilitate Sn analysis.

In April, Shea Buettner, the UGA grad student funded by this project, gave a presentation entitled "Vectors for Metal Transport in the Tim Branch/Steed Pond Watershed on the Savannah River Site" at the biannual Georgia Water Resources Conference in Athens GA, highlighting his work on this project. In July, a presentation entitled "Automated Responsive Environmental Monitoring" by J.C Seaman and J.H. Singer was given to the ACP Tech Panel.

Contaminant Bioaccumulation and Trophic Relationships in Beaver Dam Creek Biota from the D-Area Coal Combustion Waste Plume

Dean E. Fletcher, Angela H. Lindell, and J Vaun McArthur

On the Savannah River Site, coal combustion waste (CCW) has been produced by the D Area Power Plant since the early 1950's and stored in basins that discharge into Beaver Dam Creek (BDC). Beaver Dam Creek is a highly modified system. The headwaters were channelized during the early infrastructure construction of the SRS between 1951 and 1956 to transport effluents from the D Area Power plant and associated ash and coal pile runoff basins to the Savannah River. Our study sought to develop an understanding of how and to what extent aquatic organisms in BDC are at risk from contaminant bioaccumulation of CCW constituents. Furthermore, we are assessing biological and physical factors influencing contaminant accumulation in aquatic organisms inhabiting BDC. Through detailed comparative studies we are establishing an information base to aid in the design of future studies, interpret existing data, and support site-wide risk models. Stable isotope analyses ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) were employed to establish trophic relationships among study organisms. Trace element analyses (As, Ba, Be, Cd, Cr, Cu, Hg, Ni, Pb, Sb, Se, Tl, V, Zn, Cs and Sr) assessed contaminant bioaccumulation. A total of 517 trace element samples and 390 stable isotope samples were analyzed in FY11. Field collections were made at two sites in Beaver Dam Creek headwaters that differ in hydrologic regimes to make a longitudinal

comparison of accumulation. We are comparing taxa that differ in trophic position, feeding habits, habitat use, size, and longevity. Within taxa, the influence of size, sexual maturity, reproductive activity, and ontogenetic trophic shifts are being accounted for. Inclusion of two invertebrate herbivores that differ significantly in habitat use and feeding behavior is exploring a baseline of the introduction of materials into the food web from primary producers and is a critical component of stable isotope studies. Further movement through or introduction of contaminants into the food web is being addressed in a comparison of eight dragonfly genera that differ in habitat use, particularly in reference to their exposure to sediments. Additionally, detailed studies are comparing three species of bullhead catfish (*Ameiurus sp.*) that also differ in morphology and habitat use. Inclusion of four top level predatory fish is allowing comparison of elements entering the food web with those in the top predators. Exploratory stable isotope analyses compared isotopic signatures of surface sediment, biofilm, and seston with the basal herbivores and trace element analyses examined sediments and biofilm.

1) A Comparison of Two Lotic Herbivores as Bioindicators of Trace Element Bioaccumulation

One objective of our study was to compare two herbivorous invertebrates as bioindicators of bioavailable contaminants in a lotic system. While establishing a baseline of the introduction of materials into the food web from primary producers is a critical component of stable isotope studies, it also provides valuable information on the potential movement of contaminants into the food web. We examined two invertebrate herbivores with highly divergent habitat use, life cycles and feeding behavior. The Asiatic clam *Corbicula fluminea* lives shallowly buried in the substrate and is known to both filter feed particles from water and scrape deposited materials from surface sediments. The heptageniid mayfly *Maccaffertium modestum* is a dorsoventrally flattened mayfly adapted to cling to and scrape biofilm from the surface of submerged objects such as wood debris. *Corbicula* and *Maccaffertium* were collected from two sites on Beaver Dam Creek. Stable isotope analyses ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) indicated that *Corbicula* and *Maccaffertium* occupy similar trophic positions. However, the $\delta^{13}\text{C}$ of both *Corbicula* and *Maccaffertium* differed between sites indicating a spatial difference in resource use or C content of assimilated resources. *Maccaffertium* was also more enriched with $\delta^{15}\text{N}$ at the downstream site. Bioaccumulation of elements differed between both species and sites. *Corbicula* accumulated higher concentrations of several elements (e.g. Hg, Cs, Sr, Se, As, Be, Cu) than *Maccaffertium*--especially in the upstream site. However two notable elements, Pb and V, accumulated substantially higher in *Maccaffertium*. *Corbicula* inhabiting the downstream site accumulated lower concentrations of elements such as Be, Ni, Cu, Zn, As, Se, and Hg than those in the upper site. Differences in element accumulation between sites were not significant in *Maccaffertium*.

2) Contaminant Bioaccumulation and Trophic Characterization of Top-Level Predatory Fishes

Both regulatory and ecological considerations warrant evaluation of contaminant bioaccumulation in top-level predatory fishes as an essential component of aquatic

assessment efforts. However, variation within and among syntopic top predators is often not well understood. We are comparing trophic position and the magnitude of contaminant bioaccumulation in four top level highly predaceous fishes and evaluating variability within each taxa. Taxa include mid-water dwellers as well as those more closely associated with the bottom or cover. These taxa also differ in mouth structure which strongly influences feeding behavior. Channel catfish head and mouth dimensions were measured to characterize allometric growth potentially important to trophic level. Examination of muscle, liver, and gonad samples allow a more comprehensive assessment of exposure and risk of contaminant accumulation. Gut contents were archived for potential future examination. Fish were stratified across the broadest size ranges available from two sites on Beaver Dam Creek. We analyzed samples from broad size ranges of *Ictalurus punctatus* (channel catfish) and *Micropterus salmoides* (largemouth bass) that span from small juveniles to large adults, 99 to 700 mm standard length (SL) and 97 to 480 mm SL respectively. Only five bowfin (*Amia calva*) were analyzed and ranged from 367 to 506 mm SL. *Lepisosteus osseus* (longnose gar) range from 444 to 962 mm SL. Additional comparison of these predators to two herbivorous invertebrates, the Asiatic clam and a heptageniid mayfly, allowed evaluation of elements entering the food web relative to those bioaccumulating in the top-predatory fishes.

Based on stable isotope analyses, trophic position differed between species and varied within species. On average largemouth bass and longnose gars held a trophic position above channel catfish. As expected, the herbivorous invertebrates were lowest. Some elements such as Hg and Cs appeared to biomagnify in the predatory fish muscle with concentrations increasing with trophic level. Other elements such as V, Cd, Ba, Ni, and Pb accumulated in the herbivorous invertebrates, but were nearly absent from large predatory fish muscle. Consequently these elements were entering the food web, but not being transferred to large predator muscle tissue. However, further analyses found these same elements accumulating in the liver that had effectively sequestered them from accumulation in the muscle. This reduces human health risk, but given the size of the livers and the elevated concentrations, may represent an ecological risk and requires further investigation. Element concentrations in the fish livers were at times quite high. For example; Hg concentrations in the longnose gar livers averaged 59 ppm (1.3 - 626). Additionally bioaccumulation of elements in fish muscle tissue differed between and varied within fish species. Size, trophic position, and at times sex influenced patterns of bioaccumulation. Moreover, whether some elements such as Hg and Se exceeded human or ecological risk thresholds or background concentrations not only differed between species, but varied with size within some species.

3) Contaminant Bioaccumulation and Trophic Characterization of Three Bullhead Species (*Ameiurus*)

Stream and river systems are commonly inhabited by multiple species of the catfish genus *Ameiurus*. These fish generally referred to as bullhead are also prevalent among the catch of local anglers. Bullhead species are commonly incorporated into contaminant studies without rigorous consideration if risk varies among congeners. We provide a comparison of species that morphologically exhibit differences in mouth

position and body form and behaviorally tend to segregate based upon habitat use. Thus actual exposure and uptake of contaminants may differ among species. Moreover, since some aspects of their natural history such as diet and habitat use may undergo ontogenetic shifts, evaluation of variation not only between species, but within a species is essential. We compared trophic position and contaminant bioaccumulation in three species of bullheads (*Ameiurus* sp.) which included flat bullhead (*A. platycephalus*), snail bullhead (*A. brunneus*) and white catfish (*A. catus*). Fish were stratified across the broadest size ranges available from two sites on Beaver Dam Creek. No difference in trophic position ($\delta^{15}\text{N}$) or carbon sources ($\delta^{13}\text{C}$) between species within a site were observed. However, statistical comparisons were influenced by greater variability occurring in white catfish (both sites) and, flat bullhead and snail bullhead from the upstream Site A. This variability could not be attributed to body size, thus indicating either higher variety of prey ingested or more variable $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ within the prey. Fewer elements were bioaccumulating in the bullhead muscle than that of the large predatory fishes with many elements (Be, V, Cd, Sb, Tl, Pb, and As) frequently below detection limits. Some trace elements such as Zn and Cu accumulated in the bullhead muscle, but did not differ between species or sites. However, bioaccumulation of other elements (e.g. Se, Hg, Ba, Sr, and Cs) differed among species and varied within species. Which species had the highest or lowest concentration differed among elements. Element concentrations were often negatively correlated with size resulting in the smallest individuals having the highest concentrations. This pattern may have an ameliorating effect of this on human risk, but put predators of the smaller fish at greater risk. In fact, size did at times influence whether concentrations exceeded or approached ecological or human risk thresholds. When bioaccumulated concentrations differed between sites, concentrations were always higher at the upstream site suggesting reduced bioaccumulation at the downstream location. As observed in predatory fish, bullhead livers effectively sequestered many elements from incorporation into muscle tissue.

4) A Comparison of Eight Dragonfly Nymph Genera as Bioindicators of Trace Element Bioaccumulation.

Dragonfly nymphs (Odonata) are relatively long-lived predators in aquatic systems. These diverse organisms employ several different feeding strategies and habitat use that provide differential exposure to contaminants through sediments and food resources. Overall the differences in body form and habitat use among genera of aquatic nymphs of odonates make them attractive indicators of contaminant accumulation. Ecological habits range from those that burrow in the stream sediment through those that sprawl across the surface of it, to those that cling to suspended wood debris or root masses. Body form ranges from long and slender to those with broad, palmate abdomens. The morphology of the jaw-like labium used in prey capture also differs among taxa. Their predaceous behavior also renders them excellent integrators of contaminants in both space and time. Additionally odonate nymphs have relatively ubiquitous distributions making them a potential indicator in diverse habitats ranging from streams to isolated wetlands. Their relatively large size for an invertebrate also reduces collecting effort compared to some smaller taxa. Length frequencies were used to determine size categories. Examination of the nymphs confirmed that size

groups corresponded to distinct instars. Stable isotope and trace element analyses were in progress at the close of FY11 and will be completed in FY12.

In addition to the analyses previously discussed, we performed an exploratory analysis of a subset of sediment samples from our archive. Trace element analyses revealed high spatial variability, even within a site. Local habitat characteristics influenced contaminant distributions within a site. Surface biofilm was collected from each site for stable isotope analyses and exploratory comparison to the isotopic signatures of the basal herbivores. Trace element analysis results for the biofilm samples will be analyzed in FY12.

Stream System Field Condition Survey

Dean E. Fletcher, Garrett K. Stillings, and Christopher D. Barton¹

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Today a broad array of disturbances ranging from pre-Savannah River Site land use to contemporary industrial activities shapes the local landscape. Pre-SRS land use subjected streams to extensive disturbances including cattle grazing, timber harvest, channelization, and intensive agriculture. Lasting effects are evidenced by deep erosion gullies along stream valleys and incised or rerouted stream channels. Riparian corridors were fragmented by numerous dams and levees; remnants of many remain. It is becoming increasingly recognized that such legacy impacts can have long lasting effects on U.S. stream systems and at times interact synergistically with more recent disturbances. Construction of the original SRS infrastructure was a monumental task. Networks of roads and railroads, power plants, nuclear reactors as well as production and waste handling facilities were completed in only five years (1951-1956). Such intensive construction activity consequently impacted many SRS streams. Subsequent removal of land from agriculture has allowed regeneration of forests now managed by the USDA Forest Service. Present day stormwater runoff and effluent releases from SRS industrial areas and the consequent erosion and sediment deposition continue to alter some streams. Some channels were reconfigured directly (e.g. Beaver Dam Creek) to accept high volume industrial water releases, or indirectly restructured by the discharge of reactor water (e.g. Pen Branch and Fourmile Branch). Both active and abandoned structures may continue to fragment streams, alter hydrology, and provide nick points for beaver impoundment. Characterizing SRS streams will allow us to identify risks of legacy and recent disturbances as well as identify potential contaminant sources including waste sites, outfalls, and contaminated aquifers in relation to surface flow paths and seep zones. A collaborative effort among Savannah River Nuclear Solutions-Area Completion Projects, University of Kentucky, USDA Forest Service, Savannah River National Laboratory, and Savannah River Ecology Laboratory has been undertaken to establish a baseline of wetland impacts to SRS headwater streams and support SRS natural resource stewardship through a three level assessment program.

Level 1 Surveys--Level 1 assessments includes a broad scale survey of potential stream disturbances and hydrological characterization. This effort requires examining

aerial photos (1938-2010), LiDAR imagery, existing GIS data, and maps (1943 to current), as well as a field survey taken by walking entire stream drainages to identify disturbances such as flow impediments, erosion, or channelization. Documented flow impediments include active and abandoned dams, road and railroad crossings, and utilities cuts. Severe erosion, incision, and sedimentation from historical and present land use, outfalls, roads, railroads, utilities cuts, and abandoned borrow pits are being recorded. Pathways of outfalls and runoff from industrial areas reaching perennial or intermittent streams are being mapped. Disturbances are being placed into a temporal context to establish whether of pre-SRS or SRS origin. Perennial and intermittent streams are being field delineated and detailed maps created. Basin and valley characteristics are being measured for each tributary. Accuracy of stream mapping and basin characterizations are enhanced using the 2009 LiDAR imagery. Disturbed stream reaches suitable for restoration or enhancement are being identified. We are summarizing our data in GIS layers, text descriptions, and detailed tables.

Level 2 Assessments--We are examining the effects of stream alterations in a subset of those identified in the Level I surveys. Stream hydrology, geomorphology, and habitat availability at the reach level are being assessed. A total of 55 sites ranging from the least disturbed to severely altered are included. The temporal disturbance gradient ranges from pre-SRS to current. Our protocol draws upon field observations and measurements as well as GIS data. In addition to the Level I basin characteristics, features such as reach sinuosity, gradient, valley width, and valley depth are being measured. Water quality parameters are being measured at each site. Data such as canopy coverage, stream and channel dimensions, bank stability, bank vegetation coverage, substrate composition, bottom firmness, and water velocities are collected across transects. Additionally, habitat structure such as root masses, bank under cuts, aquatic macrophyte coverage, and coarse woody debris are being quantified.

Level 3 Assessments--The recently initiated Level 3 assessments are further evaluating a select subset of stream reaches by measuring additional hydrology, physicochemistry, geomorphology and biological features of each study reach. A thorough stream evaluation will allow us to prescribe, implement and monitor enhancement and restoration efforts. Additional channel characterization will include standard topographic surveying procedures to determine the rate of incision/filling. In-channel standing crop of coarse particulate organic matter will be measured periodically. Litter decay and invertebrate colonization is being determined using a standard litter bag technique. Additional macroinvertebrate community surveys are being conducted. To evaluate stream discharge patterns, monitoring stations have been established in each study reach. Monitoring stations are simultaneously and continuously recording stream temperature and stage height with the latter converted to discharge via stage-discharge rating curve. Water chemistry and suspended sediment samples are being collected at each monitoring station for select storm water events. Water quality parameters such as temperature, pH, dissolved oxygen, electric conductivity, and turbidity are measured in the field. Automatic water samplers equipped with a flow actuator (programmed to begin sampling in response to a rain event) will be installed to provide samples for laboratory evaluations. Samples will be analyzed for

alkalinity, major nutrients, total organic carbon, dissolved organic carbon, and trace elements (Ba, Be, As, Se, Tl, V, Fe, Mn, Pb, Sb, Cu, Zn, Cd, Cr, Ni). Turbidity and total suspended solids will be used to characterize suspended sediment levels. Mineralogical and elemental characterization of sediment cores will provide insight into the source of sediments and potential contamination. Over-story, mid-story and ground cover riparian vegetation layers will be characterized in each reach.

Progress To Date--Level 1 surveys have been focusing on the entire Mill Creek, Meyers Branch and McQueen Branch drainages, 19 Upper Three Runs (UTR) tributaries, six Tinker Creek tributaries and the included main stem, and the Pen Branch drainage above Indian Grave Branch. These drainages include 71 tributary basins, 164 perennial links, and over 130 km (80 miles) of perennial stream. Fieldwork for these streams was completed in FY11. Scientific products in progress include numerous GIS data layers delineating features such as perennial, intermittent stream lines, areas of sheet flow, outfall and runoff routes, head springs and seeps, and ditches and channelization. Additional layers provide the locations of structures representing potential flow impediments such as active and abandoned dams, levees and crossings, rip rap, and check dams. Data presentation tables were completed for all drainages. These include tables of 19 basin characteristics, flow impediments tabulated by stream type and time of origin, and comparisons of our field collected data to USGS blue-lines. Detailed appendices providing details of potential flow impediment structures in Mill Creek, Meyers Branch, Tinker Creek, and Pen Branch were completed in FY11. Upper Three Runs will be completed in FY12. Final report preparation began in FY11 and will be completed in FY12. Text provides a general basin description, description of pre-SRS condition, and detailed descriptions of SRS disturbances such as outfall/runoff routes and flow impediments. Data collection for Level 2 assessments were collected for 55 sites distributed across the study systems. Data processing is in progress. Level 3 assessments were initiated.

Assessing the Ecological Health of the D-Area Ash Plume Wetland

Brian Metts, Tracey Tuberville, David Scott, Kurt Buhlmann and William Hopkins¹

¹ Virginia Tech

Coal-fired facilities have been in operation on the SRS since the early 1950s. All coal combustion waste (CCW) contains a complex mixture of metals, as well as naturally occurring radionuclides, that become concentrated in the fly ash during the combustion process. Public concerns about exposure to metals and naturally occurring radionuclides are understandable, but in reality little is known concerning the biological effects of chronic exposure to mixed waste (metals/metalloids/low level radiation). Determining the long-term ecological risks from chronic exposure to contaminant mixtures is among the major challenges facing the environmental sciences.

