

**SAVANNAH RIVER  
ECOLOGY LABORATORY**

**ANNUAL TECHNICAL PROGRESS REPORT  
OF ECOLOGICAL RESEARCH FOR FY20**

Final Report: Submitted January 2021

Supported under Cooperative Agreement  
DE-EM0004391

*between*

The University of Georgia

*and*

The U.S. Department of Energy

*for the period of*

1 October 2019 – 30 September 2020

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## **SECTION I: Savannah River Ecology Laboratory – FY20 Overview of Achievements**

The Savannah River Ecology Laboratory (SREL) is a research unit of The University of Georgia (UGA). SREL has been conducting ecological research on the Savannah River Site (SRS) near Aiken, South Carolina for over 69 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 FY20 was fulfilled with the publication of 95 journal articles, proceedings articles, and book chapters by faculty, technical staff, students, and visiting scientists. One book was also authored by SREL faculty and staff. Additional journal articles and books have been submitted or are in press. Significantly, SREL conducted over 142 outreach events reaching over 23,000 people of all ages. Other noteworthy events took place as faculty members, staff, and graduate students received awards for the quality of their research. These are described in Section IX *Special Accomplishments*.

The vision, structure, and operations of SREL continue to evolve since changes in funding structure were instituted in FY07. However, the five-year Cooperative Agreement between the University of Georgia's Research Foundation and the Department of Energy for support of the Savannah River Ecology Laboratory has allowed funding from the DOE and other SRS tenants to fund SREL to meet the specific needs of DOE Environmental Management (EM) and DOE National Nuclear Safety Administration (NNSA) on the Savannah River Site. The current funding model for SREL is entrepreneurial and interdisciplinary, and seeks to pursue funding strategies that are competitive, responsive to sponsors' requirements, and based on a diverse and sustainable foundation. This model has required restructuring of research and supporting infrastructure at the laboratory.

Today, a leaner, but robust SREL presence continues to operate on the SRS. SREL's total employment in FY20 was approximately 169 faculty, technicians, students, and support staff. Although the number of employees and level of funding has been reduced relative to its height of two decades ago, SREL continues progress toward stated objectives and does not compromise safety and security. New and continuing partnerships and collaborations with the Athens campus (Warnell School of Forestry and Natural Resources, UGA Complex Carbohydrates Center, Odum School of Ecology, College of Public Health, College of Agriculture and Environmental Sciences), other universities (University of South Carolina – Aiken, University of South Carolina – Upstate, Georgia Regents University) and other agencies (US Department of Agriculture, US Army Corps of Engineers, US Department of Defense, Federal Aviation Administration) continue to be explored and developed in order to maximize the use of SREL assets. Graduate student programs have continued with funding provided by DOE, external grants, UGA, or the student's host university.

During FY20, DOE-SR funding was leveraged to acquire approximately 1.3 million dollars in salary and infrastructure investments from the University of Georgia, in addition to the 20% cost share negotiated under the terms of UGA's Cooperative Agreement with DOE. DOE funding also is being used to leverage cost-shared faculty positions with UGA units on the main campus, resulting in three tenure track faculty lines at SREL and a portion of three tenure track faculty lines on the main UGA campus that will contribute to the SREL mission on the SRS during the coming years.

SREL faculty have responded to the revised funding structure for the laboratory and have sought financial support from multiple external funding agencies, DOE-EM, DOE-NNSA, Savannah River Nuclear

Solutions-Area Closure Projects (SRNS-ACP), Savannah River Remediation (SRR) and UGA. In addition, DOE-EM has provided additional infrastructure support to SREL to help revitalize aging facilities and meet safety standards for our working environment. Establishment of a Cooperative Agreement with DOE allows SREL/UGA access to the SRS through 30 September 2021. The SREL continues to work closely with local community groups, local schools, and other area stakeholders on a number of research, environmental monitoring, education, and outreach activities.

During FY20, SREL has continued to optimize its research programs to address DOE and SRS concerns, maintain staff in critical research disciplines, and attract new personnel. SREL researchers are 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. In addition, personnel from SREL have been actively engaged in furthering DOE's SRS missions in Radioecology, Environmental Stewardship, Next Generation Cleanup Technologies and Renewable Energy.

Researchers at SREL received funding from 48 new and continuing external grants during FY20. Sources of grant awards range from private foundations to federal and state agencies, including the US Department of Interior, the US Department of Agriculture, the National Science Foundation, and the Department of Defense.

SREL faculty members hold positions in various departments at UGA. Several SREL faculty members (and emeritus faculty) have adjunct status at other colleges and universities. Faculty, staff, and students 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 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.

SREL's Education Program continues to be successful with SREL faculty and staff mentoring over 108 graduate students from numerous colleges and universities in the United States during FY20.

The SREL Outreach Program communicates scientific awareness to area schools and the general public, an audience which differs significantly from science professionals. Although restrictions caused by the COVID-19 pandemic curtailed school visits and other in-person outreach events, the SREL Outreach Program had a productive year. SREL presented over 101 talks, 12 tours, 14 exhibits, and 14 ***Ecologist for a Day*** programs reaching a total of over 23,000 people in FY20. 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 been a part of the SRS public tour program (approximately one tour per month of 20-40 attendees). 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.

Ending in FY20, SREL's DOE-funded Radiological Education, Monitoring and Outreach Program focused on increasing public awareness and education on the topic of radiation in the environment and radiological risks to the general public. The outreach coordinator for this program provided periodic talks in the local Waynesboro, GA community as well as ad hoc presentations to a variety of local audiences. This program conducted limited environmental monitoring in FY19 and FY20, and will continue providing presentations to the local community in FY21 as opportunities allow using these data to explain and inform the public about monitoring programs that currently provide data to the region.

The UGA Conference Center continues to be a valuable asset to SREL and other entities on the SRS. SREL used the facility to host numerous meetings and environmental education programs for students, teachers, and other organizations this past year. The facility is also used by DOE, the USDA Forest Service, and other site tenants when available.

In summary, it is important to note that the important roles that SREL plays on the SRS unfold prominently in several strategic areas. Such efforts by SREL staff play a critical role in helping the DOE and other SRS tenant organizations reduce costs and continue with their missions on the SRS by assisting them to maintain regulatory compliance, validating remediation efforts, providing basic research for the development of new technologies, promoting sound environmental stewardship of natural resources on the SRS, serving as an independent source of scientific expertise for reviews of technical data and monitoring programs, educating the next generation of radioecologists and nuclear biogeochemists, and conducting outreach efforts to educate local communities about the SRS, its missions, and environmental health. For example, as a critical source of scientific expertise for the Department of Energy on the SRS, SREL provides state of the art scientific support to both DOE-EM and DOE-NNSA. Examples include research on biogeochemical cycling and biological impacts of copper associated with the H-02 mitigation wetlands that provide data needed to validate regulatory compliance for the DOE-NNSA's Tritium mission on the SRS, research on the ecological impacts and potential options for recovery of function of the U-8 stream drainage associated with DOE-NNSA's construction efforts for the MOX fabrication facility on the SRS, research on the organismal effects (proteome and glycome) to aquatic organisms exposed to chronic levels of low dose radiation, research into the extent and impact of biovectors of contaminants on the SRS, and development of strategic management plans for Set Asides on the SRS to maintain the SRS designation as DOE's first National Environmental Research Park.

SREL also serves as a source of critical scientific expertise for other SRS tenant organizations, providing analytical and ecological expertise to assist these organizations with issues ranging from regulatory compliance to creative new technologies for remediation of contaminants on the SRS. Examples include research conducted in support of the SRR mission on the SRS to provide accurate and precise data for use in parameterization of models employed to comply with NRC requirements concerning long-term contaminant exposure risk from stored nuclear materials, research conducted in support of SRR and SRNS to assess biological exposure risks from leakage events involving stored nuclear materials into soil and groundwater, research on the design and efficiency of strategies for bioremediation of tritium in SRS groundwater for SRNS, and research conducted in support of SRNS ACP's remediation mission to assess radionuclide and heavy metal accumulation in long-lived vertebrates and game species to inform regulatory compliance and risk assessment issues associated with ecosystem and human health.

As a source of regional and national scientific expertise, SREL scientists attract external funding to conduct research that not only contributes to areas of national research priority, but also helps contribute to DOE's nuclear and environmental missions on the site. Examples include research on development of technologies for control of feral swine (funded by the US Department of Agriculture), which makes the SRS both a national focal point for feral swine research as well as a recipient of the technologies and strategies as they are developed, research on conservation and management of threatened and endangered species across the nation (funded by the US Department of Defense and the US Fish and Wildlife Service), which contributes to the strategies and tools available for environmental stewardship applications on the SRS, research on avian dispersal technologies (funded by the Federal Aviation Administration and the City of Augusta), which contributes to the ability of SRS tenants to manage nuisance wildlife populations, research on scavenging ecology (funded by the US Department of Agriculture and the Department of Defense), which provides insights into the role of scavengers in recycling of energy and contaminants in the environment and the potential transfer of contaminants off of SRS, and research on risk assessment models for heavy metals in avian species (funded by the US Army Corps of Engineers), which adds to the set of risk assessment modeling expertise available for study of fauna on the SRS.

## **SECTION II. Cooperative Agreement Key Tasks**

**TASK 1. SREL will assess the impact of Site operations on the environment, and will continue to provide the public and DOE with an independent view of the environmental management of the SRS**

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 quality of research conducted by SREL scientists is facilitated by their unique expertise in 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 65 years.

*The FY20 SREL research plan can be divided into three critical research areas:*

### **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.

### **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.*"

### **Remediation and Restoration**

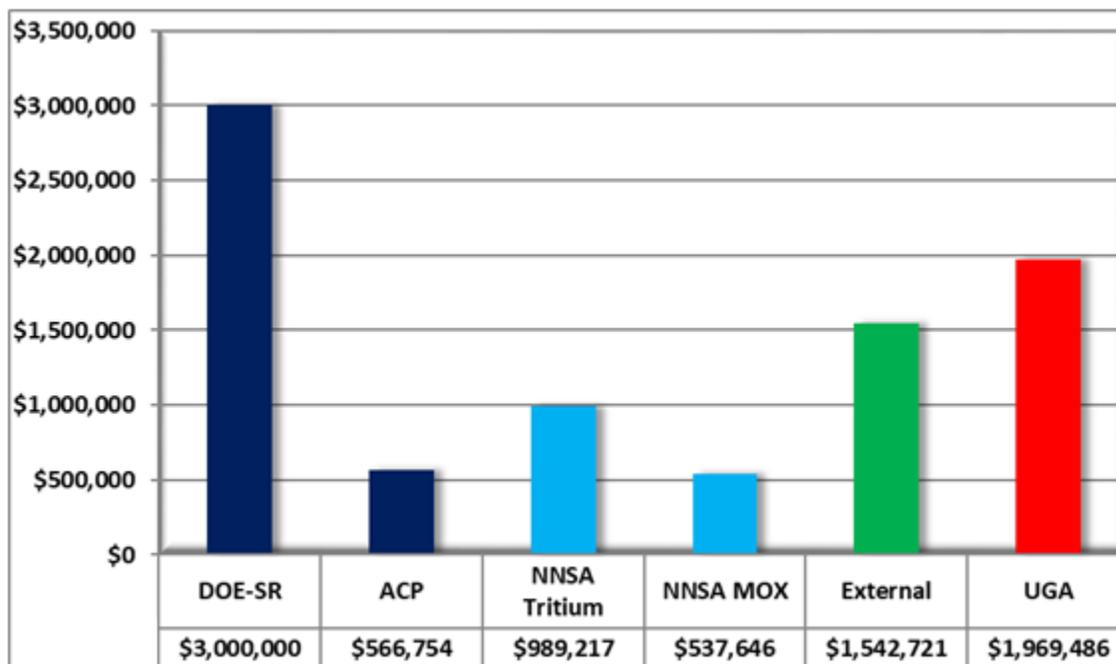
The knowledge and expertise at SREL are ideally suited to address the remediation and restoration of large land areas contaminated with relatively low levels of metals, organics, and radionuclides. SREL conducts multidisciplinary research designed to assist in the development, evaluation and stakeholder 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.

TASK 2. SREL will continue basic and applied environmental research with emphasis upon expanding the understanding of ecological processes and principles, and upon evaluating the impacts of site activities, new mission, and land use practices on the environment

In FY20, the Savannah River Ecology Laboratory received approximately 8.6 million dollars in funding from a variety of sources (Figure 2.1). These funds supported approximately 169 faculty, staff, and students conducting basic and applied environmental research for at least some portion of FY20 (Table 2.2). In total, University of Georgia funding (both direct and indirect funds returned to the lab) and external dollars received from non-SRS sources were responsible for approximately 41% of the laboratories budget. Laboratory personnel were productive and successful in attracting external funding to the site, and very active in graduate student education and service to their communities and professions (Table 2.2). SREL continues to be a productive, independent partner to DOE on the SRS and an excellent value to both stakeholders on the SRS and taxpayers.

**Figure 2.1.** Overview of funding received by SREL in FY20. Acronyms are as follows: University of Georgia (UGA), Savannah River Site Office of Department of Energy (DOE-SR), all combined sources of funding received from sources external to the Savannah River Site (External), Department of Energy National Nuclear Security Administration’s Mixed Oxide Fuel Production Facility (NNSA-MOX), Department of Energy National Nuclear Security Administration’s Tritium Facility (NNSA-Tritium), and Savannah River Nuclear Solutions Area Closures Project (ACP).

### FY20 SREL FUNDING



**Table 2.2.** SREL organizational structure for FY20. This table includes all research faculty, classified staff and Emeritus faculty in residence at the Savannah River Ecology Laboratory for any portion of the FY20 fiscal year.

<b>SREL ORGANIZATIONAL CHART – FY20</b>	
<b>Director -- Dr. Olin E. Rhodes, Jr.</b>	
<p><b><u>Associate Director, Research</u></b> Dr. T. DeVault</p> <p><b><u>Research Faculty</u></b> Dr. R. Bier Dr. J. Seaman Dr. S. Lance Dr. Xiaoyu Xu Dr. T. Tuberville Dr. G. Dharmarajan</p> <p><b><u>Tenure Track Faculty</u></b> Dr. J. Abrams Dr. J. Beasley Dr. D. Aubrey Dr. J. Martin Dr. K. Capps Dr. B. Parrott</p> <p><b><u>Emeritus Faculty in Residence</u></b> Dr. D. Adriano Dr. I. Brisbin, Jr. Dr. J.W. Gibbons Dr. K. McLeod</p> <p><b><u>Post Docs</u></b> Dr. Z. Baddar Dr. J. Hill Dr. C. Qin Dr. A. Ferreira</p> <p><b><u>Research Prof/Asst/Tech</u></b> Dr. K. Buhlmann A. Bryan      M. Mason D. Fletcher    L. Lee R. Kennamer   D. Scott A. Lindell      P. Stankus C. Fulghum     M. Baker M. Shapiro      F. Toledo K. McCallie     E. Peck J. Lott           E. Spivey K. Wilms        M. Strassburg J. Obryhim      M. Chapman</p>	<p><b><u>Assistant Director, Budget and Facilities</u></b> C. McBride</p> <p><b><u>Safety</u></b> D. Mosser R. Beasley</p> <p><b><u>Computer Service and GIS Lab Manager</u></b> W. Taylor</p> <p><b><u>Property Management</u></b> B. Morton</p> <p><b><u>Outreach Program Staff</u></b> P. Perea V. Sutton-Jackson H. DeVault J. Green-McLeod S. Poppy A. Hurst</p> <p><b><u>Research and Facilities Technical Services</u></b> R. Christie      D. Kling M. Edwards     M. Squires C. Cooper       P. Carroll D. Fraser</p> <p><b><u>Administrative Services</u></b> L. LopezdeVictoria M. Roberts J. Scott-Phillips C. Summer M. Wilburn V. Taylor M. Wead</p> <p><b><u>Temp. Research Technicians</u></b> 39 Temp. Techs were employed at SREL</p> <p><b><u>Graduate Students</u></b> 67 Graduate Students advised by SREL faculty</p>
<b>(As of 10/1/2020)* Excludes Students</b>	

**Table 2.3.** Summary of professional activities and accomplishments by Savannah River Ecology Laboratory research faculty, research professionals, postdocs and students in FY20.

<b>Publications and Reviews</b>	<b>Total</b>
Peer Reviewed Journal Articles	95
Book and Book Chapters	11
Proceedings Articles	0
Primer or Other Scientific Notes	1
Non-Peer reviewed Articles	11
Articles In Press	26
Articles In Review	59
Peer Review of Manuscripts Conducted	98
<b>External Funding (non-SRS)</b>	<b>Total</b>
External Grants Submitted as PI or CoPI	39
External Grant Funding Submitted as PI or CoPI	\$19,805,298
External Grants Funded as PI or CoPI <sup>1</sup>	48
External Grants Funded Dollars as PI or CoPI	\$5,157,122
<b>Graduate Education and Postdocs</b>	<b>Total</b>
MS Graduate Students Chaired	48
MS Graduate Students Completed	15
PhD Graduate Students Chaired	19
PhD Graduate Students Completed	6
Graduate Student Committee Memberships	41
Graduate Students Hosted at SREL	4
Post Docs Supervised	7
<b>Presentations</b>	<b>Total</b>
Invited Presentations	19
Professional Oral Presentations	71
Professional Poster Presentations	38
Extension Presentations	11
Extension Publications	2
<b>Other</b>	<b>Total</b>
Awards or Honors	22
Professional Society Committee Memberships	27
Courses Taught	26
Technical Research Consultations	35

<sup>1</sup> – includes new grants and contracts, renewals and continuations associated with funding sources external to DOE. Total includes multi-year funding commitments received in FY20 and to be received in future fiscal years.

TASK 3. SREL will use the information collected in the environmental research to develop and test hypotheses that will contribute to the scientific foundation necessary to conduct meaningful ecological risk assessments and to understand the environmental consequences of energy technologies, remediation efforts, and other SRS activities

In FY20 SREL faculty, staff, and students conducted and completed a diversity of environmental research projects on the SRS in support of the missions of SRNS, SRR, and DOE-SR, specifically in the areas of risk assessment and elucidation of the environmental consequences of energy technologies, legacy contamination, and remediation activities on the SRS. Much of this work was funded through support to the SRNS Area Closures Project, through subcontracts from SRR to perform specialized sampling or analyses, and through commitment of funds received through SREL's Cooperative Agreement with the Department of Energy to address research topics of importance to the continuing missions of DOE on the SRS. The details of these projects are outlined below:

### **Research Support to SRNS Area Closure Projects, SRR, and DOE-SR**

#### **Selective Sorbents for the In Situ Immobilization of <sup>129</sup>I and <sup>99</sup>Tc at the Four-Mile Branch Seep Line**

##### **Funding Entity**

SRNS Area Closures Projects

##### **Start Date and Funding Amount**

SREL FY20/FY21; \$100K

##### **PI and co-PIs**

Dr. John C. Seaman

##### **Objective**

The primary objective is to evaluate selective sorbents for the in situ immobilization of both <sup>129</sup>I and <sup>99</sup>Tc in an effort to enhance the natural attenuation capacity at the Four-Mile Branch seep line.

##### **Summary of Research Activities**

Based on recent literature, several potential sorbents were identified for initial lab evaluation, with an emphasis on in situ immobilization methods that do not require removal and subsequent disposal. The proposed sorbents can be placed in one of two general categories: metal-based and carbon-based sorbents.

In consultation with ACP, SREL established a limited set of test conditions that reflect specific groundwater parameters associated with important phases in the remediation process, i.e., active remediation (sorption) and post-remediation monitoring (desorption). In a previous year, batch sorption experiments were conducted using stable Iodine (<sup>127</sup>I) and Rhenium (Re as perrhenate, ReO<sub>4</sub><sup>-</sup>) as analogues for <sup>129</sup>I and <sup>99</sup>Tc, respectively, while monitoring other important components, i.e., total Fe, Fe(II), NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, etc. Care was taken to also evaluate if the proposed remediation strategies exceed regulatory limits for Ag and Cu. Such tests yielded a limited set of potential sorbents that we will focus on going forward.

##### **Conclusions**

Based on initial batch tests, the number of potential sorbents was reduced from over twenty to four that continue to be investigated. Testing has now largely shifted from batch equilibrations to long-term column flow-through experiments focused on the most promising sorbent materials. However, we continue to evaluate additional novel sorbents in batch equilibration tests. In 2020, SREL also worked with ACP to develop protocols for the field-scale evaluation of the sorbents along the Four-Mile Branch seep line, with a specific focus on the development of self-contained, removable diffusion samplers that can be used to retain the sorbents for evaluation after deployment.

##### **Major Impact(s) of Research**

Identify and evaluate in situ treatment options that reduce the need for expensive, invasive remediation efforts along the Four-Mile Branch stream.

### **Other Project Personnel**

Christina Logan, Research Professional II

Morgan Shapiro, Lab Technician

Jeffery Lott, Lab Technician

### **External Collaborators**

Dr. D. Kaplan (SRNL)

Dr. D. Li (SRNL)

### **Products**

Li, D., N. Shustova, C.R. Martin, K. Taylor-Pashow, J.C. Seaman, D.I. Kaplan, J.W. Amoroso, R. Chernikov. 2020. Anion-exchanged and quaternary ammonium functionalized MIL-101-Cr metal-organic framework (MOF) for ReO<sub>4</sub> sequestration from groundwater. *J. Environ. Radio.* <https://doi.org/10.1016/j.jenvrad.2020.106372>.

Dickson, J., N.A. Conroy, Y. Xie, B.A. Powell, J.C. Seaman, M.I. Boyanov, K.M. Kemner, and D.I. Kaplan. 2020. Surfactant-Modified Siliceous Zeolite Y for Peractin Remediation. *Chemical Engineering Journal*, <https://doi.org/10.1016/j.cej.2020.126268>.

**Tritium Distribution at the Tritiated Water Management Facility - Southwest Plume Interim Measures**

**Funding Entity**

SRNS Area Closures Projects

**Start Date and Funding Amount**

October, 2019; \$134,000

**PI and co-PIs**

Dr. John C. Seaman

**Objective**

Evaluate the efficacy of ongoing remediation efforts at the SRS Mixed Waste Management Facility (MWMF) to address the tritium and 1, 4 dioxane plume originating from the Old rad Waste Burial Ground.

**Summary of Research Activities**

In FY 2020, SREL worked collaboratively with the SRS-US Forest Service, SRNS-ACP and DOE to complete the following activities: (1) collect, process and analyze 22 soil cores collected to a depth of  $\approx 3$  m to evaluate tritiated water ( $^3\text{H}_2\text{O}$ ) distribution as an estimate of irrigation efficiency ( $\approx 220$  samples annually); and (2) maintain and update the Cornell Model for estimating water-use efficiency. ACP monitoring for 1, 4-dioxane in the surface soil of the irrigation plots was resumed in 2020 with SREL's assistance.

**Conclusions**

1. The estimated tritium evapo-transpiration efficiency for individual irrigation plots through the end of calendar year 2019 based on soil core samples ranged from  $\approx 83.1$  to  $98.4\%$  when accounting for leaching below the root zone, with increasing efficiencies observed in the Western Expansion Area as the vegetative cover improves with continued growth.
2. Monthly efficiency results derived from the Cornell 1D model from  $66.9$  to  $88.8\%$  between plots, with lower efficiencies observed for the WEA plots. The average efficiency for all plots was approximately  $86.7 \pm 1.2\%$  for the original plots,  $79.6 \pm 1.9\%$  for the EEA plots, and  $68.7 \pm 1.3\%$  for the WEA plots, resulting in an overall of annual efficiency of  $81.5 \pm 6.9\%$  for all 11 monitored plots.

**Other Project Personnel**

Christina Logan, Research Professional II

Morgan Shapiro, Lab Technician

Jeffery Lott, Lab Technician

**External Collaborators**

NA

**Products**

Logan, C., and J.C. Seaman. 2020. Estimating Evapo-Transpiration Losses for Tritium at the MWMF: 2019 End of Year Summary Report. SREL Doc.: R-19-0002. Submitted to SRNS-ACP March 18, 2020.

## Watersnakes as Ecological Receptors for Mercury Contamination and Bioaccumulation on the SRS

### Funding Entity

SRNS Area Closures Projects

### Start Date and Funding Amount

January 2018; \$105,000

### PI and co-PIs

Tracey D. Tuberville

### Objectives

The overarching objective is to explore the value of watersnakes (*Nerodia* spp.) as receptor species for mercury (Hg) contamination across the Savannah River Site (SRS) and in the Savannah River area. We are targeting three primary aquatic system types: 1) the Savannah River and its tributaries, in which the brown watersnake (*N. taxispilota*) is the dominant snake species; and 2) former nuclear cooling reservoirs, in which the green watersnake (*N. floridana*) is the dominant species; and 3) isolated ephemeral wetlands (i.e., Carolina bays), in which the banded watersnake (*N. fasciata*) is the dominant species. All three species are strictly carnivorous but they vary in the degree of specialization on fish vs amphibians – some of which may be due to prey composition in their aquatic habitat. These three systems also vary in their source and degree of Hg contamination, which will also influence Hg bioaccumulation in our target species. We will compare Hg body burdens in *Nerodia* spp. across these three system types and evaluate the effects of system type, species, body size, and sex on Hg concentrations.

### Summary of Research Activities

This fiscal year focused on quantification of total mercury (THg) in blood and tissue samples collected during the previous field season, as well as evaluating blood smears to quantify hemoparasites. We also had the opportunity to quantify methylmercury (MeHg) in samples collected in brown watersnakes as part of the current scope and compare them to archived samples collected in the 1980s. The other main focus has been data analysis and the preparation of several manuscripts.

### Conclusions

From these data the following patterns have emerged:

1. Hg concentration in both blood and tail tips is positively and significantly correlated with individual body size in both *N. taxispilota* from the Savannah River and *N. floridana* and *N. fasciata* from Carolina bays.
2. Hg concentrations in tail tips are higher than in blood samples collected from the same individuals.
3. Tail tip Hg concentrations in *N. taxispilota* vary spatially along the Savannah River, with highest concentrations near Lower Three Runs Landing, followed by Steel Creek Landing and Ellenton Bay Landing (which were similar), and finally the Jackson Landing upstream of the SRS.
4. Tail tip Hg concentrations of snakes collected from Carolina bays are low relative to published consumption limits and for values reported in literature for watersnakes from contaminated sites.
5. Historical Hg tail tip concentrations in *N. taxispilota* collected from Lower Three Runs Creek were 1.6 to 4 times higher than contemporary samples collected from the same area.

### Major Impact(s) of Research

Collectively, our research to date indicates that Hg levels in high trophic feeding watersnakes collected from the SRS overall have low concentrations relative to recognized consumption limits and no adverse effects were noted in snakes we collected. In at least one watersnake species, Hg levels were actually higher in individuals collected from isolated Carolina bays than from sites with known Hg contamination (i.e., former nuclear cooling reservoirs) – likely reflecting both Hg cycling and bioavailability in isolated temporary wetlands and dietary differences of snakes in the two major habitat types. Watersnakes, due to their propensity to bioaccumulate and their small home range sizes, appear to be excellent indicators of local contamination.

### Other Project Personnel

David Haskins, PhD Student, Interdisciplinary Toxicology Program, UGA

M. Kyle Brown, MS Student, Warnell School of Forestry and Natural Resources, UGA

Louise McCallie, Research Technician  
Xaiyou Xu, Associate Research Scientist  
Chongyang Qin, Postdoctoral Research Associate

**External Collaborators**

Robert Gogal – College of Veterinary Medicine, UGA  
Melissa Pilgrim – University of South Carolina, Upstate  
Kristina Meichner – College of Veterinary Medicine, UGA

**Products**

- Haskins, D.L., R.M. Gogal, and T.D. Tuberville. 2019. Snakes as novel biomarkers of mercury contamination: a review. *Reviews of Environmental Contamination and Toxicology* 249:133-152.
- Haskins, D.L., M.K. Brown, K. Meichner, T.D. Tuberville, and R.M. Gogal Jr. *In press*. Peripheral blood hematology, plasma chemistry, and the optimization of an *in vitro* immune-based assay in the brown watersnake (*Nerodia taxispilota*). To appear in: *Journal of Immunoassay and Immunochemistry*. <https://doi.org/10.1080/15321819.2020.1808469>
- Haskins, D.L., M.K. Brown, R.B. Bringolf, and T.D. Tuberville. *In press*. Brown watersnakes (*Nerodia taxispilota*) as bioindicators of mercury contamination in a riverine system. To appear in: *Science of the Total Environment*. <https://doi.org/10.1016/j.scitotenv.2020.142545>
- Brown, M.K. 2019. Bioaccumulation of mercury and radiocesium in *Nerodia floridana*: standard metabolic rate, hemoparasite infection, and interspecific comparisons to *Nerodia fasciata*. M.S. Thesis, University of Georgia.
- Haskins, D.L., M.K. Brown, and T.D. Tuberville. Spatial distribution and bioaccumulation of mercury in brown watersnakes (*Nerodia taxispilota*) along the Savannah River Site. Warnell Graduate Student Symposium, Athens, GA. February 2020. Platform presentation.
- Haskins, D.L., and T.D. Tuberville. Temporal trends in mercury tissue concentrations in the brown watersnake (*Nerodia taxispilota*) from the Savannah River Site. Society of Toxicology and Chemistry, Toronto, Canada. November 2019. Poster presentation.
- Research featured on “Hidden Biodiversity” episode of Coastal Kingdom environmental education television show. Full show available for viewing at: <http://coastalkingdom.com/episodes/hidden-biodiversity>

## **Hydrological Characterization Around D Area**

### **Funding Entity**

SRNS Area Completion Projects

### **Start Date and Funding Amount**

May 2019, \$25,000

### **PI and co-PIs**

Dean E. Fletcher

### **Objectives**

Establish runoff patterns from D Area into the Savannah River Swamp. Identify, map, and monitor water quality in wetlands down slope from D Area.

### **Summary of Research Activities**

Beaver Dam Creek (BDC), the D Area Discharge Canal, and channels/wetlands throughout the swamp between D Area and the Savannah River were walked in exploratory efforts to establish stream/runoff flows and wetland status in this area. When the Savannah River was not flooding, most areas identified to be potential seeps on fluvial terraces along the floodplain were dry or only damp this year. Many wetlands in the study area appeared to be ephemeral or of rather short hydroperiod. Wetlands that appeared to hold water on at least an intermittent basis were identified. Potential biomonitors were identified in most of these wetlands. Eight sites were established across six wetlands and water presence along with water temperature, pH, and conductivity recorded. Turbidity was rated 0-3.

Water quality was observed to differ among wetlands and at times differ within a wetland. Substantial temporal variability in water quality parameters were also observed within a wetland. For example, drying and refilling of a wetland near the edge of D Area resulted in high conductivity and depressed pH. The perched isolated wetland located along the road that runs from the north end of D area to the river may not be a long-term concern as it has begun succession into a forest. However, other wetlands will likely to continue to have at least an intermittent hydroperiod. Two wetlands did not entirely dry. Further investigation of these wetlands is warranted.

### **Conclusions**

1. Hydroperiod of many wetlands was short for many wetlands this year.
2. Water quality not only varied between wetlands, but substantial temporal variation was observed.

### **Major Impact(s) of Research**

1. Establishing runoff routes and hydrologic state of this area is providing a better understanding of the need for continued monitoring and the risk of present or future contaminant transfer to biota in this system.

### **Other Project Personnel**

Christina Fulghum, Research Technician - SREL

Erin Spivey, Research Technician - SREL

### **External Collaborators**

NA

### **Products**

2020 Update report and maps.

## **Hydrological Characterization of Beaver Dam Creek**

### **Funding Entity**

SRNS Area Completion Projects

### **Start Date and Funding Amount**

May 2019, \$25,000

### **PI and co-PI's**

Dean E. Fletcher

### **Objectives**

Establish the hydrologic extent of Beaver Dam Creek after pumping has ceased. Determine whether runoff from D Area is reaching perennial waters. Establish water quality sites along the entire length of BDC and examine longitudinal changes in water quality.

### **Summary of Research Activities**

For decades, water flows in Beaver Dam Creek were augmented by Savannah River water pumped through the D-Area coal-fired power plant. After water pumping from the powerhouse ceased, the hydrology of Beaver Dam Creek was poorly known. Beaver Dam Creek (BDC), the D Area Discharge Canal (DADC), and channels/wetlands throughout the swamp between D Area and the Savannah River were walked in exploratory efforts to establish stream/runoff flows and wetland status in this area. A total of 14 sites were established in BDC from the mouth of the DADC to the Savannah River confluence. Additionally, a site was established in the Savannah River and at 3 locations in the DADC for a total of 18 stream sites. On subsequent visits, water temperature, pH, and conductivity were recorded at each site with a handheld meter. Turbidity was rated on a scale of 0-3. Direction of flow was also noted. Savannah River discharge and gage heights were acquired from the USGS gage at Augusta (02197000) at the time of the downstream-most site.