At the D-Area power generating facility adjacent to the Savannah River, CCW was sluiced into fly ash settling basins <200 m from a floodplain forest and associated wetlands. In the early 1970s, CCW from one of the primary ash basins (hereafter

referred to as "Ash Basin," or AB) was released onto the bottomlands, resulting in a CCW plume that extends over 40 ha of floodplain at depths up to 2.7 m, including a natural wetland (hereafter referred to as "Ash Plume Wetland," or APW). The D-Area coal plant is still in operation and the AB continues to receive fresh sluiced CCW. The APW has not received CCW discharge for >35 years and the impacted area (including the wetland) has become revegetated and a thin organic soil layer has developed. SREL has conducted research in the D-Area system for >20 years, including investigations to: characterize the severity and spatial extent of the spill, monitor water and soil chemistry parameters, determine body burdens of contaminants in a wide variety of taxonomic groups, and conduct experimental manipulations to determine the biological effects of contaminants on aquatic organisms.

Most SREL research has focused on the active D-Area settling basins where contaminant levels are highest rather than on the floodplain where natural attenuation has occurred. Research in the AB has demonstrated that amphibians and other wildlife inhabiting the basins and discharge streams can accumulate elevated concentrations of trace elements [e.g., arsenic (As), selenium (Se)] that cause adverse effects on survival, growth and development, energy acquisition and allocation, behavior or performance, and recruitment. Metal levels in the CCW associated with the APW are generally lower than levels in recent ash. Active CCW basins may be ecological traps, but the ecological status of floodplain spill sites is unknown. For example, the contaminated D-Area floodplain site 35 years post-release appears to have an amphibian community diversity (19 documented species) and composition comparable to a nearby uncontaminated reference site, and the surrounding recovering forest exhibits species richness and basal area typical for a forest of its age.

The floodplain habitat in D-Area is critical to many amphibian species which rely on both aquatic and terrestrial habitats to complete their complex life cycles. Amphibians have been the subjects of numerous ecotoxicology studies, which have demonstrated adverse effects of CCW on amphibians. Exposure to trace metals found in CCW may decrease survivorship of larvae, increase time to metamorphosis, decrease size at metamorphosis, and alter offspring viability. Due to their biphasic lifecycle, many amphibians are susceptible to contaminants in both the aquatic and terrestrial life-stages. In addition, amphibians represent a large portion of the ecosystem biomass. One study at our reference site found that >362,000 individuals (1400 kg) of 15 species survived through metamorphosis and emigrated from the wetland in a single season. Consequently, if environmental contaminants negatively affect amphibian populations, then the whole ecosystem can be impacted.

Through a series of mesocosm experiments, we investigated the effects of CCW on hatching success, larval survival, larval growth and performance of southern toads [*Anaxyrus (Bufo) terrestris*]. Mesocosms were constructed to simulate Ash Basin (AB), Ash Plume Wetland (APW), and uncontaminated (reference) conditions. The suite of experiments was designed to distinguish among effects due to maternal, paternal, and

dietary exposure. Combined, these experiments indicated that female toads accumulated As, Cu, Ni, Se, Sr, and V in the contaminated sites, and transferred Cu, Ni, Se, and Sr to their eggs. Maternal exposure alone contributed to reduced clutch size and hatching success, resulted in slower larval growth rates, delayed metamorphosis, lower survival to metamorphosis, smaller size at metamorphosis, and reduced performance of surviving metamorphs. Although some of the negative effects on *Bufo* offspring were less pronounced under conditions simulating APW compared to AB, much of our results indicate that the sediment aging and accumulation of organic material that has coincided with the vegetation succession in the APW natural wetland have not resulted in attenuation of the biological effects of contaminated CCW sediments on amphibians.

All experiments have been completed, samples analyzed and summarized in the form of draft manuscripts. Results of the first mesocosm experiment have been accepted for publication in the journal *Environmental Pollution*. The citation is provided below. Two additional manuscripts are planned for submission during FY12.

Manuscript in press

Metts, B.S., K.A. Buhlmann, D.E. Scott, T.D. Tuberville, and W.A. Hopkins. *In press*. Interactive effects of maternal and environmental exposure to coal combustion wastes decrease survival of larval southern toads (*Bufo terrestris*). Accepted in: *Environmental Pollution*, pending revision.

Update of the Wildlife Literature Survey (WLS) GIS Database

Linda Lee

The Wildlife Literature Survey is a GIS-based literature survey designed to assist in ecological risk assessment on the SRS. The database includes relevant information on SRS wildlife, including 75 target receptor species, gleaned from ecological studies conducted on the SRS. Geographical data for receptor species are stored in a GIS.

During FY11 a new PI was brought on board to lead the project. Since that time SREL has added over 1,100 records (and five shapefiles) to the database. In FY11 SREL expanded the publication source beyond SREL and Forest Service reprints through the use of electronic search tools that did not exist when the WLS was created. SREL has also made other improvements in FY11, including data cleaning of scientific and family names in both the database and species list, and standardization of species codes. Version 20 was completed by the new PI and released in April. Version 21 was released at the end of the FY11.

Support of the SRS Trophic Transfer Modeling Effort

Larry Bryan

Significant effort has been expended by Savannah River National Laboratory (SRNL) and the Area Closure Project (ACP) group to develop a model to assess ecological risk

on DOE's Savannah River Site (SRS), including one contaminant exposure model to determine potential doses to endpoint receptor species (e.g. river otter and belted kingfisher). The model evaluates risk within geographic areas according to their location in integrator operable units (IOUs). To date, this modeling effort has been hindered by a lack of data from some areas and erroneous data for some areas. Our task was to develop a database of appropriate data from historical and ongoing SREL studies, assist SRNL in integrating this data into the model, and identify data gaps to be resolved to more effectively assess risk within components of the various IOUs. A second phase of this project includes collections and analyses to close these data gaps, resulting in a more efficient model.

SREL has identified databases containing appropriate data from Tims Branch, Fourmile Branch, Lower Three Runs, and lower Steel Creek. The databases for these IOUs contain concentrations of multiple analytes in multiple species of biota and were provided to ACP/SRNL last year.

Data gaps identified by ACP/SRNL include upper Steel Creek, Pen Branch (upper, middle and lower sections), Indian Grave Branch, Castor Creek, lower Meyers Branch, SRS Pond 2, and SRS Pond A. All sites have been sampled and aquatic biota (fish and crayfish) from these sites (25-80 individuals per site/section) have been analyzed for a suite of elements: As, Cd, Cu, Hg, Mn, Pb, Se, U, and Zn. The elemental concentration data from these sites are being compiled and summarized and a report of the findings provided to ACP/SRNL in December of 2011.

Characterization of Contaminant Levels in the P-Area Wetland System

Biota: David Scott, Larry Bryan, David Kling, and Stacey Lance

Soils: John Seaman and Julian Singer

SREL researchers have a strong history of ecological risk assessment, including the effects of coal combustion wastes (CCW) in the environment. Coal-fired facilities have been in operation on the SRS since the early 1950s and there are multiple associated ash basins, as well as areas of CCW spills. Acceptable clean-up and management of CCW impacted sites on the SRS requires knowledge of contaminant levels and quantification of the diversity and success of the biota inhabiting these areas. Most of SREL's prior research on CCW was conducted in D-Area, where CCW is sluiced into ash settling basins and was also deposited in an adjacent floodplain wetland (the D-Area Ash Plume Wetland, DAPW). The makeup of CCW varies widely due to differences in parent coal composition, combustion technology, and disposal method. As a consequence, the CCW in the D-Area system may differ from CCW deposits elsewhere on the SRS – it has not been established whether data from one basin can be applied to another. In our study we sampled the soil and biota in the P-Area system, where an historic release from the P-Area ash basin deposited CCW in a nearby wetland (Fig. 1). The P-Area Ash Plume Wetland (PAPW—Bay 96 on the SRS Carolina bays layer) appears to function as a seasonal wetland, and may be similar in key respects to the DAPW. The DAPW supports 19 species of amphibians and numerous invertebrate species. The bioaccumulation of trace elements in the D-Area

system, as well as their biological effects, has been documented in recent years. Whether similar levels and effects exist in the PAPW requires new site-specific data. This report summarizes our findings from the P-Area system; in the future we will compare the results from P-Area to similar studies in the D-Area system.

Several constituents of potential concern (COPC) to aquatic receptor species were identified in preliminary PAPW soil surveys and SRS ecological risk models. In FY-2011

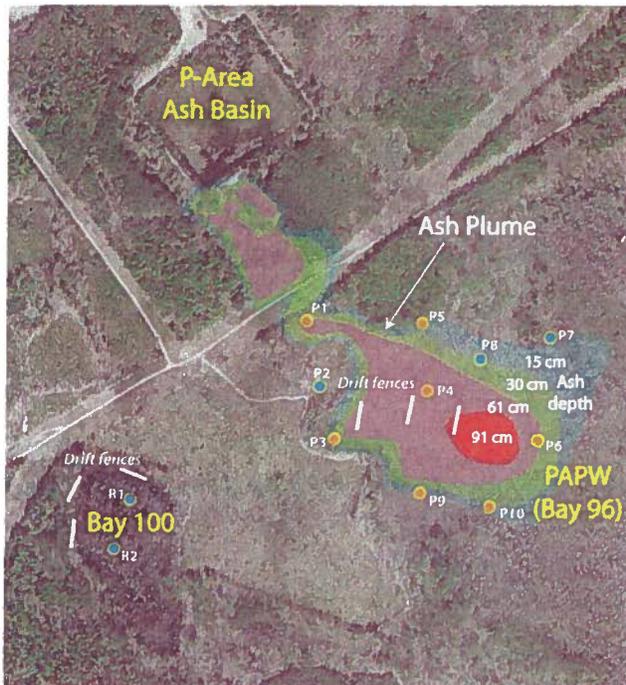


Fig. 1. Locations of sampling sites in the P-Area system. Portions of Bay 96 contain coal combustion wastes to a depth of ~91 cm—this ash plume is referred to as the P-Area Ash Plume Wetland (PAPW). Biota and soils sampled in the PAPW were analyzed for metals and compared to samples from an uncontaminated reference site (Bay 100) located 410 m to the west. Sampling locations for biota (drift fences) and soil cores are indicated as marked. Blue soil coring locations indicate sites where little or no evidence of ash deposition was found. Ash plume contours and location based on original coring and mapping conducted by Savannah River Nuclear Solutions Area Completion Project personnel.

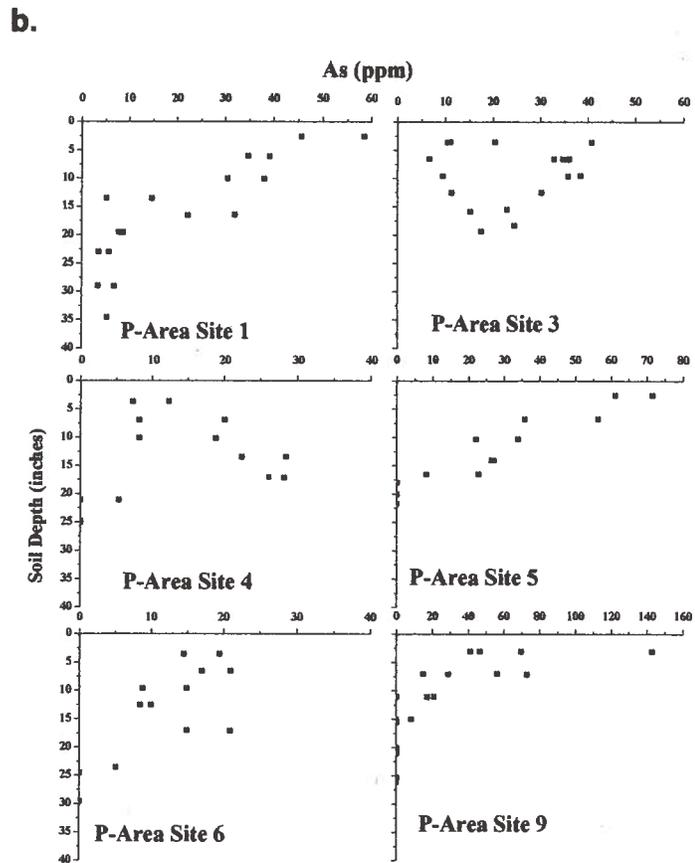
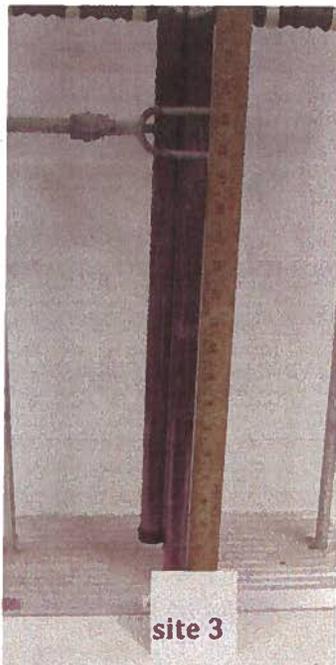
we evaluated the COPC in PAPW soil and wetland biota. In consultation with the Savannah River Nuclear Solutions Area Completion Project (SRNS-ACP) personnel, soil sampling locations were identified within the PAPW ash field and at the control site (Bay 100). Soil sampling locations were surveyed on 2 June 2011, and soil coring began on 29 June 2011 and was completed on 17 July 2011. We used a manual slide-hammer coring device to collect intact soil cores (Fig. 2a; Site P3) from the PAPW (N = 10 locations) and reference site (N = 2 locations). The intact cores were transferred to the lab and photo archived (Fig. 2).

To sample biota, in April-May 2011 we installed three 30-m partial drift fences at both the PAPW and Bay 100 (Fig. 1). Fences were checked three times per week from May-July, closed for August when no amphibian activity was occurring, and re-opened from mid-September until 31 October. We collected target species of amphibians, reptiles, small mammals, insects, spiders, millipedes, and centipedes for metal analysis. We used ICP-MS to analyze tissues, and determined concentrations of As, cadmium (Cd), chromium (Cr), Cu, mercury (Hg), nickel (Ni), Pb, Se, strontium (Sr), vanadium (V), and Zn in the biota (whole body). These estimates will be compared to levels documented in the same taxa in the DAPW, for which there is additional biological effects information. The tissue concentrations found in PAPW organisms can also be used to refine risk

assessment estimates for aquatic and terrestrial receptor species (i.e., green heron, raccoon, shrew, robin).

A minimum of two intact soil cores (e.g., Fig 2a) was collected at each sampling site, and a perched water table was not encountered at any of the sampling locations. The soil cores (N = 27 total cores) collected from the 12 sampling locations were removed from the plexiglass sleeve, and material representing the upper 30 cm of soil at each coring location was composited to create a "bulk surface soil" for subsequent chemical extraction to determine COPC levels. In many cases an obvious layer of plant litter was present on the soil surface. This material was removed and saved prior to collection of the composite soil sample. The bulk soil composites were digested using standard methods, and the COPC were analyzed using inductively coupled plasma-mass spectrometry (ICP-MS; EPA Method 6020A).

Fig. 2. a. Intact soil column from site P3 in the PAPW. **b.** Arsenic levels as a function of depth at six of the ten sampling locations within the P-Area coal ash plume.



The remaining core materials were separated into depth-discrete samples based on visual appearance, texture, and soil diagnostic horizonization. Samples representing each depth-discrete soil and/or ash deposition layer were air-dried, homogenized using an agate mortar and pestle, then loaded into x-ray fluorescence (XRF) sample holders. These depth-discrete samples were analyzed for elemental composition by XRF (EPA Method 6200). Based on XRF analysis, As and Se levels were greater in soil cores collected from the PAPW compared to the Bay 100 control site. Excluding the samples from locations with no apparent ash deposition (Sites 2, 7, and 8), As concentrations generally decreased with increasing sampling depth (Figure 2b).

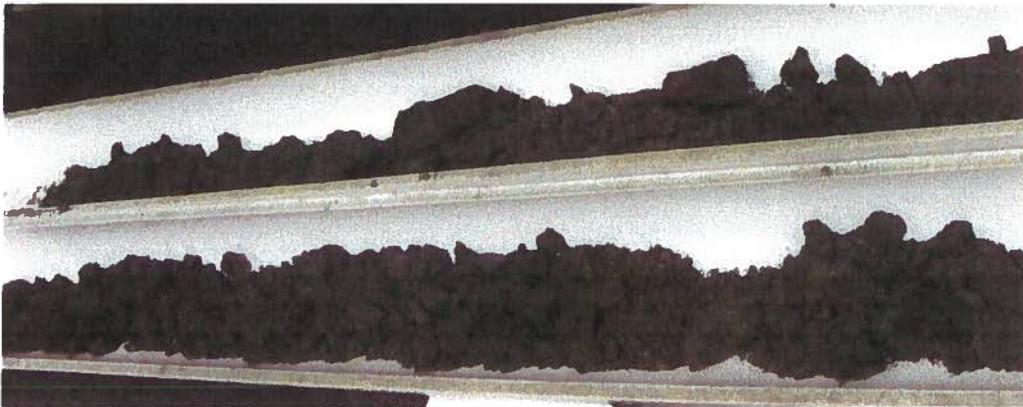
As noted above, soil cores from three of the ten sampling locations at Bay 96 showed little or no evidence for ash deposition (i.e., the locations were outside the area of the ash plume). For example (Fig. 3), Site 2 shows no evidence of CCW deposition, and appears to be outside the boundary of the wetland Bay 96, lacking diagnostics characteristics indicative of extended periods of saturation. In contrast, remnants of the CCW deposit are obvious at PAPW Site 9 with a buried plant litter horizon as well. In addition, the soil profile shows indications of extended periods of saturation. Arsenic and Se levels for the three soils outside the ash deposition zone tended to be below the method detection limit (MDL), even lower than As and Se levels observed for the two control samples from Bay 100. Mercury levels at both sites were below the MDL for ICP-MS.

Seven species of amphibians and 5 species of reptiles were found at the PAPW; comparable numbers (8 amphibian species, 5 reptile species; Table 1) were observed at the reference site. We recorded 145 amphibian captures at the PAPW, and 334 amphibian captures at Bay 100; the southern toad (*Bufo terrestris*) was the most common species at both wetlands. Averaged across taxa (i.e., lumping all samples from each location together), there are significant location differences (Fig. 4) between tissue concentrations [whole body; multivariate analysis of variance test (MANOVA); $\lambda = 0.458$, $F_{11,102} = 10.99$, $P < 0.0001$]. The univariate tests showed that location differences were due to significantly elevated Cu, As, Se, Sr at the PAPW, and elevated Hg at Bay 100. Higher Hg at the reference site may be an indicator of its longer hydroperiod. Se and Sr were more than twice as high at the PAPW compared to Bay 100 (Se: $5.41 \pm 0.42 \mu\text{g/g}$ dry mass at the PAPW vs. $2.10 \pm 0.08 \mu\text{g/g}$ at Bay 100; Sr: $222.3 \pm 29.9 \mu\text{g/g}$ vs. $95.2 \pm 6.7 \mu\text{g/g}$). Species groups also appear to differ in their bioaccumulation patterns of certain elements (Fig. 5).