Even during rather dry periods, water in Beaver Dam Creek was continuous from D Area to the Savannah River. However, whether water flowed out of BDC into the Savannah River or from the river into BDC differed on trips. Although presently based on a limited sample size, it appears that subtle changes in the discharge of BDC and the Savannah River can change whether water flows into or out of the mouth of BDC. Sedimentation in the lowest reach of BDC could also change stream gradients and consequently influence this pattern. When water flows into BDC from the Savannah River, BDC flow becomes still in the stream reach where the two water sources collide. In the reach of flow stagnation that occurred due to this collision, turbidity and conductivity substantially increased. A reach where this was observed to occur also appears to be a depositional zone where fine sediments have settled, so this pattern may be relatively common. During Savannah River flooding the area of collision can reach upstream to the mouth of the DADC. It appears that whenever BDC water is held up and not allowed to flow downstream, a rise in conductivity occurs. Despite the sometimes-elevated conductivity, water pH was never observed to be depressed in the Beaver Dam Creek channel. Overall, the greatest risk of metal discharge into the Savannah River may be when BDC floods due to a localized storm event, but the Savannah River is low.

### **Conclusions**

1. Discharge from Beaver Dam Creek is dependent upon BDC and Savannah River discharge levels.
2. Areas within Beaver Dam Creek sometimes had elevated conductivity, but pH was never observed to be unusually low.

### **Major Impact(s) of Research**

1. Establishing runoff routes and hydrologic state of this area is providing a better understanding of the risk of offsite transport of contaminants from this system.

### **Other Project Personnel**

Christina Fulghum, Research Technician - SREL

Erin Spivey, Research Technician - SREL

### **External Collaborators**

NA

**Products**

2020 Update report and maps.

## *Tracking Sources of Mercury in Contaminated Aquatic Systems on the Savannah River Site*

### **Funding Entity**

SRNS Area Closure Projects

### **Start Date and Funding Amount**

April 2017; \$31,954

### **PI and co-PIs**

Dr. Xiaoyu Xu and Larry Bryan

### **Objectives**

The Savannah River Site (SRS) has received mercury (Hg) contamination from multiple sources, including atmospheric deposition (local and non-local), off-site pollution (Savannah River – Olin Canal) and on-site activities. Accumulation of Hg by SRS biota has been documented frequently, occasionally at levels of regulatory concern, but the sources of Hg remain unclear. Our objective is to utilize a relatively new technique, Hg stable isotope analysis, in an attempt to confirm sources of Hg found in SRS biota. The isotope analysis will occur at the Center for Applied Isotope Studies at UGA after custom-fabricated accessories are installed on their existing instrumentation.

### **Summary of Research Activities**

Surface sediment samples were collected from the Savannah River (above and below Olin Canal) and Fourmile Branch; sediments were freeze dried, digested, and measured for total Hg and methyl-Hg concentrations. Both total and methyl-Hg concentrations in Olin Canal (total: 25 ng/g; methyl: 8.6 ng/g) were lower than the Fourmile Branch (total: 266 ng/g; methyl: 0.4 ng/g). The average percentage of methyl-Hg in total Hg in sediment was 3.2% in Olin Canal and 1.9% in Fourmile Branch.

Biota samples were collected from Fourmile Branch in FY17 and FY18; biota samples were identified, freeze dried, digested, measured for total Hg and methyl-Hg concentrations, and determined for the stable carbon and nitrogen isotope signatures. Biofilm, shrimp, crayfish, coastal shiner, dusky shiner, dollar sunfish, pirate perch, redbfin pickerel, spotted sunfish, yellow bullhead, and creek chubsucker were collected. The biomagnification factor of methyl-Hg in the aquatic food web was 3.3, meaning methyl-Hg concentrations increased for 3.3 times per trophic level in Fourmile Branch.

Sediment pore waters were collected with column Pore Water Sampler (peeper) from Fourmile Branch in FY18; concentrations of total Hg, methyl-Hg, DOC, sulfate, and chloride were measured. Concentrations of labile Hg in pore waters indicated variations among depths and the highest concentrations presented at around -4 to 0 cm depth (1.0 ng/g), which were much higher than total Hg concentrations (0.7 ng/g) in the surface water. Labile Hg in the sediment were highly correlated to sulfate and DOC concentrations in the pore water, but not to chloride concentrations.

All sediment and biota samples were digested with mixed acid and prepared for the future Hg isotope analysis. We had the needed accessories for Hg isotope analysis fabricated, they have been installed on the existing instrumentation, and the system has been calibrated and tested. But the accuracy of the system still needs to be improved and the QCQA of a new standard reference material also needs to be certified. We hired a research professional who is an expert in Hg stable isotope analysis who started on January 6<sup>th</sup>, 2019. But due to the Covid-19 situations and the related limitations on travel in FY20 (the instrument locates at a lab that is about 2.5 hours of driving from SREL), Hg isotopes were not analyzed yet.

### **Conclusions**

1. The relatively high concentrations of total Hg in the sediment and low concentrations in the water suggested the historical contamination of Hg in Fourmile Branch.
2. Methyl-Hg was detected in all biota samples, indicating the continuous methylation of inorganic Hg in the sediment. But the relatively low percentage of methyl-Hg in total Hg in sediment suggested low methylation rate of inorganic Hg in the sediment.
3. Biomagnification of methyl-Hg existed in Fourmile Branch though the biomagnification factor was not high.
4. Sulfate and DOC in sediment pore waters determined the microbial methylation rates of Hg.

5. A passive sampling device, such as a Pore Water Sampler, is a good approach of exploring Hg biogeochemistry in aquatic system. A Pore Water Sampler, combined with the methyl-Hg magnification model, can simplify or even substitute biomonitoring and decrease the confounding factors associated with biological variables.
6. Stable Hg isotope analysis is pending.

**Major Impact(s) of Research**

1. A passive sampling device, such as a Pore Water Sampler, should be considered as useful tools for determining environmental fluxes of Hg, understanding trophic accumulation in the food webs, and elucidating sedimentary and aqueous geochemical processes.
2. Total and methyl- Hg concentrations in the sediment and water can explain the sources of Hg in Fourmile Branch. Assuming we can isotopically differentiate among the various sources of Hg, when completed, we will have a better understanding of Hg sources in biota found on the SRS biota.

**Other Project Personnel**

Chongyang Qin, postdoctoral research associate – SREL

Erin Peck, research professional – SREL

**External Collaborators**

Sarah Jantzi– Center of Applied Isotope, UGA

**Products**

Xu, X., C. Qin, A.L. Bryan. 2020. Using pore water sampler to study mercury speciation, bioavailability, and biomagnification in contaminated streams on the Savannah River Site (SC, USA) (in preparation).

## *Contaminants in Wild Pigs and Eastern Wild Turkeys*

### **Funding Entity**

SRNS Area Closures Projects

### **Start Date and Funding Amount**

October 2017; \$176,290

### **PI and co-PIs**

Dr. Jim Beasley, Larry Bryan, and Dr. James Martin

### **Objectives**

The primary objective of this research is to quantify levels of Hg, trace elements, and radiocesium in wild turkeys and wild pigs on the SRS, and compare contaminant burdens in these species on the SRS to populations off-site to assess potential risks to hunters.

### **Summary of Research Activities**

*Wild Turkeys* – We have collected samples from 48 turkeys harvested during the Ultimate Turkey Hunts held on the SRS, as well as from an additional 17 turkeys near areas of known contamination on the SRS, for a total of 65 birds. Off-site samples (17) were collected from hunters in South Carolina and Georgia. Samples taken include breast and leg muscle, liver, and feathers. Feathers were collected from a subset of individuals that we will use to determine whether turkey feathers can be used as a non-lethal predictor of contaminant burdens within muscle or liver tissue. All analyses have been completed and we have submitted a manuscript for publication stemming from these data.

*Wild Pigs* – From 2018-2019 we collected wild pig muscle and liver samples from the SRS as well as control samples collected from the Congaree National Park. All SRS samples have been collected proximal to areas of known contamination such as D-area, the Savannah River Swamp, L-Lake Dam, Dunbarton, Par Pond, P-area, R-area, Tim's Branch, H-area, and Hunt Unit 25, which borders several SRS facilities. These samples have been freeze-dried, and we are currently in the process of prepping the samples for radiocesium and trace element testing.

### **Conclusions**

Compared to reference samples, we found turkeys inhabiting the SRS contained elevated Hg levels in both muscle and liver tissues, and lower concentrations of Chromium. However, all elements analyzed were below reference limits set by the Centers for Disease Control for safe consumption. Thus, our data suggest turkeys likely are not an important pathway of contaminant exposure on the SRS or other areas with similar contaminant distributions. We also found a positive correlation between breast muscle and feather Hg concentrations, suggesting feathers can potentially be used as a non-lethal sampling technique.

### **Major Impact(s) of Research**

1. This research will provide the first assessment of contaminant burdens in wild turkeys on the SRS, data necessary to assess potential risks to hunters consuming birds harvested during annual hunts on site.
2. Additional data on Hg, trace elements, and Radiocesium in wild pigs will be combined with data from an earlier study to provide an updated assessment of potential risks to hunters consuming pigs collected on/near the SRS.

### **Other Project Personnel**

Kaitlin Wilms, research technician – SREL

Sara Cheatham, research technician – SREL

Chris Leaphart, Ph.D. student, Warnell School of Forestry and Natural Resources, UGA

Cody Tisdale, M.S. student, Warnell School of Forestry and Natural Resources, UGA

Allison Rakowski, Research Professional, SREL, UGA

### **External Collaborators**

Robert Byrd, USDA-APHIS-WS

James Cumbee, USDA-APHIS-WS

### **Products**

Tisdale, C.A., J.A. Martin, and J.C. Beasley. 2019. Lead concentrations in muscle tissue of wild turkeys harvested with lead and copper-plated lead shot. Savannah River Ecology Laboratory Graduate Student Symposium. Aiken, SC.

Tisdale, C.A., J.A. Martin, and J.C. Beasley. *In Review*. Accumulation of contaminants by wild turkeys and potential for consumer exposure.

Tisdale, C., Martin, J., and Beasley, J. 2019. Lead contamination differences in the muscle tissue of wild turkeys harvested with lead and copper-coated lead shot. In South Carolina Chapter of the Wildlife Society Annual Meeting.

Tisdale, C., Martin, J., and Beasley, J.C. 2020. Lead contamination differences between lead and copper coated lead shot. In Warnell Graduate Student Symposium. Athens, GA.

## **Contaminants in SRS White-tailed Deer**

### **Funding Entity**

SRNS Area Closures Projects

### **Start Date and Funding Amount**

November 2019; \$60,000

### **PI and co-PIs**

Dr. Jim Beasley

### **Objectives**

Currently, white-tailed deer harvested on the SRS undergo extensive testing for presence of radionuclides. However, evaluation of the presence of Hg and trace elements is not routinely undertaken for SRS-harvested deer. Thus, the primary objective of this research is to quantify levels of Hg and trace elements in white-tailed deer inhabiting the SRS to assess potential risks to hunters.

### **Summary of Research Activities**

Field collections for this study will begin in October 2020. We will work with SRS personnel to collect tissue samples from a subset of deer harvested during annual SRS white-tailed deer hunts. Animals harvested in hunt units with known contamination will be prioritized for sampling.

### **Conclusions**

Sampling has not been completed; thus there are no conclusions at this time.

### **Major Impact(s) of Research**

1. This research will provide an extensive assessment of contaminant burdens in white-tailed deer harvested on the SRS, data necessary to assess potential risks to hunters consuming animals harvested during annual hunts on site.
2. Data will be collected across the SRS to identify whether any areas exist where more fine-scale sampling or monitoring may be beneficial.

### **Other Project Personnel**

Caitlin Kupferman, Research Professional, SREL, UGA

### **External Collaborators**

NA

### **Products**

This research has just begun; thus, there are no products at this time.

## *Contaminants in Resident Waterfowl on the Savannah River Site*

### **Funding Entity**

SRNS Area Closures Projects

### **Start Date and Funding Amount**

October 2019; \$227,063

### **PI and co-PIs**

Dr. Jim Beasley

### **Objectives**

This research seeks to improve our understanding of the bioavailability and effects of various contaminants on waterfowl species inhabiting the SRS. The primary objectives of this research are to: 1) quantify contemporary contaminant levels in wood ducks on the SRS, 2) determine the influence of prenatal mercury contamination on wood duck duckling survival, and 3) determine the influence of fine-scale habitat use on radiocesium burdens in waterfowl on the SRS.

### **Summary of Research Activities**

Due to the arrival of funds after the migratory season for the species being studied, we had to delay collection of data on contaminant burdens in SRS wood ducks by several months, as these samples must be collected in fall/winter. We began data collection during fall/winter 2019/2020, and during FY19 we were able to establish and test our protocol for dosing duck eggs with methylmercury II chloride dissolved in corn oil. During FY20 we completed data collection for studies to quantify contemporary Hg levels in free ranging wood ducks on the SRS, and are currently analyzing samples from these collections. During spring 2021 we will initiate studies to quantify the influence of prenatal Hg contamination on wood duck duckling survival, as well as determine the influence of adult habitat use on radiocesium accumulation in SRS waterfowl.

### **Conclusions**

Data analyses are incomplete; thus there are no conclusions at this time.

### **Major Impact(s) of Research**

1. This research will provide comprehensive estimates of the levels of mercury in wood ducks inhabiting the SRS, how mercury exposure varies throughout the year, and the impacts of mercury exposure on nestling survival.
2. Spatial analyses will be used to determine how fine-scale space use influences contaminant burdens in waterfowl utilizing wetlands on the SRS.
3. These findings will produce novel data on the extent to which mercury exposure could contribute to detrimental effects on wood ducks.

### **Other Project Personnel**

Chris Leaphart, Ph.D. student, Warnell School of Forestry and Natural Resources, UGA

### **External Collaborators**

NA

### **Products**

- Leaphart, C.L., and J.C. Beasley. 2019. Effects of prenatal methylmercury exposure on waterfowl hatchling phenotypic plasticity and survival. Savannah River Ecology Laboratory Graduate Student Symposium. Aiken, SC.
- Leaphart, J., and Beasley, J. 2019. Wood Ducks on the Savannah River Site. In 2019 South Carolina Bluebird Society Annual Meeting. Edgefield, SC.
- Leaphart, J., and Beasley, J. 2020. Effects of prenatal methylmercury exposure on waterfowl hatchling phenotypic plasticity and survival. Warnell Graduate Student Symposium. Athens, GA.

## **Stewardship Database of Amphibian and Reptile distribution on the SRS**

### **Funding Entity**

SRNS Area Closures Projects

### **Start Date and Funding Amount**

October 2019; \$30,000

### **PI and co-PIs**

Dr. Kurt A. Buhlmann

### **Objectives**

Our goal with this project is to compile and provide historical and current occurrence records for amphibian and reptiles. Its use should help minimize land use conflicts, guide land management, and perhaps assist in habitat restoration and species recovery, where appropriate. It is intended that this project comprise a living, dynamic database, and new distribution records for target species are still being discovered and documented on a regular basis. It is modeled after the State Natural Heritage Program data system.

### **Summary of Research Activities**

1. Development of stewardship database structure
2. Acquisition of data sources, 1951-present
3. As of this report, a total of 24,956 reptile records have been assigned lat/long points

### **Conclusions**

1. Amphibian and reptile distribution records for the 300 sq. mile Savannah River Site have been collected and documented over the period, 1951 to 2020.
2. All records known for reptiles, primarily snakes, have been assigned latitude and longitude points.
3. For rare species, the database allows for organizations operating on-site to minimize land use conflicts.
4. Although all known datasets have been located, work will continue with ACP support in 2020-through 2021 to assign distribution points (lat/long) to remaining species distribution records.

### **Major Impact(s) of Research**

1. The results of this work will help with regulatory compliance, minimize land use conflicts and guide potential habitat restoration.
2. The database serves as an ecological benchmark for future ecological studies as it provides historical context to ecological studies.

### **Other Project Personnel**

Linda Lee, Research Professional - SREL

Amanda McIntosh, Research Tech - SREL

### **External Collaborators**

Susan Blas, ACP-DOE

Charles E. Davis, U.S. Forest Service-Savannah River

Andy Horcher, U.S. Forest Service-Savannah River

### **Products**

Buhlmann, K.A. 2019. Identification of Sensitive Natural Resources on the SRS for Management, Restoration, Recovery, Stewardship, and Regulatory Compliance. Progress Report , 13 December 2019. Submitted to Mr. A. Horcher, U.S. Forest Service-SR, 17pp.

Buhlmann K.A. 2020. Identification of Sensitive Natural Resources on the SRS for Management, Restoration, Recovery, Stewardship, and Regulatory Compliance. Summary of Activities Completed, 30 September 2020. Submitted to Mr. A. Horcher, U.S. Forest Service-SR, 16pp.

**Examination of Cesium-137 Accumulation in Terrestrial and Aquatic Food Webs in the Joyce's Branch Tributary**

**Funding Entity**

SRNS Area Closures Projects

**Start Date and Funding Amount**

August 2020; \$67,000

**PI and co-PIs**

Larry Bryan, Dr. Krista Capps, and Dr. Stacey Lance

**Objectives**

Preliminary investigations conducted in 2018 suggest that levels of cesium-137 accumulation in specimens from the Joyce's Branch floodplain are among the highest recorded from the SRS. In 2018, SREL (Day and Bryan, unpublished data), explored this area and collected plant and mushroom material from multiple locations. They found concentrations ranging from below detection levels to > 200 Bq/g dry weight. The primary objectives of the current study are to assess cesium-137 concentrations in flora and fauna from both the terrestrial and aquatic food webs associated with the Joyce's Branch stream system and adjacent uplands.

**Summary of Research Activities**

1. Mapped the area using GIS and established sampling transects
2. Established fifty sampling quadrats
3. Collected plant material in the 50 quadrats and measured Cs-137 content

**Conclusions**

This project has just begun and there are no conclusions at this time.

**Major Impact(s) of Research**

This project has just begun and there are no impacts at this time.

**Other Project Personnel**

Corinne Sweeny, MS Student – UGA

Jon Skaggs, Research Assistant – SREL

**External Collaborators**

NA

**Products**

NA

**Public Consumption Risk and Alligator Mediated Transport of Contaminants: Assessing Muscle Contaminant Levels and Movement of Alligators on and off of the Savannah River Site**

**Funding Entity**

SRNS Area Closures Projects

**Start Date and Funding Amount**

November 2019; \$157,396

**PI and co-PIs**

Dr. Ben Parrott, Dr. Tracey Tuberville

**Objectives**

The American alligator (*Alligator mississippiensis*) is a high trophic level species that bioaccumulates a broad suite of environmental contaminants, ranging from persistent organochlorines to heavy metals. In addition to serving as sentinels of ecosystem health, alligators are now harvested and consumed by recreational hunters taking part in both Georgia's and South Carolina's annual alligator hunts. The primary aim of this project is to assess contaminant loads in alligator tail muscle (most commonly consumed) and also the degree to which alligators travel onto and off of the Savannah River Site. Together, project findings will inform exposure concerns associated with the public harvest and consumption of alligators in the region.

**Summary of Research Activities**

In February 2020, we recruited a masters student, Laura Kojima, to work on project objectives. Alligator trapping and data collection commenced in March. To date, 63 alligators have been sampled on the SRS, with 9 of these being fitted with GPS transmitters. Remaining transmitters will be deployed to additional alligators in March/April of 2021.

**Conclusions**

The project is in data collection stages.

**Major Impact(s) of Research**

1. Estimate the frequency of alligator movement on/off the SRS.
2. Estimate exposure risk from consuming alligator meat harvested from the Savannah River.

**Other Project Personnel**

Laura Kojima, Masters Student, Odum School of Ecology, UGA

**External Collaborators**

N/A

**Products**

- L.V. Kojima. American alligator ecotoxicology: Assessing contaminant levels and movement of alligators on and off the Savannah River Site. 2020. *Presentation*. University of Georgia Herpetological Society
- L.V. Kojima. Branches of Ecology: American alligator ecotoxicology. 2020. *Presentation*. Virtual University STEM Visit Day, Gwinnett School of Mathematics, Science, and Technology
- L.V. Kojima. American alligator ecotoxicology and the history of crocodylians. 2020. *Presentation*. Master Herpetology Class, The Amphibian Foundation
- L.V. Kojima. American alligator ecotoxicology: Assessing contaminant levels and movement of alligators on and off the Savannah River Site. 2020. *Presentation*. Herpetology Society North Carolina State University

**Sex Ratios and Frequency of Sex Reversal as Indicators of Population Health in Amphibian Species Inhabiting the Savannah River Site**

**Funding Entity**

SRNS Area Closures Projects

**Start Date and Funding Amount**

October 2020; \$151,232

**PI and co-PIs**

Dr. Ben Parrott, David Scott, Dr. Stacey Lance

**Objectives**

Balanced sex ratios are critical for long-term population health, and robust sex determining mechanisms ensure the stable maintenance of sex ratios within a population. However, skews in amphibian population sex ratios have recently been tied to anthropogenic factors, suggesting that exposure to environmental contaminants might override normal sex determining systems resulting in unbalanced sex ratios. This work is focused on two primary objectives: (1) Survey sex ratios and assess sex reversal in amphibian communities inhabiting reference and contaminated wetlands located on the Savannah River site; and (2) test if developmental exposures to coal fly ash and endosulfan, two common anthropogenic contaminants on the SRS, result in sex reversal in amphibians.

**Summary of Research Activities**

This project was just initiated. However, a master's student, Josiah Johnson, has been recruited and is currently working on project objectives. An animal use protocol for field sampling has been approved by UGA's IACUC and Josiah is establishing protocols for sexing amphibians using both gross morphology and histology. Field sampling is slated to begin in January 2021.

**Conclusions**

No conclusions can be made at this stage.

**Major Impact(s) of Research**

1. Determine sex ratio variation in amphibian communities on the SRS.
2. Advance our understanding of how anthropogenic activity and contamination can skew population sex ratios.

**Other Project Personnel**

Josiah Johnson, MS Student, Odum School of Ecology, UGA

**External Collaborators**

N/A

**Products**

None thus far.

## Controlling the Spread of Radioactive Contamination by Biotic Vectors on the Savannah River Site

### Funding Entity

DOE-SR

### Start Date and Funding Amount

October 2018; \$198,771

### PI and co-PIs

Dr. O. E. Rhodes, Jr., Dr. G. Dharmarajan, A. L. Bryan, L. Lee, and Dr. T. DeVault

### Objectives

During routine surveys at the H-Area Tank Farm (HTF), SRS, high levels of radioactivity (>100,000 disintegrations per minute (dpm) beta-gamma activity) were detected in bird feces in December 2017. Additional surveys by SRNS revealed at least three more instances of contaminated bird feces in the HTF complex (40,000-240,000 dpm beta-gamma activity). The overarching goal of this project is to identify the source(s) of radioactive materials that is (are) contributing to the contamination of facilities and other structures on the SRS so that these risks can be mitigated. This study had four objectives: (1) Identify the community of bird vectors that pose a high risk of exposure to radionuclide contaminants at the HTF and characterize the levels of contamination extant in this community; (2) Quantify spatial risk associated with the accumulation and transport of radionuclide contaminants by bird vectors within and outside the HTF; (3) Produce a spatial risk map and identify the distribution of potential radionuclide contaminant sources (soil and/or plants) in and around the HTF; (4) Evaluate mice as a potential vector of radionuclide contamination within the HTF and identify potential sources of contamination for mice within the facility.

### Summary of Research Activities

During the study period we captured a total of 506 individuals belonging to 50 bird species. We quantified whole-body radionuclide contamination levels in 225 birds belonging to 45 species using Gamma Well counter with a NaI (TI) gamma detector. We found that 3 of the 225 individuals counted (1.3%) had counts > 5000 dpm. All birds with counts > 5000 dpm and a subset of birds below the threshold were euthanized and their tissue analyzed for radionuclide contaminants using an auto-gamma counter. Importantly, no bird captured or collected showed radiation levels greater than background when scanned with Ludlum probes in the field/lab. To characterize bird habitat utilization and home range we deployed VHF transmitters on 70 individuals belonging to 9 species. We collected over 2500 radiotelemetry locations on these individuals. To characterize potential radionuclide contaminant sources we carried out intensive sampling of vegetation in and around the HTF area. We collected 588 vegetation samples that have been analyzed for radionuclide contaminants using an autogamma counter. So far only one vegetation species has shown radiation levels above background. This was the plant pokeweed/pokeberry (*Phytolacca americana*), and this sample had a count of 2500 dpm (27Bq/g dry). To evaluate rodents as a potential vector of radionuclide contamination within the HTF we trapped and whole-body-counted 75 individual rodents belonging to three species. Of the individuals counted only one individual had counts > 5000 dpm. The mouse with counts > 5000 dpm and a subset of rodents below the threshold were euthanized and their tissue analyzed for radionuclide contaminants using an auto-gamma counter. Importantly, no mouse captured or collected showed radiation levels greater than background when scanned with Ludlum probes in the field/lab.

### Conclusions

1. As in previous studies on the SRS, pokeweed was documented as an accumulator of Radiocesium and was the only plant species within HTF with counts above background levels.
2. A wide diversity of birds (53 species) and rodents (3 species) were documented and sampled on the HTF, but only 1.3% of the individuals counted had whole body counts > 5000 dpm.
3. Based on telemetry data of marked (radio transmitters) birds and rodents, these animals were found to primarily use the more vegetated peripheral sections of the facility, with low use of the more industrial (central) sections.
4. Based on these findings, the spread of contamination via biotic vectors is generally considered low.

**Major Impact(s) of Research**

We found a very low number of occurrences of Radiocesium at levels above background in plants, birds, and rodents on HTF. Therefore, the risk of spread of this contaminant by avian and rodent vectors is considered low.

**Other Project Personnel**

Mary Chapman, Research Technician – SREL

Abigail Riggs, Research Technician – SREL

Tyler Walters, Research Technician – SREL

Kaitlin Wilms, Research Technician – SREL

Elizabeth Butler, Research Technician – SREL

**External Collaborators**

NA

**Products**

Chapman, M., A. Riggs, K. Wilms, T. Walters, J. Garabedian, L. Lee, A. L. Bryan, G. Dharmarajan, O. E. Rhodes, Jr. (2019) Avian and Small Mammal Habitat Use Within a Nuclear Storage Facility on the Savannah River Site. SREL Graduate Student Symposium. July 19, 2019. Aiken, SC

Chapman, M., A. L. Bryan, L. Lee, T. DeVault, G. Dharmarajan, and O. E. Rhodes Jr. 2020.

Understanding the role of biotic vectors in the accumulation and spread of radioactive contamination on the Savannah River Site. Final project report to the DOE- Savannah River Site.

**Technical Support Provided by the Savannah River Ecology Laboratory (SREL) for R&D of Cementitious-Type Materials (i.e., Saltstone) and Soils**

**Funding Entity**

Savannah River Remediation (SRR)

**Start Date and Funding Amount**

October 2018; \$268 K for FY20

**PI and co-PIs**

Dr. John C. Seaman

**Objective**

At the Department of Energy's Savannah River Site (SRS) a mix of cementitious dry feed materials (i.e., portland cement, blast furnace slag (BFS) and fly ash) are combined with low-level radioactive saltwaste solutions to form a cementitious material known as saltstone, which is then deposited in a series of concrete vaults for long-term disposal at the Saltstone Disposal Facility (SDF). Objectives include ongoing evaluations of contaminant leaching properties (<sup>99</sup>Tc, <sup>129</sup>I, and <sup>137</sup>Cs) of saltstone simulants and additional evaluations of the proposed "cement free" saltstone formulations, both in terms of physicochemical properties (i.e., heat capacity, heat of hydration and thermal conductivity) and contaminant retention of intact monoliths.

**Summary of Research Activities**

In FY20, SREL continued testing the <sup>99</sup>Tc/<sup>129</sup>I spiked saltstone simulants using SREL's Dynamic Leaching Method (DLM). SREL continued analyzing the mineralogical and elemental composition of the saltstone dry feed materials and weathered saltstone using an x-ray diffraction and x-ray fluorescence. This information is relevant in predicting the observed variations in contaminant partitioning associated with changes in dry feed batches.

In FY20, SREL began two new research activities for SRR. The first was an evaluation of Salt Disposal Unit (SDU) liner systems that are used to address leak issues and provide chemical resistance to SDU (SDU) concrete. The test consisted immersing liner samples in simulant salt solutions at a high temperature (60 ± 2 °C) for 1,000 hours (≈42 days) to understand the effects the saltwaste can have on liner properties that impact performance. Each week, the liner specimens were removed from the heating bath, photographed, and visually inspected, with subsamples provided to SRNL for additional testing, i.e., tensile strength, seam integrity, hardness and adhesion properties.

The second new study which is ongoing consists of evaluating the factors that control mercury (Hg) partitioning in saltstone. Recent TCLP tests performed on SRNL prepared saltstone samples from the first three quarters (Q1 – Q3) of calendar year 2018 have revealed elevated mercury (Hg) concentrations relative to historical trends, despite no discernible change in Tank 50 mercury levels during the time period in question. This suggests variations in dry feed materials may be responsible for observed changes in mercury retention. Therefore, the current study evaluates the impact of saltstone dry feed materials on the variability in Hg partitioning. This study involves the preparation of mercury-spiked grout monoliths for EPA 1311- Toxicity Characteristic Leaching Procedure (TCLP) and EPA 1315- Mass Transfer Rates for Monolithic Samples testing, as well as XRD and XRF analysis of both dry feeds and the resulting saltstone grouts.

**Conclusions**

1. The tank liner systems evaluated for SDU 7 were able to withstand the harsh chemical condition present in the SDU without a significant impact on performance.
2. Preliminary Hg partitioning tests aimed at evaluating potential artifacts identified high levels of Hg(II) partitioning to labware used in conducting the tests.

**Major Impact(s) of Research**

1. Evaluated tank liner systems that can withstand the harsh chemical environment of the SDU.
2. Dry feed characterization illustrating batch to batch variability.
3. Identified potential artifacts that confound current testing practices used to evaluate mercury partitioning in saltstone.

**Other Project Personnel**

C. Logan, Research Professional II, SREL

J. Lott, Research Technician

M. Shapiro, Research Technician

**External Collaborators**

Dr. S. P. Simner (SRR Subcontractor)

Dr. D. Kaplan (SRNL)

Dr. D. Li (SRNL)

Dr. J. Mangold (SRR)

**Products**

Shapiro, M., J. Lott, C. Logan, and J.C. Seaman. 2020. SDU 7 Liner Performance Testing: Data Report. SREL Doc. No. R-20-0003. Final accepted by SRR 3-5-2020.

## *Aqueous and Solid Phase Characterization of Potential Tank Fill Materials*

### **Funding Entity**

Savannah River Remediation (SRR)

### **Start Date and Funding Amount**

June 2019; \$142K

### **PI and co-PIs**

Dr. John C. Seaman

### **Objective**

SREL completed a series of experimental tests evaluating the dynamic interactions of Savannah River Site (SRS) soil/vadose zone pore-water solutions in contact with Tank Closure Grout (TCG). Radionuclide release from residual tank waste is largely controlled by tank grout degradation in contact with water that has passed through the closure cap. Infiltration passes through the tanks and drives changes in grout mineralogy/composition, with the emerging fluid reflecting such interactions that vary over time. The current project identified changes in aqueous chemistry (i.e., Eh, pH, DO, alkalinity, solution components, etc.) that can impact long-term contaminant partitioning in both open and closed (i.e., oxic and anoxic systems) environmental systems with extended leaching.

### **Summary of Research Activities**

Dynamic column experiments and extended batch equilibrations were used to evaluate the interactions of soil backfill with cementitious materials (i.e., TCG) in a simulated leaching environment that mimics potential leachate chemistries contacting various SRS waste disposal units. In addition to monitoring eluate chemistry, changes in the properties and solid phase composition of the soil and test cement materials were characterized using appropriate analytical techniques (i.e., x-ray diffraction (XRD) spectroscopy, x-ray fluorescence (XRF) spectroscopy).

### **Major Impact(s) of Research**

1. Batch and column results will be used in updating a flow and transport model to describe radionuclide release from residual tank waste based on component solubility as controlled by tank grout degradation in contact with water that has passed through the closure cap.

### **Other Project Personnel**

C. Logan, Research Professional II, SREL

M. Shapiro, SREL Research Technician

J. Lott, SREL Research Technician

### **External Collaborators**

Dr. S.P. Simner (SRR Subcontractor)

Dr. J. Mangold (SRR)

Dr. G. Flach (SRR)

### **Products**

Seaman, J.C., S.P. Simner, and C. Logan. 2020. Aqueous and Solid Phase Characterization of Potential Tank Fill Materials. SREL. Doc. R-21-0003, submitted to SRR August 19, 2020.

Seaman, J.C., and M.R. Baker. 2019. SREL Test Plan: Aqueous and Solid Phase Characterization of Potential Tank Fill Materials. SREL. Doc. R-19-0004, submitted to SRR June 4, 2019.