Fig. 3. Representative soil cores from outside the wetland boundary and ash plume (Site 2) and within the plume (Site 9), with metal concentrations (mg/kg dry mass) from 12 locations.



PAPW Site 2



PAPW Site 9

Soil Digestion Results

Site	Depth Interval cm	V	Cr	Ni	Cu	Zn	As	Se	Sr	Cd	Hg	Pb	U
		mg/kg											
1	0-30	57.60	28.30	29.58	102.10	78.49	41.24	19.92	103.79	<MDL	<MDL	40.39	6.09
2	0-30	3.03	2.27	1.06	0.87	2.00	<MDL	<MDL	<MDL	<MDL	<MDL	8.40	<MDL
3	5-35	28.68	13.16	13.38	29.21	30.44	24.73	8.81	59.71	<MDL	<MDL	15.51	1.90
4	3.75-33.75	17.23	7.77	8.78	18.61	19.70	12.54	6.07	32.50	<MDL	<MDL	8.95	1.27
5	0-30	39.45	18.95	21.78	73.26	52.41	38.45	11.95	85.41	<MDL	<MDL	28.80	3.48
6	5-35	27.41	9.82	11.64	22.62	27.44	9.77	6.65	62.22	<MDL	<MDL	9.45	1.77
7	5-35	2.26	2.17	<MDL	0.74	<MDL	<MDL	<MDL	1.41	<MDL	<MDL	7.53	0.65
8	0-30	7.60	4.14	1.96	2.43	4.40	<MDL	<MDL	1.44	<MDL	<MDL	8.42	0.69
9	2.5-32.5	11.09	6.21	4.80	12.89	19.52	31.80	<MDL	23.45	<MDL	<MDL	13.59	1.51
9	2.5-32.5	39.51	20.51	19.96	50.01	53.68	27.21	13.66	81.19	<MDL	<MDL	29.91	2.97
10	0-30	18.74	10.12	12.11	25.26	27.91	14.88	<MDL	65.17	<MDL	<MDL	18.09	1.52
Dunbarton Bay Control Sites													
1	0-30	24.73	16.18	12.28	36.44	27.59	3.74	11.31	5.62	<MDL	<MDL	17.80	1.76
2	0-30	21.94	12.54	8.41	27.09	17.41	3.94	12.87	4.40	<MDL	<MDL	17.46	1.91

Table 1. Amphibian and reptile species list for the PAPW and Bay 100 (Apr 27 - Oct 30, 2011)



Bufo terrestris (southern toad), the most common species at both sites



Scaphiopus holbrookii (spadefoot toad), inhabits uplands at both wetlands

Species	PAPW	Bay 100
Amphibians		
<i>Ambystoma opacum</i>	X	X
<i>A. talpoideum</i>		X
<i>Bufo terrestris</i>	X	X
<i>Gastrophryne carolinensis</i>	X	X
<i>Plethodon glutinosus</i>	X	X
<i>Rana clamitans</i>	X	X
<i>R. sphenoccephala</i>	X	X
<i>Scaphiopus holbrookii</i>	X	X
Reptiles		
<i>Anolis carolinensis</i>	X	
<i>Coluber constrictor</i>		X
<i>Diadophis punctatus</i>	X	
<i>Eumeces fasciatus</i>	X	
<i>Lampropeltis getulus</i>		X
<i>Sceloporus undulatus</i>		X
<i>Scincella laterale</i>	X	X
<i>Storeria dekayi</i>	X	
<i>Virginia valeriae</i>		X

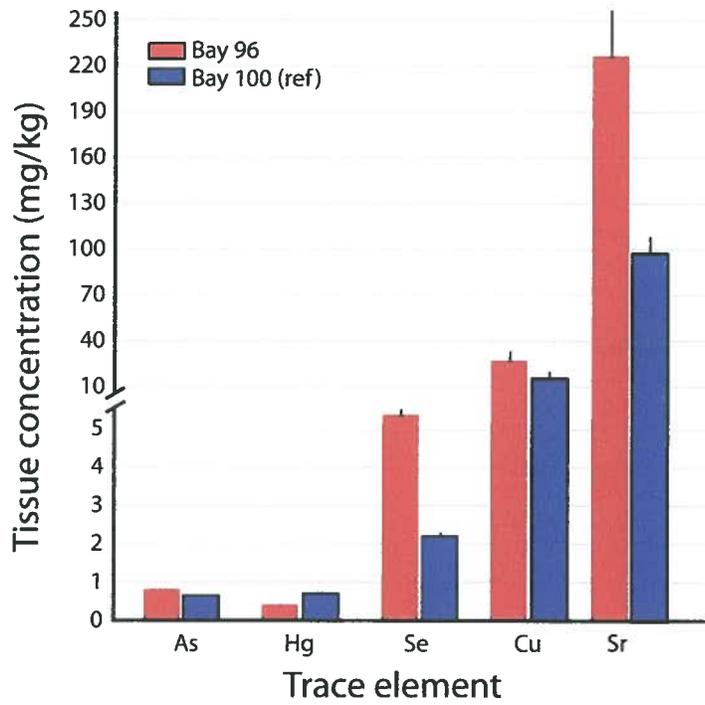
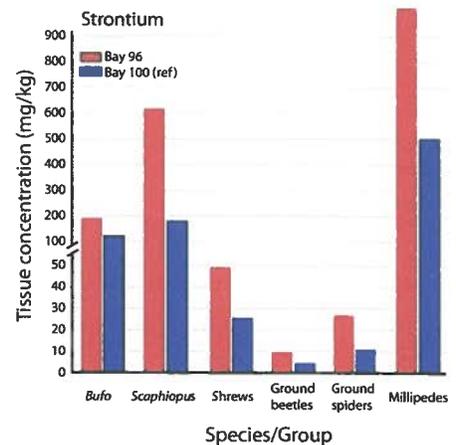
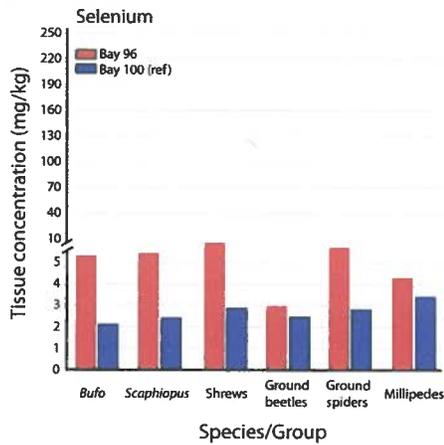
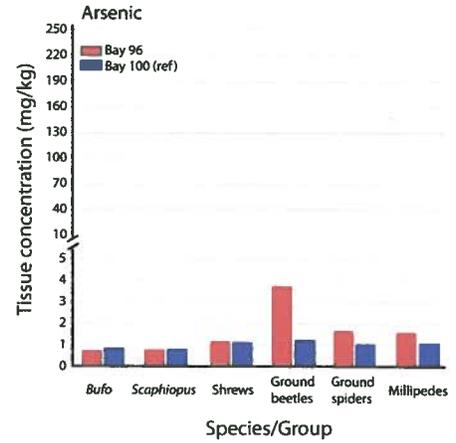
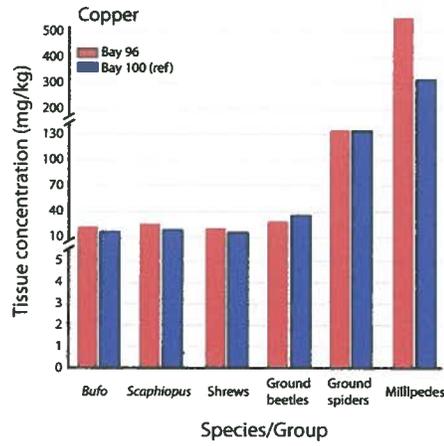


Fig. 4. Metal concentrations in biota from the PAPW and Bay 100. In general metal concentrations were higher in animals, such as *Peromyscus* (below), from the PAPW.



Fig 5. Metal concentrations (Cu, As, Se, and Sr) in biota from the PAPW and Bay 100. Body burdens depended on site, metal, and species/group.



ECOLOGICAL RISKS AND EFFECTS

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

A Comprehensive Risk Assessment Model for the Tims Branch/Steed Pond System on the Department of Energy's Savannah River Site

Larry Bryan, Karen Gaines¹, and Jim Novak¹

¹Eastern Illinois University

Various contaminants have been released into the Tims Branch/Steed Pond System on the DOE's Savannah River Site since the mid-1960s, including nickel, uranium, lead, chromium, copper, and other potential toxicants. These contaminants pose potential ecological and human health risks if they become incorporated in biological systems and get passed through the food chain. Our goal is to (1) examine historical contaminant data from this system's biota and (2) gather additional contaminant data needed to develop a comprehensive risk assessment model for the Tims Branch/Steed Pond system, determining the intrinsic ecological and human health risks to end point receptor species. The result of our examination of site-specific historical databases was that more information was needed pertaining to contaminant uptake within the lower trophic levels of this system.

To address this data gap, a graduate student from Eastern Illinois University developed a project to examine contaminant uptake in various amphibian larvae (tadpoles), biofilms, and aquatic insect larvae, specifically dragonfly larvae (Odonates). These samples were collected during the period of April through September, 2010, in a series of beaver impoundments (n=6) on the Tims Branch gradient starting above the M-Area input (as a "control") and extending down gradient to the Old Pond 25 site, immediately upstream of the confluence of Tims Branch and Upper Three Runs Creek. These samples were analyzed for contaminants and stable isotopes (to document trophic position) in the fall/winter of 2010/2011. One finding from the first year was that biofilms exhibited relatively high concentrations of certain elements of concern (e.g.; uranium in pond 2 and pond 5 averaged approximately 200 ppm and 300 ppm, respectively). In the second year of study (Spring/Summer of 2011), the project focused on the larger *Ranid* tadpoles and biofilms in the control pond, pond 2 (immediately below the historical M-Area input, and pond 5 (the old pond 25 site). These data have been collected and most have been analyzed for elements (still awaiting final stable isotope analyses of some samples).

The historical databases have been compiled and completion of the risk assessment model will occur after analyses of the remaining samples of the lower trophic level samples.

Reptiles as Long-Lived Receptors for Ecological Risk Assessment on the SRS

Tracey Tuberville, Brian Metts, and David Scott

Future management and remediation efforts within different Integrator Operable Units (IOUs) on the SRS may depend in part on predicted risks to wildlife species from contaminant exposure models. Contaminants that persist in the environment may jeopardize the reproductive fitness of long-lived organisms only after prolonged exposure. Contaminant exposure models have been developed for river otters and belted kingfishers to examine the potential effects of metal contaminants on wildlife associated with aquatic systems on the SRS. However, models for these relatively short-lived species (otters, 8-9 yrs; kingfisher, 15 yrs) may not be representative of the exposure risks for species at similar trophic levels but with much longer life spans.

American alligators and several turtles species occur in aquatic systems of the SRS, have diets composed largely of vertebrate and/or invertebrates, and have estimated longevitys of up to 40-70 years. As a result, alligators and turtles may be more appropriate ecological receptors for assessing risks associated with long-term contaminant exposure. A review of the literature to compile parameters necessary to construct the risk assessment models for these long-lived species will aid in predicting exposure risks to long-lived organisms and in identifying SRS IOUs where exposure risks are greatest. Due to their longevity, turtles and alligators may serve as reservoirs of contaminants, and because of their mobility and (at least in some species) use of rivers and streams as movement and dispersal corridors, aquatic turtles and alligators can potentially transport contaminants offsite. For example, radioactively contaminated turtles have been captured on private lands adjacent to the SRS. Both taxonomic groups are likely to be consumed by humans in surrounding communities. Indeed, alligators are now legally hunted and consumed in both South Carolina and Georgia. Therefore, body burdens of metals and radiological contaminants in wildlife are of concern not only for the wildlife but for the humans that may consume them.

By virtue of their long life spans, physiology, and trophic status as top predators, some reptile species may accumulate significant body burdens of a variety of contaminants. An assessment of tissue burdens in long-lived reptiles in SRS IOUs that have elevated levels of contaminants of potential concern would provide important validation for exposure risks models and could potentially provide opportunity to monitor long-term changes in body burdens of individuals previously sampled.

Field sampling began in April 2010 and we sampled turtles—primarily yellow-bellied sliders (*Trachemys scripta*)—and alligators (*Alligator mississippiensis*) from a series of aquatic sampling sites that differed in their contaminant types, levels and spatial scales, including references sites not known to be contaminated. Alligators were captured using Murphy traps and by hand; turtles were captured using baited aquatic hoop net

traps. Traps were set at each location for 4 days/3 nights and night time sampling for alligators was conducted once each week. We permanently and uniquely marked all animals, took standard morphometric measurements, and measured gamma radiation. We used a Canberra multi-channel analyzer and Genie 2000 spectroscopy system with a 10.16 x 15.24 cm NaI crystal to determine ^{137}Cs total body burden in subsamples of turtles and young alligators from each location. Large alligators (>40 cm) were counted with an Eberline ESP 2 Gamma Counter. The region of interest for ^{137}Cs was 596-728 keV. Count times were generally 30 minutes. The counting data were corrected for background radiation, and these data will be used to determine tissue concentrations of radioisotopes (after adjusting for the physical decay of the ^{137}Cs phantoms and animal geometry effects on counting efficiency). In addition, we collected whole blood, nail, and scute (alligators only) samples for analysis for a suite of metals.

We captured >250 turtles and 176 alligators from metals-contaminated, radiologically contaminated, and uncontaminated reference sites across the SRS. To date, 97 blood samples from yellow-bellied sliders and 44 scute samples from American alligators have been analyzed for metals. An additional 62 samples (32 blood, 30 scute samples) from alligators have been submitted for metals analysis. Our preliminary data suggest that ^{137}Cs is elevated in yellow-bellied sliders (Figure 1) and alligators (Figure 2) from Pond B and Par Pond, with the highest levels documented at Pond B. In addition, we also documented elevated gamma counts in turtles from a site not previously known to us to be contaminated – the Upper A01 Wetland. Our preliminary identification of the spectrum is a thorium isotope present at above-background levels due to SRS operations. The primary finding in regards to metals analysis were the extremely high body burdens of selenium in yellow-bellied sliders (presented as means and 95% confidence intervals; Figure 3), elevated mercury levels in sliders from PAR pond (Figure 4), and high levels of strontium from sliders collected from D-Area and A-O1 wetland (Figure 5).

Figure 1. Cesium levels in *Trachemys scripta* from various locations on the SRS.

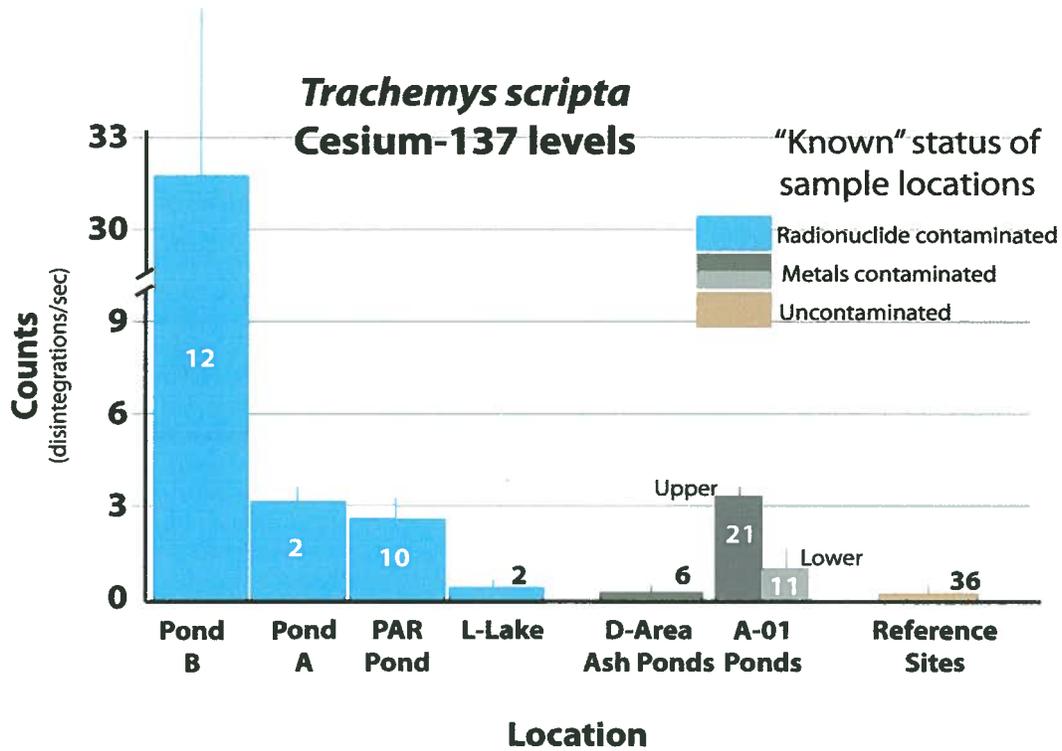


Figure 2. Cesium levels in *Alligator mississippiensis* from various locations on the SRS.