TASK 4. SREL public outreach and communication programs will focus on the SRS environment and ecological research to increase the public's understanding of scientific issues affecting the Site and to increase general ecological awareness

**SREL Outreach Activities in FY20**

SREL's public outreach and communication programs focus on habitats and environments on the SRS and the ecological research that is conducted by SREL, with the purpose of increasing public understanding of scientific issues affecting the site and bringing general ecological awareness to the general public. Historically, the program's mission has been to educate the public about ecological research and environmental issues. SREL has also worked with the Citizens Advisory Board, various onsite organizations, state and federal regulatory authorities, and other stakeholder groups to raise awareness of the SRS and of regional ecological issues and opportunities for environmental stewardship. The program highlights SREL's ecological research on the SRS through oral presentations, exhibits, tours, and various electronic media. Outreach programs facilitate and encourage participation by students, regional teachers, resident and visiting faculty and training programs. SREL actively communicates information to the media via UGA Public Affairs and local and regional media outlets. To accomplish these goals and provide an overall educational outreach program, SREL has focused on the following specific objectives since its inception:

- A. Publish articles on environmental issues and ecological research in popular press outlets including newspaper columns, popular magazines, University of Georgia publications, Department of Energy publications, encyclopedias, special publications such as alumni magazines and ancillary publications of scientific societies.
- B. Provide news releases to newspapers and other appropriate media that relate to environmental activities of SREL, with particular emphasis on the SRS.
- C. Develop and present an on-site tour program that focuses on the environments of the SRS and the ecological projects of SREL—conveying SREL's role as an independent evaluator.
- D. Give presentations to the public, including schools, civic groups, and other organizations that focus on environments of the SRS region and on SREL's ecological projects.
- E. Develop portable and permanent exhibits appropriate for use at special presentations at SREL, schools, other organizations, and special events.
- F. Develop video and multimedia shows for presentations to groups or for use by onsite organizations, emphasizing SREL environmental programs and projects on the SRS.
- G. Investigate opportunities for broadcast programs that focus on environmental issues, SREL's ecological research, and ecological projects on the SRS.
- H. Develop and distribute brochures and publications that are informative to the public and on-site tenants of SREL's ecological research, and the environments on the SRS.
- I. Develop and establish displays of SREL research projects in appropriate areas of the SREL facilities.
- J. Publish an internal newsletter (The GrapeVine) as a means of enhancing internal communications—promoting individual as well as organizational achievement.
- K. Develop the UGA conference center as a focal site for environmental education.

L. Establish a photograph collection that tells SREL's story, is informative of plants, animals, and habitats of the SRS region, and that emphasizes current ecological projects of SREL.

M. Maintain a collection of live plants and animals that can be used to educate the public about environmental issues and ecological research.

N. Maintain an area of the website for education on wildlife native to the SRS to include identification of regional species and information on wildlife safety.

O. Develop and present SRS wildlife safety talks for site tenants and visitors.

In accomplishing the goal of communicating ecological information to non-scientists, the Outreach program has 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. The Outreach program has conducted workshops and training sessions and has attended SRS monthly safety meetings to deliver PowerPoint presentations and introduce live animals and native plants. The Outreach program has also developed and distributed safety materials (protocol badge cards and safety fact sheets) to SRS employees, and has managed an educational section on the SREL website. While the primary focus of most of these wildlife safety programs has been on snakes and alligators, the programs have also provided information on plants, insects, spiders, snapping turtles, and mammals of concern. During the 2020 fiscal year, the Outreach program hosted two safety talks for SRS employees and contractors.

The Outreach Program historically has been a participant in SRS's outreach to the general public via the *SRNS Public Tours* program, with SREL providing a 45 to 60-minute presentation bimonthly year-round (24 scheduled and up to 20 additional lab tours and impromptu presentations). These presentations provide a general introduction about the history and ongoing mission of SREL and the lab's involvement with research, teaching, and community service. The programs conclude with a question and answer period for participants on wildlife identification, site environmental research programs, safety, and other ecological matters of public interest. During the 2020 fiscal year, the Outreach Program hosted 12 tours, including eight SRNS public tours and four lab tours and presentations for SRS partners.

SREL also hosts a series of scientific seminars, which are open to SRS employees, on a variety of research and educational topics which are pertinent to the SRS mission. Speakers include SREL research scientists, invited scientists from other university or agency programs, and graduate students who are conducting research on the SRS. During the 2020 fiscal year, the Outreach Program hosted five seminars, featuring current research from the SRS to the latest research in genetics, toxicology, herpetology, invasive species management, environmental monitoring, and radioecology.

Other programs in which Outreach personnel participate include: *REMOP*, a community outreach program designed to educate Burke County, Georgia, residents about environmental monitoring, including metals and radioactive elements; the *Let's Grow Together* pollinator program, a collaboration with the USDA Forest Service that provides pollinator gardens and education programs to local schools as well as encourage families to participate in outdoor adventures at National Parks, Historic Sites and federal properties through the *Every Kid in a Park* free pass program; *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; the *Ecologist for a Day* program allows 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 the off-site UGA Conference Center. The conference center also hosts civic group presentations and ecological tours. All school programs incorporate science standards and curricula for particular school

districts. Most of these programs provide an opportunity for participants to work with SREL staff as they catch, mark, and measure various species of reptiles, amphibians, fish, small mammals, and invertebrates.

During the 2020 fiscal year, the **REMOP** program held nine stakeholder education and general public events in Burke County. The program brought together the Burke County community, Georgia Power, Savannah River Site scientists, local educators, and special interest groups. The **Let's Grow Together** program created four pollinator gardens and curriculum at local schools in areas that are traditionally underrepresented in STEM education. In addition, the Outreach program distributed more than 1,000 **Every Kid in a Park** passes in the CSRA region. The outreach staff presented 57 **Ecotalks** in area classrooms, and hosted 14 **Ecologist for a Day** programs. The educational programs reached more than 23,000 students, teachers and members of the public in the CSRA region.

The communications program has worked to increase the visibility of the SREL and its role on the SRS through traditional media such as newspapers, magazines, and regional/local TV and radio outlets. In addition, the communications program has greatly increased its digital presence on social media such as Facebook, Twitter, Instagram, and other emerging social media platforms. During the 2020 fiscal year, the SREL outreach programs, press releases, media advisories, and staff appeared in 293 newspapers, magazines, television shows, and radio programs, and made 20.7 million impressions across all media. The media coverage spanned local, regional, national and international outlets reaching millions of people. The SREL Facebook community grew to more than 1,521 followers and had more than 68,000 views of its content. The SREL Twitter community grew to 455 followers and had more than 172,800 views of its content. The SREL Instagram community grew to more than 1,267 followers and generated more than 103,400 impressions of its content.

The Outreach section of the SREL website, <https://srel.uga.edu/outreach>, receives many visitors, as it has links to the popular **Ecologist for a Day** program, Outreach fact sheets and educational products, the **Ecoviews** weekly newspaper column. It also invites questions about wildlife native to the SRS that are answered by the Outreach personnel. This website is frequented by educators, bloggers, researchers and news outlets from all over the world, who use the materials in their classrooms, blogs, research and news platforms. SREL distributes thousands of educational products and materials worldwide to schools, organizations, and the general public. The website is consistently ranked in the top 10 among UGA's research affiliated websites.

TASK 5. SREL will maintain ecological databases for use by the public, SRS, governmental, academic, and private organizations. These databases incorporate more than 60 years of data collection on the SRS and provide a resource for understanding changes impacting ecosystems on the SRS and elsewhere in the southeastern United States

## **SREL Data Management Activities in FY20**

### **IT Infrastructure**

The IT department at SREL is dedicated to providing a reliable IT network that effectively stores and protects our data, providing our employees with an efficient set of platforms with which to perform their job-related functions, and keeping us connected with the greater community with which we interact. To that end, we made several improvements to our IT capabilities over the last year. Perhaps the most significant of these improvements was the replacement of all of our computers that were over five years old. A number of personnel were using dated computers that were not as efficient as newer technology, and thus, using UGA funds, we completed the purchase and deployment of over thirty notebooks and desktops to our personnel. This has upgraded the IT hardware for our staff as a whole and now all of our machines are capable of efficiently working with the latest platforms and software.

We also have strived over the last few years to integrate our IT functions into the University of Georgia's IT network. This has historically been an issue since we were not located on campus and actually located on a secure DOE facility. Although we have been working on this task for a few years, this year we took increased steps to integrate all of our IT switchgear to the UGA system, and this allows UGA IT to help use manage our network and solve issues remotely. Other recent integrations with campus include a direct high-speed fiber connection to campus that provides SREL with internet, firewall and data security protection that is managed by campus; wireless internet located through our facility that is managed by UGA; and the establishment of virtual servers provided by UGA that allow us to reduce the need to purchase and maintain expensive hardware.

Another significant event for the SREL IT department was the transition from the Dropbox cloud storage platform to the Microsoft OneDrive platform to maintain all of our SREL data storage needs. SREL moved to the Dropbox cloud storage platform a few years ago to help us more efficiently store, access, protect, and share our data. While this solution has worked well, it is not inexpensive, and causes us to set aside slightly over \$30,000 annually to pay for this service. The University recently made Microsoft's OneDrive storage platform available to us for free, so we transitioned to this platform this year to take advantage of a comparable product that resulted in a large annual cost savings for us.

Efficient IT systems and networks are a necessary part of successfully operating in today's world and the Savannah River Ecology Lab remains committed to using our funds to develop the systems and platforms that we need to effectively serve our stakeholders. Our recent accomplishments are significant steps in the directions of accomplishing that objective.

### **Database Management**

Responsible management of research data plays an important role in preserving SREL's institutional memory. Data archiving supports DOE's mission, contributes to future ecological research, and is often required by funding agencies. SREL continues to build the technological and policy infrastructure to support an active archiving program, as well as to address existing legacy data.

In FY20, SREL continued to gather new data submissions via checkout and manuscript notification processes. We also continued to archive legacy data, including examination and digitization of hard copy

materials. Efforts to convert snake capture locations descriptions to GIS begun in FY19 and have yielded over 24,000 successfully plotted point records to date. Freeman station IDs 1-103 are also complete, and Freeman data for all herpetological species have been digitized and assigned spatial coordinates.

Materials continue to be stored via the Excel data archiving template until the data entry portal is complete. Web forms for entering and revising metadata and uploading data files to the server were completed on a testing server at UGA in 2018, but some unexpected technical issues (e.g., deprecation of software components and tools), minor changes in application requirements (e.g., the need for more automation and fewer manual steps by SREL personnel), and changes in personnel status at UGA (e.g., Wade Sheldon retirement) have delayed fully deploying the new features to the SREL data server. The technical and application scope issues have been largely resolved, and we are working diligently to finish the last stage of implementation. Our current goal is to have the data submission and review web site fully usable in January 2021.

New features that have been tested by UGA/LTER and are ready to be deployed include self-registration and validation of new database users, including secure password hashing and password resets based on emailed security codes; data file upload forms for new metadata submissions; data submission review forms with automatic emailing of data manager decisions or requests for revisions to metadata submitters; automatic registration of approved submissions for display in the data catalog, including archiving of submitted data files to the primary data directory organized in subdirectories by metadata ID; and management forms for revising status and release dates of archived data sets. Remaining tasks include testing of user self-registration forms on the SREL server; adding fields to the personnel update page for adding new personnel entries and revising active/inactive status by SREL data managers; finishing MS Access 365-compatible forms for managing rarely-updated content in the database without UGA/LTER assistance (porting from deprecated Access 2010 forms); and user testing/feedback.

TASK 6. SREL will serve as the point-of-contact for the “DOE Research Set-Aside” areas that are protected from site impacts so that they are available for environmental research and can serve to establish representative standards for comparison to impacted areas on the SRS. Currently SRS has 30 “set-aside” areas. SREL will also continue to promote the role of the SRS as a National Environmental Research Park.

### **SREL Set Aside and National Environmental Research Park Activities in FY20**

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 what was formerly known as the Savannah River Plant and to secure study sites to conduct 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 sites from 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-Asides provide sites for long-term research, habitat for sensitive species, and protection for several archaeological sites.

*Administration and Management of the Set-Aside Areas* – 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. SREL serves as the representative for the Set-Aside program in the SRS Site Use process and in the military training coordination meetings, reviewing activities in both venues for potential impacts.

*Set-Aside Oversight* – In recent years SREL has taken a more active approach to managing these areas, with wider application of prescribed fire, as well as some timber management. Management is conducted with an adaptive approach that gives the flexibility to address changing environmental conditions and research needs.

- In FY20 several Set-Aside Areas were damaged by the “Easter tornados” that touched down on SRS property just before dawn on April 13. The most damage occurred in the E. P. Odum Wetland Set-Aside, where the tornado approximately followed the Tinker Creek drainage down from the Williston area, with the bulk of the damage on the ridge at the confluence with Upper Three Runs. The decision was made not to salvage the damaged areas of the SA, as a contrast to the areas outside, which will be salvaged by USFS. Some limited damage occurred in the northwest corner of the Sandhills Fire Site; it is a small area even relative to the size of this relatively small SA. It will not be salvaged. Significant damage also occurred in the Mona and Woodward Bays SA. The young stand on the hill on the north side of Woodward Bay, which had already lost some timber due to fire damage in 2011, was reduced to a basal area of perhaps 10 square feet per acre. The northwest side of Woodward Bay also took a direct hit, removing all canopy in some parts of the ecotone and basin; over the summer the new combination of water and light revealed an abundant herbaceous seedbank, including a few individuals of a rare species, *Croton elliotii*. The older mixed stand in the southwest corner of the Set-Aside also sustained some significant damage. Discussions with USFS-Fire indicated that salvage was not necessary to maintain prescribed fire at this site. At the close of FY20, management discussions are ongoing.
- Mona and Woodward Bays SA, Craig’s Pond and Sarracenia Bay SA, Sandhills Fire Site SA, and Little Cypress Bay SA are all part of the upcoming Par Pond East timber prescription. Initial discussions have begun and will continue in FY21. Both Mona/Woodward and

Craig/Sarracenia contain stands of young plantation that may provide improved habitat if thinned. Discussions also included fire management of Craig's Pond ecotone.

- Slash pine thinning prescribed at Flamingo Bay has occurred.
- High water levels have been observed in Craig's Pond and Sarracenia Bay this year that may complement efforts to manage these areas with prescribed fire. Late in the year water levels were high enough in the two bays to meet, inundating the ecotones where loblolly pine invasion occurs.

#### Current research in SRS Set-Asides

- SRARP continues to catalog artifacts previously recovered from Flamingo Bay SA. No additional excavations have occurred there this FY. Long term archaeological research at Flamingo Bay has provided a wealth of information on early inhabitants of the CSRA, as well as information on bay formation.
- Partial drift fences at Craig's Pond were closed due to limited gopher frog activity. They may be reopened in 2021.
- The E. P. Odum Wetland Set-Aside was the site of a box turtle release study. A large group of confiscated box turtles was released into a 2.5-acre silt fence enclosure in October of 2019, and allowed to overwinter in the pen. In April 2020 a subset was given radio-transmitters, and the gate to the pen was opened. Dispersal has been monitored continually since then. Another cohort of 25 will be added to the pen in early FY21 and tracked.
- Researchers from SREL, USFS-SR, and the University of Kentucky continue stream characterization in the E.P. Odum Wetland Set-Aside. This research will be used to inform future DOE restoration and mitigation efforts.
- A tributary in the E.P. Odum Wetland Set-Aside was used as a reference site to assess effects of excessive stormwater runoff on aquatic insect emergence.
- Dragonfly nymphs from Flamingo Bay were used in studies assessing the effects of contaminated constructed wetlands on aquatic insects.
- The E. P. Odum Wetland, Organic Soils, and Mature Hardwood Set-Asides provided field sites for a study of ecological factors affecting the success of rabies elimination in the southeastern US.
- Craig's Pond, Sarracenia Bay, Mona Bay, and Thunder Bay, as well as nine non-SA wetlands in the central and northeast regions of the SRS, continue to be monitored as egg-laying sites for the state-endangered gopher frog, *Lithobates capito*, and as part of a regional southeastern population-status study. Egg masses were observed at Bay 58 and Mona Bay in spring of 2020. Efforts are also underway to identify habitat restoration priorities in those regions and the Crackerneck area of the SRS.
- 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 D-Area Ash Basin system.
- The amphibian community at the Rainbow Bay Amphibian Reserve Set-Aside has been monitored for 42 consecutive years, during which time local species extinctions, colonizations, and dramatic

population fluctuations have occurred. Researchers are currently investigating how amphibian community changes and hydroperiod fluctuations over time have influenced nutrient fluxes between the wetland and upland habitats, as well using telomeres as indicators of cellular aging and stress in the marbled salamander.

- SREL researchers continue collecting amphibian tissue samples from multiple wetlands for studies of amphibian landscape genetics and effects of future climate change. Samples from eight species have been collected from approximately 43 isolated wetlands across the SRS, including the following Set Asides: Rainbow Bay Amphibian Reserve, Cypress Bay, Dry Bay, Ellenton Bay, Mona Bay and Woodward Bay, Flamingo Bay, Thunder Bay, Craig's Pond and Sarracenia Bay, Ginger's Bay, and Road 6 Bay.
- Amphibian species in bay Set-Asides and other site wetlands are being monitored for two amphibian diseases of concern, chytrid and ranavirus, to determine disease prevalence on the SRS and possible relationships to contaminant distributions.

#### National Environmental Research Park Support

SREL serves as the official SRS point of contact for the DOE National Environmental Research Park System. In its role as a point of contact, SREL conducts a variety of functions, one of which is the improvement and archiving of critical historical research data on the SRS. For more information on this subject, see the Data Management section elsewhere in this report.

TASK 7. Through general research and public outreach programs, SREL will increase scientific understanding in the general areas of environmental characterization, ecological risk assessment, and environmental remediation and restoration. This will require research on topics such as terrestrial and aquatic ecology, environmental chemistry, molecular ecology and genetics, microbial ecology, radiation ecology, and ecotoxicology. SREL will also continue to communicate and coordinate with SRS contractors and the public on these issues

In FY20 SREL faculty, staff, and students conducted and completed a diversity of outreach and education programs for the public and environmental research projects on the SRS in support of the missions of DOE (EM & NNSA) on the site. Specifically, outreach programs were conducted for local community residents on behalf of DOE as part of ongoing community education programs to increase environmental awareness of citizens and provide independent information to community residents relative to the activities of site tenants. In addition, specific research programs were conducted for NNSA to assess the environmental consequences of the former Mixed Oxide Fuel Fabrication Facility construction site on local stream quality and function as well as to assess the function, performance, and environmental consequences of constructed wetland treatment systems for metal sequestration associated with the NNSA Tritium facility on the SRS. These programs were funded by NNSA and the details of these projects are outlined below:

### **Research Support to DOE National Nuclear Security Agency (NNSA)**

#### **H-02 Constructed Wetland Studies—Metal Biogeochemical Processes**

##### **Funding Entity**

NNSA

##### **Start Date and Funding Amount**

October 2019; \$275,000

##### **PI and co-PIs**

Dr. Xiaoyu Xu

##### **Objectives**

The goal of this research is to support, assess and improve operations of the NNSA constructed wetlands to maintain treatment efficiency and ensure long-term sustainability. Our primary objectives are to study the removal efficiency of metals, primarily copper (Cu) and zinc (Zn), for the wetland system, the chemical speciation and bioavailability of metals in surface waters, the stability of sediment accumulated metals and the potential for remobilizing sediment metals, the overall biogeochemistry of metals in the sediment and water, and the relative environmental impacts of free water surface constructed wetland.

##### **Summary of Research Activities**

###### *1. Water quality and metals in the water*

Water samples were collected monthly over FY20 in the primary discharge pipes, retention basin, influent, effluent, both treatment cells, and the discharge stream that connects to regulatory Upper Three Runs. Water quality parameters (temperature, pH, oxidation-reduction potential (ORP), alkalinity, dissolved organic carbon (DOC), sulfate, and chloride) and metal concentrations (total and dissolved Cu and Zn) were measured. Generally, the H-02 wetland system still functioned well in FY20. The overall metal removal efficiencies were 70.6% for Cu and 64.4% for Zn and were not statistically different from values in FY19. Concentrations in the influent (Cu: 20.8 µg/L; Zn: 30.6 µg/L) were higher than those in the effluent (Cu: 5.8 µg/L; Zn: 8.5 µg/L) and stream (Cu: 5.9 µg/L; Zn: 8.0 µg/L), and the effluent concentrations were lower than the NPDES discharge permit (Cu: 12.3 µg/L; Zn: 110 µg/L). Water quality was improved after water being treated by the wetland cells; the alkaline water became nearly neutral after running through the wetland cells with noticeable decreased pH (influent: 9.5; effluent: 6.8) and the ORP in the pipe (330 mV) was lowered by 60% compared to the effluent (134

mV). In summary, a relatively stable performance of the H-02 wetland was indicated by the similar metal concentrations and water quality parameters in the effluent between FY19 and FY20.

#### 2. *Metals in the sediment*

Sediment cores were collected from treatment cells in the cool and warm seasons of FY20. Cores were sectioned and processed to monitor Cu, Zn, Mn, Fe, and Ni concentrations in different sediment layers. In general, and except for Fe, the top layer had considerably higher Cu and Zn concentrations than the other layers. Seasonal variation was present with the top layer comprising about 90% of Cu and Zn in the warm season and 65% in the cool season. The observations bring up a possibility that in the future the metal removal efficiencies maybe lowered due to the saturation of the top sediment layer.

#### 3. *Using passive samplers to explore metal biogeochemistry*

Multiple passive samplers were applied to understand metal biogeochemistry in the wetland. In surface water, piston Diffusive Gradients in Thin Film (DGT) was deployed monthly. The percentage of DGT-indicated bioavailable Cu in dissolved Cu was much higher than the summed percentages of free and inorganic Cu in the wetland cells, effluent, and stream, but the percentage of DGT-Zn in dissolved Zn was not different from the summed percentages of free and inorganic Zn, which was explained by their dissimilar speciation in the water, such as binding capabilities of organic ligands. Zn indicated higher lability and/or bioavailability in the wetland water than Cu by its higher percentages and concentrations of free and inorganic Zn. Comparing DGT-Cu percentages among sites, the increase of Cu bioavailability was suggested in the effluent than that those in the wetland cells, revealing a possible negative environmental impacts of the free water surface constructed wetland.

In the sediment, probe DGT and plate Pore Water Sampler (peeper) were deployed in both wetland cells in March, July, and September of FY20 to measure bioavailability of Cu and Zn in sediment pore waters. The concentration of DGT-Zn is almost one order of magnitude higher than DGT-Cu. The concentration patterns of DGT-Cu and DGT-Zn were different among months, across depths, and between cells, which were attributed to the seasonal change of water chemistry, the disturbance in the sediments, heavy rain, and/or routine wetland maintenance. Meanwhile, sulfide DGTs were deployed in the sediment coupling with the probe DGT of metals to explore sulfur dynamics in the sediment, since sulfur cycling primarily controls metal removal mechanisms and directly relates to the good performance of the wetland. There was a visible difference in bioavailable sulfide with higher concentrations in the H-02 wetland in comparisons with the A-01 wetland, however the sulfide depth gradient of DGT has not been digitized in the software.

#### 4. *Mercury (Hg) in the wetland cells*

Surface water samples were collected monthly in FY20 in the pipes, retention basin, influent, treatment cells, effluent, and stream for analysis of total Hg concentrations. Sediment cores were collected and probe DGTs were deployed monthly in both wetland cells to study total Hg in bulk sediment and bioavailable Hg (DGT-Hg) in sediment pore waters. Total Hg concentrations in the water averaged 0.16 µg/L that was lower than the averaged DGT-Hg concentrations (cell 1: 0.40 µg/L; cell 2: 0.37 µg/L), suggesting Hg deposition from the atmosphere. Similar to DGT-Cu and DGT-Zn, the highest DGT-Hg concentration was found in the sediment water interface and the surface sediment. Sediment cores will be processed in the future, so Hg geochemistry will be demonstrated by the combined results of Hg in the water, sediment, and DGT, as well as the possible negative environmental impacts of the wetland being a trap and sink of the atmospheric Hg. Sediment cores will be processed in the future. The current results implied the possible negative environmental impacts of the wetland being a trap and sink of the atmospheric Hg.

### **Conclusions**

1. The H-02 constructed wetland effectively reduces total Cu and Zn concentrations in the surface water released by Tritium Facility to achieve NPDES regulatory limits, and water quality was improved after being treated by the wetland cells.
2. Metal bioavailability and toxicity were reduced due to the decreased concentrations of labile metals, such as DGT-measured labile and/or bioavailable metals.

3. Most deposited metals were retained in the top sediment layer, which may lead to decreased removal efficiency and performance in the future when the surface sediment is saturated.
4. The metal-removal processes in both wetland cells are related to seasonal water chemistry, sulfur cycling in the sediment, biomass productivity, and routine maintenance.
5. The wetland treatment cells are a sink of the atmospheric Hg and a source of methyl-Hg to the surrounding environment.
6. Passive sampling devices, such as DGTs and Pore Water Samplers, and geochemical modeling are great approaches as the surrogates for biomonitoring and they should be included in routine monitoring. But care needs to be taken on their appropriate uses.

#### **Major Impacts of Research**

1. This research supports the use of cost-effective constructed wetlands for the treatment of metal contaminated wastewater and supports DOE's goal of employing "green technologies" for waste cleanup and remediation. Constructed wetlands play an important role in the SRS environmental plan to achieve both federal and state regulatory compliance for the discharge of effluent waters.
2. This research evaluates the potential transport of contaminants from constructed wetlands to surrounding environments and supports DOE commitment to good ecological stewardship.
3. Results of our studies support the EPA's goal of advancing our understanding of metal biogeochemistry in wetland systems and developing better tools for predicting the fate and effects of metals in aquatic ecosystems.

#### **Other Project Personnel**

Erin Peck, research professional – SREL

Zeinah Elhaj Baddar, postdoctoral research associate – SREL

Chongyang Qin, postdoctoral research associate – SREL

Cher Nicolson, MS graduate student – SREL

#### **External Collaborators**

NA

#### **Products**

Harris, S., X. Xu, G.L. Mills. 2020. Metal-sulfide dynamics in a constructed wetland in the Southeastern United States. *Wetlands Ecology and Management*. doi: 10.1007/s11273-020-09749-6.

Xu, X., E. Peck, D. Fletcher, A.M. Korotaza, and J. Perry. 2020. Limitations of applying DGT to predict bioavailability of metal mixtures in aquatic systems with unstable water chemistries. *Environmental Toxicology and Chemistry*. doi: 10.1002/etc.4860.

Qin, C., X. Xu, E. Peck. Metal removal by a free surface constructed wetland: insights on metal speciation and bioavailability indicated by DGT and biota ligand model (BLM). *Ecological Engineering* (in review).

Elhaj Baddar, Z, X. Xu, E. Peck. Effect of time and seasonality on Cu and Zn concentration in the sediments of constructed wetlands. *Ecological Engineering* (in review).

## **H-02 Constructed Wetland Studies: Amphibian Ecotoxicology**

### **Funding Entity**

NNSA

### **Start Date and Funding Amount**

October 1, 2019; \$324,498

### **PI and co-PIs**

Dr. Stacey Lance and David Scott

### **Objectives**

Our research at the H-02 constructed wetland complex focuses primarily on several questions related to these treatment wetlands: 1) Over time, what amphibians, reptiles, and plants have become established in the wetlands? 2) Do the elevated trace metal levels (e.g., copper and zinc) in the wetlands affect amphibian reproductive success, disease ecology, and population dynamics? 3) How do the amphibian diversity and numbers compare to other, more natural, wetlands? and 4) As the constructed wetlands age, how will the amphibian community respond?

### **Summary of Research Activities**

This report summarizes our amphibian studies related to the H-02 treatment wetlands from October 2019 to September 2020. Following up on previous research, during this year we continued analyzing data on amphibian communities and disease prevalence from 20 wetlands on the SRS. We expanded our analyses to include boosted regression trees to deal with the very large data set and are currently writing a manuscript for submission to *Ecology*. Similarly, we continued work to examine the landscape genetics of two species, the Southern toad and Southern leopard frog, from across the SRS. We are currently analyzing the genetic data for ~600 individuals. From these data, we will be able to establish baseline levels of genetic diversity in both species and examine patterns of gene flow across the SRS. These data are critical for establishing whether the contaminated wetlands on the SRS, such as the H-02 wetlands, are acting as ecological sinks or traps. In addition to these site-wide studies, we undertook two new pilot studies to determine whether environmental DNA techniques can be used to assess the relative abundance and genetic diversity of amphibians in wetlands. To accomplish this, we conducted controlled laboratory studies with known abundances of southern leopard frogs and southern toads in aquaria and will compare how quantitative PCR and SNP analysis methods perform in estimating abundance. We also conducted a complementary study with southern leopard frogs in the H-02 wetlands where we used traditional catch per unit efforts to estimate abundance and we will compare how quantitative PCR and SNP analysis methods perform in estimating abundance. We have continued to collect eDNA and trapping data from the H-02 wetlands to compare techniques across time and amphibian communities. We also expanded the eDNA work to assess its sensitivity to identifying wetlands with the at-risk Carolina gopher frog. When trapping in the wetlands at times we inadvertently capture water snakes and are using this opportunity to examine uptake of metals and distribution of metals across body parts. Finally, we completed the 42<sup>nd</sup> year of monitoring at Rainbow Bay, and finished analyzing the data in the context of community shifts in response to environmental change and subsequent impacts on nutrient cycling.

### **Conclusions**

1. Ranavirus prevalence is driven more by environmental factors, including canopy cover, air and water temperature than by community level factors, such as species richness.
2. *Ambystoma talpoideum* continues to be present in the H-02 wetlands and remains the only salamander species present.
3. At the D-Area wetlands there is evidence that amphibians can locally adapt to a mixture of heavy metals.
4. The amphibian community at Rainbow Bay has shifted from long- to short-hydroperiod species over four decades in response to drought and associated shortened wetland hydroperiods.
5. Ignoring adult mortality in models of terrestrial flux of biomass leads to erroneous conclusions and the probability of terrestrial flux of nutrients due to amphibian movements is tightly linked to wetland hydroperiod and amphibian biomass, but not specific species.

6. Concentration of eDNA captured from aquaria is more strongly correlated to amphibian biomass than abundance.

#### **Major Impact(s) of Research**

1. Our continued time series of metal concentrations in the H-02 system (in sediments, water, and biota) will enable informed assessment of how this type of constructed treatment wetland functions, and whether it provides suitable wildlife habitat in addition to enhancing water quality.
2. Our *in situ* mesocosm and laboratory studies demonstrate the importance of looking a) at multiple stressors, b) beyond the larval period, and c) at multiple source populations. We have found significant latent effects that lead to completely different conclusions than the larval study alone—effects on juvenile survival were apparent five months later, largely due to Cu effects on body size at metamorphosis.
3. Our disease studies are ongoing but are demonstrating the complexity of variables involved with disease incidence and prevalence in amphibians. The nature of the wetland—metal-contaminated vs. clean, permanent vs. ephemeral, and constructed treatment wetland vs. natural— impacts disease prevalence and variables are confounded with each other.
4. Our combined research at H-02, A-01 and D-Area are providing insight into how amphibian populations may locally adapt to metal contamination. Results from A-01 and D-Area suggest that within a few more generations amphibians at H-02 may be more tolerant of copper, however this may come with tradeoffs that reduce fitness in non-contaminated environments.
5. Our understanding of the factors that drive the population dynamics of amphibians in natural systems, based on the long-term RB study, will allow predictions of the effects of climate change on isolated wetlands and provide insights to land managers who may need to design protective measures for rare species.

#### **Other NNSA-supported Project Personnel and Students**

Cara Love, PhD student, SREL

Dr. Jason O'Bryhim, Research Professional, SREL

Adam McFall, Research Technician, SREL

#### **External Collaborators**

Dr. Scott Weir, Queens University, Charlotte, NC

Dr. Krista Capps, Odum School of Ecology, UGA, Athens, GA

Gabriela Rodriguez, undergraduate intern, University of South Carolina Aiken, Aiken, SC

#### **Products**

Flynn, R.W., A. Welch, and S.L. Lance (Accepted with revision) Phenotypic divergence in heritable life history traits suggests adaptive divergence and trade-offs associated with a coal ash disposal site. Evolutionary Applications.