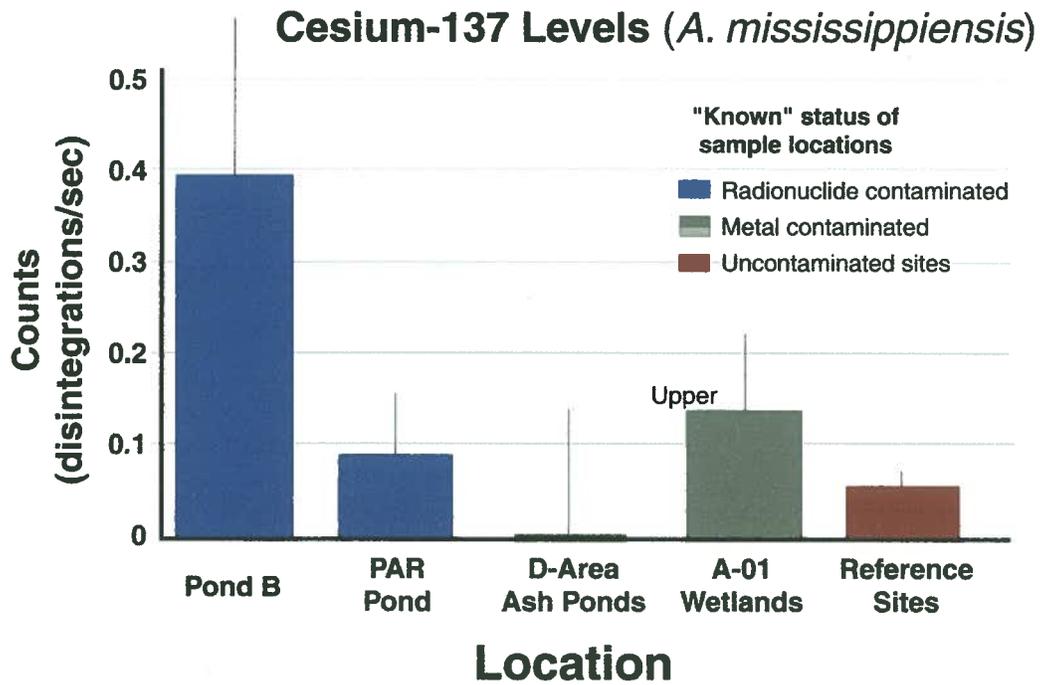


Figure 3. Selenium levels in *Trachemys scripta* from various locations on the SRS.

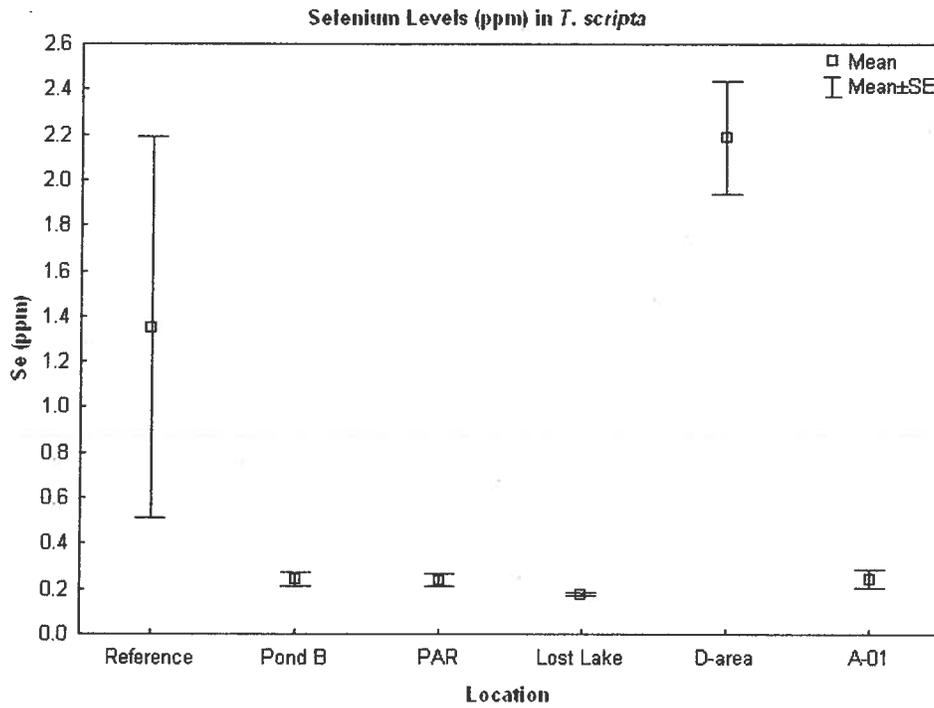


Figure 4. Mercury levels in *Alligator mississippiensis* from various locations on the SRS.

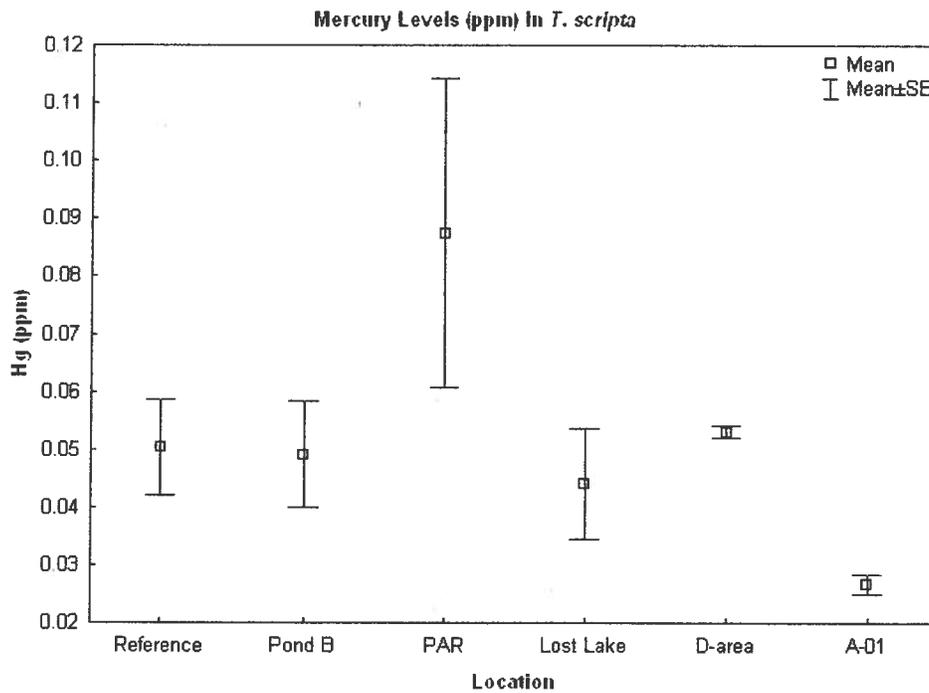
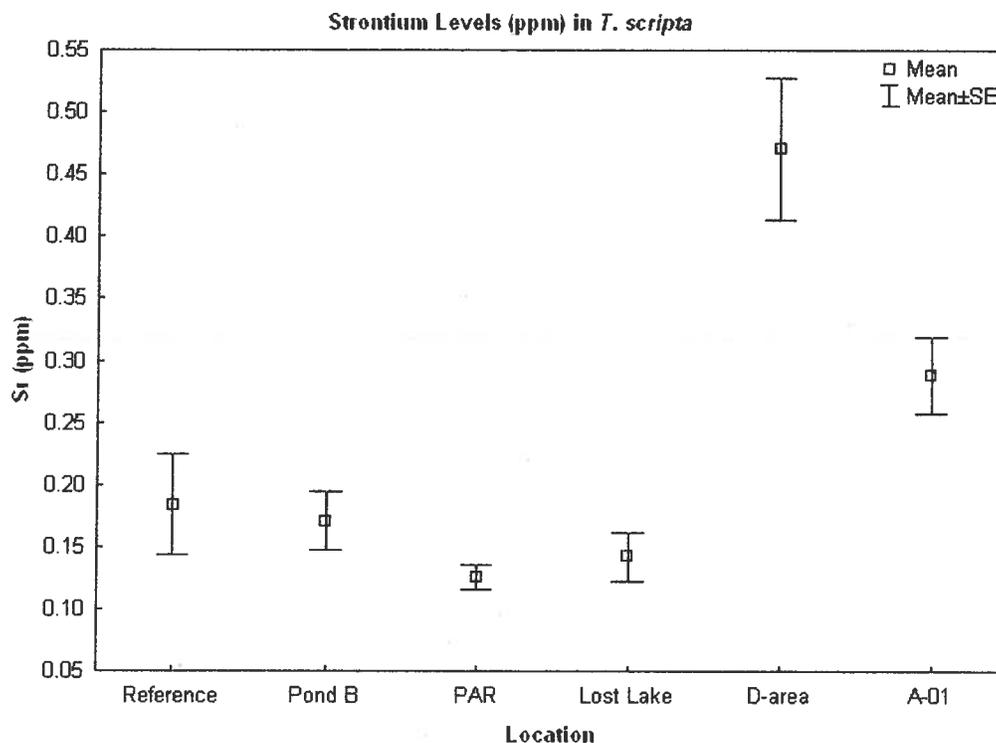


Figure 5. Strontium levels in *Alligator mississippiensis* from various locations on the SRS.



We also initiated a mesocosm experiment to investigate the effects of dietary exposure to contaminant mixtures on health and performance of hatchling alligators. Thirty-six hatchling alligators are exposed to one of four dietary treatments that vary in trace metal levels. Hatchlings are weighed and measured monthly and biological samples are collected every three months for subsequent analysis for trace metals concentrations, blood profiles, organ function, and other measures of individual health and performance. Upon completion of this study we will be able to relate contaminant concentrations in non-destructive (blood) samples to organ/tissue/whole body burdens of contaminants that are likely to affect health and reproduction (as determined by gene expression analyses and other health-related endpoints). Preliminary analysis of the first blood samples collected since initiating dietary contaminant exposure indicate low levels of trace element accumulation. However, continuation of this experiment and analysis of additional samples is contingent on continued project funding.

We are currently in the process of preparing our final report for our long-lived reptile field efforts. Preliminary results have been presented at scientific meetings and one manuscript has been published to date (see below). Additional presentations and manuscripts will be prepared following completion of the final report.

Poster presentations:

Harris, B.B., A.M. Grosse, R.V. Horan III, D.E. Scott, B.S. Metts, and T.D. Tuberville. Alligators and freshwater turtles as long-lived bioindicators in aquatic ecosystems of the southeastern U.S.

Presented at:

- Turtle Survival Alliance, Orlando, FL. August 2011.
- Savannah River Site 60th Anniversary Symposium, USC-Aiken, Fall 2011.

Manuscripts published:

Davis, A.K., R.V. Horan III, A.M. Grosse, B.B. Harris, B.S. Metts, D.E. Scott, and T.D. Tuberville. 2011. Gender differences in haemogregarine infections in American alligators (*Alligator mississippiensis*) at Savannah River, South Carolina. *Journal of Wildlife Diseases* 47:1047-1049.

Environmental Protection of DOE Lands: Studies in Long-Term Stewardship

J Vaun McArthur, Cary Tuckfield, Rebecca Sharitz, and John Morse¹
¹Clemson University

After more than 30 years of site operation the biodiversity of one group of pollution sensitive macroinvertebrates found in Upper Three Runs Creek is actually higher now than when the first survey was conducted in the early 1970s. Upper Three Runs has the highest species richness and diversity of any stream in the USA and probably the world based on the survey in 1970s. This is tremendously valuable PR for DOE. It shows that DOE has been a very good steward of an extremely valuable biotic resource in the United States. DOE should use this information for PR purposes. We have yet to process the other important taxa collected because of lack of funds but expect to see similar results.

Although there has been extensive development in the Upper Three Runs Creek Watershed above the SRS in Aiken County the water quality entering the site has not been seriously impacted. Indeed water quality of UTRC is primarily affected by Tinker Creek, a major tributary that originates off site. The chemistry of UTRC below the confluence with Tinker Creek is more similar to Tinker than to the water coming down the mainstream. Tinker Creek chemistry appears to be controlled by offsite processes. Once again this is excellent news for DOE because there are a number of site operations that have runoff into UTRC and these processes are not significantly altering stream chemistry.

Baseline Studies of Water and Soil Quality, Aquatic Macroinvertebrate Communities, and Vegetation in an Area Adjacent to the MOX Fuel Fabrication Facility

J Vaun McArthur, Rebecca Sharitz, and John Seaman

We concluded investigations into the disturbance ecology of the stream draining the MOX facility. This stream system experienced a major environmental impact when construction sand was washed into it following an exceptional rain event. The deposition of the sand has caused mortality of riparian vegetation and was expected to have adverse affects on the macroinvertebrates found in the stream. This report documents continued research on this environmental event and presents new data.

Specifically we have found:

- 1) Macroinvertebrate abundance and diversity is very different from either a control stream or from Upper Three Runs Creek.
- 2) Interestingly, the macroinvertebrates show similar patterns above the sand impacted area indicating that the distribution and abundance of macroinvertebrates in this stream are affected by something other than the sand disturbance.
- 3) Seven mayfly, eight stonefly and twelve caddisfly taxa were identified from all streams. Of these only the most ecologically tolerant species were found in the MOX stream.
- 4) We collected or attempted to collect fish from the control stream and from the MOX stream. Four species of fish were collected from the control stream but no fish were collected from the MOX stream. Considering the MOX stream drainage is seven times larger than the control stream, this result is astonishing.
- 5) There was extensive tree mortality in the slope area adjacent to the stream where sediment deposition was heaviest, especially of tulip poplar trees, the former canopy dominant species.
- 6) By 2010, there was recruitment of small tulip poplar saplings (1 cm or less in diameter), as well as saplings of other woody species including sweetgum and black willow. Thus, the wetland forest appears to be on a trajectory toward recovery.

REMEDIATION AND RESTORATION

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

Alternative Vegetation Studies for Waste Cap Covers

John Seaman, Rebecca Sharitz, Linda Lee, Julian Singer, and Hyun-shik Chang

The burial of waste material and the stabilization of the overlying soil is an important part of waste management at any operational facility. Maintaining a healthy vegetative cover is an important aspect in the long-term management of such systems. At the Department of Energy (DOE), with low-level radioactive waste often being a component of the buried material, maintaining a stable landfill cap has even greater urgency. Originally, the current project was designed to make use of the Bentonite Mat Demonstration site, a set of test covers constructed in 1993 to evaluate the effectiveness of geosynthetic materials as an alternative cover system configuration for the containment of radiological waste. However, the project scope was expanded to include developing specification standards for the installation and maintenance of ACP Closure Caps, including the development of an automated system for evaluating cap performance behavior (buried sensors, remote sensing, real-time systems), recommendations for optimizing the current management regime, and the potential use of native plant species that are well-suited to the SRS environment and require less maintenance (i.e., mowing, fertilization, etc.).

The waste caps at the Sanitary Landfill 740G (SL) south of Road C and the Old Radiological Waste Burial Ground (ORWBG) are being used as field sites to understand soil and vegetation conditions on existing caps. In 2011, A final vegetation survey was conducted on both field sites in March, completing the one-year seasonal sampling effort begun in FY 2010. Nutrient analysis was conducted on a set of shallow soil samples collected from both the SL and ORWBG. The results were provided to ACP in early spring, along with fertilizer and lime recommendations for both sites. Soil test results indicated that ACPs scheduled fertilizer application was sufficient to promote adequate turf growth. Instead of annual lime applications, SREL recommended the application of gypsum (CaSO_4) as an alternative means of addressing subsoil acidity and improving rainfall infiltration. Based on such recommendations, gypsum was applied to portions of the ORWBG cap at a rate of 1 ton/acre. Sample for soil fertility analysis and Infiltration measurements will be taken in 2012 to determine the efficacy of gypsum applications compared to similar waste caps receiving only lime applications.

Native alternatives to turf grasses are being explored via a soil mesocosm experiment intended to reproduce soil conditions typical of the waste caps, i.e., soil depth, slope, etc. The mesocosms will be used to evaluate rainwater infiltration and runoff, and evapotranspiration on moderate slopes under native and non-native vegetation. Results will indicate whether low-maintenance native vegetation provides comparable water management relative to higher-maintenance turfgrasses under meteorological conditions present on the SRS.

In 2011, SREL completed construction of the six mesocosms to be used in the experiment, with each mesocosm having four treatment cells (Fig. 1). Mesocosms were built of plywood, lined with watertight high-density polyethylene (HDPE) plastic sheeting, and the exposed portions of HDPE were coated with metal to protect them from UV damage. The mesocosms and a solar-powered weather station have been installed behind SREL. Extensive soil sampling on the two SRS caps was used to determine criteria for mesocosm soil selection.

Modeling to determine sensor placement was conducted using the HYDRUS3D code. SREL has also used HYDRUS3D with prior data from the SRS in simulations to predict drainage and vegetation response of the mesocosms. Based on these simulations, mesocosms may not drain at all in dry years; the vegetation layer may completely transpire or evaporate all precipitation.

Seeds for the experiment were germinated in soil obtained from spoil piles left over from caps on the SRS. Germination requirements for the native species were determined experimentally. Seeding rates were determined via the scientific literature and discussions with growers. In late winter, seeds of the native species and two common turfgrasses (centipede and bahia) were planted in the greenhouse. Germination was successful, and vegetation of all treatments is well-developed.

During 2011, SREL also gave two presentations on the mesocosm experiment: a poster at the SRS Heritage Symposium in August, and an invited oral presentation at the Cullowhee Native Plant Conference in July.

Fig.1. Mesocosm treatment block containing mixed-native grasses, centipede, bahia, and non-vegetated control (left to right). The mesocosm treatments were transplanted in February 2012.



Continued Research at the Mixed Waste Management Phytoremediation Facility Julian Singer and John Seaman

In support of remediation efforts at the Mixed Waste Management Phytoremediation Facility, generally referred to as the MWMF, SREL continued working in collaboration with the DOE, USDA Forest Service, and ACP to assist with site management and provide the evapotranspiration efficiency estimates required for the Corrective Action Report (CAR). SREL representatives met with the MWMF management team on a regular basis to coordinate all activities, and maintain, update and report the results from the one dimensional (1D) tritium efficiency model originally developed by researchers from Cornell (Drs. K. Rebel and S. Riha). Efforts in FY 2011 focused on assisting with expansion of the irrigation area, including the installation of electronic flow meters for each of the twelve new plots and the collection of soil data for parameterization to update the Cornell Model to estimate water-use efficiency for the eastern expansion area. As in previous years, SREL also collected and analyzed two sets of bi-annual three-meter soil cores from the six original instrumented plots for the mid-year 2011 water-use efficiency calculations and 2011 end-of-year CAR. To assess tritium use efficiency for the expanded irrigation area, SREL collected and analyzed an additional two sets of 4 soil cores, the results of which are included in the CAR.