## **H-02 Constructed Wetland Studies—Ecosystem Health**

### **Funding Entity**

NNSA

### **Start Date and Funding Amount**

October 1, 2019

\$152,811.78

### **PI and co-PIs**

Dr. Gene Rhodes and Dean Fletcher - SREL

### **Objectives**

The H-02 wetland water treatment system was constructed as a green technology initiative to remove metals and buffer pH in discharge waters from the NNSA Tritium Processing facility. Constructed wetlands have been widely employed to reduce nutrients and prevent eutrophication of surface waters. Their application for treatment of metal contaminated wastewater is still being refined. Our overall goal is to assess wetland effectiveness and develop strategies to maintain or improve treatment efficiency of the NNSA constructed wetlands to ensure long-term sustainability. Information is needed to evaluate: (1) the distribution of biologically available contaminants throughout and downstream of the system and how this changes over time, (2) effects of disturbance events (e.g., storms) on wetland effectiveness, (3) the potential export of metals to terrestrial or downstream food webs by aquatic biota, (4) effects of daily discharge fluctuations on wetland efficiency, (5) management options for the retention basin and wetland cells to improve wetland efficiency. Previous work indicated considerably higher levels of biologically available Cu below the wetland than expected. Efforts are focusing on identifying the conditions under which metals leave the wetland system and on developing strategies to improve wetland treatment efficiency.

### **Summary of Research Activities**

#### **Metal Transport by Emerging Macroinvertebrates**

Aquatic insects commonly exhibit complex life cycles with both terrestrial and aquatic phases. Terrestrial adults lay eggs that hatch into aquatic larvae that feed and grow for from a few months to several years before emerging as flying adults. The aquatic larvae of many species have been shown to accumulate both essential (e.g. Cu, Zn, Mn, Ni) and nonessential (e.g. Cd, Hg, Pb) trace elements to toxic levels when exposed to diverse pollutant sources. The aquatic larvae of many species tend to have relatively small home ranges, thus accumulate contaminants from their local environment. Consequently, aquatic invertebrates have been extensively used as biomonitors of diverse contaminants. Metamorphosis of aquatic insect larvae ultimately results in emergence of flying juvenile or sexually mature adult insects from a stream or wetland. Emergence of these aquatic insects from a wetland provide a food source to terrestrial predators that live along stream or wetland margins and cause increased productivity near the wetland. This linkage of aquatic to terrestrial food webs also presents a potential of exporting contaminants accumulated in the aquatic larvae from the contaminated wetland or stream to terrestrial food webs. Evaluating the impacts of biovectors exporting specific contaminants from aquatic into terrestrial food webs is not simple to assess and mechanisms driving this export remain poorly known. Ameliorating factors can reduce the flux of contaminants from contaminated habitats by emerging insects. For example, changes in the body burden of insects can occur during metamorphosis and emergence into flying adults/subadults. Concentrations can decrease or increase depending upon contaminant. Thus, aquatic larvae body burdens do not necessarily reflect what terrestrial predators may be exposed to by emerging insects.

We have been using dragonflies and damselflies to evaluate the potential export of contaminants from the H-02 constructed wetland where aquatic nymphs have previously been shown to accumulate elevated levels of metals. Capture of dragonflies as they emerged from the wetland along with their shed exuviae allows evaluation of metals leaving the wetland in the emerging dragonfly (teneral) versus shed in the exuviae and returned to the wetland. Elements may be bound to the exuviae surface, incorporated into it, or expelled onto the inner surface by the larvae during metamorphosis. We found that some

elements (e.g. Al, Fe, Mn, Pb) tend to be largely shed in the exuviae and returned to the wetland, whereas others tended to leave the wetland in the teneral (e.g. Cu, Zn, Mg, B) in larger proportions. However, the proportion of trace elements accumulating in/on exuviae versus teneral differed among species and elements in both dragonflies and damselflies. Monitoring of water quality in the wetlands has shown seasonal changes in water chemistry. Consequently, the potential also exists of the amount of metals being transported from the wetlands by emerging insects to vary over the course of the extended flying season. We analyzed a total of 280 teneral dragonflies and 196 exuviae distributed across 4 species. Samples were distributed across the months from April through August. Some species such as *Plathemis lydia* emerged and were collected throughout the season, whereas *Pachydiplax longipennis* had early and late season emergence pulses.

Temporal variability in teneral body burden and the proportion shed with the exuviae occurred throughout the flying season. However, for a single element, the range within a species was most often < 25%, but a few exceptions occurred. Elements such as Pb, Mn, V, Al, Fe, and Ba were consistently largely shed in the exuviae throughout the season. Similarly, greater proportions of Cu, Zn, Mg, and B generally left the wetland in the teneral throughout the season. In *P. lydia*, the average proportion of Cu shed in the exuviae ranged from 33 to 59%. This illustrates that care should be taken when interpreting data that represents “snap shots” of a single moment in time, because things do change. However, differences among species did tend to be greater than those among collection weeks within a species. In all species, body mass differed throughout the growing season. Differences in teneral metal mass body burden among species or weeks within a species appeared often driven more by differences in body size than metal concentrations. This illustrates the complexity of such assessments because not only the level of contaminant exposure influence contaminant flux from wetlands in biovectors, but also factors that influence the size and abundance of emerging insects. Our work is contributing to a better understanding of these contaminant fluxes.

Tetragnathid spiders construct webs horizontally above the water’s surface and capture diverse taxa of smaller emerging insects. It has been shown that their diet is composted primarily of emerging insects. Thus, contaminant accumulation in tetragnathid spiders can be an indicator of contaminant flux from the wetland. Moreover, the physical condition of these spiders provides an indicator of the number of emerging insects and thus physical condition of the wetlands. We collected tetragnathid spiders from the retention basin, both wetland cells, and effluent pool to assess contaminant flux throughout, especially downstream of the constructed wetlands. Additionally, for comparison we collected spiders from wetland Fire Pond as a non-contaminated reference system and from impacted wetlands in D Area and E Area. Over 300 spiders were collected in all, but compositing for metal analyses will likely be necessary due to small body size. Sample preparation will begin in FY21.

#### **Factors Influencing Metal Accumulation in Macroinvertebrates**

Different taxa of dragonfly nymphs stratify vertically in wetlands. Our work conducted in streams and wetlands frequently found taxa associated with sediments, particularly fine sediments rich in organic matter, tend to accumulate higher concentrations of metals/metalloids. For example, the genus *Anax* that typically lives in aquatic vegetation well above bottom sediments accumulated lower Cu concentrations than *Erythemis* that is more closely associated to bottom sediments. However, it cannot be ruled out that differences in physiology or biology drive these differences rather than habitat use. We collected 100 *Anax longipes* and 100 *Erythemis simplicicollis* from uncontaminated reference wetlands and held them in cages on frames in H-02 retention basin. Equal numbers of each species were held at four water depths ranging from on the sediment to 75 cm above sediment which corresponded to just under the water’s surface. Nymphs were held for two months and then removed for trace element analyses. Water was sampled at each depth with water diffusion samplers, and surface sediments were sampled. Water and most dragonfly samples were processed in FY20. Sediment and the remaining dragonfly samples will be analyzed in FY21. Evaluation of these samples will clarify whether previously observed differences are due to exposure to contaminated sediment or biological/physiological differences between taxa.

In collaboration with Guha Dharmarajan, a laboratory dosing experiment was conducted that is comparing the contribution of metal exposure in water versus dietary exposure to nymph metal body burden. These samples will be processed in FY21. These studies will contribute to a better understanding of how contaminants enter and pass through aquatic communities.

#### **Metal Mobilization by Stormwater Flows**

Previous work using automated samplers found significant transport of metals during storm events. We found that substantial spikes of suspended solids were being mobilized during the first flush of severe storm events. During these episodes, fluxes of total metals were elevated during the first flush, followed by periods of elevated dissolved metals. In FY20 we modified our sampling strategy to work within lab personnel usage limits necessary during the pandemic as it was not possible to process the large number of samples over a short period of time. Rather than using the laboratory time intensive automated samplers, 13 stations were established and water grab samples were taken at base flow and during storm events. A primary goal of these stations is to establish whether the elevated suspended solids are being mobilized in the retention basin and/splitter box or entering through the outfall pipes that flow into the retention basin. Additionally, the potential of solid mobilization in the wetland cells and effluent pool are being assessed. The sampling is also providing information on where in the system metal concentrations increase during storm events. Total suspended solids in the water samples are being quantified along with total and dissolved copper and zinc. Preliminary results suggest metals being mobilized in the retention basin, but sample collection will continue to better establish metal mobilization patterns.

Experimental sampling was also initiated to examine the effects of vegetation cleared along the edges of the wetland cells. Water velocity and water depth were measured in the cleared area as well as in the vegetation at 7 locations along each side of the wetland cell to evaluate any potential differences in water movements. Water samples were also collected and metal concentrations analyzed. Techniques are being refined based on our initial samples.

#### **Conclusions**

1. Severity of a storm can influence outfall discharge, metal concentrations, and consequently fluxes of metals that enter and leave the treatment cells.
2. During more severe storm events, elevation of both metal concentrations and discharge rate can cause a more substantial metal load to enter and leave the wetlands. Wetland efficiency is reduced during these more severe events.
3. Metal concentrations and fluxes were generally most elevated in a “first flush” that occurred early in a storm event. Higher concentrations of suspended solids leaving the wetland during the first flush resulted in a greater portion of tCu being associated with particulate material. During later hours, a larger portion of the tCu was dissolved. Wetland metal removal efficiency also changed over the course of the storm events.
4. Even though substantial amounts of metals left the wetlands, overall greater masses of metals entered the treatment cells than left in the effluents. Thus in the evaluated storm events, the H-02 wetlands functioned to lessen impacts on the receiving stream. Preliminary analyses indicate mobilization of sediments in the retention basin during storm events. Future work will continue evaluating whether the heavy loads entering the treatment cells during storms are originating from the retention basin or water entering it.
5. Elements differed in their propensity to accumulate in or on dragonfly and damselfly exuviae that are shed during emergence and remain in the wetland versus being transferred to terrestrial food webs by the emergent. The amounts leaving the wetland in the emergent insects can vary over the flying season. Shedding of metals in their exuviae can result in a substantial portion of a nymph’s body burden being left in the wetland where we want it.
6. Overall, concentrations of trace elements accumulating in nymphs are indicative of trophic doses when preyed upon directly, but not necessarily to that of predators preying on emerging dragonflies.

#### **Major Impacts of Research**

1. This research supports the use of cost effective constructed wetlands for the treatment of metal contaminated waste water and supports DOE’s goal of employing “green technologies” for waste

cleanup and remediation. Constructed wetlands play an important role in the SRS environmental plan to achieve regulatory compliance for the discharge of effluent waters.

2. Our research evaluates the potential transport of contaminants from constructed wetlands to downstream waters or terrestrial environments and supports DOE commitment to good ecological stewardship. Evaluations are aimed at developing management strategies to maximize wetland effectiveness.
3. Overall, identification of factors reducing constructed wetland efficiency will allow us to prescribe and ultimately implement management strategies to maximize wetland efficiency.

#### **Other Project Personnel**

Christina Fulghum, Research Technician - SREL

Erin Spivey, Research Technician - SREL

Dr. Xiaoyu Xu, Assistant Research Scientist - SREL

Dr. Guha Dharmarajan, Assistant Research Scientist - SREL

Paul Stankus, Research Professional - SREL

Angela Lindell, Research Professional - SREL

#### **External Collaborators**

Brian Bledsoe, Director of Institute for Resilient Infrastructure Systems, University of Georgia, College of Engineering

#### **Products**

Fletcher, D.E., A.H. Lindell, P.T. Stankus, N.D. Fletcher, B.E. Lindell, and J.V. McArthur. 2020. Metal Accumulation in Dragonfly Nymphs and Crayfish as Indicators of Constructed Wetland Effectiveness. *Environmental Pollution* 256:113387.

Xu, X, E. Peck, D.E. Fletcher, A. Korotasz, and J. Perry. (in press). Limitations of Applying Diffuse Gradients in Thin Films to Predict Bioavailability of Metal Mixtures in Aquatic Systems with Unstable Water Chemistry. *Environmental Toxicology and Chemistry*.

Fletcher, D.E., C.M. Fulghum, D.B. Pitt, A.H. Lindell, and P.T. Stankus. 2019. Trace elements shed in exuviae versus exported from a contaminated wetland by emerging damselflies. Annual Meeting of the Society of Environmental Toxicology and Chemistry, Toronto, ON, November 2019 [poster].

Fletcher, D.E., C.M. Fulghum, D.B. Pitt, A.H. Lindell, and P.T. Stankus. 2020. Trace elements shed in exuviae versus exported from contaminated wetlands by emerging damselflies. Annual Meeting of the Society of Freshwater Science. [poster]

## Assessing Effects of Copper Exposure in an Environmental Fish Model

### Funding Entity

NNSA

### Start Date and Funding Amount

October 2019; \$68,000

### PI and co-PIs

Dr. Ben Parrott, Dr. Olin Rhodes

### Objectives

Metal contamination resulting from industrial use has the potential to impair water systems throughout the United States. Impacts to biota in these systems are often predicted using toxicological exposure models that do not fully capture complex interactions between environmental factors (e.g. temperature variability) and/or are limited to predicting bioavailability and uptake. Further, these models rely on a limited suite of endpoints that do not adequately assess sublethal impacts that have the potential to influence population dynamics in more subtle ways than lethality. The H-02 treatment wetland system on the Savannah River Site is designed to remove metals from waters discharged from the NNSA Tritium Processing Facility. Copper and zinc levels remain elevated in this system and understanding their sublethal effects on organismal health and fitness remain a concern.

Sub-lethal effects of contaminant exposure can alter health and survival of organisms and ultimately populations. In order to understand the biological impact and risk of contaminant exposures in nature, we must first determine how reliable markers of biological health relate to contaminant exposure under controlled conditions. Medaka fish (*Oryzais latipes*) are increasingly used as a model system in environmental toxicology applications due to the availability of genetic resources, stereotypical development, and their experimental tractability. Because fertilization of medaka eggs occurs externally, eggs can be collected shortly after fertilization, staged, and subsequently used for exposure assays incorporating a high degree of developmental resolution (e.g., hour time scale). The project objectives are centered on using the medaka fish model to investigate the sublethal impacts of copper exposure. Specifically, the project is aimed at revealing how exposures occurring during sequentially ordered temporal windows during development influence subsequent physiological function. The work will develop and validate biomarkers of organismal health in medaka fish and these tools will be used to test the hypothesis that developmental exposure to copper will exert negative impacts on organismal health in adult animals. More specifically, exposures occurring earlier in development are hypothesized to disproportionately impact organismal health relative to those exposures occurring later in life.

### Summary of Research Activities

Two independent cohorts of medaka embryos were exposed to copper sulfate (10 ppb and 100 ppb) across a range of ambient temperatures (27°C, 30°C, and 33°C). Hatch rates, time to hatch, and absorbed Cu levels were tested and recorded. Further, subsets of exposed fish were reared to 4 months post fertilization at which point growth rates were recorded and fish underwent fin regeneration challenges. Fish were subsequently necropsied and gonadal, hepatic, and CNS tissues were collected for telomere measurements, gene expression assays, and gonadal histology. Data collection and analysis is complete and is currently being prepared for publication. Briefly, embryonic exposure to copper interacted with temperature to affect developmental rate, with copper accelerating developmental rate at lower temperatures (27°C, 30°C), but not at the warmer temperature (33°C). Embryonic exposure also resulted in persistent, dose-dependent effects on growth, with higher exposure concentrations resulting in smaller fish. This effect persisted for at least 4 months post exposure. An effect of embryonic copper exposure on fin regeneration rate was not detected in fish exposed to Cu as embryos or adults. Additional experiments in which Medaka embryos were exposed to copper sulfate (0 ppb, 10 ppb, 100 ppb) at three distinct developmental windows is currently underway. After exposure, fish are reared to maturity (~90 days). Upon reaching maturity, fish will be necropsied and telomere length, epigenetic age, and gene expression will be assessed. These experiments are also accompanied by the development and validation of biomarkers of organismal health. An epigenetic clock has now been developed and validated

in the Parrott Lab. In addition, a qPCR based telomere length and fin regeneration assays have also been developed. These tools will serve as valuable resources for assessing sublethal effects of copper exposure.

### **Conclusions**

1. Temperature interacts with copper exposure to affect developmental dynamics in a model teleost.
2. Copper exposure does not appear to exert latent or acute effects on regeneration rates in medaka.

### **Major Impact(s) of Research**

1. The work addresses the impacts of copper exposure under ecologically relevant temperature regimens on endpoints associated with health and reproduction in aquatic vertebrates.
2. This work advances our understanding regarding how exposures during development can have lasting effects on adult physiology.

### **Other Project Personnel**

Emily Bertucci, PhD student – SREL

Marilyn Mason, Research technician – SREL

### **External Collaborators**

None

### **Products**

Mason, M.W., Bertucci, E.M., Leri, F.M., Rhodes, O.E., Parrott, B.B. Developmental temperatures interacts with copper exposure to affect developmental rate in a model teleost. *In Prep.*

B.B. Parrott. Invited Seminar. January 14, 2020. Department of Physiological Sciences Seminar Series, University of Florida. Gainesville, FL, USA

Bertucci EM, Parrott BB. Characterization of the age-related DNA methylome and development of an epigenetic age predictor in medaka (*Oryzias latipes*). January 31, 2020. Oral Presentation. Odum School of Ecology Graduate Student Symposium, Athens, GA

Bertucci EM, Parrott BB. Characterization of the age-related DNA methylome and development of an epigenetic age predictor in medaka (*Oryzias latipes*). January 7, 2020. Oral Presentation. Society for Integrative and Comparative Biology Annual Meeting. Austin, TX, USA

Bertucci, E.M., Parrott, B.B. Is CpG density the link between epigenetic aging and lifespan? *Trends in Genetics* 36(10):725-727

Bertucci, E.M., Parrott, B.B. Characterization of the age-related DNA methylome and development of an epigenetic age predictor in medaka (*Oryzias latipes*). 2019. University of Alabama Birmingham Integrative Aging Symposium, Birmingham, AL, USA

Bertucci, E.M., Parrott, B.B. Characterization of the age-related DNA methylome in medaka (*Oryzias latipes*). 2019. Savannah River Ecology Laboratory Graduate Student Symposium, Aiken, SC, USA

Bertucci, E.M., Parrott, B.B. Effects of environmental stressors on aging trajectories in medaka. 2019. Odum School of Ecology Graduate Student Symposium, UGA, Athens, GA, USA

Parrott, B.B., Bertucci, E.M. Epigenetic aging clocks in ecology and evolution. 2019. *Trends in Ecology and Evolution* 34(9): 767-770

## **Support for SREL Environmental Missions on the SRS**

### **Funding Entity**

NNSA

### **Start Date and Funding Amount**

March 2019; \$285,355

### **SREL PI and Co-PIs**

Dr. Olin E. Rhodes, Jr. and P.J. Perea

### **Objectives**

SREL will assist the SRS NNSA mission by educating the public through community outreach activities that include organizing tours and exhibits featuring the local ecology and associated research; conducting environmental education workshops for teachers, students, and the general public, as well as for site personnel; development of a variety of environmental education materials for diverse audiences; increasing internet accessibility of information; distributing ecological information; presentation of data and reports on the local and regional environment; and assisting in educational efforts about the importance of environmental stewardship and National Environmental Research Park (NERP) programs at the SRS. Accomplishments relative to these tasks will be summarized in an annual report.

### **Summary of Program Activities**

The SREL Environmental Outreach Program utilizes information from SREL's ongoing research and long-term research efforts to provide training and service for SRS employees. The program also educates the public locally, regionally, and nationally about ecological research findings associated with on-site activities. NNSA has continued to provide critical funding that has facilitated SREL's ability to achieve the goal of maintaining informative outreach programs for SRS personnel and stakeholders, as well as programs that educate the public through outreach. These programs enhance an individual's understanding of environmental issues affecting the SRS and increase general ecological awareness. In fiscal year 2020, the COVID-19 pandemic greatly affected SREL Outreach's ability to deliver programs. Schools were closed starting in March 2020 in favor of virtual learning, much of the public were sent home under quarantine and stay-at-home orders, and most public events were canceled or rescheduled to reduce the spread of COVID-19. The SRS also closed public programs and non-essential employees teleworked at home. These conditions continued through the spring, summer and into the fall. While there has been some easing of restrictions and schools have returned to a combination of in-person and virtual learning, most schools are reluctant to bring in outside educators as they are struggling with many challenges during the pandemic.

In fiscal year 2020 and before the COVID-19 pandemic, SREL conducted engaging educational presentations to K-12 schools and adult audiences. These outreach efforts achieved DOE and NNSA goals of enhancing the public's knowledge and understanding of the ecological health of the SRS, and the importance of environmental stewardship and the NERP programs on the SRS. SREL also distributed educational resource materials to facilitate these goals. To address the challenges, the NNSA has continued to provide critical funding that has facilitated SREL's ability to achieve the goal of maintaining informative outreach programs for SRS personnel and stakeholders, as well as programs that educate the public through outreach. These programs enhance an individual's understanding of environmental issues affecting the SRS and increase general ecological awareness.

The Outreach Program was readily available to conduct Lunch and Learn presentations to site personnel, provided tours for DOE site interns, new SRS employees, and children of SRS personnel. SREL developed and distributed literature and developed displays on animals and plants native to the SRS and the surrounding communities, and produced materials on specific research programs. The SREL Outreach Program is designed to enhance SREL's overall mission of acquiring and communicating environmental knowledge and to highlight NNSA's and DOE's focus on environmental issues on the SRS. To accomplish these goals, education initiatives are used that include 1) Environmental Safety on the SRS, 2) On-site outreach to the general public and site personnel, and 3) Off-site outreach activities at schools, civic organizations, and community events. Issues as diverse as

wildlife safety in the field, wildlife population declines, potential responses of organisms to contamination, distribution of abundance of sensitive species, impacts of invasive species, wetland dynamics and remediation, water quality, and dispersal of organisms from radioactively or chemically contaminated sites are all important to on-site personnel and the general public.

### **Major Impact(s) of Program**

SREL's Outreach program has consistently communicated information about the environmental activities on the SRS based on ecological research conducted on the site by SREL scientists. Regardless of format — presentations, tours, or exhibits, the SREL Outreach program's numerous efforts continue to succeed in raising awareness through education of the rich ecological diversity of the SRS area and the region. The program continues to succeed in developing the general public's knowledge of and appreciation for the fauna and flora native to the area and in so doing develops an active interest in environmental stewardship, and an interest in protecting the integrity of the environment on the SRS and the communities that surround it.

### **Other Project Personnel**

Vicky Sutton-Jackson, Public Relations Coordinator – SREL

Sean Poppy, Outreach Coordinator – SREL

Amanda Hurst, Animal Caretaker – SREL

Judy Greene-McLeod, Research Professional – SREL

Holly DeVault, Outreach Coordinator – SREL

### **Products**

1. Conducted 12 scheduled tours; number of attendees – 273 (includes 8 SRS Public Tours, attendees – 188; 4 tours for on-site employees/visitors, attendees – 85)
2. Provided 2 Wildlife Safety talks; number of attendees – 3,000 (includes 2 talks to SRS employees at SRS Safety Expo, number of attendees – 3,000)
3. Presented 57 STEM classroom education programs for elementary and secondary students; number of attendees – 4,174
4. Presented 33 environmental programs for college, professional and adult audiences; number of attendees – 1,529
5. Provided 14 STEM exhibits at local and regional events; number of attendees – 13,536
6. Conducted 14 Ecologist for a Day programs (school field trips to SREL's Conference Center); number of attendees – 420
7. Provided 9 REMOP programs for the public; number of attendees – 267

**\*Total Outreach events: 142; total estimated attendance: 23,199**

## **Restoration of NNSA Related Streams: Pretreatment Assessment**

### **Funding Entity**

NNSA

### **Start Date and Funding Amount**

October 2019; \$213,645

### **PI and co-PIs**

Dr. Olin E. Rhodes Jr. and Dean E. Fletcher

### **Objectives**

Our overall goal is to provide a comprehensive assessment of the Upper Three Runs tributary that drains the former MOX construction site (here after referred to as tributary U8). Contaminants accumulating in stream sediments and biota are being assessed as well as impact on hydrologic, geomorphic, and biologic stream features. The potential of restoration or enhancement strategies that would improve and better protect the integrity of the system are being evaluated.

### **Summary of Research Activities**

Integration of our work on tributary U8 into additional SRS stream studies has expanded the geographic scope as well as incorporated additional critical expertise into our collaborative effort. The NNSA funded study, while providing a nucleus for the U8 work to be built around, has benefited by contaminant analyses that placed it into a broader spatial perspective with comparisons to other stressed SRS streams. Use of the same study taxa and environmental media along with identical laboratory and field protocols has allowed seamless comparison of data among these efforts. Overall, our contaminant assessments have included five streams in the Upper Three Runs basin along with the main stem of Upper Three Runs. The latter evaluates whether U8 is increasing contaminants in the main stem of Upper Three Runs. Geomorphic and hydrologic analyses of these streams have also been conducted in collaboration with the University of Kentucky and funded by the USDA Forest Service-SR in a project aimed at setting up a mitigation bank for SRS headwater streams. The restoration potential of U8 is being evaluated and a pre-treatment baseline being established.

The U8 channel is severely eroded and unstable and will continue to degrade until the excessive runoff from impervious surfaces is slowed. As additional land development occurs in the watershed, this disturbance will not only continue, but likely be worsened without appropriate management. Buffering the stream channel of excessive storm flows over short periods of time will be the first step of improving and protecting this tributary from effects of industrial operations and construction. We have been shifting efforts from environmental assessments to collect data critical for the design of remediation strategies. Collection of high-resolution discharge data will provide an improved understanding of pollutant loading and erosive flows that cause instability in receiving stream channels, as well as assessment of hybrid gray and green stormwater control measures to mitigate these influences. New collaborations were established to bring in expertise from the Institute for Resilient Infrastructure Systems in the University of Georgia's College of Engineering to make these calculations and model how different water retention scenarios will protect the integrity of the downstream stream channel. Future efforts will provide a design for the needed structures.

Locations were identified to place stream gaging stations in tributaries U8 and U6 in 2020. The latter site below outfall F-02 was added to our evaluations to determine whether remediation activities in the U8 tributary could be utilized to improve more than one Upper Three Runs tributary. A detailed evaluation of stormwater flows from these stations will provide the necessary assessment to allow calculation of the size of sedimentation basins required to sufficiently protect these streams from further structural damage. High resolution discharge data are being collected at culverts in U8 and U6 and will be used to assess the magnitude of stormwater runoff in the watersheds and to calibrate hydrologic models. At U8, an area-velocity sensor was installed on 3/10/2020 and has been recording flowrate at 5-minute intervals for 253 days, while a supplementary ultrasonic sensor was installed on 10/2/2020 and recorded depth for 47 days. There was constant measurable flow in the U8 culvert. The mean and median flowrates observed were 7.50 and 1.87 L/s (0.265 and 0.066 ft<sup>3</sup>/s), respectively. Over 20 storms have been captured

at U8, with a maximum flowrate of 3,131.3 L/s (110.6 ft<sup>3</sup>/s) occurring during remnants of Tropical Storm Sally on September 17. Mean monthly flows were lowest in August (2.23 L/s; 0.079 ft<sup>3</sup>/s) and highest in September (23.8 L/s; 0.840 ft<sup>3</sup>/s).

Area-velocity and ultrasonic depth sensors were installed at U6 on 3/11/2020 and have also continuously been collecting data at 5-minute intervals, for a total of 252 days (as of 11/17). Flow was present in the culvert on only 25% of monitored days and less than 10% of the monitoring period. The mean flowrate was 0.91 L/s (0.032 ft<sup>3</sup>/s) over the entire monitoring period. Mean and median flowrates were 9.97 L/s (0.352 ft<sup>3</sup>/s) and 0.64 m/s, respectively, when analyzing instances with measurable flow. Over 25 storm events were observed, with a maximum flow of 912 L/s (32.2 ft<sup>3</sup>/s) also occurring during remnants of Tropical Storm Sally on September 17. Mean monthly flows varied from a minimum of 0.03 L/s (0.001 ft<sup>3</sup>/s) in October to a maximum of 2.45 L/s (0.086 ft<sup>3</sup>/s) in September across all data points. When considering only times with measurable flow, mean monthly flows ranged from 1.13 L/s (0.040 ft<sup>3</sup>/s) in November to 22.71 L/s (0.802 ft<sup>3</sup>/s) in September. Data collection at these monitoring stations will continue in FY21. Model simulations will be used to estimate baseline hydrology in the U8 watershed and to design cost-effective stormwater control measures to capture runoff from the facility. Various stormwater control options will be evaluated and a mix of nature-based and conventional solutions will be designed to mitigate excess runoff from the former MOX facility construction site across a wide range of storm magnitudes and frequencies. These solutions will protect the water quality and geomorphic stability of receiving stream U8, while demonstrating techniques that can be transferred to other watersheds.

We are also further evaluating the impact of stream incision on riparian groundwater level. Depression of riparian ground water has the potential to have significant impacts to stream hydrology and floodplain geochemical processes. Monitoring equipment was ordered and sites selected to begin evaluating the relationship between stream incision and ground water level. Sites were selected in U8 in addition to tributary U36 that does not have a history of industrial disturbance as a reference stream. In addition to monitoring groundwater elevation at approximately 5-minute intervals, grab samples of water will be collected approximately monthly or during significant rain events for analysis of nitrogen concentrations and byproducts indicative of nitrate removal by denitrification. This expanded water quality analysis can improve understanding of how implementing stormwater controls can enhance downstream water quality.

We have continued monitoring impacts of excessive runoff on tributary U8 biota. Many aquatic insects have complex life cycles that involve an immature aquatic nymph or larval stage that emerges from the water as a flying adult. This emergence connects aquatic and terrestrial food webs and results in aquatic habitats subsidizing food resources to terrestrial organisms. Consequently, stream disturbances that impact stream macroinvertebrate communities have the potential of also impacting the surrounding terrestrial food webs. Our previous work established impacts to the aquatic biota. We are now assessing whether these impacts are influencing the number and taxonomic composition of aquatic insects emerging from tributary U8. Traps to capture insects emerging from streams were first designed and trapping initiated in FY19. Additional traps were constructed in early FY20 so that 15 traps were deployed in each tributary U8 and Tinker Creek reference tributary TC5. A trapping strategy was employed to assess the effects of strong storm events on aquatic insect emergence during the time following storms. Traps were deployed over a total of 31 days. Preliminary analysis indicates that over the trapping period, 48% of the reference stream traps contained insects as opposed to 31% of the U8 traps. Captured insects will be identified and counted in FY21.

Tetragnathid spiders construct webs horizontally above the water's surface and capture diverse taxa of smaller emerging insects. It has been shown that their diet is composed primarily of emerging insects. Thus, contaminant accumulation in tetragnathid spiders can be an indicator of contaminant flux from the wetland. Moreover, the physical condition of these spiders provides an indicator of the number of emerging insects and thus physical condition of the wetlands. In coordination with funded research efforts on the H-02 wetland system, we collected spiders from four H-02 wetland sites, Fire Pond

as a reference stream, a D Area wetland, and a basin in the headwaters of U8 to assess condition of and contaminant flux from these systems. Sample preparation will begin in FY21.

### **Conclusions**

1. U8 is severely degraded with impaired hydrology, channel form, substrate composition, and biological communities; impacts on stream macroinvertebrate communities and life histories have been observed.
2. Scouring by excessive flows has reduced organic matter content in the streams, influencing contaminant dynamics and potentially stream energetics.
3. Stream disturbance is reducing aquatic insect emergence, and thus aquatic to terrestrial food subsidies.
4. Evaluation of sediments throughout the U8 drainage indicated no concern of Cs-137 contamination.
5. Trace elements were scoured from the perennial stream, but upstream sources exist.
6. Highest concentrations of many trace elements were generally found upstream of the previous MOX construction site and associated with the area around the coal combustion waste basin and an older sedimentation basin.
7. Restoration efforts will be designed to reduce further redistribution of contaminants from this area.

### **Major Impact(s) of Research**

1. We are assessing potential physical and biological impacts of the site construction and other activities in the U8 drainage area and providing a comprehensive baseline of its present condition including contaminant distributions.
2. Extensive contaminant analyses combined with the physical characterization are helping establish that observed biological impairments are presently, primarily the result of excessive runoff rather than contamination.
3. A group of onsite and offsite collaborators are completing necessary evaluations to develop a successful restoration plan for tributary U8.
4. Overall, identification of specific stream impairments and establishment of baseline conditions will provide the foundation to restore the U8 system and subsequently monitor restoration effectiveness.

### **Other Project Personnel**

Paul Stankus, Research Professional - SREL  
Christina Fulghum, Research Technician - SREL  
Erin Spivey, Research Technician - SREL

### **External Collaborators**

Brian Bledsoe, Director of Institute for Resilient Infrastructure Systems, University of Georgia, College of Engineering  
Roderick Lammers, Post-doctorate Associate, University of Georgia, College of Engineering.  
Daniel Buhr, Doctorate Student, University of Georgia, College of Engineering  
Christopher Barton, Professor of Watershed Management, University of Kentucky, Department of Forestry, President, Green Forest Works  
James Fudge, SRNS  
Andy Horcher, USDA Forest Service

### **Products**

- Fletcher, D. E., A. H. Lindell, J. C. Seaman, P. T. Stankus, N. D. Fletcher, C. D. Barton, R. A. Biemiller, and J V. McArthur. 2019. Sediment and Biota Trace Element Distribution in Streams Disturbed by Upland Industrial Activity. *Environmental Toxicology and Chemistry* 38:115-131.
- Fletcher, D. E., B. E. Lindell, A. H. Lindell, P. T. Stankus, N. D. Fletcher, J V. McArthur, and J. C. Seaman. 2019. Basins, beaver ponds, and the storage and redistribution of trace elements in an industrially impacted coastal plain stream on the Savannah River Site, SC. *Environment International* 133: 105174.
- Fulghum, C.M., P.T. Stankus, Fletcher, D.E. 2019. A closer evaluation of contaminant distributions in sediments of a coastal plain stream impacted by industrial runoff. Annual Meeting of the Society of Environmental Toxicology and Chemistry, Toronto, ON, November 2019 [poster].

- Buhr, D., B. Bledsoe, R. Lammers, and D. Fletcher. 2020. An innovative field approach to inform modeling watershed-scale riparian nitrogen dynamics in incised stream networks. Annual Meeting of the Society of Freshwater Science. [poster].
- Fulghum, C.M., P.T. Stankus. Fletcher, D.E. 2020. A closer evaluation of contaminant distributions in sediments of a coastal plain stream impacted by industrial runoff. Annual Meeting of the Society of Freshwater Science. [poster].

## Assessing Environmental Health Using Invertebrate Models

### Funding Entity

NNSA

### Start Date and Funding Amount

October 2019; \$67,903

### PI and co-PIs

G. Dharmarajan

### Objectives

Metal contamination resulting from industrial use has the potential to impair water systems throughout the United States. Impacts to biota in these systems are often predicted using toxicological exposure models that do not fully capture complex interactions between environmental factors (e.g. temperature variability) and/or are limited to predicting bioavailability and uptake. The H-02 treatment wetland system on the Savannah River Site is designed to remove metals from waters discharged from the NNSA Tritium Processing Facility. Copper and zinc levels remain elevated in this system and understanding the dynamics of their bioaccumulation and trophic transfer remain a concern. Work described in this proposal will investigate how organismal traits affect copper absorption and trophic transfer under ecologically relevant conditions

### Summary of Research Activities

1. **Copper accumulation dynamics in insects** – Aquatic insects form a basal link in natural foodwebs, and can be important for moving contaminants between aquatic and terrestrial foodwebs. During their aquatic life stages insects (like mosquitoes) absorb contaminants (like copper) from the surrounding water. To test the relative efficacy with which insects can transfer metals from aquatic to terrestrial systems we exposed mosquito larvae to 5 levels of copper (0, 10, 30, 50 and 100 ppb) in replicated 300 ml microcosms. To quantify metal accumulation profiles we sampled larvae, pupae and pupal exuvia. Copper concentrations in these tissue were analyzed using ICP-MS.
2. **Trophic transfer of copper in aquatic systems** – Organisms in aquatic environments can be exposed to heavy metal contaminants through two distinct routes: direct absorption from surrounding contaminated water and dietary uptake. To discriminate the importance of these routes we established a simple two trophic-level laboratory food chain (i.e., mosquito larvae and dragonfly nymphs). Mosquitoes and dragonflies were maintained under standard insectary conditions (27°C; 80% humidity; 12:12 hour day-light cycle). To control for exposure route we used a unique experimental design with four treatments: (a) Dragonflies maintained in uncontaminated water and fed uncontaminated larvae (control); (b) Dragonflies maintained in uncontaminated water and fed larvae raised in 100 ppb Cu; (c) Dragonflies maintained in contaminated water (100 ppb Cu) and fed uncontaminated larvae; (d) Dragonflies maintained in contaminated water (100 ppb Cu) and fed larvae raised in 100 ppb Cu. Using this experimental design, we will be able to effectively quantify levels of metals in dragonfly tissue that are derived directly from the water vs. through the diet. At the end of experiment all dragonflies were sacrificed and pooled insect samples and water from each microcosm were analyzed using ICP-MS. Data for all experiments have been collected and currently are being analyzed.

### Conclusions

Our data show copper concentrations  $>600 \mu\text{g/L}$  reduced both development rate and the proportion of mosquitoes pupating ( $\chi^2$  (DF) = 194.989 (5);  $P < 0.001$ ). We also found significant main and interactive effects between mosquito life-history stage/tissue (material) and copper dose (Copper:  $\chi^2$  (DF) = 209.527 (2);  $P < 0.001$ ; Material:  $\chi^2$  (DF) = 130.117 (4);  $P < 0.001$ ; Copper  $\times$  Material:  $\chi^2$  (DF) = 78.508 (8);  $P < 0.001$ ). The highest level of copper contamination can be seen during the larval stages of development, which was expected due to rapid nutrient acquisition during this stage, with sequential decreases in copper burden in pupal and adult stages. However, not all copper is excreted into the exuvia of the mosquitoes and a substantial portion is carried into the adult stage. Interestingly, adult female mosquitoes had higher copper concentrations compared to adult male mosquitoes and female mosquitoes had a seemingly proportional copper accumulation respective to copper dose.

Data for the trophic transfer experiment are currently being analyzed and conclusions are not yet available.

**Major Impact(s) of Research**

1. This work also will improve our understanding of the impacts of heavy metals on invertebrate populations, and can further refine our understanding of the parameters that need to be assessed when using invertebrates as bioindicators of ecosystem health
2. The study will quantify the relative importance of insects in transferring copper from aquatic to terrestrial systems
3. The study will also help better understand the dynamics of trophic transfer of contaminants in natural systems.

**Other Project Personnel**

Erik Neff, MS student – SREL

Jennifer Dirks, Research technician – SREL

Sarah Ebert, Research technician – SREL

**External Collaborators**

None

**Products**

Neff, E, and G. Dharmarajan. In Review. Effects of copper exposure on mosquito fitness and vectorial capacity. *Environmental Pollution*

## **H-02 Constructed Wetlands Studies—Terrestrial Food Web Monitoring**

### **Funding Entity**

NNSA

### **Start Date and Funding Amount**

January 2020; \$80,000

### **PI and co-PIs**

Dr. O. E. Rhodes, Jr.; Dr. T. L. DeVault

### **Objectives**

The objective of this project is to develop and assess methods for determining the extent to which metals sequestered in the H-02 treatment wetlands are transferred into the surrounding terrestrial environment via aquatic to terrestrial food webs. Such information would allow for a more complete understanding of the potential impacts (or lack thereof) of Tritium Facility discharge effluent to the terrestrial environment surrounding Crouch Branch and Upper Three Runs to meet the regulatory compliance and DOE ecological stewardship goals.

### **Summary of Research Activities**

The H-02 treatment wetland was constructed as a green technology initiative to remove metals, especially copper (Cu) and zinc (Zn), in discharge waters from the NNSA Tritium Processing facility. Constructed wetlands have been widely employed to reduce nutrients and prevent eutrophication of surface waters. However, their application for treatment of metal contaminated wastewater is relatively novel and it is unclear how much of the sequestered metals are transferred through the aquatic food web into the surrounding terrestrial environment. Despite meeting regulatory compliance, a previous study showed that elevated levels of trace elements were becoming bio-available downstream, as seen in bottom dwelling dragonfly nymphs, showing the importance of assessing the extent of potential contamination to other species within and surrounding the wetlands.

We were delayed in starting field sampling until 15 June 2020 due to restrictions on field research at SRS caused by the COVID-19 pandemic. Our ongoing research involves capturing a subset of the insectivorous bird community in several locations across three seasons (Breeding, Fall, Winter) (N=180 birds total). Birds are euthanized and samples of blood, liver, feathers, and muscle are analyzed at the Savannah River Ecology Laboratory with an ICP-Mass spectrometer for a selection of metal contaminants, including Cu and Zn. We also quantify mercury (Hg) contamination in the samples using a mercury analyzer. Birds are captured both from the H-02 wetlands and a wetland downstream from H-02. Metals from bird tissues collected from both sites will be compared to bird samples obtained from a reference site with no known contamination. Metal contamination levels will also be compared across seasons.

### **Conclusions**

This study is ongoing and no conclusions are available at this time.

### **Major Impact(s) of Research**

1. Determine whether H-02 contaminants are being transferred to the terrestrial environment through the avian community.

### **Other Project Personnel**

Mary Chapman, Research Technician – SREL

Shayna Munoz, Research Technician – SREL

### **External Collaborators**

N/A

### **Products**

No publications, presentations, or reports have been prepared yet.

TASK 8. SREL will continue to serve as a regional resource for scientific expertise and environmental research. SREL staff scientists will continue to provide special technical assistance to other site contractors, area stakeholders, other researchers, and the public. SREL will also continue to collaborate with scientist from other institutions

In FY20, SREL faculty, staff, and students conducted a diversity of environmental research projects both on and off of the SRS in support of their mission to pursue collaborations and funding to serve as a regional source of scientific expertise and to provide technical assistance to other site contractors, stakeholders, other researchers, and the public. Due to both the specific technical expertise represented by research faculty and staff at SREL and the unique opportunities for scientific research represented on the Savannah River Site, SREL scientists are often sought out as potential collaborators by researchers across the globe. SREL staff served as collaborators on both funded and non-funded research involving environmental remediation, ecotoxicology and environmental stewardship and, as Principal Investigators or co-Investigators on funded research all over the United States and internationally. In addition, SREL faculty, staff and students served as hosts for over 138 researchers from other universities, federal and state agencies, and non-governmental organizations to discuss and conduct collaborative research and funding. Research, external funding, and requests for technical assistance conducted in support of this task are outlined below:

### **Collaborations and Externally Funded Research on the SRS**

#### ***Suitability of Confiscated Box Turtles for Release Back into the Wild***

##### **Funding Entity**

SREL

##### **Start Date and Funding Amount**

August 2019; NFP

##### **SREL Collaborators**

Tracey D. Tuberville, Kurt A. Buhlmann, and Olin E. Rhodes Jr.

##### **Objectives**

The overall goal of this research is to determine whether turtles confiscated from illegal wildlife trafficking trade can be successfully released back into the wild, using box turtles released onto the SRS as a case study.

##### **Summary of Research Activities**

North American turtles are increasingly being collected from the wild and exported to other countries through the illegal wildlife trade. Even when these exports are seized, confiscated turtles are often euthanized as there is limited capacity in captive facilities to absorb the growing number of animals seized. In August 2019, South Carolina Department of Natural Resources (SCDNR) seized over 200 box turtles and transferred them to SREL for temporary holding until their final disposition could be determined. Ultimately, SREL, SCDNR and U.S. Forest Service Savannah River forged a collaboration to release the turtles on the Savannah River Site and to conduct research and monitoring that would allow us to evaluate the success of the translocation and inform future similar efforts.

All turtles were permanently marked, subjected to visual health assessment, and had biological samples collected for future genetic analysis and pathogen screening. In late October 2019, turtles were placed in a temporary enclosure at the release site, where they were held until April 2020. Prior to release from the pen, radio-transmitters were placed on 40 adult turtles and turtles will be radio-tracked through the activity season, into winter dormancy, and until turtles emerge from dormancy in Spring 2021. Temperature dataloggers (ibuttons) were also attached to telemetered turtles to monitor winter dormancy behavior. As released turtles were lost from the study, resident box turtles encountered at the release site were added to the telemetry study. Resident turtles from across the SRS are also marked, measured, and undergo a physical exam that includes collection of genetic and health samples. A master's

student was recruited to lead the data collection effort and develop a thesis focused on movement, survival, and health of released and resident turtles based on radio-telemetered animals.

### **Conclusions**

This research has just begun, thus there are no conclusions at this time.

### **Major Impact(s) of Research**

1. Novel data on the fate of confiscated box turtles released back into the wild
2. Insight into the broader issue regarding the suitability of confiscated turtles for repatriation back to the wild and how they can contribute to the conservation of wild populations
3. A better understanding of the resident box turtle population on the SRS, which has received little attention despite long-term studies of aquatic turtles

### **Other Project Personnel**

Emma Browning, M.S. Student – UGA

Amelia Russell, M.S. Student – UGA

Pearson McGovern, M.S. Student – UGA

David Lee Haskins, PhD Student – UGA

M. Kyle Brown, M.S. Student – UGA

### **External Collaborators**

Will Dillman (SCDNR)

Andrew Grosse (SCDNR)

Charlie Davis (USFS-SR)

### **Products**

Browning, E.A., K.A. Buhlmann, J.W. Dillman, A.M. Grosse, C.E. Davis, and T.D. Tuberville.

Evaluating the suitability of confiscated eastern box turtles (*Terrapene carolina*) for release into the wild. Southeastern Partners in Amphibian and Reptile Conservation Annual Meeting, Nauvoo, AL. February 2020.

**Development of Innovative Biostimulation and Bioaugmentation Strategies for In-Situ Uranium Bioremediation of DOE Contaminated Ecosystems**

**Funding Entity**

DOE - Minority Serving Institutions Partnership Program (MSIPP)

**Start Date and Funding Amount**

SREL FY20/FY21; \$25K

**SREL Collaborators**

Drs. John C. Seaman

**Objectives**

This project focused on the SRS Steeds Pond/Tims Branch ecosystem in an effort to reduce solubility, bioavailability and toxicity of U and Ni co-contaminants. The current research addresses the following objectives: 1a) evaluate the efficacy of P sources (phytate, glycerol phosphate, phosphate rock) as biostimulants, in aerobic soil slurry microcosms; 1b) identify and isolate the microbial “first-responders” to U+phytate and Ni individually and in combination; 2) evaluate U/ Ni bioremediation via biostimulation, bioaugmentation, or a combination thereof using the “first-responder” bacterial and fungal isolates; 3) conduct genomics and proteomics studies on isolated strains; and 4) train a steady stream of under-represented students in the field of environmental biotechnology, genomics, proteomics, chemistry and environmental restoration.

**Summary of Research Activities**

This project is a continuation of ongoing collaboration between FAMU and SREL. Large-scale legacy of environmental contamination with U resulted from years of nuclear materials production in the U.S. Towards this end, we continue to obtain innovative strategies for remediation and restoration of uraniumiferous soils and sediments of the Savannah River Site (SRS). Remediation of such environments using conventional excavation-disposal or pump-and-treat approaches has been deemed unfeasible. Less expensive in-situ remediation, by applying chemical and/or microbial additives, can alter contaminant speciation, thus enhancing solid-phase partitioning to reduce migration, bioavailability and associated toxicity. Towards this end, we have successfully demonstrated the effectiveness of PO<sub>4</sub> compounds, such as phytate, glycerol phosphate and phosphate minerals (e.g., apatite), as biostimulants to stabilize heavy metals and radionuclides through sorption and/or the formation of secondary phosphate precipitates. However, detrimental impacts of P amendments in soils has also been demonstrated, in that nickel (Ni) became more labile relative to U. Therefore, in sites where both U and Ni occur as mixed contaminants, such as the SRS Steed Pond soils, further research is required. A supplemental technique, called bioaugmentation, which entails isolation of native U-bioremediative microbiota and evaluation of their efficacy as bioremediative amendments with or without additional P biostimulants, is being developed.

**Conclusion**

1. Demonstrated the effectiveness of PO<sub>4</sub> based amendments in immobilizing U in SRS soils.
2. Identified potential problems associated with the use of soluble PO<sub>4</sub> sources for U immobilization.
3. Identified additional SRS microbial isolates that are insensitive to U and Ni contamination, and developed a U biosensor.

**Major Impact of Research**

The current research has the potential to impact the long-term disposition of U contaminated areas within Steeds Pond/Tims Branch watershed on the SRS, and other U contaminated sites associated with DOE Facilities.

**Other Project Personnel**

C. Logan, Research Professional II, SREL

**External Collaborators**

Dr. Victor Ibeanusi, Florida A&M University

Dr. Ashvini Chauhan, Florida A&M University

Dr. Charles Jagoe, Florida A&M University

Dr. Dan Kaplan, SRNL

**Products**

Li, D., N. Shustova, C.R. Martin, K. Taylor-Pashow, J.C. Seaman, D.I. Kaplan, J.W. Amoroso, R. Chernikov. 2020. Anion-exchanged and quaternary ammonium functionalized MIL-101-Cr metal-organic framework (MOF) for ReO<sub>4</sub> sequestration from groundwater. *J. Environ. Radio.* <https://doi.org/10.1016/j.jenvrad.2020.106372>.

Dickson, J., N.A. Conroy, Y. Xie, B.A. Powell, J.C. Seaman, M.I. Boyanov, K.M. Kemner, and D.I. Kaplan. 2020. Surfactant-Modified Siliceous Zeolite Y for Per technetate Remediation. *Chemical Engineering Journal*, <https://doi.org/10.1016/j.cej.2020.126268>.

Kaplan, D.I., R. Smith, C. Parker, T. Cabrera, B. Ferguson, K.M. Kemner, M. Laird, C. Logan, J. Lott, D. Montgomery, L. Manglass, N. Martinez, J.C. Seaman, and B.A. Powell. 2020. Uranium Attenuated by a Wetland 50 Years After Release into a Stream. *ACS Earth and Space Chemistry*.

## Ecological Factors Affecting the Success of Rabies Elimination in the Southeastern US

### Funding Entity

USDA-APHIS

### Start Date and Funding Amount

December 2019; \$132,822

### SREL Collaborators

O. E. Rhodes, Jr., G. Dharmarajan and J. C. Beasley

### Objectives

(1) Quantify variance in raccoon and opossum densities among three major habitat types found commonly in the Southeastern US; (2) Evaluate the role of isolated wetlands surrounded by presumably inhospitable upland pine habitats in supporting raccoon and opossum populations; (3) Quantify realized levels of rabies vaccine bait uptake by raccoons in the presence of bait competition from opossums and other mesopredators and mammals in the Southeastern US; (4) Evaluate the relative densities of raccoons measured by camera and live traps in the Southeast US.

### Summary of Research Activities

Trapping for raccoons (*Procyon lotor*) and opossums (*Didelphis virginiana*) in 2020 was carried out at 24 sites across the SRS in each of four major habitat types: pine, riparian, isolated wetland and bottomland swamp. We trapped each of the 24 sites for ten days on two separate occasions. In the Spring, trapping was carried out from February-March and from May. A total of 52 unique raccoons and 31 unique opossums were captured during the spring 2020. For raccoons, the estimated densities (Mean  $\pm$  SE animals/km<sup>2</sup>) in the various habitat types for females were: Bottomland swamp (6.265  $\pm$  0.831), isolated wetlands (3.131  $\pm$  0.648), riparian hardwood (3.666  $\pm$  0.831), upland pine (4.003  $\pm$  0.718). Alternatively, for male raccoons estimated abundances (Mean  $\pm$  SE) in the various habitat types were: Bottomland swamp (11.027  $\pm$  0.869), isolated wetlands (6.580  $\pm$  0.593), riparian hardwood (6.506  $\pm$  0.879), upland pine (6.480  $\pm$  0.713). Estimated densities of opossums (Mean  $\pm$  SE animals/km<sup>2</sup>) were highest in sites designated as bottomland swamp (1.2  $\pm$  0.18) and riparian hardwood (D = 1.3/km<sup>2</sup>, SE = 0.20), whereas upland pine (0.72  $\pm$  0.14) and isolated wetland (0.56  $\pm$  0.12) had the lowest estimated densities. To better understand the spatial ecology of opossums, we fitted a total of 71 unique individuals with GPS transmitters. Analyses of the telemetry data revealed that opossum home ranges estimated by 99% LOCoH differed significantly between the sexes ( $\beta = 0.36$ , SE = 0.176, P = 0.04) with larger home ranges observed for males (Mean  $\pm$  SE = 115.9  $\pm$  104.0 ha) compared to females (76.7  $\pm$  75.0 ha). We also used placebo bait with the biomarker Rhodamine-B to quantify bait consumption rates by raccoons and opossums (by quantifying the proportion of live trapped animals that have a Rhodamine-B in their whiskers). Our analyses revealed that proportion of trapped animals marked by Rhodamine-B differed significantly between species (i.e., raccoons vs. opossums;  $\chi^2 = 6.58$ , P = 0.01) but not habitat type ( $\chi^2 = 4.12$ ; P = 0.25). Data from camera trapping revealed similar patterns of bait competition. Thus, while raccoons took the most bait overall, compared to other competitors, non-target bait competitors accounted for 9% of bait taken in bottomland hardwood and 7% of bait taken in upland pine. There was a significant negative effect of bait competitors on bait uptake by raccoons, with a 1% increase in bait taken by bait competitors leading to a 2% decrease in bait taken by raccoons.

### Conclusions

This study is ongoing and no conclusions are available at this time.

### Major Impact(s) of Research

The major impacts of this research will be to: (1) Obtain baseline ecological information for making disease management decisions regarding important rabies reservoir (raccoon) and nontarget (opossum) species in the Southeastern US; (2) Quantify levels of oral rabies vaccine (ORV) bait uptake by raccoons and levels of ORV bait competition due to other non-target species (e.g., opossums); (3) Additionally, this project will investigate and validate an index of raccoon density using passive methods of detection in an effort to reduce field personnel requirements during future program monitoring and planning efforts.

### Other Project Personnel

Jacob Hill, Postdoc - SREL  
David Bernasconi, MS student - SREL  
Wesley Dixon, MS student - SREL  
Lonnie Helton, MS student - SREL  
Karen Beatty, Research Technician - SREL  
Marianne Kelso, Research Technician - SREL  
Emily Masterton, Research Technician - SREL  
Anita Michalak, Research Technician - SREL  
Makala Knox, Research Technician - SREL  
Austin Rife, Research Technician - SREL  
Kaileigh Smith, Research Technician - SREL  
Tyler Walters, Research Technician - SREL

**External Collaborators**

Richard B. Chipman, USDA-APHIS  
Amy T. Gilbert, USDA-APHIS  
Amy J. Davis, USDA-APHIS

**Products**

Bernasconi, D. A., M. T. Hamilton, R. B. Chipman, A. T. Gilbert, J. C. Beasley, G. Dharmarajan, O. E. Rhodes, Jr. (2019) A multiyear analysis of the influence of landscape attributes on Virginia Opossum abundance. Joint Conference of the American Fisheries Society and The Wildlife Society. Sept. 29-Oct. 3, 2019. Reno, Nevada.

Dixon, W., R. Chipman, A. Davis, A. Gilbert, J. Beasley, G. Dharmarajan and Rhodes O. E., Jr. (2020) Interspecific Oral Rabies Vaccine Bait Competition in the Southeastern United States. The Wildlife Society 2020 Annual Conference. Sept. 27-Oct. 1, 2020

**Restoring Headwater Streams and Riparian Corridors at the Savannah River Site, SC: Part A- Mitigation Plan Proposal Supporting Documentation**

**Funding Entity**

USDA Forest Service-Savannah River

**Start Date and Funding Amount**

Project renewal date June 2017; \$27,042

**SREL Collaborators**

Dean E. Fletcher

**Objectives**

Our overall goal has been to provide assessments of legacy and current stream disturbances to enable Savannah River Site management organizations and regulatory oversight agencies to move forward with specific restoration/enhancement treatments and a monitoring plan for a stream restoration-mitigation project.

**Summary of Research Activities**

Stream restoration and enhancement provides opportunity to correct or improve previous alterations that have destroyed, diminished, or impaired the character and function of stream systems. The Savannah River Site (SRS) provides an ideal research opportunity for restoration of coastal plain streams. SRS stream disturbances span a temporal range from pre-SRS legacy impacts, through the early infrastructure development in the early 1950s, to more recent and current industrial activities. In a collaborative effort, a multiphase program was conducted to characterize SRS streams, identify risks of legacy and recent disturbances, and identify disturbed stream reaches with potential for restoration. Three levels of assessments were completed with each level providing an increased level of detail and scientific rigor. A Level I assessment involved a broad scale survey of potential stream disturbances and stream basin characterization. Level II assessments assessed the effects of stream alterations on physical stream condition in a subset of Phase I identified streams. Level III assessments further evaluated a selected subset of stream reaches by measuring additional hydrology, physicochemistry, biology, and geomorphology features. This comprehensive stream evaluation identified management options and is guiding prescriptions for potential restorative actions.

Our work has identified enhancement/restoration opportunities that span a broad range of complexity, cost, and risk. Current plans are being developed for some of the more cost-effective opportunities with lower levels of risk of environmental disturbance by the management activities. Experience gained by these restoration and enhancement efforts in less complex situations, may be applied to more costly systems in the future. The headwaters of Tinker Creek offer several suitable areas/reaches for restoration and enhancement that could be performed cost effectively, in a timely fashion and with overarching goals that could improve stream and riparian condition. Restoration opportunities in the Tinker Creek watersheds include ditch filling, in-stream habitat enhancement, and dam removal. Riparian enhancement would involve opening of the closed-canopy forest to release native cane in the understory. Use of fire to manage forest understory including cane stands will be evaluated. Prescriptions for these specific stream enhancements as well as required permit materials are being assembled.

**Conclusions**

1. Amount of industrial area in a drainage has a strong influence on instream geomorphology, chemistry, and macroinvertebrate communities; streams receiving excessive stormwater runoff are generally the most disturbed streams within our study systems.
2. Basin and valley characteristics interact with landscape disturbance to influence level of stream disturbance.
3. Enhancement of tributaries of Tinker Creek represent cost-effective options for establishment of a mitigation bank.

### **Major Impact(s) of Research**

1. We have identified effects of legacy and current disturbances on stream chemistry, hydrology, geomorphology, and biology on select SRS streams.
2. Through an effort with onsite and offsite collaborators, we are proposing restoration plans including post-treatment monitoring for four headwater streams while using a fifth as a reference system. These efforts may establish better management strategies for the riparian zones of sandhills streams.
3. A framework upon which a headwater stream mitigation bank can be built is being developed and restoration prospectus written.

### **Other Project Personnel**

Paul Stankus, Research Professional - SREL

Christina Fulghum, Research Technician - SREL

### **External Collaborators**

Christopher Barton - University of Kentucky

Andy Horcher - USDA Forest Service-SR

John Blake (retired) - USDA Forest Service-SR

### **Products**

Fletcher, D. E., A. H. Lindell, J. C. Seaman, P. T. Stankus, N. D. Fletcher, C. D. Barton, R. A. Biemiller, and J V. McArthur. 2019. Sediment and Biota Trace Element Distribution in Streams Disturbed by Upland Industrial Activity. *Environmental Toxicology and Chemistry* 38:115-131.

Fletcher, D. E., B. E. Lindell, A. H. Lindell, P. T. Stankus, N. D. Fletcher, J V. McArthur, and J. C. Seaman. 2019. Basins, beaver ponds, and the storage and redistribution of trace elements in an industrially impacted coastal plain stream on the Savannah River Site, SC. *Environment International* 133: 105174.

Prospectus, Savannah River Site Mitigation Bank, Savannah River Site, Aiken and Barnwell Counties, South Carolina. In preparation.

## **Spatial Ecology, Reproduction, and Social Structure of Invasive Wild Pigs**

### **Funding Entity**

SREL

### **Start Date and Funding Amount**

January 1, 2018; NFP

### **SREL Collaborators**

Dr. James C. Beasley

### **Objectives**

The goal of this study is to evaluate a broad suite of underlying factors contributing to the spatial ecology, reproductive dynamics, and social structure of wild pigs. Specific objectives include: 1) quantifying fine-scale movement behavior, home range structure, and resource selection of wild pigs, 2) quantifying biotic and abiotic factors contributing to reproductive rates of female wild pigs, 3) determining survival of neonate wild pigs, and 4) assessing the social structure of wild pigs using genetics and genomics and factors contributing to variance in reproductive rates among individuals.

### **Summary of Research Activities**

This research builds upon previous spatial and genetic data collected for wild pigs on the SRS to advance our understanding of the ecology of this invasive species. We are currently analyzing GPS spatial data from nearly 50 wild pigs to quantify the underlying factors contributing to the movement behavior and resource selection of wild pigs in the southeastern US. In addition, since 2019 we have been working with collaborators at USDA's NWRC to elucidate the social structure of wild pigs using SNP markers collected for several hundred individuals. Lastly, since 2018 we have been collecting reproductive data from female wild pigs as well as quantifying survival of neonate wild pigs. Analyses of all of these data are ongoing.

### **Conclusions**

This research is still in progress; there are no conclusions at this time.

### **Major Impact(s) of Research**

This research will provide some of the most comprehensive data to date on the social structure and reproductive ecology of wild pigs, as well as novel assessments of the underlying factors contributing to the movement ecology of this important invasive species. Data derived from this study will be used to better understand the ecology of wild pigs to improve the efficiency of management programs to mitigate damages caused by this destructive invasive species.

### **Other Project Personnel**

Ms. Sarah Chinn, PhD Student, SREL

Ms. Lindsay Clontz, MS student, SREL

Ms. Chelsea Titus, MS student, SREL

### **External Collaborators**

Dr. Tim Smyser (USDA-APHIS-WS-NWRC)

Dr. Stephen Webb (Noble Research Institute)

Dr. Kurt VerCauteren (USDA-APHIS-WS-NWRC)

Dr. Kim Pepin (USDA-APHIS-WS-NWRC)

### **Products**

Chinn, S., and Beasley, J. 2020. Parental investment strategies in a highly polytocous species: maternal attributes and resource availability modulate litter size and sex ratio. In Warnell Graduate Student Symposium. Athens, GA.

Chinn, S., and Beasley, J. 2020. Parental investment strategies in a highly polytocous species: maternal attributes and resource availability modulate litter size and sex ratio. In Society for Integrative and Comparative Biology Annual Meeting 2020. Austin, TX.

Chinn, S., and Beasley, J. 2020. Parental investment strategies in a highly polytocous species: maternal attributes and resource availability modulate litter size and sex ratio. In 2020 "Virtual" Wild Pig Conference.

## **Feral Swine Field Studies**

### **Funding Entity**

USDA-APHIS-Wildlife Services-National Wildlife Research Center

### **Start Date and Funding Amount**

September 5, 2019; \$200,000

### **SREL Collaborators**

Dr. James C. Beasley

### **Objectives**

The goal of this study is to evaluate a broad suite of attractants and food-based baits across multiple seasons and geographic locations to identify the most effective feral swine attractants for improving the efficiency and efficacy of wild pig control efforts under the National Feral Swine Program. This research will encompass three overarching objectives: 1) evaluate the performance (i.e. visitation rates) of a broad suite of lures/attractants/baits in attracting feral swine, and whether seasonal or geographic differences in performance exist, 2) determine differences in performance of lures/baits between male and female feral swine, and 3) provide recommendations for specific lure/bait combinations to maximize trapping efficacy.

### **Summary of Research Activities**

This research was initiated in spring 2020, with additional field sampling planned for fall 2020-summer 2021. During spring 2019 we conducted extensive field trials of >60 baits/attractants in South Carolina, resulting in a suite of baits/lures to be incorporated into further studies in 2020-2021.

### **Conclusions**

This research is still in progress; there are no conclusions at this time.

### **Major Impact(s) of Research**

1. This research will provide a robust evaluation of a broad suite of attractants for improving efficacy of trapping efforts to control wild pig populations.
2. Data derived from this study will be integrated into national-level management of wild pigs by the USDA to mitigate damages caused by this invasive species.

### **Other Project Personnel**

Ms. Caitlin Kupferman, Research Professional, SREL

Ms. Kayla Goodman, research technician, SREL

Ms. Alexa Murray, research technician, SREL

Mr. Stephen Graber, research technician, SREL

### **External Collaborators**

Dr. Kim Pepin (USDA-APHIS-WS-NWRC)

Dr. Nathan Snow (USDA-APHIS-WS-NWRC)

Dr. Kurt VerCauteren (USDA-APHIS-WS-NWRC)

Mr. Mike Lavelle (USDA-APHIS-WS-NWRC)

### **Products**

This research has just begun; there are no products at this time.

## **Effect of Carcass Type on Vertebrate Scavenging Dynamics**

### **Funding Entity**

SREL

### **Start Date and Funding Amount**

January 1, 2020, NFP

### **SREL Collaborators**

Dr. James C. Beasley, Dr. Travis DeVault, Dr. Olin Rhodes, Jr.

### **Objectives**

The objective of this study is to evaluate the influence of carcass type on the composition and efficiency of vertebrate scavenging communities in the southeastern U.S. Specifically, this study aims to quantify differences in vertebrate scavenging dynamics between predator and prey carrion, to understand how carrion type influences the flow of nutrients among decomposer and scavenging guilds, as well as the underlying factors contributing to carcass use and the occurrence of indirect cannibalism among vertebrate scavengers.

### **Summary of Research Activities**

This research was initiated in spring 2020 and is still ongoing at this time.

### **Conclusions**

This research is still in progress; there are no conclusions at this time.