The 2010 end-of-year report for the CAR and the 2011 mid-year efficiency calculations also included the Cornell Model estimates of tritium use efficiencies and mass balance tritium use efficiencies based on soil data collected January 2011, and June 2011, respectively. Remediation efficiencies for the original irrigation area were calculated based on irrigation schedules, climate data, and soil core analysis from select plots within the facility since operation began in 2001. The lab results for the 2010 CAR report documenting the soil tritium data, evapotranspiration efficiency estimates, and updated results from the Cornell model were provided to ACP in February 2011. Average tritium use efficiencies for the six monitored plots within the original irrigation area were estimated to be 88% and 85% for tritium mass balance calculations and the Cornell model estimates, respectively. Increased rainfall in late November and December of 2010 influenced tritium concentrations sampled in the soil core samples and decreased irrigated water applications, but did not substantially affect yearly efficiencies when compared to efficiencies of previous years. Similar tritium-use efficiency rates were observed for the 2010 mid-year efficiency calculations.

SREL researchers completed the development of an alternate 1D model for describing tritium uptake by vegetation and downward movement through the soil profile based on the HYDRUS-1D code developed by researchers at the USDA. HYDRUS-1D numerically solves Richards' equation for variably saturated water flow and Fickian-based advection dispersion equations (ADE) for heat and solute transport. Results focusing on the effects of climate variability on phytoremediation efficiency were presented by SREL researchers in a paper titled "Evaluating Long Term Water Use Efficiency at the Field Scale for Phytoremediation Management" at the Georgia Water Resources Conference in Athens, Georgia in April of 2011. The research demonstrated that efficiency results obtained with the more mechanistic HYDRUS-1D model based on available soil characterization data were consistent with the Cornell water budget model when evaluating various irrigation management strategies based on 30-years of site meteorological data. The results will be beneficial in adapting current irrigation management strategies to additional areas planned for the MWMF. Additional work in the expansion areas continued to evaluate the importance of soil and vegetation variability on system efficiency. Preliminary tritium data taken in March 2011 from 3 year old planted pines trees in the Eastern Expansion Area were analyzed and showed no trends associated with soil tritium depth profile. Evaluation of the relationships between tritium application and root uptake in growing trees will continue.

A set of surface soil samples (≈ 15 cm soil depth) were collected from the six instrumented plots and from the Eastern Expansion Area to evaluate soil nutrient content. Numerous soil samples from within a plot were homogenized to create a given representative plot sample. The resulting soil fertility data was summarized and provided to the MWMF working group along with potential fertilizer application recommendations based on loblolly pine management strategies. The expansion area plots tested higher in terms of pH, K, Ca, and Mg, likely the result of base cation release associated with timber harvesting and initial site preparation. All of the plots tested below the threshold for P based on the establishment or maintenance of a pine

plantation (i.e., < 10 lb/ac), indicating that the irrigation site should positively respond to P fertilization.

Table 1. Soil pH, Mehlich I extraction results, and texture for the ORWBG.

Sample Plot	pH	*Soil Texture				
		Phosphorus	Potassium	Calcium	Magnesium	
		lb/Ac				
4	4.5	6	35	86	30	LS
13	4.4	9	33	88	25	LS
16	4.4	7	32	122	32	LS
19	4.5	8	26	78	23	LS
22	4.2	9	35	116	35	LS
27	4.5	7	54	230	65	LS
Avg.	4.4	7.7	35.8	120.0	35.0	
Sd. Dev.	0.1	1.1	8.7	51.7	14.0	
Expansion Area						
31/32	5.2	5	57	540	100	
36	5.5	8	78	666	108	

*Soil Texture Classifications (Hydrometer Method)
LS = Loamy Sand

In 2011, the MWMF began work on expanding the irrigation system into the area immediately west of the original treatment area, hereafter referred to as the Western Expansion Area. SREL initiated work on a new sampling analysis plan for soil core sampling in the Western Expansion Area as part of pre-construction site characterization. This sampling will coincide with scheduled bi-annual soil core sampling in the two existing areas.

SREL continued assisting the USFS in maintaining the irrigation system and automating various monitoring system components. In FY 2011 this included the routine maintenance of the weather station, pond-depth gauge, and automated data logging system that provides data to USFS personnel for the water deficit calculations used to determine irrigation rates.

H-02 Constructed Wetland Biogeochemical Studies

Gary Mills, Elizabeth Burgess, and Michele Harmon¹

¹ Department of Biology, University of South Carolina-Aiken

The H-02 wetland system was designed to treat building process water and storm water runoff from multiple sources associated with the Tritium Facility at the DOE-Savannah River Site, Aiken, S.C. Modeled after the A-01 system, which has been operating

successfully at the Savannah River Site for approximately a decade, the H-02 system was constructed in 2007. Both wetland systems were designed for the removal of trace metals from treatment water, in particular Cu and Zn. The wetland construction included the addition of gypsum (calcium sulfate) to foster a sulfate-reducing bacterial population, and thus a source of sulfides for the sequestration of Cu and Zn into sulfide mineral phases within the sediments. Conceptually, the wetland functions as follows:

- Cu and Zn initially bind to both dissolved and particulate organic detritus within the wetland.
- A portion of this organic matter is subsequently deposited into the surface sediments within the wetland.
- The fraction of Cu and Zn that is discharged in the wetland effluent is organically complexed, less bioavailable, and consequently, less toxic.
- The Cu and Zn deposited in the surface sediments are eventually sequestered into insoluble sulfide minerals in the sediments.

Development of the H-02 system has been closely monitored; sampling began in August 2007, shortly after its construction. This monitoring has included the measurement of water quality parameters, Cu and Zn concentrations in surface water and sediments, as well as, characterization of the prokaryotic (e.g., bacterial) component of wetland biogeochemical processes. The objective of this study was to determine changes in biogeochemical parameters as the H-02 wetlands progressed to a mature, steady-state system and monitor how seasonal variations in these parameters affect wetland function over time.

Data from water samples taken biweekly at several sites within the wetland system and upstream and downstream reference locations in Upper Three Runs Creek (UTR) include total and dissolved metals, and standard water quality constituents including dissolved organic carbon, pH, temperature and redox potential. These data indicate that significant quantities of Cu and Zn discharged from the Tritium Facility are removed from the water in the wetland. Since the beginning of the study, the mean influent Cu concentration was 31.5 ± 12.1 ppb and the mean effluent concentration was 11.9 ± 7.3 ppb, corresponding to an average Cu removal of 64%. Zn concentrations were more variable, averaging 39.2 ± 13.8 ppb in the influent and 25.7 ± 21.3 ppb in the effluent. Average Zn removal was 52%. In addition, the wetland ameliorated high pH values associated with the influent water, occasionally greater than 10, to values similar to those measured at reference sites in Upper Three Runs Creek. Water sample data have also been used to support amphibian bioaccumulation and toxicity studies at SREL (Scott, et al. 2011).

Seasonal variations in DOC concentration corresponded to seasonal variations in Cu and Zn removal efficiency. Water samples were also collected seasonally for standard EPA toxicity assays using *Ceriodaphnia dubia*. The results of these assays demonstrated that toxicity of Cu was reduced in wetland effluent waters as compared to

standard laboratory water, and that constituents in both particulate and dissolved phases played a role in reduction of toxicity.

The water chemistry data were used in parameterizing the biotic ligand model (BLM) to predict the bioavailable forms of Cu and Zn and assess the toxicity to receptor organisms. The model predictions were compared with the EPA water effects ratio calculated using results from the *C. dubia* assays. It was concluded that the water effects ratio is a better predictor of toxicity in the H-02 wetland system, because the BLM results overestimated the toxicity of the water as compared to results from the *C. dubia* assays. This is likely due to the acidic pH and low hardness values of the blackwater streams of the SRS.

Sediment core samples have been collected approximately biannually since wetland construction to monitor changes in trace metals, sulfides, and organic C and N. The concentration of Cu and Zn in the surface layer of the sediments has increased over the lifetime of the wetland and, like removal efficiency, demonstrated seasonal variation. By design, sulfate-reduction in the wetland should contribute to mineralization of Cu as copper sulfides within the sediments over time. We measured sulfide in vertical profiles in sediment cores to determine potential for this process and the depth at which it occurs. Black layers within sediment cores, indicating sulfide formation, were observed with increasing frequency since wetland construction. Reduced sulfur concentrations in the sediments ranged from below detection limits (<200 ppm) to 1480 ppm; however, the highest concentrations did not necessarily correspond to sediment depth or color. Determination of organic C and N provides an estimate of the nutrient quality of the sediment organic matter. C and N did not vary considerably in the H-02 samples analyzed thus far, but comparison with A-01 suggested some additional changes to be expected in the wetland over time which are being examined more closely with recently collected sediment samples.

The bacterial communities associated with wetland sediments were also examined. Within its first year, the H-02 wetland showed biomarkers for sulfate-reducing bacteria, and estimates of bacterial biomass were comparable to A-01. The presence of Bacteria and Archaea at all sediment depths examined has been confirmed and sequencing is underway to enable identification of the most abundant community members, as well as, how the communities have changed since wetland construction. During a recent core sampling event, sulfate-reduction and methane-oxidation rates in the sediments were determined using radiotracer techniques. Sulfate-reduction was detected in all depths of sediment cores, even in surface detritus layers. Gas measurements from H-02 sediments demonstrated that methane is available to support a methane oxidizing community, and active methane-oxidation was detected in the sediments and overlying water.

Our results demonstrate that the H-02 wetlands are functioning successfully to remove Cu and Zn from influent waters. The continued success and long-term sustainability of the functioning H-02 system is predicated on maintaining *in situ* biogeochemistry; however, the relative importance of various biogeochemical cycles remains unclear. For

example, the Cu and Zn deposited in the sediments are associated with organic detritus at the sediment surface. The extent and rate at which the metals will redistribute to more recalcitrant sulfide mineral phases remain to be determined. In a larger context, these aspects of constructed wetlands are important considerations that should influence selection, design and operation of future constructed wetlands for treatment of metals in processing facility discharge waters. Thus, the H-02 wetland system is a valuable resource not only for metal removal at SRS, but also for elucidation of the conditions that can further enhance the understanding of wetland function within the scientific and regulatory communities.

H-02 Constructed Wetland Studies: Amphibians and Plants

Stacey Lance, David Scott, Wes Flynn, Rebecca Sharitz, Tracey Tuberville, Linda Lee

An artificial wetland complex (H-02) was constructed in H-Area on the Savannah River Site (SRS) in 2006-07 to treat process and storm water discharge from H-Area industrial facilities. The H-02 wetland complex is a series of surface flow constructed wetlands designed to reduce copper (Cu), zinc (Zn), and pH levels before H-Area effluent enters Upper Three Runs Creek tributaries. The ability of artificial wetlands and stormwater ponds to improve many aspects of water quality is well known. Trace metals such as Cu, lead (Pb), and Zn are removed by adsorption to organic matter and clay particles, and sulfate-reducing bacteria enable the precipitation of metal ions in the anaerobic soils.

Constructed wetlands that are engineered primarily for water quality improvement provide new habitat for local plant and animal species, but these artificial wetlands may also have negative consequences for biota. For aquatic-breeding animals such as amphibians, if high levels of trace metals limit recruitment then these wetlands could become population sinks. In addition, there is potential for non-native invasive plant species to become established in artificial wetlands. The H-02 treatment complex consists of a retention pond, which receives the process water, and two constructed wetland cells. The two treatment cells were planted with giant bulrush, *Schoenoplectus californicus*, in 2007. Water in the retention pond has the highest levels of Cu, Zn, and pH, and after a residence time of several days water exiting the wetland cells has lower levels of these variables. The Savannah River Ecology Laboratory (SREL) initiated amphibian and vegetation surveys at the H-02 wetlands in May 2008. We are monitoring amphibian and plant species colonization and establishment in the wetlands, and evaluating the effects of elevated metal levels on amphibian success.

Copper concentrations in the H-02 system can vary spatially throughout the system, ranging as high as 31-37 ppb in the influent in summer months to 7 ppb in the effluent exiting the treatment wetlands. Levels in portions of the retention pond have reached 340-590 ppb. These concentrations may be of concern for normal amphibian development. The ecological research conducted by SREL at the site focuses primarily on four questions related to these treatment wetlands: 1) Over time, what amphibians, reptiles, and plants have become established in the wetlands? 2) Is there any evidence that elevated trace metal levels (e.g., Cu and Zn) in the wetlands affect amphibian

reproductive and recruitment success? 3) How do the amphibian diversity and numbers compare to other, more natural, wetlands? 4) As the constructed wetlands age, how will the amphibian and plant communities respond?

This report summarizes our amphibian and vegetation sampling at the H-02 treatment wetlands from October 2010 to September 2011. We used permanent plots established for vegetation monitoring and aquatic trapping to characterize biota of the treatment wetlands. Adjacent drift fence arrays with pitfall traps (Fig. 1) were used to estimate breeding amphibian numbers and juvenile production. In addition to monitoring amphibian and reptile use of the wetlands, we collected data on metal burdens of amphibians inhabiting the wetlands, and we are conducting field and laboratory tests on effects of Cu concentration on amphibian development in three species (the southern toad, *Anaxyrus (Bufo) terrestris*, the eastern narrowmouth toad, *Gastrophryne carolinensis*, and the southern leopard frog, *Lithobates (Rana) sphenoccephalus*). We are also monitoring vegetation colonization and succession patterns, with a special interest in how vegetation structure, species richness, proportional abundances, and invasive species numbers change over time.

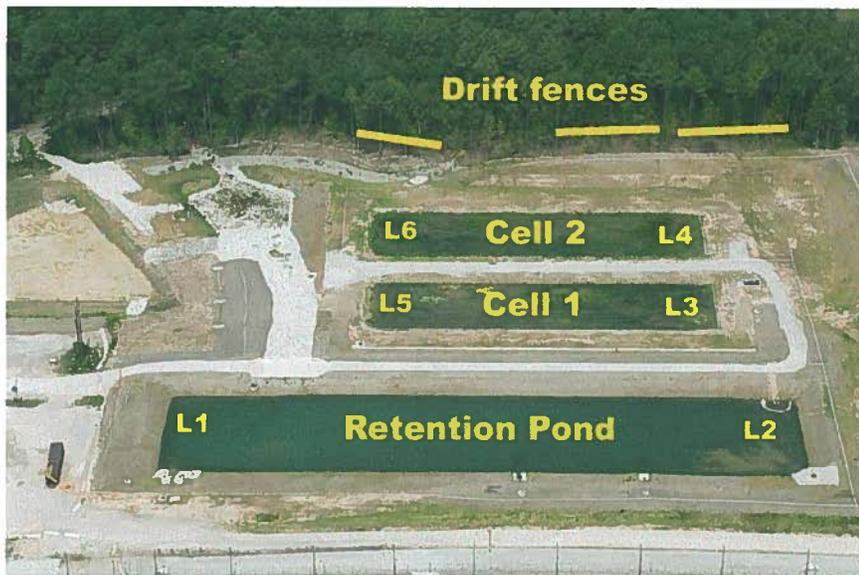


Figure 1. Drift fence/pitfall trap and egg/larval field experiment locations within the H-02 wetland system. Locations L1 & L2 (retention pond), L3 & L4 (influent end of wetland cells), and L5 & L6 (effluent end of wetland cells) represent a gradient of water chemistry along which the “bucket studies” have been conducted. Highest pH, Cu, and Zn levels occur in the retention pond, and progressively lessen throughout the treatment wetlands.

Amphibian Analyses

In previous years some of our findings include:

1. Metamorphs from our laboratory experiments have Cu body burdens comparable to those in metamorphs from the H-02 wetlands suggesting our lab conditions accurately approximate field exposures
2. Embryo and early hatchling survival is reduced by Cu exposure in three species: *Lithobates (Rana) sphenoccephalus*, *Anaxyrus (Bufo) terrestris*, and *Gastrophryne carolinensis*.
3. There is a lot of among species variation in sensitivity to Cu with *L. sphenoccephalus* being the most tolerant and *G. carolinensis* the least tolerant.
4. Preliminary data suggests there is also among and within population variation in tolerance.

Based on these findings our objectives in FY2010 included 1) further examining within and among population variation in tolerance, 2) examining genetic components of Cu exposure tolerance, 3) conducting mesocosm studies to explore more realistic exposure pathways and 4) further examining *G. carolinensis*.

Within and among population variation in tolerance

In addition to the potential effects of water chemistry on life history traits such as survival, there are concerns that contaminant-induced genetic changes may negatively impact both individuals (through increased mutation rates) and populations (through loss of genetic diversity). Among population differences suggest that there may be the potential for some species to adapt to elevated levels of metals. Adaptation to one stressor typically comes with negative trade-offs and may increase the risk that the H-02 wetlands become population sinks. Thus we wanted further examine variation in tolerance with the long-term goal of identifying genetic components to tolerance. To accomplish this we analyzed our leopard frog and southern toad data with an emphasis on among clutch variation (Fig. 2). There is a highly significant clutch effect in both species and in southern toads there is a significant source population effect with animals from the H-02 wetlands performing worse than those from a reference site (Ellenton Bay). In the spring of 2011 we examined more populations of southern toads and found additional significant among population variation in Cu tolerance.

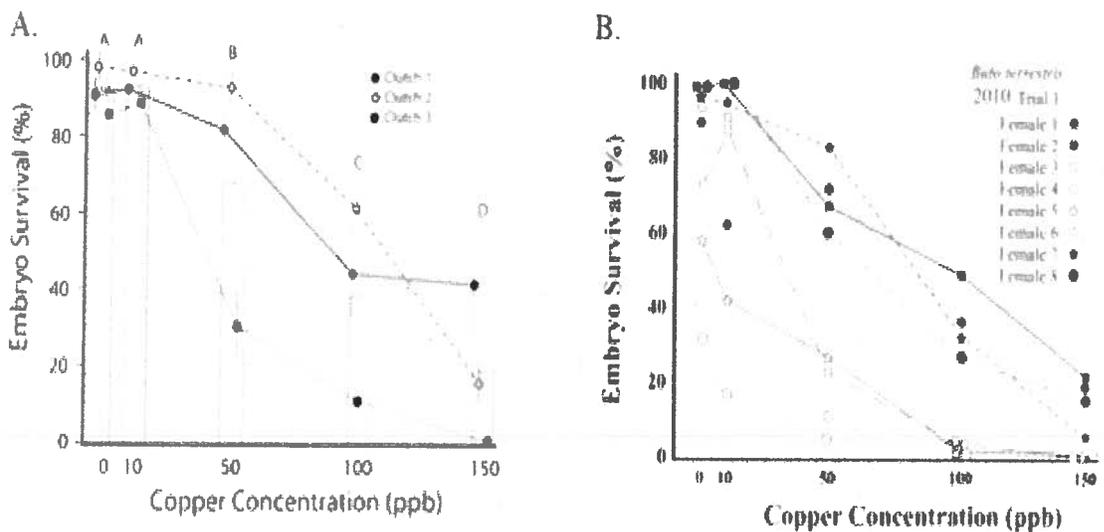


Figure 2. Effect of aqueous copper concentration on embryo survival (Gosner stage 25 feeding stage) in A) *Lithobates sphenoccephalus* and B) *Anaxyrus terrestris*. For *L. sphenoccephalus* three clutches with four replicates per clutch were used. Columns represent treatment means (across clutches) and those marked with different letters are significantly different from each other. For *A. terrestris* eight clutches from two populations (open symbols from H-02, closed symbols from Ellenton Bay) were used. Lines indicate the differential survivorship patterns for each clutch.