### **Major Impact(s) of Research**

1. This research will produce novel insights into factors contributing to underlying differences in carcass use among vertebrate scavengers, as well as the mechanisms contributing to patterns of carrion use among vertebrate consumers.
2. This research will advance our understanding of the prevalence of indirect cannibalism among vertebrate scavengers.

### **Other Project Personnel**

Ms. Miranda Butler-Valverde, M.S. Student, Warnell School of Forestry and Natural Resources – UGA

### **External Collaborators**

NA

### **Products**

This research has just begun; there are no products at this time.

## **Stewardship Database of Amphibian and Reptile Distribution on the SRS**

### **Funding Entity**

USFS-Savannah River

### **Start Date and Funding Amount**

January 2019; \$32,000

### **SREL Collaborators**

Dr. Kurt A. Buhlmann

### **Objectives**

Our goal with this project is to compile and provide historical, as well as current occurrence records for amphibian and reptiles. Its use should help minimize land use conflicts, guide land management, and perhaps assist in habitat restoration and species recovery, where appropriate. It is intended that this project comprise a living, dynamic database, and new distribution records for target species are still being discovered and documented on a regular basis. It is modeled after the State Natural Heritage Program data system.

### **Summary of Research Activities**

Development of Stewardship database structure

Acquisition of data sources, 1951-present

As of this report, a total of 24,956 reptile records have been assigned lat/long points

### **Conclusions**

1. Amphibian and reptile distribution records for the 300 sq. mile Savannah River Site have been collected and documented over the period, 1951 to 2020.
2. As of this report, all records known for reptiles, primarily snakes, have been assigned latitude and longitude points.
3. For rare species, the database allows for organizations operating on-site to minimize land use conflicts.
4. Although all known datasets have been located, work will continue with ACP support in 2020-through 2021 to assign distribution points (lat/long) to remaining species distribution records.

### **Major Impact(s) of Research**

1. The results of this work will help with regulatory compliance, minimize land use conflicts and guide potential habitat restoration
2. The database serves as an ecological benchmark for future ecological studies as it provides historical context to ecological studies.

### **Other Project Personnel**

Linda Lee, Research Professional - SREL

Amanda McIntosh, Research Tech - SREL

### **External Collaborators**

Susan Blas, ACP-DOE

Charles E. Davis, U.S. Forest Service-Savannah River

Andy Horcher, U.S. Forest Service-Savannah River

### **Products**

Buhlmann, K.A. 2019. Identification of Sensitive Natural Resources on the SRS for Management, Restoration, Recovery, Stewardship, and Regulatory Compliance. Progress Report , 13 December 2019. Submitted to Mr. A. Horcher, U.S. Forest Service-SR, 17pp.

Buhlmann K.A. 2020. Identification of Sensitive Natural Resources on the SRS for Management, Restoration, Recovery, Stewardship, and Regulatory Compliance. Summary of Activities Completed, 30 September 2020. Submitted to Mr. A. Horcher, U.S. Forest Service-SR, 16pp.

## *Forest Health Metrics: DNA Methylation Age Predictors in Loblolly Pine*

### **Funding Entity**

USFS-Savannah River

### **Start Date and Funding Amount**

September 2019; \$19,098

### **SREL Collaborators**

Dr. Ben Parrott, Dr. Doug Aubrey

### **Objectives**

The overall goal of this research is to assess age-associated DNA methylation patterning and develop a DNA methylation based epigenetic clock for Loblolly pine.

Aging is amongst the most universal biological processes, as organisms generally experience declines in molecular and physiological function over time, ultimately resulting in increased risk of death.

DNA methylation is perhaps the best-studied epigenetic modification and when genomic patterning of the DNA methylome is resolved at the nucleotide level, the stereotypical nature of age-associated changes can be modeled to generate “epigenetic clocks”. Epigenetic clocks summarize the readout of age-associated hyper- and hypo-methylation from a selection of loci across the genome which are collectively capable of predicting chronological age with high accuracy. Interestingly, the degree to which an individual’s “epigenetic age” exceeds their true chronological age (also called epigenetic-to-chronological age discordance) is associated with life-history traits (e.g., growth rate, size at birth) in humans as well as risk for a number of age-associated disease including cardiovascular disease, cancer, and even all-cause mortality (e.g., risk of dying). Thus, there is exciting potential for measures of epigenetic-to-chronological age discordance to advance our understanding of trait development in other species.

DNA methylation has well-studied roles in plants including the repression of transposable elements, as well as regulating gene expression, organismal development, and adaptive responses to environmental cues. However, the dynamics of epigenetic aging have not been fully described nor have attendant epigenetic clocks been developed in the plant kingdom. The overarching goal of the proposed work is to discover broadly conserved epigenetic signatures of aging in economically important tree species and to develop epigenetic clocks capable of predicting chronological age that can be used in ecological, conservation, and management contexts. Findings will provide insights into the basic biology underlying fundamental aging processes and will also provide a framework for exploring the consequences of accelerated epigenetic aging on traits associated with stand productivity, wood quality, and ecosystem processes and attributes, including resilience.

### **Summary of Research Activities**

Tree tissue was sampled from a cohort of known-aged trees and subsequent DNA extraction, library preparation, and sequencing of the DNA methylome is now complete for 24 individuals. Reads were aligned to the Loblolly pine genome and 3,700 CpGs (cytosines immediately preceding guanines represent potential methylation sites) were covered across all individuals. Of these, only 5 CpGs acquire statistically significant age-dependent methylation patterns (all but one lose methylation with age). This is generally a weaker signal than observations in vertebrates. When coverage filtering requirements are relaxed to include CpGs that are covered in at least 2/3 samples in each age group (instead of 3/3), we increase the number of CpGs in our dataset to 27,000. Of these, the methylation status of ~400 CpGs have correlation coefficients  $> \pm 0.5$  with age, indicating considerable age-related DNA methylation pattern in the Loblolly pine.

However, the modeling techniques typically used to develop age predictors (i.e., elastic net regularized regression) do not tolerate missing data, so an imputation technique that borrows data from other samples and nearby CpGs to substitute for missing data is being developed. Although results are in preliminary stages, initial efforts suggest this method will yield a DNA methylation based estimate of chronological age which can be further related to commercially important traits (see above).

Also of note, plants are unique in that they also have high levels of CHG methylation (where H corresponds to either an A, T, or C). CHG is relatively low in animals, but is much more frequent in

plants. CHG methylation in our dataset appears variable (not all methylated or all unmethylated), which is interesting and means there is potential that CHG methylation might also correlate with age. To date, CHG methylation has not been examined in the context of age or age-related traits. Thus, the Parrott Lab is developing a bioinformatic pipeline to analyze this dataset as well.

#### **Conclusions**

1. Age-associated DNA methylation patterns are observed in the Loblolly pine.
2. Age-associated DNA methylation patterns, when assessed in the context of CpG methylation, are not as widespread as observed in animals.
3. Age-associated DNA methylation patterns, when modeled using penalized regression techniques, are likely capable of predicting chronological age.
4. Age-associated CHG methylation represents a promising path for investigation.

#### **Major Impact(s) of Research**

Establishing an epigenetic aging clock for trees will open the door for applications relevant to assessing commercial traits, forest productivity, plant disease, as well as the basic biology and evolution of life history traits.

#### **Other Project Personnel**

Emily Bertucci, PhD Student, Odum School of Ecology

#### **External Collaborators**

Dr. Andy Horcher, USDA USFS-SRS

Randall Sutton, USDA USFS-SRS

#### **Products**

Products are not yet available.

## **Environmental and Ecological Determinants of Epigenetic Aging**

### **Funding Entity**

National Science Foundation

### **Start Date and Funding Amount**

September 2020; \$853,295

### **SREL Collaborators**

Dr. Ben Parrott

### **Objectives**

Because of its near universal occurrence, understanding the causes and consequences of aging is central to broad biological, ecological, and evolutionary questions. Recent advances demonstrate a central role of the epigenome in mediating biological aging as it provides a molecular context for integrating both intrinsic and extrinsic forces. The purpose of this project is to discover fundamental mechanisms by which the environment directs epigenetic aging trajectories to ultimately impact organismal function. The project will utilize the tractable and ecologically relevant fish, Japanese medaka as a model system as much of their genome and physiology are broadly conserved across the animal kingdom. The study will support the training and mentoring of a postdoctoral researcher, two graduate students, and an undergraduate researcher, with recruiting efforts aimed at under-represented groups in the sciences. The project will develop a primer that introduces fundamental concepts in life history ecology and evolution, and will highlight variable life histories observed across the animal kingdom. The primer and project findings will be presented to students at area schools as part of an established outreach program. Together, this work will advance current understanding of how interactions between organisms and their environment affect biological aging and will provide training opportunities for the next generation of scientists.

### **Summary of Research Activities**

The work will focus on recently developed epigenetic aging clocks to advance an ontogenetic- and endocrine-based understanding of how ecological and environmental challenges shape biological aging and attendant variation in the timing of life history events (e.g., age at maturity). Epigenetic aging clocks are based on age-dependent DNA methylation patterning and summarize the readout of age-associated hyper- and hypo-methylation from a selection of loci across the genome which are collectively capable of predicting chronological age with high accuracy. Despite their unprecedented accuracy, the age indicated by epigenetic clocks can differ from an individual's actual age. This is referred to as epigenetic-to-chronological age discordance, and the magnitude and directionality of this discordance are associated with physiological function and life history traits. The origins of epigenetic-to-chronological age discordance are not resolved, and this project aims to investigate the role of ecological and environmental dynamics in driving this discordance. Along with other ecologically relevant stressors, fish will be exposed to ionizing radiation at the SREL's Low Dose Irradiation Facility. The project will also test the role of specific endocrine signaling pathways in mediating the influence of the environment on epigenetic aging. Interactions between organisms and their environments can result in different outcomes depending on when during life they occur. Age-dependent epigenetic patterning appears especially dynamic during early life and experiments will test if environment-organism interactions exert disproportionate influences during specific life stages. Collectively, project findings will provide a novel perspective regarding the proximal mechanisms by which environmental factors are translated into aging trajectories to affect organismal function and produce variable life histories.

### **Conclusions**

DNA methylation clocks have been developed and are highly accurate.

### **Major Impact(s) of Research**

1. Findings will advance our understanding by demonstrating cause-and-effect relationships between age-associated methylation patterning and ecological and environmental conditions.
2. Findings will reveal the extent to which biological aging mechanisms influence life history traits.

**Other Project Personnel**

Emily Bertucci, PhD Student, Odum School of Ecology

**External Collaborators**

Kristen Navara, UGA

**Products**

This project was recently initiated, and no products are available yet.

## **Impact of Long-term Chronic Exposure to Low Dose Ionizing Radiation on Organismal Health**

### **Funding Entity**

SREL

### **Start Date and Funding Amount**

NFP

### **SREL Collaborators**

Dr. Ben Parrott, Dr. Olin E. Rhodes

### **Objectives**

The overall goal of this research is to assess the effects of chronic low dose ionizing radiation over long periods of time on and the associated epigenome.

### **Summary of Research Activities**

Environmental stressors influence developmental and aging trajectories leading to either health or disease. Ionizing radiation (IR) represents perhaps the most universal ecological stressor. With background levels present since the dawn of evolution, highly conserved pathways act to repair DNA damage caused by UV and other sources of IR. This project aims to assess how long-term chronic exposures to low doses of IR impact organismal health through altering epigenetic and genomic processes in a model fish (*Oryzias latipes*).

Epigenetic mechanisms mediate genome-by-environment interactions, and due to recent advances in sequencing technologies, the molecular modifications underlying these interactions can be assessed at genomic scales. DNA methylation is the best characterized epigenetic modification with roles in regulating gene expression and promoting chromosomal stability, and alterations to DNA methylation patterning characterize specific malignancies and in some cases, predict cancer prognosis. We will resolve epigenetic responses and genome-wide changes to gene regulation across IR doses and across different dose lengths. Further, we will assess the influence of IR exposure on the transcriptome. In addition to identifying biomarkers of IR exposure, these findings are expected to provide fundamental insights into how IR affects genomic processes associated with disease in environmentally, occupationally, and ecologically relevant exposure contexts.

### **Conclusions**

Fish exposed to IR in the LoDIF system (ranging from 3-6 months, and 3 months recovery) have been collected and necropsied. Effects of IR exposure are observed on reproductive function and overall body condition. Global shifts in DNA methylation in response to ionizing radiation are not observed. RNA-seq analysis on hepatic tissue reveals an influence of IR exposure on gene expression patterns. Ongoing analyses are focused on identifying molecular pathways and markers that underlie organismal responses to IR.

### **Major Impact(s) of Research**

1. Establishing an IR model to assess impacts of chronic low-dose exposure. Once established, the model can be extended to examine impacts of combinatorial exposures.
2. The project will produce findings that are relevant to both wildlife as well as human health.

### **Other Project Personnel**

Emily Bertucci, PhD Student, Odum School of Ecology

Marilyn Mason, Technical Staff

### **External Collaborators**

N/A

### **Products**

Bertucci, E.M., Mason, M.W., Camus, A.C., Rhodes, O.E., Parrott, B.B. Chronic low dose irradiation alters the hepatic transcriptome, but not the global DNA methylome in medaka (*Oryzias latipes*). 2020. *Science of the Total Environment* 729:138680

Bertucci, E.M., Mason, M.W., Camus, A.C., Rhodes, O.E., Parrott, B.B. Effects of low dose irradiation on the global DNA methylome in medaka (*Oryzias latipes*). 2019. Annual meeting of the Society for Integrative and Comparative Biology, Tampa, FL

Topolski, C.R., M.W. Mason, E.M. Bertucci, O.E. Rhodes, Jr., and B.B. Parrott. Impacts of chronic exposure to gamma radiation on the DNA methylome using the medaka fish model. July 25, 2017. Savannah River Ecology Laboratory NSF REU symposium. Savannah River Ecology Laboratory, Aiken, SC, USA

Topolski, C.R., M.W. Mason, E.M. Bertucci, O.E. Rhodes, Jr., and B.B. Parrott. Impacts of chronic exposure to gamma radiation on the DNA methylome using the medaka fish model. July 27, 2017. University of South Carolina Summer Research Symposium; University of South Carolina, Columbia, SC, USA

Bertucci, E.M. and B.B. Parrott. Using small fish to understand environmental impacts of ionizing radiation. Poster presented at SREL's Touch An Animal Day. 2017.

## **Addressing Reproductive Dysfunction in an Environmental Model of Endocrine Disruption**

### **Funding Entity**

SREL

### **Start Date and Funding Amount**

NFP

### **SREL Collaborators**

Dr. Ben Parrott

### **Objectives**

The overall goal of this research is to determine how ecologically relevant thermal dynamics impact developmental responses to endocrine disrupting contaminant exposures.

### **Summary of Research Activities**

The etiology of many reproductive disorders is complex and likely involves interactions between an individual's genetics and external environmental factors, including exposures to anthropogenic stressors. Environmental contaminants that interfere with the native functioning of the endocrine system have been linked to reproductive abnormalities and population declines in wildlife and humans globally. Alligators from environments contaminated by endocrine disrupting compounds (EDCs) display disorders of the reproductive system including alterations in circulating sex hormone levels, a decreased robustness of sexually dimorphic gene expression, and morphological abnormalities of ovarian follicles. Investigations into a population of alligators inhabiting a contaminated system in Florida, Lake Apopka, have uncovered the roots for a subset of these abnormalities in altered estrogen signaling during embryonic development, including shifts in ovarian function and transcription that persist into later life stages.

Using a model in which juvenile alligators, collected as eggs from Lake Apopka and a reference site, were raised under identical laboratory conditions and challenged with either a vehicle control or a gonadotropin hormone (FSH) that stimulates ovarian function, we employed targeted gene expression analyses and a non-biased RNAseq-based method to uncover the depth and possible etiology of population-level differences in ovarian function associated with contaminant exposure. After identifying core transcriptional networks shared between both populations, including FSH-responsive genes involved in steroid hormone production, cell proliferation, and oocyte development, we uncovered a cohort of responsive genes unique to each site that are putatively linked to developmental contaminant exposure. Furthermore, in the non-challenged ovary, we identified a large proportion (~40%) of transcripts that differ by population. Collectively, these findings indicate a substantial role for developmental contaminant exposure in shaping future ovarian function. Given prior evidence in the alligator linking similar functional shifts to precocious estrogen signaling, next steps entail identifying cohorts of dysregulated genes at Apopka that are recapitulated in estrogen-exposed reference animals and functional pathways enriched in population-specific responses.

### **Conclusions**

To date, findings show widespread transcriptomic divergence between ovaries from a contaminated site and a nearby reference site. Further, we show that treating embryos from the reference site with estrogen recapitulates alterations observed in those animals from a contaminated site, suggesting that exposure to estrogenic contaminants during developmentally sensitive windows underlies reproductive abnormalities observed in alligators from Lake Apopka.

### **Major Impact(s) of Research**

1. Establish fundamental insights into how EDC exposure during development affects reproductive development and reproductive health
2. Provides a basic understanding of how variable environmental conditions interact with contaminant exposures in biological systems

### **Other Project Personnel**

Samantha Bock, PhD Student, SREL

Chris Smaga, MS Student, SREL

## External Collaborators

N/A

## Products

- Hale, M.D., Parrott, B.B. Precocious estrogen signals underlie altered ovarian function in a model of environmental health. 2020. *Environmental Health Perspectives* 128(11):117003
- Parrott, B.B., Hale, M.D. Precocious estrogen signaling during embryonic development underlies persistent alteration of ovarian transcriptional networks in an environmental model of endocrine disruption. 2019. Invited Talk. North American Society for Comparative Endocrinology Conference. Gainesville, FL, USA
- Parrott, B.B. Wildlife as models for understanding mechanisms of reproductive disorders. 2019. Invited Seminar. University of Georgia, Department of Cellular Biology Seminar Series, Athens, GA, USA
- Parrott, B.B. Adaptive and disruptive mechanisms underlying epigenome-by-environment interactions. 2019. Invited Seminar, University of South Carolina- Aiken, Department of Biology Seminar Series, Aiken, SC, USA
- Galligan, T.M., Hale, M.D., Bermudez, D., Cloy-McCloy, J.A., Guillette L.G., Parrott, B.B. 2019. Assessing impacts of precocious steroid exposure on thyroid physiology and gene expression patterns in the American alligator. *General and Comparative Endocrinology* 271(15):61-72
- Hale, M.D., Cloy-McCoy, J.A., Doheny, B.M., Galligan, T.M., Guillette, L.G., Parrott, B.B. 2019. Embryonic estrogen exposure recapitulates persistent ovarian transcriptional programs in a model of environmental endocrine disruption. *Biology of Reproduction* 100(1): 149-161
- Hale, M.D., McCoy, J., Galligan, T., Bangma, J., Nilsen, F., Doheny, B., Guillette, L.J., Parrott, B.B. 2018. Precocious estrogen signaling during sex determination leads to persistent alterations in ovarian function in an environmental model of endocrine disruption, the American Alligator. Poster Presentation. 8<sup>th</sup> International Symposium on Vertebrate Sex Determination (VSD); Kona, HI.
- Hale, M.D., Galligan, T., Guillette, L.J., Parrott, B.B. 2018. Linking historical exposures to modern-day signaling: dioxin and the American alligator. Oral presentation. 22<sup>nd</sup> Odum School of Ecology Graduate Student Symposium; Athens, GA.
- Hale, M.D., Cloy-McCoy, J.A., Doheny, B.M., Parrott, B.B. 2018. Reproductive Biology of Crocodilians, published in *Encyclopedia of Reproduction* (2<sup>nd</sup> edition)

## Costs of Incubation: Linking Incubation-Induced Alterations in Phenotype to Changes in Fitness

### **Funding Entity**

National Science Foundation

### **Start Date and Funding Amount**

October 2019; NFP

### **SREL Collaborator**

Robert Kennamer - SREL

### **Objectives**

Our overall goals have been to examine the importance of incubation temperature during early development, and to provide a better understanding of how reproductive tradeoffs made by females influence their fitness. In one recent part of the project, we collected eggs from SRS wood duck nests and artificially incubated the eggs at different temperatures known to influence phenotype. Following hatching, the ducklings were monitored for feeding behaviors, consumed food amounts, and body condition. Larger ducklings from higher temperature incubated eggs acquired more food even though there was no difference in feeding behaviors.

### **Summary of Research Activities**

In FY20, manuscripts were published.

### **Conclusions**

1. Our studies have determined how the early thermal and social environments interact to influence offspring behavior/performance and suggested that these factors may play an important role in shaping offspring metrics that are critical for survival.
2. Our studies have documented that significant variability in egg temperatures exists within incubated wood duck clutches, enough so that duckling phenotypes, including important survival-related traits, can be impacted.

### **Major Impacts of Research**

1. Illustrates the importance of incubation as related to offspring quality/performance.

### **Other Project Personnel**

John Hallagan, Research Technician - Virginia Tech

Sydney Hope, PhD student - Virginia Tech

A. Grimaudo, Masters student – Virginia Tech

### **External Collaborators**

Dr. Bill Hopkins - Virginia Tech University

Dr. Sarah DuRant – University of Arkansas

### **Products**

Grimaudo, A., S.F. Hope, S.E. DuRant, R.A. Kennamer, J.J. Halagan, and

W.A. Hopkins. 2020. Ambient temperature and female body condition are related to night incubation behavior in wood ducks (*Aix sponsa*). *Journal of Avian Biology*. doi: 10.1111/jav.02379.

Hope, S.F., R.A. Kennamer, A. Grimaudo, J.J. Halagan, and W.A. Hopkins. 2020. Incubation temperature affects duckling body size and food acquisition despite no effect on associated feeding behaviors. *Integrative Organismal Biology* 2: 1-19. doi: 10.1093/iob/obaa003.

Hope, S.F., S.E. DuRant, F. Angelier, J.J. Halagan, I.T. Moore, C. Parenteau, R.A. Kennamer, and W.A. Hopkins. 2020. Prolactin is related to incubation constancy and egg temperature following a disturbance in a precocial bird. *General and Comparative Endocrinology* 295, doi: 10.1016/j.ygcen.2020.113489.

Hepp, G.R., R.A. Gitzen, and R.A. Kennamer. 2020. Relative importance of vital rates to population dynamics of Wood Ducks. *Journal of Wildlife Management* 84: 320-330. doi: 10.1002/jwmg.21792.

## **Canopy Complexity, Physiological Function, and Ecosystem Resilience of Longleaf Pine**

### **Funding Entity**

Tall Timbers Research Inc.

### **Start Date and Funding Amount**

08/01/2018; \$23,188

### **SREL Collaborators**

D. Aubrey

### **Objectives**

The goal of this project is to better understand how canopy architectural complexity of old-growth longleaf pine trees may influence physiological function and, ultimately, ecosystem resilience. Briefly, we hypothesize that individual with complex canopies may maintain positive water relations during periods of moderate drought because of lower branches with foliage that maintain higher water potentials.

### **Summary of Research Activities**

We instrumented a series of old-growth longleaf pine trees representing complex and simple canopy architecture in spring 2019 and measured physiological processes throughout the spring of 2020. Measurements are complete. The student's MS thesis and resulting publications are planned for FY21.

### **Conclusions**

1. Lower branches exhibited higher water potential than upper branches within the same continuous canopies; however, water potential was higher in discrete canopies compared to continuous canopies measured at the same height.
2. Photosynthetic assimilation in discrete canopies was higher than continuous canopies at the same height, and there were no differences between upper and lower continuous canopy positions.
3. Whole-tree water use was higher in continuous than discrete canopies, with the majority of water use occurring in upper portions of continuous canopies.

### **Major Impact(s) of Research**

Our data suggest lower strata of complex canopies are less water stressed than upper portions of continuous and discrete canopies; however, upper portions of both canopy types maintain similar or higher photosynthetic rates than lower portions of continuous canopies.

### **Other Project Personnel**

Laura Fowler, MS Student, Warnell School of Forestry and Natural Resources, UGA

### **External Collaborators**

J.K. Hiers, Tall Timbers Research Station

### **Products**

- Fowler, E., K. Hiers, J. O'Brien, D. Johnson, and D.P. Aubrey. 2019. Does canopy complexity influence physiological function and contribute to ecosystem resilience of old-growth longleaf pine? Warnell Graduate Student Symposium. Athens, GA.
- Fowler, E., J.K. Hiers, J.J. O'Brien, E.L. Loudermilk, S. Pokswinski, E. Rowell, and D.P. Aubrey. 2019. Does canopy complexity promote resilience in old-growth longleaf pine forests? Ecological Society of America. Louisville, KY.
- Fowler, E., K. Hiers, J. O'Brien, S. Pokswinski, E. Rowell, and D.P. Aubrey. 2020. Does canopy complexity influence physiological function and contribute to ecosystem resilience of old-growth longleaf pine (*Pinus palustris*)? Warnell Graduate Student Symposium. Athens, GA.

## Comparative Hydrologic Budgets and Water Use Efficiencies of Developing Bioenergy Plantations

### Funding Entity

USDA National Institute of Food and Agriculture

### Start Date and Funding Amount

09/01/2019; \$498,663

### SREL Collaborators

D. Aubrey

### Objectives

Our overarching goal is to improve our understanding of how the major outputs of the forest hydrologic cycle change through stand development and manipulate those dynamics through silvicultural approaches to increase water use efficiency (WUE).

### Summary of Research Activities

We will construct hydrologic budgets by quantifying the hydrologic inputs (precipitation and interception) and outputs ( $E_t$  and  $E_s$ ) in experimental plots of the most promising alternative woody bioenergy crop species for production in the southeastern US (i.e., coppiced sweetgum and *Eucalyptus*) and compare them with the current management system for this region (i.e., loblolly pine). In doing so, we will gain the most comprehensive species comparisons of water use and WUE to date that consider dynamic changes in  $E_s$  and  $E_t$  that occur as forest stands transition through stand development from the point of establishment when leaf area is low and the soil surface is bare to the point of canopy closure when leaf area has maximized and soil surfaces contain years of litter inputs that impede  $E_s$ . In addition to comparing species, we will compare silvicultural approaches that impose drastically different densities (i.e., coppice vs. standard row plantings of sweetgum; 6,364 seedlings ha<sup>-1</sup> and 1,336 seedlings ha<sup>-1</sup>, respectively). Different densities will result in different rates of canopy leaf area development and, therefore, different dynamics in hydrologic budgets. We will also perform a series of manipulative experiments to gain a mechanistic understanding of how litter type, litter depth, shading, and boundary layer mixing influence  $E_s$ . We will also measure fine- and coarse-root dynamics among species and silvicultural approaches to calculate WUE, not only with respect to aboveground production and bioenergy production, but also with respect to belowground carbon sequestration. Our proposed research will provide rigorous data accounting for dynamic shifts in key hydrologic processes that occur through stand development and will demonstrate silvicultural approaches to increase WUE, thereby informing decisions on how to manage bioenergy production systems.

### Conclusions

This research has just begun and was postponed a year due to the pandemic, thus there are no conclusions at this time.

### Major Impact(s) of Research

By taking a systems approach to quantifying evapotranspiration and its components (soil evaporation and transpiration) in woody feedstock (loblolly pine, American sweetgum, and *Eucalyptus*) across entire harvest rotations, we will capture dynamic shifts occurring through stand development and provide the most comprehensive assessment of WUE to date. We hypothesize that inherent differences in leaf habit and area among species, coupled with silvicultural options related to density and resource availability, provide opportunity to reduce soil evaporation during early stand development, thereby increasing rotation-length WUE and reducing the overall ecological footprint of woody bioenergy production systems. Our empirical approach, assessing rotation-length hydrologic budgets and generating integrated measures of WUE using a systems framework, will provide data that can be used directly by landowners in the Southeast US as a decision support tool for selecting woody bioenergy crop species and silvicultural approaches. Our conceptual framework, which relies broadly on functional traits of potential woody bioenergy crop species, formulates a hypothesis that will be tested with our empirical data and thus, generalized patterns, processes, and controls can be transferrable to other forestry and agriculture production systems. This project addresses the urgent need for quantitative information regarding trade-offs between maximizing forest production and maintaining sustainable water yields.

**Other Project Personnel**

C.R. Jackson (CoPI), Warnell School of Forestry and Natural Resources, UGA

Fabio Toledo, Research Professional, SREL, UGA

Tyler McIntosh, Research Technician, SREL, UGA

**External Collaborators**

None

**Products**

No publications, presentations, or reports have been prepared yet.

## **Managing Forests for Ecological Services and Environmental Sustainability**

### **Funding Entity**

USDA Forest Service

### **Start Date and Funding Amount**

05/13/2019; \$479,480

### **SREL Collaborators**

D. Aubrey (PI)

### **Objectives**

Our overarching goal is to improve our understanding of how carbon inputs to soil influence soil organic carbon (SOC) accrual and storage dynamics through stand development and across multiple harvest rotations, thereby demonstrating how manipulating inputs through silvicultural approaches can influence the magnitude and timing of SOC equilibrium.

### **Summary of Research Activities**

We developed a conceptual model, based on carbon inputs to soil and their decomposition rates, and parameterized it with empirical data to test the hypothesis that changing the silvicultural approach to increase carbon inputs to soil can result in increased equilibria of SOC inputs, and ultimately, increased SOC accrual and storage. To challenge/validate our model, we will establish plots of intensively managed loblolly pine and sweetgum and construct belowground carbon budgets that account for all biomass inputs to soil, decay rates of those inputs, soil CO<sub>2</sub> efflux, and subsequent changes in SOC across multiple rotations.

### **Conclusions**

This research has just begun and was postponed a year due to the pandemic, thus there are no conclusions at this time.

### **Major Impact(s) of Research**

Our empirical approach, assessing biomass production and carbon inputs to soil through early stand development and across multiple harvest rotations, will provide data that can be used directly by landowners in the Southeast US as a decision support tool for selecting production forest species and silvicultural approaches that simultaneously maximize productivity, increase atmospheric CO<sub>2</sub> sequestration, improve soil health, and enhance ecosystem resilience. Our conceptual framework, which relies broadly on silvicultural decisions and the functional trait of resprouting, formulates a hypothesis that will be tested with our empirical data and thus, generalized patterns, processes, and controls can be broadly transferrable to other production forestry systems nationally and globally.

### **Other Project Personnel**

Fabio Toledo, Research Professional, SREL, UGA

Tyler McIntosh, Research Technician, SREL, UGA

### **External Collaborators**

None

### **Products**

No publications, presentations, or reports have been prepared yet.

## **MWMF Forest Growth**

### **Funding Entity**

USDA Forest Service

### **Start Date and Funding Amount**

05/20/2020; \$290,000

### **SREL Collaborators**

D. Aubrey (PI)

### **Objectives**

The main objective of this work is to: (1) quantify the potential increase in transpiration that may occur through fertilization of a closed canopy loblolly pine forest; (2) quantify the potential increase in soil evaporation that may occur from raking pine straw; and (3) determine the relative importance of leaf litter accumulation versus shading on soil evaporation under a closed canopy.

### **Summary of Research Activities**

We purchased instrumentation, materials, and supplies and also selected sites, delineated plots, and collected baseline measurements to describe plot structure and identify potential measurement trees for future work.

### **Conclusions**

This funding was primarily to purchase equipment and materials for future work, thus there are no conclusions.

### **Major Impact(s) of Research**

We predict that our results will demonstrate the potential for expedited phytoremediation of contaminated water through improvement/intensification of forest management.

### **Other Project Personnel**

Fabio Toledo, Research Professional, SREL, UGA

Tyler McIntosh, Research Technician, SREL, UGA

### **External Collaborators**

None

### **Products**

No publications, presentations, or reports have been prepared yet.

**Characterizing Multiscale Feedbacks Between Forest Structure, Fire Behavior and Effects: Integrating Measurements and Mechanistic Modeling for Improved Understanding of Pattern and Process**

**Funding Entity**

US Department of Defense

**Start Date and Funding Amount**

01/23/2020; \$329,320

**SREL Collaborators**

D. Aubrey (PI)

**Objectives**

The ultimate goal of this research is to provide a mechanistic understanding of surface fire behavior to increase managers' ability to manipulate fire behavior during prescribed fire operations to meet ecological objectives in an effective, efficient, and safe manner. The specific objective for SREL is to characterize dose-dependent fire effects of vegetative thermal environment on plant tissues and represent this characterization in a new modeling tool that predicts fire effects based on spatially explicit outputs of the FIRETEC model.

**Summary of Research Activities**

Preliminary measurements have been made in the field and preliminary experiments have been begun in the laboratory.

**Conclusions**

This research has just begun and was postponed a year due to the pandemic, thus there are no conclusions at this time.

**Major Impact(s) of Research**

The results of this research should aid in the development of spatially explicit management tools that mechanistically link vegetation structure, fuels, fire behavior and fire effects, which will result in novel knowledge and tools that will enhance fire and fuel managers' ability to develop effective, efficient, and safe fire management strategies.