Genetic components of Cu tolerance

In order to further explore these differences and the potential for a genetic component to tolerance we have undertaken several studies. First, we have developed microsatellite markers for southern toads (we developed markers for leopard frogs the previous year) and will begin conducting population genetic comparisons next year. Second, to identify potential candidate loci for selection we initiated gene expression studies. Because none of our species are model organisms we started by building baseline transcriptomes for southern toads and southern leopard frogs. We then conducted a gene expression study on southern toads and identified a suite of genes that are up regulated and down regulated in response to embryonic exposure to Cu. There is a clear pattern in which initially the toad embryos up regulate genes need to deal with the metal stress but then as gene regulation is negatively affected they become unable to express the genes needed for normal development (Fig. 3). We will build upon these findings in the upcoming year.

Genes UP-regulated in Cu Tx

75 genes	156 genes	2 genes	0 genes	4 genes
24 hours	30 hours	40 hours	48 hours	55 hours
1 gene	5 genes	174 genes	19 genes	815 genes

Genes DOWN-regulated in Cu Tx

Up regulated in response to Cu

- Lysosome transport
- Oxidative stress pathways
- Apoptosis pathways
- Gene regulation altered
(kinases, transcription factors)

Down regulated in response to Cu

- Cell cycle
- DNA replication
- Spindle formation
- Circadian cycle

Figure 3. Effect of aqueous copper exposure on gene expression in *Anaxyrus terrestris* embryos. Eggs from one clutch were exposed to Cu after fertilization and then sub-sampled from 24-55 hours post exposure. From 24 to 30 hours post treatment many genes are up regulated. These genes represent pathways involved in metal stress and gene expression regulation. From 40 to 55 hours many genes are down regulated and all concern pathways needed for normal development.

Mesocosm studies

In our laboratory experiments to date we have only examined aqueous Cu exposure. In nature amphibians would be exposed to both aqueous and dietary Cu. In the spring of 2011 we conducted mesocosm studies in which southern toad tadpoles were reared in outdoor cattle tanks. We had three Cu treatments: 0, 15 and 30 ppb. The tanks were seeded with organic material, plankton, and algae to create a natural food supply for the tadpoles. We reared animals from two source populations to metamorphosis. This first attempt at maintaining Cu levels in large tanks was extremely challenging. With the assistance of John Seaman we tested Cu levels weekly and even with regular additions of Cu we were unable to maintain Cu levels at the desired concentration. As a result our 30 ppb treatment was closer to 15 and our 15 ppb treatment was not different from the controls. Perhaps because of this we did not see a significant effect of Cu on several endpoints (Fig. 4). However, once again we did see a significant effect of source population. This year we will fine-tune our ability to maintain desired Cu levels in mesocosms.

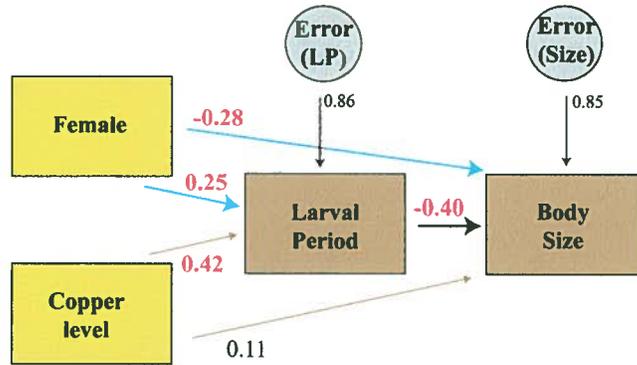


Figure 4. Diagram of path analysis used to evaluate the relative contributions of female, copper concentration, and duration of larval period on body mass at metamorphosis in *Anaxyrus terrestris*. Arrows indicate causal paths, and line width indicates magnitude of the effect. Path coefficients for rectangles (manifest variables) are standardized partial regression coefficients; circles represent all unmeasured variables that affect larval period and body size (i.e., the error variances).

Additional *Gastrophryne carolinensis* studies

Our earlier results indicated that *G. carolinensis* is very sensitive to embryonic Cu exposure. We replicated our studies to narrow the range of Cu treatments and find that at Cu levels above 15 ppb the embryos are not able to survive to the free-swimming stage (Fig. 5). Based on observations we also hypothesized that Cu exposure delays the development of *G. carolinensis*. Working with Wendy Kuhne from SRNL we conducted a study to create a detailed developmental stage map for the species and to experimental determine that Cu does significantly delay development rates. Finally, in previous studies we have been unable to rear this species to metamorphosis. We experimented with several different rearing conditions and diets and are now able to rear tadpoles to metamorphosis. Based on these findings we can conduct future experiments looking at the effect of Cu on larval and transgenerational effects.

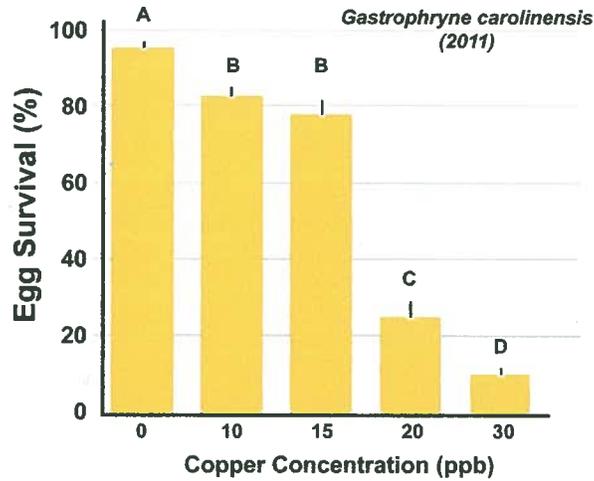


Figure 5. Effect of aqueous copper concentration on embryo survival (Gosner stage 25 feeding stage) in *Gastrophryne carolinensis*. Columns marked with different letters are significantly different from each other.

Vegetation Surveys

Vegetation was sampled in August 2011, making this the fourth annual plant survey. As in previous years, surface coverage of each plant species within 12 circular plots in each of the wetland cells was estimated, and living stems of the planted *Schoenoplectus californicus* (giant bulrush) were counted. The dominant plant species were generally similar between the two cells of the wetland complex; most differences in species composition reflected differences in the less abundant species.

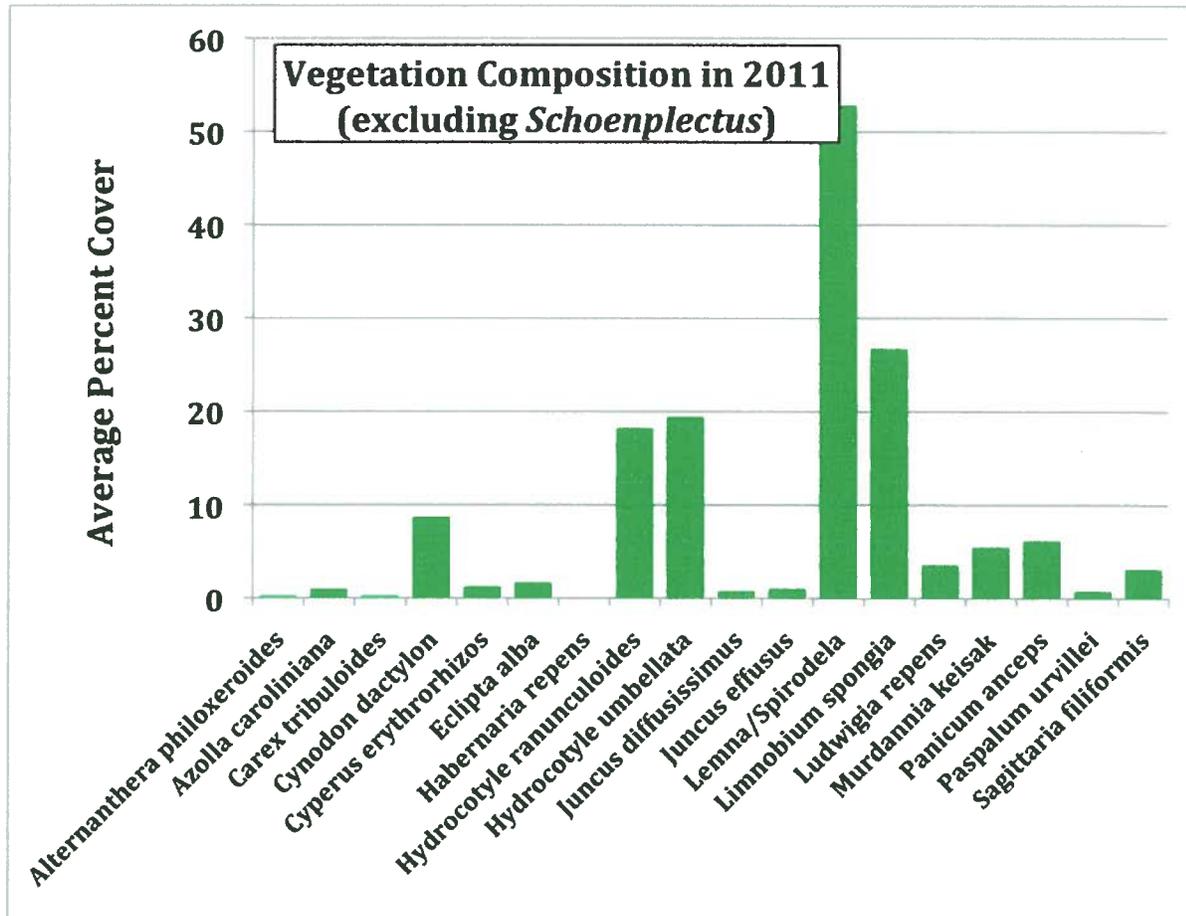


Figure 6. Average percent cover of naturally established plant species in the H-02 constructed wetlands in 2011 (both cells combined), not including the planted *Schoenplectus californicus*. Species with no data shown were present but had less than 1% coverage.

Several floating aquatic species, especially *Limnobium spongia* (spongeplant), *Lemna minor* and *Spirodela polyrrhiza* (the duckweeds), and *Hydrocotyle ranunculoides* and *H. umbellata* (marsh pennyworts) dominated the aquatic flora in both cells (Fig. 6). Spongeplant and the duckweeds have been abundant in the two wetland cells since the first sampling in 2008. *Azolla caroliniana* (mosquito fern) increased in 2009 and 2010, but declined from 19% coverage in 2010 to less than 1% coverage in 2011, likely due to intense shading by the dominant floating aquatics.

Giant bulrush was planted into the H-02 wetland cells in 2007. Over the years it has become the dominant plant species, although there remains considerable variability in stem density (Fig. 7). Occasional declines in some of the subplots resulted from high stem mortality where densities were quite high. Areas of extensive ‘blow-downs’ of dead bulrush stems were observed in both wetland cells.

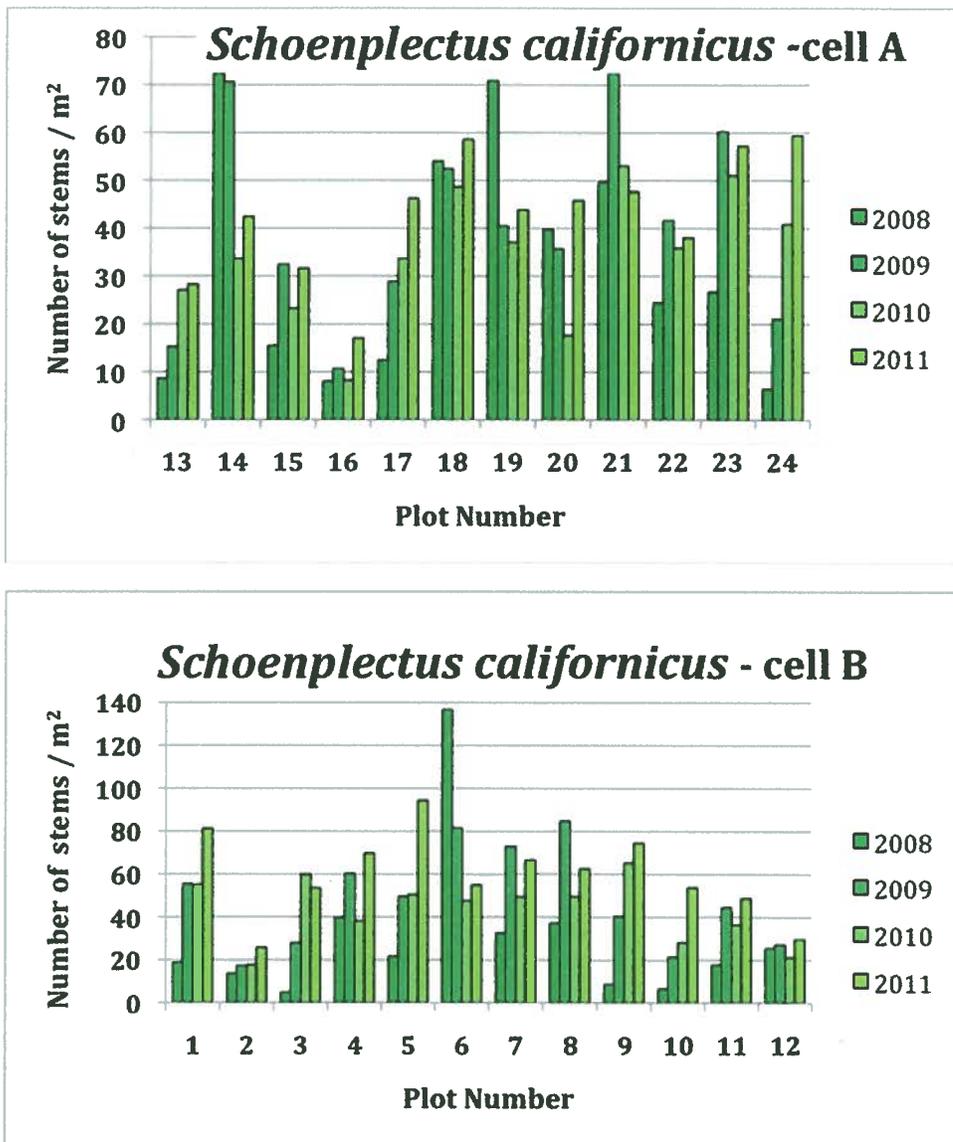


Figure 7. Stem density of the planted giant bulrush, *Schoenplectus californicus*, by plot within each wetland cell in 2011. (Note that cell A contains plots 13-24 and cell B contains plots 1-12.)

Altogether, 20 species of vascular plants were recorded in the sampled plots in 2011 (Fig. 6). A non-native and potentially invasive species found in previous years, *Pistia stratiotes* (water lettuce) was not seen in the wetland cells, and attempts to remove this species appear to have been successful. Two additional species, *Typha latifolia* (common cattail), and *Alternanthera philoxeroides* (alligatorweed) were not encountered in the plots, although occasional stems of these species were seen in the cells. These

two species are somewhat invasive in nature, but occur elsewhere on the SRS. Attempts to remove species also appear to be successful.

Continued studies –

We will continue to build on our previous data to gain a better understanding of the effects of Cu on amphibian populations. Our laboratory studies to date have only explored aqueous Cu exposure. We will add a layer of complexity by examining both aqueous and dietary exposure. We are also rearing southern toads that were exposed to Cu as larvae to reproductive maturity. The groups of toads are divided into two treatments and are either being fed a diet of clean or Cu contaminated fruit flies. This experiment represents a pilot study (and learning experience) to guide future studies looking at transgenerational effects of Cu exposure. We will also be conducting additional field experiments in the H-02 wetlands and retention pond. We are improving upon enclosures used in previous attempts in order to successfully rear tadpoles in the cells. Additional genetic studies are being planned for several species. Together, these genetic endpoints along with the life history endpoints will allow us to infer whether contaminant exposure causes genetic damage and whether this damage is likely to have long-term population-level consequences.

We also will continue studies on wetland plant community development and on focal amphibian species to better understand any water quality or contaminant thresholds that may negatively affect local populations. Because body burdens of trace elements acquired during the aquatic larval phase are retained through metamorphosis, these metals may be transferred from the wetland system (where they were acquired) into terrestrial food webs. This study will ultimately give us an understanding of how amphibians are an important pathway in trace element accumulation and elimination, and the extent to which they transfer metals from the H-02 wetlands to terrestrial food webs.

RESEARCH SUPPORT PROGRAMS

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

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

Environmental Health and Safety (EH&S) Program

Donald Mosser, Environmental Health and Safety (EH&S) Manager

The Savannah River Ecology Laboratory (SREL) continues to operate successfully under safety and environmental requirements and standards established by The University of Georgia and the SREL Safety Manual. These standards continue to address the hazards associated with SREL operations by permitting a focused effort on the health and safety issues most pertinent to SREL operations. SREL supports and promotes an integrated approach to SRS environmental health and safety issues as a signatory to the SRS Workplace Safety, Health and Security Policy and the SRS Environmental Management System Policy Statement.

SREL maintains a commitment of one, full-time position (SREL EH&S Manager) dedicated to the support of the SREL EH&S Program. The SREL EH&S Manager interfaces with other SRS Contractor Environmental Health and Safety Programs and Professionals through participation in site level management Committees (ISM Integration Council and the SRS Senior Environmental Managers Council).

The SREL EH&S Manager functions as an interface with other SRS organizations in receiving and distributing applicable Lessons Learned information. By integrating with other SRS organizations to share Lessons Learned information, SREL takes advantage of the collective experience and improvements identified by other organizations for similar work processes and controls at SREL. SREL's internal computer network was used to provide targeted safety information to specific groups in the laboratory. The SREL EH&S Manager electronically distributed 25 (twenty-five) lessons learned notices in FY 2011 to targeted groups at SREL. Additionally, the SREL EH&S Manager electronically communicated in excess of 100 (one-hundred) SRS operational safety and environmental related announcements and notices to all SREL personnel.

The SREL EH&S Manager provided weekly reports of recordable personnel accidents or injuries to DOE-SR line management. SREL also provided monthly, SREL personnel work hour statistics to DOE-SR. SREL personnel reported 0 (zero) work related recordable injuries/illnesses during FY2011.

The SREL EH&S Program continues to place an emphasis on safety and environmental training of SREL personnel. All new SREL personnel receive a two-hour SREL-specific orientation on the topic of SREL safety and environmental programs, policies, and procedures in addition to the SRS required General Employee Training (GET). New SREL personnel also receive job specific safety training provided for by their SREL supervisor. Approximately 14 (fourteen) new SREL personnel received this required training during FY2011. Additionally, SREL personnel received EH&S related training during FY2011 in the following functional areas as their job tasks required:

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

- Remote worker training in accordance with SRS remote worker requirements
- Georgia Right-To-Know Law (GRTK- HAZCOM equivalent) chemical specific training for UGA/SREL employees who utilize hazardous chemicals in the work place

SREL waste minimization and chemical disposal issues continue to be refined to promote sound environmental practices and support SRS environmental initiatives. 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. SREL generated very small amounts of hazardous wastes in FY2011. As part of SREL waste minimization efforts and to ensure that chemical hazards are addressed prior to purchasing chemicals, the SREL EH&S Manager reviewed and approved 87 (eighty-seven) purchase requisitions that included chemicals purchased by SREL personnel.

SREL received no Notices of Violation in FY2011 as the result of external or internal reviews, inspections, or assessments. SREL conducted assessments in the areas of chemical and radiological air emissions, community right-to-know, and the Georgia Right-to-Know law in compliance with state and federal requirements. SREL also participated in the SRS's annual, comprehensive review and declaration process for Integrated Safety Management Systems (ISMS). As part of the annual ISMS declaration, SREL revised its Integrated Safety Management System Description Document, reviewed its FY2011 safety performance, and established its FY2012 safety performance goals.

Quality Assurance Program

SREL has attempted to maintain the U.S. Department of Energy (DOE)-approved Quality Assurance (QA) program. This QA Program is devoted to assuring the continued quality of research data, benefitting both the DOE and the research community. SREL's "Good Research Practices" highlight research concepts and context, research logistics, and the conduct of research and are available to all SREL personnel.

Research Data Archive Activities

Responsible management of research data plays an important role in preserving SREL's institutional memory. SREL has built a centralized repository of research data files and associated "metadata" necessary to make these data fully accessible. 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. A web-based SREL data archive system allows users to upload metadata information and actual data files directly from their office desktop computers. Anyone at SREL or on the SRS can search for data using this web-based system; however,

decisions about releasing original data to third parties are retained by the principal investigators.

Loss of access to a secure SREL server reduced our ability to interactively access this system. While the computer files still exist, they are not as conveniently linked and searchable as before and retrieval of these data would be quite time consuming. SREL has secured some additional funding late in FY10 from SRNS to begin to return these data archive files to their previous condition. It is anticipated that this effort will assist in Decontamination and Decommissioning (D and D) of facilities on the SRS and interacting with relevant state and federal regulators.

In this project, the data archive was restored, which contained 6 decades of environmental data collected by Savannah River Ecology Laboratory (SREL) scientists and staff. This forms the basis for the development of a *bona fide* relational database, the SREL Database, that will serve two purposes, viz., to

1. provide support knowledge regarding past characterizations of, and potential future impacts to, Savannah River Site (SRS) ecosystems in response to new DOE initiatives such as creating an "Energy Park"
2. promote the continued data importation by SREL scientists to this repository that can be "mined" in future ecological research contexts by SREL and SRS environmental professionals.

The initial design model for this new database (DB) was provided in a technical report to DOE and SRNS personnel entitled "SREL Database Assessment Project – Final Report" dated 1/13/2011 for consideration and future funding.

SREL Undergraduate and Graduate Education Program

Gary Mills, Stacey Lance, and Tracey Tuberville

Objectives of the SREL Education Program are to (1) recruit and develop additional professionals to the environmental sciences and (2) enhance environmental awareness and research opportunities among undergraduate and graduate students with emphasis on conducting ecological research important to the DOE and Savannah River Site mission. We have made special efforts in the recruitment from under-represented minority groups and our faculty have worked with both students and faculty from Historically Black Colleges and Universities (HBCU) throughout the Southeast.

SREL has a long history of graduate and undergraduate education, training over 400 graduate and over 600 undergraduate researchers since 1967. Undergraduate students from more than 275 different colleges and universities have coauthored more than 170 peer reviewed research publications and more than 200 of these students have gone on to pursue careers in science. SREL offers students state-of-the-science laboratory facilities, a wide variety of natural and impacted habitats for field research, a diversity of faculty expertise, and more than fifty years of experience in ecological research. Since 1967, an average of six students per year have completed graduate studies at SREL,

resulting in a total of more than 335 dissertations and theses. Since 1985, our graduate students have won over 200 awards from regional, national, and international competitions at numerous professional societies and foundations. In recent years, SREL has undergone significant changes in administrative infrastructure and transitioned to self-supporting funding model. Throughout this transition period, SREL has maintained its commitment to student research and education as an integral component of its mission. In fact, many of the current graduate students initiated their program of study in 2011.

Although we had no funded program in the summer of 2011, SREL faculty and staff mentored and supervised 10 undergraduate students (Table 1) from several universities. These students were supported from several funding sources including DOE supported projects and other extramural grants and projects. In addition, SREL faculty provided support for 28 graduate students (Table 2) from universities across the country in 2011. In many cases, this included formal involvement by serving as major advisors/co-major advisors and committee members for M.S. and Ph.D. candidates and in a few cases students received stipend support. However, support for students also included various activities in less formal relationships such as assistance at and access to field sites, use of field equipment, temporary lab space, as well as analytical and GIS resources for their studies.

Finally, SREL offered a 10 week lecture and training program entitled Applications of GC-MS in the Environmental Sciences, Hands-on training course for undergraduate students at South Carolina HBCU funded by DOE which was taught by Gary Mills.

Table 1. SREL Undergraduate Student Program Participants, FY 11

<u>Student</u>	<u>Academic Institution</u>	<u>Faculty Advisor/Supervisor</u>
Traci Jones	Tennessee Technological University	G. Mills
Zach Ross	New York University (Abu Dhabi)	J.W. Gibbons
Nicholas Scobe	Michigan State University	K. Buhlmann
Frances Owens	University of Iowa	K. Buhlmann
Nicole Gerrard	Rutgers University	K. Buhlmann
Jeff Slocum	University of Connecticut	K. Buhlmann
Jared Green	University of Massachusetts	K. Buhlmann
Diana Nelson	Clemson University	S. Lance
Shelby Weathersbee	University of South Carolina-Aiken	G. Mills
Aubrey Danielson	University of South Carolina-Aiken	G. Mills

Table 2. SREL Graduate Student Program Participants, FY 11

<u>Student</u>	<u>Degree</u>	<u>Institution</u>	<u>SREL Faculty Advisor or Collaborator</u>
Shea Beuttner	M.S	University of Georgia - Crop and Soil	J. C. Seaman
Larchinee Turner	M.S.	University of Georgia - Crop and Soil	J. C. Seaman
Julian Singer	Ph.D.	University of Georgia - Crop and Soil	J. C. Seaman
Gabielle Robinson	M.S.	University of Georgia - Warnell	G. Mills
Tom Luhring	Ph.D.	University of Missouri	G. Mills
Paul Edwards	M.S.	U. of Eastern Illinois	L. Bryan/ G. Mills
Sean Sterrett	Ph.D	University of Georgia	T. Tuberville
Bess Harris	Ph.D.	University of Georgia - Warnell	T. Tuberville
Melia Nafus	Ph.D.	University of California, Davis	T. Tuberville
Jess McGuire	Ph.D.	University of Georgia - Warnell	T. Tuberville
Brett DeGregorio	Ph.D.	University of Illinois, Champaign-Urbana	T. Tuberville
Kaetlyn Kerr	Ph.D.	Hofstra University	T. Tuberville
John Finger	Ph.D.	University of Georgia - Environmental Health Science	T. Tuberville
Daniel Gaillard	M.S.	University of Southern Mississippi	T. Tuberville
Kim Sonderman	M.S.	University of Georgia - Warnell	T. Tuberville
Steve Kimble	Ph.D.	Purdue	T. Tuberville
Johnathan Walls	M.S.	Auburn	R. Kennamer
Maureen McClintock	M.S.	Auburn	R. Kennamer
Sarah DuRant	Ph.D.	Virginia Tech	R. Kennamer
Brian Crawford	M.S.	University of Georgia - Warnell	K. Buhlmann
Brett Moule	Ph.D.	Clemson University	K. Buhlmann
Melia Nafus	Ph.D.	University of California, Davis	K. Buhlmann
Brian Metts	Ph.D.	University of Georgia - Ecology	K. Buhlmann
Marcus Zokan	Ph.D.	University of Georgia - Ecology	R. Sharitz
Schyler Nunziata	M.S.	Eastern Kentucky University	S. Lance
Justin Henningsen	Ph.D.	U. of Massachusetts, Amhurst	S. Lance/T. Tuberville
Jason O'Bryhim	Ph.D.	George Mason University	S. Lance
Bryan Falk	Ph.D.	American Museum of Natural History	S. Lance
Shane Campbell-Staton	Ph.D.	Harvard University	S. Lance

Environmental Outreach Program

J. Whitfield Gibbons

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

The Savannah River Ecology Laboratory (SREL) Outreach Program uses information from SREL long-term research efforts to provide training and services to Savannah River Site employees and to educate the public locally, regionally, and nationally. The

Outreach Program is designed to enhance SREL's overall mission of acquiring and communicating environmental knowledge and to highlight NNSA's and the U.S. Department of Energy's (DOE) focus on environmental issues on the SRS. We accomplish these goals via education initiatives focusing on 1) Environmental safety on the SRS, 2) On-site outreach to the general public, and 3) Off-site outreach activities at schools and community events. Issues as diverse as wildlife safety in the field, amphibian and reptile population declines, potential responses of organisms to contamination, distribution and abundance of sensitive species, wetland dynamics and remediation, water quality, and dispersal of organisms from radioactively or chemically contaminated sites all are important beyond SREL. Outreach initiatives during FY10 were accomplished through a variety of programs and materials funded predominately from NNSA MOX and SRNS.

During the past year SREL scheduled and completed the following:

- 1) 65 Public Tours; estimated number of attendees - 1497.
- 2) 9 Wildlife safety talks for SRS employees; estimated number of attendees - 472.
- 3) 263 in the classroom education programs for elementary and secondary students; estimated number of attendees - 14,917.
- 4) 70 Presentations to college, civic, and professional groups; estimated attendees - 3,700.
- 5) 15 Exhibits at local and regional events; estimated number of attendees - 11,682.
- 6) 1 workshop conducted for adults; Subject included Turtle Mark-Recapture and SCDNR (South Carolina Department of Natural Resources) Master Naturalist at Palmetto Bluff - estimated number of attendees - 16.
- 7) 29 Ecologist for a Day Programs (school field trips to SREL's Conference Center); estimated number of attendees - 729.

Total events: 452; Total estimated attendance: 33,013

Consistent with the goals of the MOX project, SREL provided information and presentations to schools and programs in addition to resource materials to demonstrate the ecological health of the SRS, and the importance of environmental stewardship and National Environmental Research Park (NERP) programs on the SRS. Accordingly, we conducted Lunch and Learn presentations, provided tours for site interns, and developed slideshows suitable for use in the lobby. Lastly, we produced literature on native SRS plants and animals specifically associated with the MOX site, and continued to distribute the NERP booklet to visitors.

We also provided on-site training and services to demonstrate the potential sources of injury from animals and plants found on the SRS and the CSRA that could occur to remote workers engaged in field activities or to employees and their families at home. We conducted workshops and training, attended site monthly safety meetings where we delivered live animal and PowerPoint presentations. We also developed and distributed safety materials (protocol badge cards, safety fact sheets, and website). While the primary focus of most of these programs has been on snakes and alligators, we are also able to provide information on plants, insects, spiders, snapping turtles, and mammals of concern.

We participated in SRS outreach to the general public via the SRNS Public Tours program with SREL providing a 45-60 minute presentation to two or three tours each month year-round (30 presentations). Our presentations provide a general introduction about the history and ongoing mission of SREL and the lab's involvement with research, teaching, and community service. We concluded these programs by fielding questions from participants on wildlife identification, site environmental research programs, safety, and other ecological matters.

Public Outreach programs include: *Ecotalk*, an opportunity for students to have nature brought into their classroom for a face-to-face lesson on a variety of live animals found in local habitats; *Ecologist for a Day* visits allow students to spend the day in the field gaining hands-on knowledge of the plants and animals of the unique Upper Three Runs Creek area at our conference center; civic group presentations; and ecological tours. All school programs incorporate science standards and curricula for particular school districts. In many of these programs participants get an opportunity to work with SREL staff as they catch, mark, and measure various species of reptiles, amphibians, fish, small mammals, and invertebrates. In addition, we offer an annual free program, *Touch an Animal Day*, to the CSRA community at the SREL Conference Center that allows the public of all ages to interact with live animal and plant species, to meet site researchers, and to learn more about SRS efforts. Lastly, the Outreach Program offers tours of SREL facilities, as well as exhibits and workshops for the general public.

The main SREL Outreach site receives numerous hits, as it has links to the popular *Ecologist for a Day* program, Outreach fact sheets and products, and the *Ecoviews* newspaper column. SREL also continues the website for *Kids Do Science* that provides all the necessary materials for 10 hands-on activities originally developed as part of the hands-on science program with the AHF (American Honda Foundation). This site is frequented by teachers from throughout the country who use the materials in their own classes.

SREL distributes thousands of copies of educational products and materials nationwide to schools, organizations, and the general public. Educational materials include two six-foot-long full-color posters describing the importance of wetlands, along with teachers' guides. The full-color brochure *Snakes of Georgia and South Carolina* (currently in its fifth printing) has proved to be an extremely successful educational product that reflects positively on DOE and the SRS and has additional value as a safety education tool. 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 other organizations such as the Boys and Girls Clubs in Aiken and Augusta. Articles referencing the book have appeared in numerous local newspapers and magazines including publications in Florida and Texas.

The Outreach Program also continued to distribute educational materials including fliers on *Carnivorous Plants and Their Habitats*; the national version of the Partners in Amphibian and Reptile Conservation (PARC) poster produced by SREL; the brochure *American Alligator* that discusses all safety, ecological, and conservation aspects of alligators; *An Amphibian's Eye View of Wetlands*; and *Is it a Water Moccasin?*; a children's comic book entitled *Stepping into Ecology: the Ecological Adventures of Mud E. Boot*; a sticker on *Chemistry – it's all about the nature of things*, and the *Metric System Rap* bookmark, as well as numerous fact sheets available through the website. These products are extremely popular with educators and their students, and thousands of copies have been distributed during the past year. Previously created full-color fact sheets and research "snapshots" on a wide variety of research topics were distributed as well. The SREL copies of *Carnivorous Plants and Their Habitats*, *American Alligator*, and the PARC poster are now depleted and reprinting will be necessary.

The Outreach Program continues to respond to inquiries from the press, directing reporters to the most appropriate researchers for their stories. In addition, SREL sends press releases to media contacts on a regular basis as well as submits research information to appropriate audiences. From 2010-2011 a wide diversity of national and regional outlets contacted researchers at SREL and reported information about research, education, and outreach programs. These included Aiken Standard, Athens Banner-Herald, Atlanta Journal-Constitution, Augusta Chronicle, Bluffton Today, Charlotte Observer, Discovery Channel, Georgia Public Broadcasting, Harvard Film Crew, KMOX Radio (St. Louis, MO), Oryx, Post and Courier, Reptiles Magazine, Science Daily, Tuscaloosa News, UGA Columns, USA Today, U.S. Fish and Wildlife Service, Educational video. Topics in the news included regional Outreach presentations and exhibits: animal behavior, animal release protocols, biodiversity, preserving data sets, conservation, genetics, stream and Carolina bay wetland research, and SREL researcher profiles.

DOE Research Set-Aside Areas

Linda Lee

The SRS's Set-Aside Program began in the 1960s when the Atomic Energy Commission (AEC) established 10 relatively small *SREL Reserve Areas* to represent the various habitats on the SRP and to secure study sites for conducting long term ecological research. The Program was expanded in the 1980s to 30 *DOE Research Set-Aside Areas* to better protect sensitive species habitats, preserve the biological integrity of Upper Three Runs Creek, and to buffer SREL's long term research from

encroaching forest management activities. These Areas are a significant component of the SRS landscape (7% of SRS totaling 14,560 acres/5,892 ha) and are found in 43 of the Site's 89 timber resource compartments. There are approximately 275 miles (443 km) of posted boundary line.

Set-Aside Areas are critical to the DOE's Environmental Stewardship mission: they provide for long term study sites as well as sanctuary and protection to much of the SRS's sensitive flora and fauna, including many archaeological sites. They also serve as benchmarks or baseline controls for conducting ecological risk assessments, contaminant transport studies, and site remediation and restoration work. They exist today in strong support of the SRS being a National Environmental Research Park.

Administration and Management of the Set-Aside Areas

Under the existing Cooperative Agreement with the DOE, SREL serves as the point of contact for the 30 Set-Asides and provides custodial oversight of the SRS Set-Aside Program. SREL chairs the DOE's Set-Aside Task Group which approves management prescriptions, evaluates proposed ecological research, and ensures protection from onsite land use activities. Since FY07, DOE funding support for this program has ceased. However, SREL continues the day-to-day administration of the program.

Boundary maintenance and the development and implementation of stewardship management plans are not currently supported (i.e. funded) despite the need for such efforts in order to maintain ecological integrity and future research value of the Areas. However, SREL continues critical, day-to-day functions such as SRS site use permit application reviews, research coordination, and leadership in addressing basic management issues.

Management Initiatives in FY2011

The Set-Aside Task Group met with USFS Fire regarding fire policy for Upper Three Runs and Meyers Branch set-aside areas. The Task Group approved back-in, dormant season fire in these drainages. These areas will not be intentionally ignited, but neither will they be purposefully excluded from burns by the use of plowed firebreaks.