**Other Project Personnel**

Ream Thiomas, MS Student, Warnell School of Forestry and Natural Resources, UGA

Jennifer McDaniel, PhD Student, Warnell School of Forestry and Natural Resources, UGA

**External Collaborators**

C. Hoffman, Colorado State University

J.J. O'Brien, USDA Forest Service-Southern Research Station

J.K. Hiers & M. Varner, Tall Timbers Research Station

R. Lynn, Los Alamos National Lab

**Products**

Thomas, R., J. O'Brien, L. Loudermilk, and D.P. Aubrey. 2020. Energy dose thresholds for causing tree stem tissue mortality for certain south eastern functional groups. Warnell Graduate Student Symposium. Athens, GA. 2/14/2020

McDaniel, J. and D.P. Aubrey. 2020. Integrating tissue-specific mortality thresholds to predict fire-caused tree mortality. Warnell Graduate Student Symposium. Athens, GA. 2/14/2020

**Characterizing Dose-dependent Fire Effects of Vegetative Thermal Environment on Tree Stems**

**Funding Entity**

USDA Forest Service

**Start Date and Funding Amount**

08/01/2020; \$22,280

**SREL Collaborators**

D. Aubrey (PI)

**Objectives**

The objective of this project is to characterize dose-dependent fire effects of vegetative thermal environment on tree stems.

**Summary of Research Activities**

Preliminary dose-dependent mortality experiments have been performed, but much of the work was delayed to due to pandemic.

**Conclusions**

This research has just begun and was postponed a year due to the pandemic, thus there are no conclusions at this time.

**Major Impact(s) of Research**

Results from this study will be used to develop dose-dependent relationships between energy transfer and tree stem, which will be integrated into models of fire behavior to predict mortality.

**Other Project Personnel**

Ream Thomas, MS Student, Warnell School of Forestry and Natural Resources, UGA

**External Collaborators**

J.J. O'Brien USDA Forest Service-Southern Research Station.

**Products**

No publications, presentations, or reports have been prepared yet.

## **Spatial and Temporal Patterns and Ecological Implications of Leaf Litter Dispersal**

### **Funding Entity**

The Jones Center at Ichauway

### **Start Date and Funding Amount**

08/01/2020; \$24,024

### **SREL Collaborators**

D. Aubrey (PI)

### **Objectives**

The goal of this project is to analyze an existing long-term dataset of leaf litter dynamics in longleaf pine forests, explore the spatial and temporal patterns of that dataset, and use the dataset to parameterize a model predicting spatial patterns of leaf litter dispersal from stem mapping.

### **Summary of Research Activities**

Preliminary analysis of leaf litter dataset has begun. We have also delineated plots for additional research related to the ecology of fuels.

### **Conclusions**

This research has just begun, thus there are no conclusions at this time.

### **Major Impact(s) of Research**

Results from this study will provide information regarding forest structure, fuel dynamics, fire behavior, and seedling regeneration in longleaf pine stands.

### **Other Project Personnel**

Suzanne Henderson, MS Student, Warnell School of Forestry and Natural Resources, UGA

### **External Collaborators**

J. Cannon, Assistant Scientist, The Jones Center at Ichauway

### **Products**

No publications, presentations, or reports have been prepared yet.

## **Managing Forest Ecosystem Processes through Physiology**

### **Funding Entity**

USDA National Institute of Food and Agriculture McIntire Stennis

### **Start Date and Funding Amount**

08/01/2020; \$350,561

### **SREL Collaborators**

D. Aubrey (PI)

### **Objectives**

The overarching goal of our proposed research is to improve our understanding of how forest management decisions influence tree physiology, ecosystem processes, and ecosystem services. Our specific objectives are to: (1) develop nuanced conceptual models of forest water and carbon cycles and parameterize them with empirical data; (2) apply model results to identify sensitive processes of forest water and carbon cycles that require empirical investigation; and (3) challenge/validate conceptual models with empirical data collected in a high density sweetgum (*Liquidambar styraciflua*) coppice system. Though we focus our research on regionally appropriate tree species, the fundamental processes we explore can be applied to forest systems across the globe.

### **Summary of Research Activities**

We have delineated research plots for seedling planting in early 2021. Initial draft conceptual models have been developed.

### **Conclusions**

This research has just begun, thus there are no conclusions at this time.

### **Major Impact(s) of Research**

This project addresses McIntire-Stennis Forestry Research Topics 1) Reforestation and management of land for the production of crops of timber and other related products of the forest. It also addresses two high priority issues identified in the current McIntire-Stennis strategic plan: 1) science of integration and 5) technological advancements, productivity, and forest applications. In addition to directly addressing a specific Forestry Research Topic and the “Ecosystem Service” Priority Area, our project will also strengthen the McIntire-Stennis Cooperative Forestry Research Program 2017 Strategic Plan critical goal to “create the future generation of forestry educators, scientists, and practitioners through graduate education” by training multiple graduate students in forest ecology and tree physiology at the highest levels through direct involvement in research activities and through the PI’s graduate level courses on these topics (i.e., Advanced Forest Ecology and Advanced Tree Physiology).

### **Other Project Personnel**

Fabio Toledo, Research Professional, SREL, UGA

Tyler McIntosh, Research Technician, SREL, UGA

### **External Collaborators**

D. Johnson (co-PI), Warnell School of Forestry and Natural Resources, UGA

### **Products**

No publications, presentations, or reports have been prepared yet.

**Capture-Mark-Recapture Studies of Large Mouth Bass on Par Pond Reservoir**

**Funding Entity**

DOE-EM Support to SREL

**Start Date and Funding Amount**

October 2017; \$30,000

**SREL Collaborators**

Dr. Olin E. Rhodes, Jr. and Matt Hamilton

**Objective(s)**

To estimate the population size and characterize movement patterns of largemouth bass, *Micropterus salmoides*, in relation to the distribution of contaminants on Par Pond reservoir.

**Summary of Research Activities**

We have individually marked >1400 largemouth bass from Par Pond with a RFID (Radio-frequency identification) PIT (Passive Integrated Transponder) tag. With each capture, morphometric data (e.g., length, weight, sex), GPS coordinates, and physical condition (i.e., health status) are recorded before each individual is released at the point of capture. This initial capture effort is ongoing and will provide baseline data for characterizing movement patterns and estimating largemouth bass abundance on Par Pond.

**Conclusions**

This research is in the initial stages of data collection.

**Major Impact(s) of Research**

This research will provide insight into the seasonal distribution and the potential for contaminant exposure and transport of largemouth bass on a man-made reservoir.

**Other Project Personnel**

Chris McBride, Assistant Director – SREL

Megan Winzeler, Project Coordinator II – SREL

Brian Morton, Property Management Coordinator – SREL

Austin Coleman, MS Student – UGA

**External Collaborators**

NA

**Products**

No publications, presentations, or reports have yet been prepared.

## **Testing a Novel Bait for Large-scale Control of Wild Pigs**

### **Funding Entity**

USDA-APHIS – Wildlife Services – National Wildlife Research Center

### **Start Date and Funding Amount**

September 2018; \$338,960

### **SREL Collaborators**

Dr. James C. Beasley, Lindsay Clontz, and Allison Rakowski

### **Objectives**

The objectives of this study are 1) to assess the efficacy of Kaput® feral hog bait for controlling wild pigs and the behavioral and physiological impacts to wild pigs following consumption, 2) quantify the accessibility of feeders designed for use with Kaput® feral hog bait by pigs and non-target species, 3) determine the impacts of Kaput® feral hog bait on non-target species, and 4) determine warfarin levels in tissues of wild pigs following consumption of Kaput® feral hog bait.

### **Summary of Research Activities**

During fall 2018 through spring 2019 we conducted pen trials where we quantified the response of pigs fed Kaput® feral hog bait. Following the pen trials, we captured, tagged, and attached radio collars to wild pigs, raccoons, opossums, and mice within select areas of the SRS. During summer 2019 we deployed pig-specific feeders containing corn, followed by Kaput® feral hog bait, to determine the accessibility of the bait to both wild pigs and non-target species. These trials are currently ongoing through fall 2019.

### **Conclusions**

This research is still in progress; there are no conclusions at this time.

### **Major Impact(s) of Research**

1. This research will provide the first independent evaluation of Kaput® feral hog bait for use in controlling invasive wild pigs.
2. Data derived from this study will be integrated into national-level management of wild pigs by the USDA to mitigate damages caused by this invasive species.
3. Results from this study also will be used by individual states to further develop management plans for controlling invasive wild pigs.

### **Other Project Personnel**

Ms. Amy Hilger, Research Technician - SREL

Ms. Sara Cheatham, Research Technician - SREL

Mr. Lexington Belyeu, Research Technician - SREL

Mr. Chad Argabright, Research Technician - SREL

### **External Collaborators**

Dr. Kim Pepin (USDA-APHIS-WS-NWRC)

Dr. Nathan Snow (USDA-APHIS-WS-NWRC)

Dr. Kurt VerCauteren (USDA-APHIS-WS-NWRC)

### **Products**

Beasley, JC. Evaluation of Kaput for use in wild pigs. 2019. National Wild Pig Task Force Meeting, Starkville, MS (oral presentation)

Beasley, JC. Evaluation of Kaput for use in wild pigs. 2019. Georgia Wildlife Services Annual Meeting, Athens, GA (oral presentation)

## **Collaborations and Externally Funded Research Non - SRS**

### **Molecular Diet Analysis of Coyote Scat through Implementation of DNA Metabarcoding**

#### **Funding Entity**

SCDNR

#### **Start Date and Funding Amount**

July 2019; \$80,059

#### **SREL Collaborators**

Dr. Stacey L. Lance

#### **Objectives**

The overall goal of this research is to use DNA metabarcoding to determine the diet of coyotes in three ecoregions of South Carolina. An emphasis is placed on assessing the percentage of coyotes that are feeding on turkeys and deer.

#### **Summary of Research Activities**

In spring of 2020 we collected scats from eight locations across South Carolina and have extracted DNA. We have identified an organization that will do the metabarcoding for a fee.

#### **Conclusions**

The data are still being collected.

#### **Major Impact(s) of Research**

1. Data will be acquired to estimate coyote diet in different ecoregions
2. Data will be used to inform deer and turkey management policies

#### **Other Project Personnel**

Dr. Jason O'Bryhim, Research Professional, SREL

Adam McFall, Research Technician, SREL

Gabriela Rodriguez, Undergraduate researcher, SREL

#### **External Collaborators**

Dr. Gino D'Angelo, UGA Warnell School of Forestry and Natural Resources, Athens, GA

Dr. Karl Miller, UGA Warnell School of Forestry and Natural Resources, Athens, GA

Dr. John Kilgo, USFS, Aiken, SC

Jordan Youngman, UGA Warnell School of Forestry and Natural Resources, Athens, GA

Charles Ruth, SCDNR

#### **Products**

Youngmann, J.L., S.L. Lance, J.C. Kilgo, C. Ruth, J. Cantrell, and G.J. D'Angelo. 2020. DNA metabarcoding of coyote (*Canis latrans*) scat to assess diet in South Carolina, USA. 2020 Warnell Graduate Student Symposium, Athens, GA. (platform presentation)

Youngmann, J.L., S.L. Lance, J.C. Kilgo, C. Ruth, J. Cantrell, and G.J. D'Angelo. 2020. Diet analysis of coyote scat in South Carolina, USA through DNA metabarcoding. 27th Wildlife Society Conference. (poster presentation)

Youngmann, J.L., S.L. Lance, J.C. Kilgo, C. Ruth, J. Cantrell, and G.J. D'Angelo. 2020. DNA metabarcoding of coyote (*Canis latrans*) scat to assess diet in South Carolina, USA. 43rd Annual Meeting of the Southeast Deer Study Group, Auburn, Alabama. (poster presentation)

## Conservation and Management of Gopher Frogs in South Carolina

### **Funding Entity**

Longleaf Alliance; Greenville Zoo; National Fish and Wildlife Foundation, United States Fish and Wildlife Service

### **Start Date and Funding Amount**

September 2020 \$107,287

### **SREL Collaborators**

Dr. Stacey Lance

### **Objectives**

Gopher frogs (*Lithobates capito*) are an uncommon species historically distributed throughout the southeastern coastal plain of the United States. Gopher frog populations have been declining due to loss of both their terrestrial uplands and their breeding sites. They are now listed as endangered at the state level and being considered for listing at the federal level. Within SC, gopher frog conservation is a high priority of the Department of Natural Resources and the population strongholds are the Savannah River Site and Francis Marion National Forest. It is unclear whether populations exist between these two widely separated areas. Our objectives are to 1) identify private lands with suitable gopher frog wetland and upland habitat, 2) survey for gopher frogs on these lands, 3) make management recommendations, 4) optimize larval and juvenile headstarting protocols and 4) establish a rapid wetland assessment protocol.

### **Summary of Research Activities**

To date we have compiled a list of potential landowners to work with based on habitat suitability models for the gopher frogs. We have met with land managers at numerous plantations and surveyed wetlands on six different plantations. We provided support to the Orangeburg National Fish Hatchery and helped them rear and release 350 metamorphs onto the SRS. We are currently selecting properties for 2021 surveys, establishing experimental protocols for headstarting in 2021, and have a draft rapid wetland assessment protocol.

### **Conclusions**

None at present.

### **Major Impact(s) of Research**

None at present

### **Other Project Personnel**

Dr. Jason O'Bryhim, Research Professional, SREL

Adam McFall, MS student, SREL

Tucker Stonecypher, MS student, SREL

Kiersten Nelson, PhD student, SREL.

### **External Collaborators**

Lisa Lord – Longleaf Alliance

Melanie Olds – USFWS

Jason Ayers – USFWS

Jarrett Hill – Orangeburg National Fish Hatchery

### **Products**

Lance, S.L. 2020 Conservation and restoration of the Carolina gopher frog: taking steps toward a longleaf forest with diverse wetland and upland ecosystems. 13<sup>th</sup> Biennial Longleaf Conference. Virtual meeting. (platform)

**Use of Fecal Genotyping and Spatial Capture-recapture Modeling to Investigate Coyote Abundance in South Carolina**

**Funding Entity**

SCDNR

**Start Date and Funding Amount**

January 2017; \$50,000

**SREL Collaborators**

Dr. Stacey L. Lance

**Objectives**

The overall goals of this research are to estimate coyote densities among regions in South Carolina and to evaluate densities relative to landscape composition and other variables.

**Summary of Research Activities**

Coyote scats from a previous study on the SRS were collected over four seasons for two years on the SRS. To date we have extracted DNA from all scats have completed an initial screen of all samples across 9-10 microsatellite loci. We have confirmed recaptures of individuals across seasons and years. The new project is an extension of that work and will include those samples. A PhD student, Jordan Youngmann, has now led a team to collect scat along transects in 8 different locations representing three ecoregions within the state in both 2019 and 2020. We have extracted DNA from all scats and optimized a multiplex PCR reaction. Currently we are genotyping all of the samples.

**Conclusions**

The data are still being collected.

**Major Impact(s) of Research**

1. Data will be acquired to estimate coyote abundance in different ecoregions
2. Data will be used to inform deer management policies

**Other Project Personnel**

Dr. Jason O'Bryhim, Research Professional, SREL

Adam McFall, MS Student, SREL

Gabriela Rodriguez, Undergraduate researcher, SREL

**External Collaborators**

Dr. Gino D'Angelo, UGA Warnell School of Forestry and Natural Resources, Athens, GA

Dr. Karl Miller, UGA Warnell School of Forestry and Natural Resources, Athens, GA

Dr. John Kilgo, USFS, Aiken, SC

Jordan Youngman, UGA Warnell School of Forestry and Natural Resources, Athens, GA

**Products**

Youngmann, J. L., G. J. D'Angelo, J. C. Kilgo, S. L. Lance, K. V. Miller, and C. Ruth. 2019. Use of non-invasive genetic sampling to estimate population density, resource selection, and genetic structure of coyotes in South Carolina. Georgia Department of Natural Resources Annual Meeting. (Poster presentation).

Youngmann, J.L., G.J. D'Angelo, J.C. Kilgo, S.L. Lance, K.V. Miller, and C. Ruth. 2019. Use of fecal genotyping and spatial capture-recapture to investigate population density, resource selection, and genetic structure of coyotes in South Carolina. 26th Annual Conference of The Wildlife Society, Reno, Nevada. (Poster presentation)

Youngmann, J.L., G.J. D'Angelo, J.C. Kilgo, S.L. Lance, K.V. Miller, and C. Ruth. 2019. Use of fecal genotyping and spatial capture-recapture modeling to investigate coyote abundance in South Carolina. 42nd Annual Meeting of the Southeast Deer Study Group, Louisville, Kentucky. (Poster presentation)

## *The Zoonotic Origin of Major Human Infectious Diseases*

### **Funding Entity**

SREL

### **Start Date and Funding Amount**

June 2020; NFP

### **SREL Collaborators**

G. Dharmarajan

### **Objectives**

The purpose of this study was to improve our understanding of the factor driving zoonotic disease emergence in human populations.

### **Summary of Research Activities**

Zoonotic diseases, infections that are transmissible to humans from other vertebrate animals, constitute about three quarters of all emerging infectious diseases, and represent an important public health issue. We gathered an international group of scientists to critically review the origins of past zoonotic epidemics globally and to provide recommendations to reduce the emergence of such epidemics in the future.

### **Conclusions**

Most emerging and novel zoonoses are due to sporadic host switching events that are inherently stochastic and unpredictable. Thus, we argue that future countermeasures against zoonotic disease pandemics require a holistic approach that focuses on several mechanisms, including: (a) Interruption of transmission chains; (b) Reinvigorated and sustained vector control; (c) Improved surveillance; (d) Source identification and spatial spread; (e) International collaboration.

### **Major Impact(s) of Research**

1. Our study showed that the prevention of future pandemics requires a critical examination of the evolutionary drivers influencing zoonotic disease emergence in human populations and the characteristics associated with past pandemics.
2. Emergence of novel diseases is stochastic, future countermeasures against zoonotic pandemics requires a holistic approach that focuses on several mechanisms, including breaking transmission chains, improved pathogen, host/vector surveillance, and enhanced international collaboration

### **Other Project Personnel**

Pooja Gupta, PhD Student – SREL

### **External Collaborators**

Emmanuel Chanda, World Health Organization

Katharine R. Dean, Norwegian Veterinary Institute, Norway.

Rodolfo Dirzo, Stanford University

Kjetill S. Jakobsen, University of Oslo, Norway.

Imroze Khan, Ashoka University, India

Leirs Herwig, University of Antwerp, Belgium

Zhengli Shi, Wuhan Institute of Virology, China

Nathan Wolfe, Metabiota, California

Ruifu Yang, Beijing Institute of Microbiology and Epidemiology, China

Nils C. Stenseth, University of Oslo, Norway

### **Products**

White Paper on Emerging Infectious Diseases. Working Group on Zoonotic Disease, International Union of Biological Sciences (<https://www.iubs.org/iubs-activities/new-initiatives/zoonotic-diseases.html>)

Dharmarajan, G., E. Chanda, K.D. Dean, R. Dirzo, I. Khan, S.J. Kjetill, H. Leirs, Z. Shi, N. Wolfe, R.

Yang and N.C. Stenseth. In Review. The zoonotic origin of major human infectious diseases. *Nature Ecology and Evolution*

## **Ecological and Evolutionary Dynamics of Avian Malaria, an Emerging Infectious Disease in Wild Birds**

### **Funding Entity**

SREL

### **Start Date and Funding Amount**

October 2016; NFP

### **SREL Collaborators**

G. Dharmarajan

### **Objectives**

Emerging infectious diseases are considered to be one of the greatest challenges of our times, and their recent proliferation has been associated with anthropogenic factors such as global climate change. Avian malaria (AM) – a vector-borne disease caused by protozoan parasites *Plasmodium* spp. (Plasmodiidae) and *Haemoproteus* spp. (Haemoproteidae) is an important emerging disease in bird populations. This study will characterize patterns of avian malaria in a natural bird community in India to improve our understanding of the disease. The objective of this study is to elucidate the ecological and evolutionary dynamics of avian malaria in bird communities in the tropical sky-islands of the Western Ghats, India

### **Summary of Research Activities**

Blood, sampled (50-100 µl) from the ulnar vein of birds was used to extract genomic DNA and screen for avian malaria infection. A nested Polymerase chain reaction (PCR) approach was employed to amplify the partial mitochondrial cytochrome b gene (478bp) of avian haemosporidian parasites. Positive samples were sequenced and analyzed in Geneious 9.1.5 for analyses.

### **Conclusions**

A total of 1177 birds from 28 bird species were sampled, and our data revealed that 24 out of 28 species were infected with avian malaria parasites (*Plasmodium* spp. and *Haemoproteus* spp.) with an overall prevalence of 41.6% (490 individuals). We identified a total of 47 different mitochondrial cytb lineages, with 18 *Plasmodium* and 29 *Haemoproteus* parasites, but only six *Plasmodium* and four *Haemoproteus* lineages showed identity with sequences available in the Genbank/Malavi database. *Haemoproteus* lineages showed greater levels of species specificity as compared to *Plasmodium*.

### **Major Impact(s) of Research**

1. Our data reveal that relatively few lineages of avian malaria are shared between India and areas where the parasite has been newly introduced, thus indicating that disease emergence is associated with rare *Plasmodium* lineages
2. Our data also reveal that *Plasmodium* is generally less species specific compared to *Haemoproteus*, which could explain why EIDs are generally associated with the former rather than the latter pathogen.

### **Other Project Personnel**

P. Gupta, PhD student, SREL and Warnell School of Forestry, UGA

### **External Collaborators**

R. V. Vijayan – Indian Institute of Science Education and Research Tirupati

### **Products**

One grant has been submitted to Department of Biotechnology, Govt. of India

Gupta, P., V.V. Robin, and G. Dharmarajan. 2020. Towards a more healthy conservation paradigm:

Leveraging disease ecology to aid biological conservation. *Journal of Genetics* 99 (Article 65): 1-26

Dharmarajan, G., P. Gupta, C.K. Vishnudas and V.V. Robin. In Review. Anthropogenic disturbance increases disease emergence risk through predictable changes in parasite community structure. *Ecology Letters*

Gupta, P., C.K. Vishnudas and V.V. Robin and G. Dharmarajan. In Review. Host phylogeny matters: Examining sources of variation in infection risk by blood parasites across a tropical montane bird community in India. *Parasites and Vectors*

## **Levels of Alpha-gal in Ticks: Implications for Red Meat Allergy**

### **Funding Entity**

SREL

### **Start Date and Funding Amount**

October 2016; NFP

### **SREL Collaborators**

G. Dharmarajan

### **Objectives**

The purpose of this study was to characterize  $\alpha$ -gal in *Amblyomma americanum* and *A. maculatum* ticks reared in the lab and those collected naturally from hunter killed deer on the SRS and adjoining areas.

### **Summary of Research Activities**

Delayed anaphylaxis linked red meat is a newly recognized allergic disease. Individuals bitten by the lone star tick (*Amblyomma americanum*) may develop IgE antibodies to the carbohydrate galactose- $\alpha$ -1,3-galactose ( $\alpha$ -Gal; a carbohydrate moiety). Upon exposure of sensitized subjects to mammalian meat containing  $\alpha$ -Gal on glycoproteins or glycolipids, delayed anaphylaxis may ensue, often three to six hours after ingestion. Tick saliva and salivary gland samples were processed to recover N-glycans. The N-linked glycans were permethylated for structural characterization by mass spectrometry. Briefly, the permethylated glycans were reconstituted in 100% MeOH and introduced to the mass spectrometer (Thermo Fusion Tribrid Orbitrap) with offline emission. These data were used to search for a Hex-Hex-HexNAc signature, and when glycoforms matching a Hex-Hex-HexNAc signature were found samples were manually fragmented at masses corresponding to each of the possible N-linked glycoforms.

### **Conclusions**

A large number of N-glycans was found in each of the tick samples, and these varied greatly from extended high mannose structures to highly complex and hybrid structures. No indications of the  $\alpha$ -Gal glycoforms were found in any of the samples from the *Amblyomma maculatum* (Gulf Coast Tick) samples, either by not finding a mass corresponding to the glycoform, or if the mass was found, MS/MS fragmentation indicated that the glycoform did not contain the Hex-Hex-HexNAc signature fragment. No indications of the  $\alpha$ -Gal glycoforms were found in the salivary glands from unfed *Amblyomma americanum*. However, multiple alpha-Gal glycoforms were found in salivary glands and saliva from partially or fully blood fed *Amblyomma americanum*.

### **Major Impact(s) of Research**

1. Our preliminary data reveal  $\alpha$ -Gal glycoforms are only associated with saliva and salivary glands of partially or fully fed *A. americanum*.
2. The presence of  $\alpha$ -Gal in *A. americanum* has significant implications for red meat allergy in South Carolina, especially amongst individuals that have high risk of tick bite (e.g., hunters)

### **Other Project Personnel**

Jennifer Dirks, Technician – SREL

### **External Collaborators**

P. Azadi – Complex Carbohydrate Research Center, UGA

S. Karim – University of Southern Mississippi

### **Products**

Two grants (to NSF and NIH) have been submitted on the basis of these data.

## **Factors Affecting the Vectorial Capacity of Mosquitoes**

### **Funding Entity**

SREL, Filariasis Reagent and Resource Center UGA, Department of Infectious Diseases UGA

### **Start Date and Funding Amount**

August 2017; NFP

### **SREL Collaborators**

G. Dharmarajan (SREL)

### **Objectives**

Recent decades has seen the reemergence of many mosquito-borne diseases in human and animal populations (e.g., filariasis and malaria). The evolution of resistance against anti-parasitic drugs is one of the most important drivers of reemergence. The focus of this study is to test if mosquitoes act to enhance or reduce the risk of a drug resistant parasite genotype spreading in the vertebrate host population, and the role of the microbiome in affecting mosquito vectorial capacity.

### **Summary of Research Activities**

We have completed three main aspects of this study, including understanding the natural variation in resistance and tolerance to parasites in mosquitoes, the role of the mosquito in the spread of drug resistance, and the role of the mosquito microbiome in shaping vectorial capacity.

### **Conclusions**

Our data indicates that there is considerable genetic variation between drug resistant strains of *D. immitis*. However, drug resistance does not reduce mosquito vectorial capacity. This may explain the rapid spread of drug resistant traits among *D. immitis* populations in the US.

### **Major Impact(s) of Research**

1. The study has important implications for our understanding of the ecological and evolutionary factors affecting vectorial capacity in mosquitoes, and hence has implications for the control of vector-borne diseases
2. The study has uncovered the vital role the mosquito microbiome plays in parasite development and thus mosquito vectorial capacity
3. This study has elucidated the role of the mosquito vector in the rise and spread of drug resistant genotypes of mosquito-borne parasites in natural populations, and will thus improve the control of these diseases.

### **Other Project Personnel**

Erik Neff, MS student – SREL

Jennifer Dirks, Research Technician – SREL

Sarah Ebert, Research Technician – SREL

### **External Collaborators**

A. Moorehead – Filariasis Reagent and Resource Center UGA

R. Kaplan – Department of Infectious Diseases UGA

S. Karim – University of Southern Mississippi

### **Products**

Adegoke, A., E. Neff, A. Geary, M.C. Husser, K. Wilson, S.M. Norris, G. Dharmarajan, S. Karim. 2020. Laboratory colonization by *Dirofilaria immitis* alters the microbiome of female *Aedes aegypti* mosquitoes. *Parasites and Vectors* 13: 349

Sanchez, J., G. Dharmarajan, M.M. George, C. Pulaski, A.J. Wolstenholme, J.S. Gilleard and R.M. Kaplan. 2020. Using population genetics to examine relationships of *Dirofilaria immitis* based on both macrocyclic lactone-resistance and geography. *Veterinary Parasitology* 109125

Neff, E., R.M. Kaplan and G. Dharmarajan. In Review. The potential role of mosquito vectors in the spread of drug-resistant filarial parasites. *Pathogens*

## *Evaluation of Head-starting as a Recovery Tool for the Mojave Desert Tortoise*

### **Funding Entity**

National Park Service, California Energy Commission

### **Start Date and Funding Amount (If no funding involved indicate No Funding Provided (NFP))**

Nov 2010; \$450,000 (NPS); \$313,000 (CEC, Phase I); \$493,089 (CEC, Phase II); \$299,658 (National Fish and Wildlife Foundation)

### **SREL Collaborators**

Tracey Tuberville, Kurt Buhlmann

### **Objectives**

1. Determine behavior, survivorship, and habitat use of head-started juvenile desert tortoises compared to direct-release hatchlings (i.e., juveniles released shortly after hatching).
2. Develop habitat suitability models for juvenile desert tortoises to identify optimal habitat.
3. Evaluate the efficacy of indoor rearing as a head-starting technique.

### **Summary of Research Activities**

Our research activities included monitoring of hatchling and juvenile desert tortoises in outdoor rearing pens, indoor rearing facilities, and radio-tracking of juveniles released into the wild.

### **Conclusions**

Based on preliminary data analysis, most movement occurred within 30 days of release, and those with the greatest movements during that time were less likely to survive through the winter dormancy period. Animals head-started for 1 year in outdoor pens exhibited greater post-release survivorship than animals released immediately after hatching, but rearing animals for longer than 1 year in the enclosures may significantly reduce native forage inside pens. Initial attempts at indoor head-starting was effective in increasing growth, but did not result in increased survival compared to smaller outdoor head-starts when released at 1 yr of age. However, combination head-starting (involving 1 year of indoor head-starting to increase growth followed by 1 yr outdoor head-starting to harden shells and provide natural cues) proved to be an efficient approach to produce juveniles of a size more resistant to predation and with high post-release survival.

### **Major Impact(s) of Research**

1. Important life history data for a poorly understood life stage of a federally listed species
2. Improve management for species by factoring juvenile requirements into management and policy decisions, such as identifying habitats likely to serve as important areas of juvenile recruitment. These data will be helpful when selecting potential solar development sites by identifying areas to avoid.
3. Continued advancement of the technique has resulted in a more time and cost-efficient head-starting protocol, increasing its likelihood of implementation as part of a recovery program for the species.

### **Other Project Personnel**

Pearson McGovern, M.S. Student, Warnell School of Forestry and Natural Resources, UGA

Carmen Candal, M.S. Student, Warnell School of Forestry and Natural Resources, UGA

### **External Collaborators**

Brian Todd, University of California, Davis

Collin Richter, Research Technician, University of California-Davis

Susanna Mann, Research Technician, University of California-Davis

Mark Peadar, Rogers State University

Kristin Navarra, University of Georgia

Nicole Stacy, University of Florida School of Veterinary Medicine

Taylor Edwards, University of Arizona

Clint Moore, University of Georgia

## Products

- McGovern, P.A., K.A. Buhlmann, B.D. Todd, C.T. Moore, J.M. Peaden, J. Hepinstall-Cymerman, J.A. Daly, and T.D. Tuberville. *In press*. The effect of size on post-release survival of head-started Mojave desert tortoises. To appear in: Journal of Fish and Wildlife Management.
- McGovern, P.A., K.A. Buhlmann, B.D. Todd, C.T. Moore, J.M. Peaden, J. Hepinstall-Cymerman, J.A. Daly, and T.D. Tuberville. *In press*. Comparing husbandry techniques for optimal head-starting of the Mojave desert tortoise (*Gopherus agassizii*). To appear in: Herpetological Conservation and Biology.
- Nowakowski, A.J., J.M. Peaden, T.D. Tuberville, K.A. Buhlmann, and B.D. Todd. 2020. Thermal performance curves based on field movements reveal context-dependence of thermal traits in a desert ectotherm. *Landscape Ecology* 35:893-906.
- McGovern, P.A. 2019. Changing the survival formula for the Mojave desert tortoise (*Gopherus agassizii*) through head-starting. M.S. Thesis, University of Georgia.
- Todd, B., P. McGovern, J.M. Peaden, K.A. Buhlmann, and T.D. Tuberville. Evaluating techniques for optimal head-starting of the Mojave desert tortoise (*Gopherus agassizii*). Desert Tortoise Council, Las Vegas, NV. February 2020.
- Tuberville, T.D. Conservation lessons from 25+ years with turtles. Invited seminar at University of Miami, October 2019.

## *Head-starting to Augment Gopher Tortoise Populations on Protected Areas in Georgia*

### **Funding Entity**

Georgia Department of Natural Resources

### **Start Date and Funding Amount**

October 2013; \$141,158; currently NFP

### **SREL Collaborators**

Tracey Tuberville, Kurt Buhlmann, Jesse Abrams

### **Objectives**

Evaluate the effectiveness of head-starting as a means of establishing viable populations of gopher tortoises on protected lands in Georgia.

### **Summary of Research Activities**

Activities during this year focused on quantifying corticosterone in archived samples collected from head-started gopher tortoises at time of release, as well as analyzing movement and survival data as part of a graduate student's thesis research. We also added a new component that included conducting structured interviews of species biologists and natural resource managers across Georgia to identify potential barriers to implementing land management activities that promote ideal gopher tortoise habitat.