Research and Outreach in Set-Aside Areas

- The amphibian community at the Rainbow Bay Amphibian Reserve Set Aside has been monitored for 32 consecutive years, during which time dramatic changes in species composition and population sizes have occurred. Some species have gone locally extinct at the site, while others have become more abundant. The data series is now sufficiently long to begin examining long-term trends related to environmental changes over time. Researchers are also investigating genetic changes over time for mole and marbled salamanders to better understand how population size relates to genetic diversity, which is important for conservation efforts.
- Intensive studies of marbled salamanders, the species most ecologically similar to the federally endangered flatwoods salamander, have been conducted at Ginger's Bay since 1986. Recent modeling of pond-breeding salamanders at Ginger's Bay

showed that a large proportion of the population utilizes habitat well beyond the Set Aside boundary. Researchers are also using molecular techniques to examine mating behavior and reproductive success.

- SREL researchers continue collecting amphibian tissue samples to gather pilot data for future studies of amphibian landscape genetics and effects of future climate change. Samples from eight species have been collected from ~35 isolated wetlands across the SRS, including the following Set Asides: Rainbow Bay Amphibian Reserve, Dry Bay, Ellenton Bay, Mona and Woodward Bays, Flamingo Bay, Thunder Bay, Craig's Pond and Sarracenia Bay, Ginger's Bay, and Road 6 Bay.
- Researchers at SREL and a collaborating institution are examining predator-prey interactions between snakes and songbirds in Field 3-412. The research includes using videography to document nest predators and radio telemetry to explore links between snake movement patterns and predation rates of songbird nests.
- Rainbow Bay, Ellenton Bay, Ginger's Bay, and Flamingo Bay continue to serve as reference sites for several amphibian ecotoxicology studies, including effects of copper in the Tritium Facility's H-02 Treatment Wetlands and metals uptake in the P-Area ash plume.
- Researchers at several institutions are using the Dry Bay Set-Aside to study the ecology of ticks and their hosts. Dry Bay is one of a network of sites in a NSF-funded initiative to understand the ecological and/or evolutionary drivers for the geographic variation in Lyme disease risk in eastern North America. The disease is virtually absent from the Deep South, despite the presence of its common vector, the black-legged tick. Three alternative hypotheses are being examined: climate, host community, and vector genetic structure.
- A mark-recapture population analysis of greater sirens and two-toed amphiumas continues in Dry Bay. This study examined the distribution of species and individuals among microhabitats and depth levels in the bay and focused on the ability of these species to sense and respond to chemical cues from predators.
- Research funded by SC Department of Natural Resources is examining habitat use of state-endangered gopher frogs in the Craig's Pond and Sarracenia Bay Set-Aside. Four sections of drift fence have been installed at the north end of Craig's Pond to intercept adults and metamorphs. Metamorphs were head-started over winter and released this spring with radiotransmitters. Data will be useful in management of this Set-Aside as well as Mona Bay (another gopher frog habitat).
- Mark-recapture studies of black swamp snakes continue at Ellenton Bay. Long-term monitoring of these aquatic snake populations and their community dynamics will aid in understanding their response to environmental variation (drought) and amphibian prey availability.
- Researchers continue to maintain duck boxes at Ellenton Bay, Steel Creek Bay, and Flamingo Bay, as part of a long-term (since the 1970s) study of the breeding ecology of wood ducks on the SRS.
- Researchers from SREL and SRNL are using Set-Aside streams in a SERDP-funded project to develop ecological reference models and an assessment framework for other southeastern coastal plain stream systems common to the DOD's military reservations.

- Researchers from SREL, USFS-SR, and University of Kentucky continue stream characterization in the UTRC/Tinker Creek and Meyers Branch set-asides. This research, part of the ACP Stream Restoration Baseline Project, will be used to inform future DOE restoration and mitigation efforts.
- Archaeologists with the USC-Savannah River Archaeologist Research Program continued their investigations examining the occupational use of the Flamingo Bay sand rim. Artifacts dating to the Clovis period have been found.
- UGA researchers are examining zooplankton assemblages in several Set-Asides containing seasonal wetlands. The scientific literature indicates that the SRS zooplankton community is the most species-rich of any comparable system yet studied. Their research attempts to uncover the reasons behind this remarkable diversity.
- SREL's Outreach Program continues to use the E.P. Odum Wetland Set-Aside as an outdoor classroom during its enormously popular "Ecologist for a Day" programs. These programs give K-12 students hands-on experiences in ecological research, foster understanding of environmental issues, promote environmental stewardship, and encourage students to consider careers in science.

EXTERNALLY FUNDED GRANTS

PI
Project Title
Funding Agency
Period
Budget

I. Lehr Brisbin
UGA Foundation Canine Research
UGA Foundation
Open
\$28,505

PI
Project Title
Funding Agency
Period
Budget

Kurt Buhlmann
Development of Amphibian Monitoring Methodologies for the Gulf Coast Network (CESU) Phase 3
CESU – Piedmont
October 2008 – September 30, 2013
\$29,253

PI
Project Title
Funding Agency
Period
Budget

Kurt Buhlmann
Increasing Juvenile Recruitment of Bog and Wood Turtles on Managed Lands at the Great Swamp National Wildlife Refuges: Building on Past Success
CESU – Piedmont
May 1, 2011 – May 31, 2012
\$13,000

PI
Project Title
Funding Agency
Period
Budget

Kurt Buhlmann
A Partnership for Continued Research and Monitoring of the Gopher Tortoise Reintroduction Project at the Aiken Gopher Tortoise Heritage
SC Dept of Natural Resources
January 1, 2010 – August 31, 2011
\$22,428

PI
Project Title
Funding Agency
Period
Budget

Kurt Buhlmann
Habitat use by the Carolina Gopher Frog at Craigs Pond (SC Heritage Preserve and Savannah River Site)
SC Dept of Natural Resources
February 20, 2010 – September 30, 2011
\$15,000

PI
Project Title
Funding Agency
Period
Budget

Kurt Buhlmann
A Habitat Assessment and Dip-Net Surveys for Flatwood Salamanders on NAS Pensacola, Nolf Bronson and NETPDTCSaufley Field
CESU – Piedmont
May 15, 2010 – September 30, 2011
\$16,944

PI Whit Gibbons
Project Title Scientific Oversight and Web Site Enhancement for the North American Reporting Center for Amphibian Malformations (NARCAM) and National Biological Information Infrastructure (NBII)
Funding Agency USDI – USGS
Period August 23, 2010 – July 31, 2011
Budget \$30,000

PI Robert Kennamer
Project Title Cost of Incubation: Linking Incubation-Induced Alterations in Phenotype to Changes in Fitness
Funding Agency Virginia Polytechnic Institute
Period September 1, 2006 – August 31, 2011
Budget \$83,832

PI Robert Kennamer
Project Title Ecological Study of Birds in the Vicinity of Augusta Regional Airport at Bush Field
Funding Agency Augusta-Richmond County Consolidated Government
Period October 1, 2010 – September 30, 2011
Budget \$90,905

PI Stacey Lance
Project Title Schistosomiasis Consortium for Operational Research and Evaluation (SCORE)
Funding Agency B and M Gates Foundation
Period December 1, 2008 – December 1, 2013
Budget \$16,048

PI Stacey Lance
Project Title US-Mexico Workshop on Evolutionary Genomics of Non-Model Species: Next-gen Sequencing, Data management and Hypothesis Testing
Funding Agency National Science Foundation
Period May 1, 2011 – April 30, 2012
Budget \$4,792

PI Stacey Lance
Project Title US-Mexico Workshop on Evolutionary Genomics of Non-Model Species: Next-gen Sequencing, Data management and Hypothesis Testing
Funding Agency National Science Foundation
Period May 1, 2011 – April 30, 2012
Budget \$45,049

PI John Seaman
Project Title Polarized Light Microscopy (PLM) and Chemical Analysis
Funding Agency Savannah River Nuclear Solutions
Period January 25, 2010 – September 30, 2011
Budget \$1,499

PI Tracey Tuberville
Project Title Genetic Mating System of Translocated Gopher Tortoises
Funding Agency Riverbanks Zoo
Period June 1, 2008 — December 31, 2010
Budget \$4,804

PI Tracey Tuberville
Project Title Using Individual Behavior-Based Modeling to Predict Population Response and Long-Term Viability of “Species At Risk”
Funding Agency US Department of Army
Period August 26, 2008 — August 31, 2010
Budget \$76,749

PI Tracey Tuberville
Project Title South Carolina Reptile and Amphibian Conservation
Funding Agency SC DNR
Period July 15, 2011 – September 30, 2011
Budget \$1,500

PI Tracey Tuberville
Project Title Stream Ecological Reference Model Development
Funding Agency Savannah River Nuclear Solutions
Period September 14, 2009 – September 3, 2011
Budget \$94,000

PI Tracey Tuberville
Project Title Direct and Indirect Effects of Exurbanization on the Eastern Diamondback Rattlesnake
Funding Agency Riverbanks Zoo
Period January 1, 2001 – January 1, 2011
Budget \$9,200

PI Tracey Tuberville
Project Title Juvenile Survivorship of Gopher Tortoises
Funding Agency American Museum of Natural History
Period April 1, 2011 – December 31, 2011
Budget \$5,407

PI Tracey Tuberville
Project Title Landscape Scale Conservation Genetics of *Heterodon platirhinos* and *H. simus*: A Comparison of two closely related sympatric species with contrasting spatial ecologies
Funding Agency Project Oriannne Ltd
Period January 31, 2011 – January 31, 2013
Budget \$8,000

PI Tracey Tuberville
Project Title Biological Impacts of Solar Energy Projects in the Desert
Funding Agency Subcontract from University of California at Davis
Period November 1, 2010 – October 31, 2011
Budget \$22,252

PI Tracey Tuberville
Project Title Vulnerability Assessment for Sandhills Fauna: Development of Ranking Criteria, Sensitivity Models, and a Decision Framework for Long-Term Installation Management
Funding Agency CESU – Piedmont
Period March 28, 2011 – March 27, 2012
Budget \$12,249

PI Tracey Tuberville
Project Title A Survey of NAS Whiting Field and Associated Properties for Gopher Tortoises
Funding Agency CESU – Piedmont
Period May 15, 2010 – September 30, 2011
Budget \$21,337

PUBLICATIONS

Journal Articles Published In FY2011 (publication noted with an asterisk were previously published but never assigned an SREL publication number)

- 3088 Bolan, N. S., D. C. Adriano, A. Kunhikrishnan, T. James, R. McDowel, and N. Senesi. 2011. Chapter 1. Dissolved Organic Matter: Biogeochemistry, Dynamics, and Environmental Significance in Soils. pp. 1-75. In *Advances in Agronomy*, Volume 10. D. L. Sparks (ed.) Elsevier, Inc. Newark, Delaware,.
- 3155 Bhupathy, S. and K. A. Buhlmann. 2010. Le Trionyx á clapets de L'Inde. *Chéloniens* 18: 24-28.
- 3156 Lance, S. L., J. E. Light, K. L. Jones, C. Hagen, and J. C. Hafner. 2010. Isolation and characterization of 17 polymorphic microsatellite loci in the kangaroo mouse, genus *Microdipodops* (Rodentia: Heteromyidae). *Conservation Genetics Resources* (2010)2:139-141.
- 3157 Flanagan, S. P., W. H. Wilson, K. L. Jones, and S. L. Lance. 2010. Development and characterization of twelve polymorphic microsatellite loci in the Bog Copper, *Lycaena epixanthe*. *Conservation Genetics Resources* (2010)2:159-161.
- 3158 Erickson, M. R., D. E. Scott, K. L. Jones, C. Hagen, and S. L. Lance. 2010. Development and characterization of ten microsatellite loci for the eastern spadefoot toad, *Scaphiopus holbrookii*. *Conservation Genetics Resources* (2010)2:143-145.
- 3159 Kwiatkowski, M. A., C. M. Somers, R. G. Poulin, D. C. Rudolph, J. Mstino, T. D. Tuberville, C. Hagen, and S. L. Lance. 2010. Development and characterization of 16 microsatellite markers for the Louisiana pine snake, *Pituophis ruthveni*, and two congeners of conservation concern. *Conservation Genetics Resources* (2010)2:163-166.
- 3160 Henningsen, J. P., S. L. Lance, K. L. Jones, C. Hagen, J. Laurila, R. A. Cole, and K. E. Perez. 2010. Development and characterization of 17 polymorphic microsatellite loci in the faucet snail, *Bithynia tentaculata* (Gastropoda: Caenogastropoda: Bithyniidae). *Conservation Genetics Resources*(2010)2:247-250.
- 3161 Jones, K. L., J. R. Henkel, J. J. Howard, S. L. Lance, C. Hagen, and T. C. Glenn. 2010. Isolation and characterization of 14 polymorphic microsatellite DNA loci for the endangered Whooping Crane (*Grus americana*) and their applicability to other crane species. *Conservation Genetics Resources* (2010)2:251-254.

- 3163 Pinder III, J. E., T. G. Hinton, B. E. Taylor, and F. W. Whicker. 2011. Cesium accumulation by aquatic organisms at different trophic levels following an experimental release into a small reservoir. *Journal of Environmental Radioactivity* 102(2011):283-293.
- 3165 Jackson, N. D., T. C. Glenn, C. Hagen, S. L. Lance, and C. C. Austin. 2011. Microsatellites isolated from the North American ground skink (*Scincella lateralis*). *Conservation Genetics Resources* (2011)3:95-97.
- 3166 Tuberville, T. D., T. M. Norton, B. J. Waffa, C. Hagen, and T. C. Glenn. 2011. Mating system in a gopher tortoise population established through multiple translocations: Apparent advantage of prior residence. *Biological Conservation* 144(2011):175-183.
- 3168 De Steven, D., R. R. Sharitz, and C. D. Barton. 2010. Ecological Outcomes and Evaluation of Success in Passively Restored Southeastern Depressional Wetlands. *Wetlands* 30:1129-1140.
- 3169 Byers, J. E., I. Altman, A. M. Grosse, T. C. Huspeni, and J. C., Maerz. 2010. Using Parasitic Trematode Larvae to Quantify an Elusive Vertebrate Host. *Conservation Biology* 25(1): 85-93.
- 3170 Coghill, L. M., J. Chaves-Campos, F. E. Espinoza, S. L. Lance, T. C. Glenn, and S. G. Johnson. 2011. Microsatellite markers isolated from the Mexican banded spring snail *Mexipyrgus churinceanus*. *Conservation Genetics Resources* (2011)3: 29-31.
- 3171 Breton, J. S., K. Oliveira, R. E. Drew, K. L. Jones, C. Hagen, and S. L. Lance. 2011. Development and characterization of ten polymorphic microsatellite loci in the yellowtail flounder (*Limanda ferruginea*). *Conservation Genetics Resources* (2011)3: 369-371.
- 3172 McKee, A. M., S. L. Lance, K. L. Jones, C. Hagen, and T. C. Glenn. 2011. Development and characterization of 18 microsatellite loci for the Southern Leopard Frog, *Rana sphenocephala*. *Conservation Genetics Resources* (2011)3: 267-269.
- 3173 Purcell, K. M., S. L. Lance, K. L. Jones, and C. A. Stockwell. 2011. Ten novel microsatellite markers for the western mosquitofish. *Conservation Genetics Resources* (2011)3: 361-363.
- 3174 Somers, C. M., K. Neudorf, K. L. Jones, and S. L. Lance. 2011. Novel microsatellite loci for the compost earthworm *Eisenia fetida*: A genetic comparison of three North American vermiculture stocks. *Pedobiologia - International Journal of Soil Biology* 54(2011): 111-117.

- 3175 Dorcas, M. E., J. D. Willson and J. W. Gibbons. 2011. Can invasive Burmese pythons inhabit temperate regions of the southeastern United States? *Biological Invasions* 2011(13):793-802.
- 3176 Jin, V. L., C. S. Romanek, L. A. Donovan, and R. R. Sharitz. 2010. Soil nitrogen availability and in situ nitrogen uptake by *Acer rubrum* L. and *Pinus palustris* Mill. in the southeastern U.S. Coastal Plain" *Journal of the Torrey Botanical Society* 137(4):339-347.
- 3177 Willson, J. D., C. T. Winne, and B. D. Todd. 2011. Ecological and Methodological Factors Affecting Detectability and Population Estimation in Elusive Species. *Journal of Wildlife Management* 75(1):36-45.
- 3178 Durso, A. M., J. D. Willson, and C. T. Winne. 2011. Needles in haystacks: Estimating detection probability and occupancy of rare and cryptic snakes. *Biological Conservation* 144(2011):1508-1515.
- 3179 Hepp, G. R. and R. A. Kennamer. 2011. Date of Nest Initiation Mediates Incubation Costs of Wood Ducks (*Aix sponsa*). *The Auk* 128(2):258-264.
- 3180 Bozarth, C. A., S. L. Lance, D. J. Civitello, J. L. Glenn and J. E. Maldonado. 2011. Phylogeography of the gray fox (*Urocyon cinereoargenteus*) in the eastern United States. *Journal of Mammalogy* 92(2):283-294.
- 3181 Luhring, T. M., J. D. Willson, and C. T. Winne. 2011. *Nerodia Fasciata Fasciata* (Banded Watersnake). Inter-Wetland Movement. *Herpetological Review* 42(1):100-101.
- 3182 Willson, J. D., M. E. Dorcas, and R. W. Snow. 2011. Identifying plausible scenarios for the establishment of invasive Burmese pythons (*Python molurus*) in Southern Florida" *Biol Invasions* 2011(13):1493-1504.
- 3183 Willson, J. D. and W. A. Hopkins..2011. Prey morphology constrains the feeding ecology of an aquatic generalist predator. *Ecology* 92(3):744-754.
- 3185 Grosse, A. M., J. C. Maerz, J. Hepinstall-Cymerman and M. E. Dorcas. 2011. Effects of Roads and Crabbing Pressures on Diamondback Terrapin Populations in Coastal Georgia. *The Journal of Wildlife Management* 75(4):762-770.
- 3186 Todd, B. D., D. E. Scott, J. H. K. Pechmann and J. W. Gibbons. 2011. Climate change correlates with rapid delays and advancements in reproductive timing in an amphibian community. *Proceedings of the Royal Society B* 2011(278):2191-2197.

SREL ORGANIZATIONAL CHART – FY11

Interim Director
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John C. Seaman

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Donald R. Mosser

**Computer Service and GIS Lab
Manager**

William Finney, Jr.

Education Program

Gary L. Mills
Tracey D. Tuberville

Outreach Program

J. Whitfield Gibbons

**Research and Facilities Technical
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Mark Edwards
Malcolm Squires

Administrative Services

Marie Roberts
Cherie Summer
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(As of 10/1/2011)