### **Conclusions**

Head-starting appears to be an effective way of increasing juvenile survivorship, although site-specific predator pressures from raccoons and fire ants can exert strong influence on outcome. Analysis of structured interviews has not yet been completed.

### **Major Impact(s) of Research**

This work is among the first to evaluate post-release site fidelity and survival of released head-started gopher tortoises. Our results will help determine whether head-starting can be used as a potential recovery tool for the species. Several agencies have now asked for technical assistance and advice regarding initiating head-starting programs for tortoises.

### **Other Project Personnel**

Amelia Russell, M.S. Student, Warnell School of Forestry and Natural Resources, UGA

Louise McCallie, Research Technician, SREL

### **External Collaborators**

Terry Norton, Georgia Sea Turtle Center, Jekyll Island, Georgia

Kristen Navarra, University of Georgia

Nicole Stacy, University of Florida School of Veterinary Medicine

### **Products**

Tuberville, T.D., D.P. Quinn, and K.A. Buhlmann. *In press*. Movement and survival to winter dormancy of fall-released hatchling and head-started yearling gopher tortoises. To appear in: Journal of Herpetology.

Russell, A.L., K.A. Buhlmann, K.J. Navara, N.I. Stacy, and T.D. Tuberville. Characterizing stress in head-started gopher tortoises (*Gopherus polyphemus*) reared for an extended duration. Southeastern Partners in Amphibian and Reptile Conservation, Nauvoo, AL. February 2020. Platform presentation.

Russell, A.L., K.A. Buhlmann, D.P. Aubrey, J.B. Abrams, and T.D. Tuberville. Determining perceptions and operations restrictions on gopher tortoise (*Gopherus polyphemus*). Southeastern Partners in Amphibian and Reptile Conservation Annual Meeting, Nauvoo, AL. February 2020. Poster.

Russell, A.L., K.A. Buhlmann, K.J. Navara, N.I. Stacy, and T.D. Tuberville. Evaluating stress in head-started gopher tortoises (*Gopherus polyphemus*). Warnell Graduate Student Symposium, Athens, GA. February 2020. Platform presentation.

## Can Waifs be Used to Restore Viability of Gopher Tortoise Populations?

### **Funding Entity**

South Carolina Department of Natural Resources, US Fish and Wildlife Service, Riverbanks Zoo Conservation Support Fund, Animal Welfare Institute

### **Start Date and Funding Amount**

May 2017; \$90,424; currently NFP

### **SREL Collaborators**

Tracey D. Tuberville, Kurt A. Buhlmann, Rebecca McKee

### **Objectives**

Wild gopher tortoises are frequently translocated when they are displaced by development and these animals have been successfully used to augment depleted wild populations elsewhere. However, waif tortoises – formerly captive tortoises, rehabilitated, or those of unknown origin – are rarely considered for population augmentation due to heightened disease and genetic concerns. However, for peripheral populations that do not have available wild donor populations, waif tortoises may represent the only option for stabilizing populations. We have been releasing waif gopher tortoises at the Aiken Gopher Tortoise Heritage Preserve since 2006. The purpose of this project is to evaluate the survivorship, site fidelity, and health metrics of translocated waif gopher tortoises used to build a viable population of state-protected land in South Carolina.

### **Summary of Research Activities**

The primary activities during FY20 centered on preparing manuscripts for publication in peer-reviewed journals and submitting our final report to the granting agency. The only new research activity undertaken this year was initiating parentage analysis based on genotypes obtained from released waif gopher tortoises and resulting offspring produced on site following translocation.

### **Conclusions**

Waifs exhibited remarkably high survivorship on par with that documented for wild, unmanipulated populations as well as wild-to-wild translocated populations. In addition, only two pathogens were detected – both *Mycoplasma* species known from wild populations. We suggest that waifs can be used to build isolated viable populations of gopher tortoises when other options are not available. However, we still recommend use of waifs only in select circumstances, when released waifs are unlikely to interact with individuals from wild, stable populations. Conclusions regarding social integration of waifs from multiple release groups will be made once parentage analysis is completed.

### **Major Impact(s) of Research**

Waif adult tortoises represent valuable animals from a species recovery perspective, but methods need to be explored that use them for species recovery, even when these individuals cannot be returned to their original populations of origin. Our research also helps address what to do with the growing waif population in captivity.

### **Other Project Personnel**

Rebecca McKee, Masters student, University of Georgia

### **External Collaborators**

Will Dillman, SCDNR

Hunter Young, SCDNR

Andrew Grosse, SCDNR

Matt Allender, University of Illinois

Nicole Stacy, University of Florida Department of Veterinary Medicine

Clint Moore, UGA

Jeff Hepinstall-Cymerman, UGA

### **Products**

McKee, R.K., K.A. Buhlmann, C.T. Moore, J. Hepinstall-Cymerman, and T.D. Tuberville. *In revision*.

Waif gopher tortoise survival and site fidelity following translocation. *Journal of Wildlife Management*.

Tuberville, T., K. Buhlmann, and R. McKee. Can waif gopher tortoises be used to restore viability of gopher tortoise populations? Final report to SCDNR for USFWS Section 6 funding. 23 March 2020.

**Assessment of Changes in Population Size, Agricultural Damage, and Environmental Impacts of Wild Pigs in Response to Control**

**Funding Entity**

NRCS, SCDNR, Newberry Soil and Water Conservation District

**Start Date and Funding Amount**

June 1, 2020; \$220,000

**SREL Collaborators**

Dr. James C. Beasley

**Objectives**

Despite the extensive and widespread damages caused by wild pigs, currently there is a paucity of data on assessments of damage reduction related to population control efforts. Therefore, the overarching goal of this study is to work in collaboration with USDA-APHIS-WS, NRCS, and private landowners to quantify changes in wild pig population size and damages associated with wild pigs in response to control efforts. Specifically, this research will address the following research objectives to assess the impacts of wild pig removal efforts: 1) Quantify changes in wild pig population size across targeted areas in response to wild pig removal effort, 2) Quantify changes in wild pig damage to common agricultural crops (e.g., corn, peanuts, pasture/hay land) in response to wild pig removal efforts, and 3) Quantify changes in environmental damages caused by wild pigs across targeted areas in response to wild pig removal efforts.

**Summary of Research Activities**

This research was initiated in spring 2020 and is still ongoing at this time.

**Conclusions**

This work has just begun, there are no conclusions at this time.

**Major Impact(s) of Research**

This research is part of a broader effort by USDA and NRCS to quantify the efficacy of wild pig control on private lands at reducing economic and agricultural damages. Data from this research will be incorporated into national-level management decisions regarding the management of invasive wild pigs under the national Feral Swine Damage Management Program.

**Other Project Personnel**

Joseph Treichler, M.S. Student, Warnell School of Forestry and Natural Resources – UGA

**External Collaborators**

Dr. Kurt VerCauteren (USDA-APHIS-WS-NWRC)

Mr. Noel Myers (USDA-APHIS-WS)

**Products**

This research has just begun; there are no products at this time.

**Wild Pig Management in Sumter and Francis Marion National Forest**

**Funding Entity**

USDA-USFS

**Start Date and Funding Amount**

May 21, 2018; \$43,759

**SREL Collaborators**

Dr. James C. Beasley

**Objectives**

The objective of this study is to assist USDA-USFS personnel working on the Long Cane Ranger District in South Carolina in the management of wild pigs through collection of biological data needed to inform management activities.

**Summary of Research Activities**

During summer 2018-summer 2020 we collected samples from wild pigs captured by USFS personnel on the Long Cane Ranger District in SC. Samples will be incorporated into ongoing wild pig studies in South Carolina to elucidate the genetic structure of wild pig populations in the state. In addition, we deployed remote cameras throughout the study area to determine the relative abundance and distribution of wild pigs in the region.

**Conclusions**

This research is still in progress; there are no conclusions at this time.

**Major Impact(s) of Research**

This research will provide data needed to inform management activities targeting invasive wild pigs on the Long Cane Ranger District.

**Other Project Personnel**

Mr. Jakob Kemp. Amy Hilger, research technician, SREL

Mr. Kyle Cockrell, research technician, SREL

**External Collaborators**

Mr. Donny Ray (USFS)

**Products**

This research has just begun; there are no products at this time.

## **Evaluation of Crop Damage by Wild Pigs**

### **Funding Entity**

USDA-APHIS, Wildlife Services, National Wildlife Research Center

### **Start Date and Funding Amount**

February 20, 2017; \$58,883

### **SREL Collaborators**

Dr. James C. Beasley

### **Objectives**

The objectives of this study are 1) to quantify timing and spatial distribution of wild pig damage to corn and peanut fields in SC, as well as 2) assess the utility of UAV's for quantifying wildlife damage to agricultural crops.

### **Summary of Research Activities**

We selected crop fields and conducted walking surveys for fields adjacent to the Congaree National Forest, the Savannah River Site in SC, and in South-Central South Carolina during the planting seasons of 2017-2019. During 2018 and 2019 we also conducted aerial surveys of fields using UAV's to evaluate the efficacy of UAV's for quantifying wildlife damage to crops. All field work has been completed and we are currently finalizing publications from this research.

### **Conclusions**

Results of this research suggest the majority of wild pig damage to corn fields occurs shortly after planting, followed by pulses of damage during development and maturation of ears. Damage to peanuts occurred almost exclusively following planting. Thus, targeted management of wild pigs immediately prior to crop planting will have the most substantial impact in reducing crop losses from this species. Damage to both crops increased in areas with greater area of forest and wetlands adjacent to crop fields, while fields surrounded by agriculture and the presence of paved roads were associated with lower damage estimates. Although analyses of aerial survey data are ongoing, drone surveys were effective at distinguishing wildlife damage to both corn and peanut fields.

### **Major Impact(s) of Research**

1. This research provides novel and important data on the timing and spatial distribution of wild pig damage to corn and peanut fields, two important agricultural crops in the southeast.
2. Data derived from this study will be integrated into national-level management of wild pigs by the USDA to mitigate damages caused by invasive wild pigs.

### **Other Project Personnel**

Chris Boyce, M.S. Student, Warnell School of Forestry and Natural Resources - UGA

### **External Collaborators**

Dr. Kurt VerCauteren (USDA-APHIS-WS-NWRC)

Steve Smith (USDA-APHIS-WS)

### **Products**

Beasley, J.C. Wild Pig Research on the Savannah River Site. 2018. South Carolina Wild Pig Task Force Meeting, Columbia, SC (oral presentation)

Boyce, C., and J.C. Beasley. 2018. Agricultural damage timing and extent by wild pigs along the Congaree River. Warnell Graduate Student Symposium. Athens, Georgia (oral presentation)

Boyce, C. 2019. Timing and extent of crop damage by wild pigs (*Sus scrofa*) to corn (*Zea mays*) and peanuts (*Arachis hypogaea*). Thesis. University of Georgia, Athens GA. 85pp.

Boyce, C., K. VerCauteren, and J.C. Beasley. 2019. Agricultural damage timing and extent by wild pigs along the Congaree River. Savannah River Ecology Laboratory Graduate Student Symposium. Aiken, South Carolina (poster presentation)

Boyce, C.M., K.C. VerCauteren, and J.C. Beasley. 2020. Timing and extent of crop damage by wild pigs (*Sus scrofa*) to corn and peanut fields. *Crop Protection* 133:105131.

Beasley, J., Boyce, C., and VerCauteren, K. 2020. Timing and extent of crop damage by wild pigs (*Sus scrofa*) to corn (*Zea mays*) and peanuts (*Arachis hypogaea*). In International Wild Pig Conference. Jacksonville, FL; virtual.

***Distribution and Relative Abundance of Wildlife in Fukushima along a Gradient of Contamination and Human Land-use Intensity, and Spatial Ecology of Invasive Civets***

**Funding Entity**

University of Georgia Office of the Vice President for Research, Office of International Partnerships

**Start Date and Funding Amount**

June 1, 2016; \$15,350

**SREL Collaborators**

Dr. James C. Beasley

**Objectives**

The overall objectives of this project are to quantify the distribution and relative abundance of mammals and other scavengers in Fukushima, Japan across a gradient of radiation contamination and level of human disturbance. In addition, we will deploy radio collars on civets and potentially wild boar to assess their movement behavior and radiation exposure, which will be used to assess potential sub-lethal effects of chronic radiation exposure, as well as the influence of human presence on their spatial ecology.

**Summary of Research Activities**

During spring 2016 we travelled to the Fukushima exclusion zone and conducted 120 2-month remote camera trials. Data from these cameras have now been analyzed, and a manuscript highlighting the key findings has been published. During fall 2016 and summer 2017 we returned to Fukushima to conduct additional camera trials and attached GPS transmitters to civets and wild boar in the exclusion zone. Data from these studies have been analyzed and we are currently preparing publications stemming from this research.

**Conclusions**

Results of this research suggest numerous species of mammals are abundant within the exclusion zone, and that the density of some species, especially wild boar, differs considerably between the exclusion zone and areas that remain occupied by humans. Further, resource selection patterns by civets and raccoons show selection of abandoned croplands and urban areas. Radiation was not found to be a factor driving occupancy, abundance, or movement patterns among the wildlife species we have studied. Rather, human presence, habitat availability, and elevation appear to be the underlying driving force influencing the distribution and abundance of mammals in Fukushima.

**Major Impact(s) of Research**

This research represents the first assessment of the occupancy and abundance patterns of wildlife communities inhabiting the Fukushima Exclusion Zone. Further, this research is one of the first studies of the spatial ecology of civets and raccoons in Japan. Thus, data generated from this research will provide important insights into the management and conservation of wildlife in Fukushima.

**Other Project Personnel**

Phillip Lyons, M.S. Student – Warnell School of Forestry and Natural Resources, UGA

Matt Hamilton, Research Professional – Warnell School of Forestry and Natural Resources, UGA

Helen Bontrager, M.S. Student – Warnell School of Forestry and Natural Resources, UGA

**External Collaborators**

Dr. Thomas Hinton (Fukushima University)

Dr. Kei Okuda (Fukushima University)

**Products**

Lyons, P., T.G. Hinton, K. Okuda, M. Hamilton, and J.C. Beasley. 2018. Fukushima's Wildlife: Camera Analysis of Species in and Around the Exclusion Zone. Georgia Chapter of the Wildlife Society. Tifton, GA (oral presentation)

Lyons, P., T.G. Hinton, K. Okuda, M. Hamilton, and J.C. Beasley. 2018. Fukushima's Wildlife: Mammalian Species in and Around the Exclusion Zone. Warnell Graduate Student Symposium. Athens, Georgia (oral presentation)

- Beasley, J.C. 2018. Radioactive Wildlife: The Secret Life of Wildlife Inhabiting Chernobyl and Fukushima. SREL Research Experiences for Undergraduates. Aiken, SC (oral presentation)
- Beasley, J.C. 2018. Radioactive Wildlife: The Secret Life of Wildlife Inhabiting Chernobyl and Fukushima. Colorado State University. Fort Collins, Colorado (oral presentation)
- Beasley, J.C. 2018. Radioactive Wildlife: The Secret Life of Wildlife Inhabiting Chernobyl and Fukushima. Warnell Seminar. Athens, GA (oral presentation)
- Webster, S., and Beasley, J. C. (2017). Ecological effects of the Chernobyl accident. Grovetown Middle School (oral presentation)
- Beasley, J.C. 2018. <sup>137</sup>Cs Accumulation in Wildlife. International Atomic Energy Agency Mission to Fukushima Prefecture, Japan. Fukushima City, Japan (oral presentation)
- Beasley, J.C. 2018. Radioactive Wildlife: The Secret Life of Animals in Chernobyl and Fukushima. NASA Goddard Space Flight Center. Greenbelt, Maryland (oral presentation)
- Beasley, J.C. 2018. Radioactive Wildlife: The Secret Life of Animals in Chernobyl and Fukushima. Citizens for Nuclear Technology Awareness Up and Atom. Aiken, South Carolina (oral presentation)
- Beasley, J.C. 2018. Radioactive Wildlife: The Secret Life of Wildlife Inhabiting Chernobyl and Fukushima. Reactor Tech Reunion. North Augusta, South Carolina (oral presentation)
- Beasley, J.C. 2019. Radioactive Wildlife: Impacts of the Chernobyl and Fukushima nuclear accidents on wildlife. Auburn University. Auburn, Alabama
- Beasley, J.C. 2019. Radioactive Wildlife: The Secret Life of Animals in Chernobyl and Fukushima. Mississippi State University. Starkville, Mississippi (oral presentation)
- Beasley, J.C. 2019. Radioactive Wildlife: The Secret Life of Animals in Chernobyl and Fukushima. Cedar Shoals High School. Athens, GA (oral presentation)
- Beasley, J.C. 2019. Radioactive Wildlife: The Secret Life of Animals in Chernobyl and Fukushima. University of South Carolina – Aiken. Aiken, SC (oral presentation)
- Beasley, J.C. 2019. Radioactive Wildlife: The Secret Life of Animals in Chernobyl and Fukushima. University of South Carolina – Aiken. Aiken, SC (oral presentation)
- Beasley, J.C. 2019. Radioactive Wildlife: The Secret Life of Animals in Chernobyl and Fukushima. Midland Valley High School. Aiken, SC (oral presentation)
- Beasley, J.C., T. Hinton, K. Okuda, P. Lyons, H. Gerke, and S. Chinn. Ecology, health, and radiation exposure of wildlife inhabiting the Fukushima Evacuation Zone. US DOE-HQ Annual Site Environmental Report Workshop. Aiken, SC (poster presentation)
- Lyons, P.C., K. Okuda, M. Hamilton, T.G. Hinton, and J.C. Beasley. 2020. Rewilding of Fukushima's human evacuation zone. *Frontiers in Ecology and the Environment* 18:127-134.
- Beasley, J., Lyons, P., Okuda, K., Hamilton, M., and Hinton, T. 2020. Wild Boar and the Rewilding of Fukushima's Human Evacuation Zone. In International Wild Pig Conference. Jacksonville, FL; virtual.
- Beasley, J. 2020. Radioactive Wildlife: Impacts of the Chernobyl and Fukushima Nuclear Accidents Large Mammals. In 21st Ecological Integration Symposium: Ecology in the Anthropocene. College Station, TX; virtual. Plenary Speaker
- Beasley, J. 2020. Radioactive Wildlife: Impacts of the Chernobyl and Fukushima Nuclear Accidents Large Mammals. 105th Annual Meeting of the Ecological Society of America.
- Beasley, J., Lyons, P., Okuda, K., Hamilton, M., and Hinton, T. 2020. Wild Boar and the Rewilding of Fukushima's Human Evacuation Zone. In International Wild Pig Conference. Jacksonville, FL; virtual.

***<sup>137</sup>Cs Activity Levels, Movement Behavior, and Efficiency of Vertebrate Scavengers in the Fukushima Exclusion Zone***

**Funding Entity**

University of Georgia, Office of International Education

**Start Date and Funding Amount**

May 1, 2018; \$5,000

**SREL Collaborators**

Dr. James C. Beasley

**Objectives**

The objectives of this project are to 1) quantify the composition and efficiency of vertebrate scavengers within the Fukushima Exclusion Zone, 2) deploy GPS transmitters on rat snakes within the Exclusion Zone to assess their movement behavior, and 3) quantify activity levels of <sup>137</sup>Cs in snakes throughout Fukushima Prefecture, Japan.

**Summary of Research Activities**

During summer 2018 we travelled to the Fukushima exclusion zone and conducted remote camera trials, as well as attached GPS transmitters and dosimeters to snakes. We also collected <sup>137</sup>Cs activity data from both live and road-kill collected snakes. Data from these efforts have been analyzed and numerous publications have been produced from this work.

**Conclusions**

Whole-body radiocesium levels for snakes were highly variable among individuals (16 to 25,000 Bq/kg, FW), but were influenced more by levels of local contamination than species, sex or size.

Short-term home ranges included more areas close to streams, buildings, and roads, as well as more grassland and less evergreen forest than expected given the availability of these habitat components on the landscape. The limited distance snakes moved compared to more mobile species suggests snakes could be useful bioindicators of local contamination. Species richness and carcass removal rates of vertebrate scavengers were similar between the evacuated exclusion zone and the inhabited reference area, although slight differences existed in scavenging rates of some species (e.g., wild boar, fox), which may have implications for nutrient distribution. Our results suggest that despite radiological contamination, the FEZ supports a highly diverse, efficient scavenger community, lending support to studies which identified abundant and diverse wildlife communities in radiologically contaminated areas with limited human activity.

**Major Impact(s) of Research**

This research represents one of the most extensive evaluation of snake radioecology to date, including the first assessment of the efficiency of vertebrate scavengers within the Fukushima Evacuation Zone.

Collectively, these data suggest snakes are most influenced by external sources of radionuclide exposure, and are useful bioindicators of local contamination. These data also represent some of the first information on the spatial ecology of Japanese rat snakes, providing novel insights into the landscape factors influencing the movement behavior of these species. Lastly, results of scavenging experiments associated with this research support previous studies that have demonstrated an abundance of a broad diversity of wildlife within the Fukushima Evacuation Zone.

**Other Project Personnel**

Hannah Gerke, M.S. Student – Warnell School of Forestry and Natural Resources, UGA

Wes Dixon, Research Technician – SREL

**External Collaborators**

Dr. Thomas Hinton (Fukushima University)

Dr. Kei Okuda (Fukushima University)

Donovan Anderson (Fukushima University)

Dr. Kenji Nanba (Fukushima University)

Dr. T. Takase (Fukushima University)

## Products

- Gerke, H., and J.C. Beasley. 2018. Radiocesium Accumulation and Spatial Ecology of *Elaphe* spp. in Fukushima, Japan. 2018 Annual Southeastern Partners in Amphibian and Reptile Conservation. Helen, Georgia (poster presentation)
- Gerke, H., and J.C. Beasley. 2018. Effects of Anthropogenic Activity on the Efficiency and Composition of Vertebrate Scavenging Communities in Fukushima, Japan. Warnell Graduate Student Symposium. Athens, Georgia (poster presentation)
- Gerke, H.C., T.G. Hinton, T. Takase, and J.C. Beasley. 2019. Radiocesium accumulation and spatial ecology of rat snakes (*Elaphe* spp.) in the Exclusion Zone in Fukushima, Japan. Savannah River Ecology Laboratory Graduate Student Symposium. Athens, Georgia (poster presentation)
- Gerke, H.C., T.G. Hinton, T. Takase, D. Anderson, K. Nanba, and J.C. Beasley. 2020. Radiocesium concentrations and GPS-coupled dosimetry in Fukushima
- Gerke, H.C., T.G. Hinton, and J.C. Beasley. *In Review*. Movement behavior and habitat selection of snakes (*Elaphe* spp.) in the Fukushima Exclusion Zone.
- Gerke, H.C., T.G. Hinton, K. Okuda, and J.C. Beasley. *In Review*. Effects of anthropogenic activity on the efficiency and composition of vertebrate scavenging communities in Fukushima, Japan.
- Gerke, H., Beasley, J., Hinton, T., and Okuda, K. 2019. Effects of Anthropogenic Activity on the Efficiency and Composition of Vertebrate Scavenging Communities in Fukushima, Japan. In AFS and TWS Joint Conference. Reno, Nevada.
- Gerke, H.C., Hinton T.G., and Beasley, J.C. 2020. Radiocesium accumulation and spatial ecology of rat snakes (*Elaphe* spp.) in Fukushima Japan. World Congress of Herpetology 9. Dunedin, New Zealand.

## **Effects of Chronic Radiation Exposure on the Health of Wild Boar in Fukushima Prefecture, Japan**

### **Funding Entity**

Fukushima University, Institute of Environmental Radioactivity; University of Georgia, Office of International Education; University of Georgia, Graduate School; Environmental Radioactivity Research Network Center

### **Start Date and Funding Amount**

May 1, 2018; \$17,936

### **SREL Collaborators**

Dr. James C. Beasley

Sarah Chinn, Ph.D. Student – Warnell School of Forestry and Natural Resources, UGA

### **Objectives**

The overall goal of this project is to assess the health impacts of chronic radiation exposure in wild boar inhabiting the area surrounding the Fukushima Daiichi nuclear accident. This work is being conducted in collaboration with researchers at Fukushima University, Colorado State University, Northern Michigan University, Fukushima Prefecture, and the University of Illinois, where we are testing captured wild boar for a broad suite of potential health impairments at the molecular through individual level.

### **Summary of Research Activities**

During summer 2018 we travelled to the Fukushima exclusion zone to begin capturing boar and collecting samples to be used for this research. Additional research expeditions were conducted in 2019 to collect additional samples to be used for this research. During 2019-2020 we have been conducting laboratory analyses and analyzing the results of the research for preparation of peer-reviewed manuscripts.

### **Conclusions**

This research is incomplete; there are no conclusions at this time.

### **Major Impact(s) of Research**

This research will produce novel data on the impact of chronic radiation exposure on a large mammal. Thus, data generated from this research will provide important insights into the management and conservation of wildlife in Fukushima, as well as other sites impacted by radiological contamination.

### **Other Project Personnel**

Shannon Gregory, SREL

### **External Collaborators**

Dr. Thomas Hinton (Fukushima University)

Dr. Kei Okuda (Fukushima University)

Ms. Aryn Bordman (Colorado State University)

Dr. Thomas Johnson (Colorado State University)

Dr. Sami Pederson (Colorado State University)

Ms. Maggie Roberts (Colorado State University)

Dr. Rao Veeramachaneni (Colorado State University)

Dr. Jodi Flaws (University of Illinois)

Dr. Yui Nemoto (Fukushima Prefecture)

Dr. Diana Lafferty (Northern Michigan University)

### **Products**

S. Pederson, M. Li Puma, J. Hayes, T. Hinton, K. Okuda, J.C. Beasley, T. Johnson, L. Li Puma, and K. Freeman. 2018. Cataracts in Boar following the Fukushima Dai-ichi Nuclear Disaster. Radiation Research Society Conference. Chicago, Illinois (Poster presentation)

Chinn, S.M., T.G. Hinton, K. Okuda, H. Ishiniwa, Y. Nemoto, and J.C. Beasley. 2019. Reproductive health of wild boar in response to chronic radiation exposure. Savannah River Ecology Laboratory Graduate Student Symposium. Athens, Georgia (poster presentation)

- Pederson, S.L., M.C. Li Puma, J.M. Hayes, K. Okuda, C.M. Reilly, J.C. Beasley, L.C. Li Puma, T.G. Hinton, T.E. Johnson, and K.S. Freeman. 2020. Effects of chronic low-dose radiation on cataract prevalence and characterization in wild boar (*Sus scrofa*) from Fukushima, Japan. *Scientific Reports* 10:4055.
- Nanba, K., Chinn, S., Beasley, J., Ambai, K., Nemoto, Y., McKenney, E., Gillman, S., Hinton, T., and Lafferty, D. 2020. Effects of radionuclide exposure on the microbiome community within the gastrointestinal tract of wild boar. In Environmental Radioactivity Research Network Center 2020 Web Kickoff Meeting. Fukushima, Japan; virtual.
- Beasley, J., and Wakiyama, Y. 2020. Influences of Rooting Activity by Wild Boar on <sup>137</sup>Cs Bioavailability. In Environmental Radioactivity Research Network Center 2020 Web Kickoff Meeting. Fukushima, Japan; virtual.

**Panmixia, Promiscuity, and Nest Parasitism among Wood Storks (*Mycteria americana*)**

**Funding Entity**

University of South Carolina

**Start Date and Funding Amount**

May 2016; \$14,988

**SREL Collaborators**

A. Lawrence Bryan Jr. and Dr. Stacey Lance

**Objectives**

To sample US Wood Storks (nestlings) from selected colonies throughout their range and use a subset of storks to develop a panel of SNP (single nucleotide polymorphism) capture probes. These probes will then be used for capturing loci from additional stork DNA samples for genotyping via next generation sequencing to provide a robust examination of genetic structure and mating system in US WOST colonies as well as in other parts of their range.

**Summary of Research Activities**

Blood samples were obtained from > 300 total stork nestlings from multiple southeastern U.S. colonies and will be compared to archived samples from 2 additional colonies. Additionally, samples from Brazil have been acquired. A subset of 24 stork samples was used to create genomic libraries for sequencing and identification of SNP loci. A total of 12,228 capture probes were designed to target 6114 loci. Currently, library preps have been made and sequenced and we are analyzing the data.

**Conclusions**

None at present, pending completion of genetic analyses.

**Major Impact(s) of Research**

When completed, will have a thorough analyses of the genetic structure of the US population of Wood Storks and should know the prevalence, if any, of non-standard mating strategies.

**Other Project Personnel**

NA

**External Collaborators**

Dr. Kristina Ramstad (USC-Aiken)

Dr. Natalia Bayona (UGA)

**Products**

Ramstad, K.M., N. Bayona-Vasquez, S. Del Lama, S. Lance, A. Bryan. 2020. Genomic population structure of American wood storks - how many populations are there? North American Ornithological Conference, held virtually August 2020.

**Wood Stork (*Mycteria americana*) Species Status Assessment (SSA)**

**Funding Entity**

U.S. Fish and Wildlife Service

**Start Date and Funding Amount**

June 2019; \$20,000

**SREL Collaborators**

A. L. Bryan and O. E. Rhodes

**Objectives**

We were asked to assist in the drafting of a Species Status Assessment (SSA) for the wood stork, a species listed as federally “threatened” under the Endangered Species Act. An SSA utilizes historical and current data (habitat use, population distribution and trends, recovery efforts, etc.) to examine threats to the species as well as project best- and worst-case future scenarios for the species (e.g., different projected global climate change scenarios and landscape level development).

**Summary of Research Activities**

We initiated the drafting of the SSA in June of 2019, which involved acquisition of literature and data from regional sources. All chapters associated have been drafted (project complete for SREL).

**Conclusions**

The current draft is being reviewed by state regulatory agencies. It is expected that the draft will be finalized in early 2021. Generally, it is presumed that the adaptability of the species will allow it to maintain itself in all but the worst negative impacts.

**Major Impact(s) of Research**

The Species Status Assessment summarizes known information regarding habitat use, population trends and potential treats to the recovery of the species. It is a compliance step for federally threatened and endangered species.

**Other Project Personnel**

NA

**External Collaborators**

NA

**Products**

Draft chapters of SSA being handled by USFWS.

## **Population Augmentation of Rare Turtles to the Great Swamp National Wildlife Refuge**

### **Funding Entity**

Friends of the Great Swamp National Wildlife Refuge, New Jersey

### **Start Date and Funding Amount**

February 2019; \$16,500

### **SREL Collaborators**

Dr. Kurt A. Buhlmann, Senior Research Associate

### **Objectives**

1. Recovery of a regionally threatened turtle species in New Jersey.
2. Initiated in 2011, this project seeks to establish a viable, recovered population of a threatened species using various population augmentation methods.

### **Summary of Research Activities**

1. Adult female turtles are tracked with radio telemetry.
2. Nests are protected from predators.
3. Hatchlings are headstarted in collaboration with a high school that specializes in offering a natural resource management curriculum.
4. Headstarts are returned to the Great Swamp National Wildlife Refuge and monitored for survival, growth, and site fidelity.
5. Habitat restoration activities are also implemented in collaboration with USFWS Refuge staff.

### **Conclusions**

1. Headstart survival is greater than survival of directly-released hatchlings of the same cohort.
2. Headstarted turtles behave normally and display site fidelity.
3. In 2019 and again in 2020, individuals from the 2011 headstarted cohort reached reproductive maturity and produced hatchlings of their own.

### **Major Impact(s) of Research**

1. Headstarting can help recover depleted natural populations of threatened turtle species if the causes of initial decline are satisfactorily addressed.
2. Headstarting puts more juvenile turtles on a trajectory to maturity than would happen naturally.
3. Headstarting reduces the number of years required to attain maturity, thus population recovery can, in theory, be sped up.

### **Other Project Personnel**

NA

### **External Collaborators**

Marilyn Kitchell, U.S. Fish and Wildlife Service, Great Swamp NWR, NJ

Colin Osborn, U.S. Fish and Wildlife Service, Great Swamp NWR, NJ

Brian Bastarache, Bristol County Agricultural High School, Massachusetts

James Angley, New Jersey DEP

Brian Zarate, New Jersey DEP

### **Products**

Wood turtles on the verge at Great Swamp | by U.S. Fish & Wildlife Service Northeast Region |  
Conserving the Nature of the Northeast | Medium Newsletter-July-2020.pdf  
(friendsofgreatswamp.org)

**Headstarting as a Species Population Augmentation and Recovery Tool for a Threatened Turtle Species**

**Funding Entity**

Longleaf Alliance

**Start Date and Funding Amount**

August 2019; \$49,999

**SREL Collaborators**

Dr. Kurt A. Buhlmann, Senior Research Associate

Dr. Tracey D. Tuberville, Associate Research Scientist

**Objectives**

The objectives of the research are designed to increase the efficacy of headstarting as a species population augmentation and recovery tool for threatened turtle species, specifically the Gopher tortoise.

**Summary of Research Activities**

Nests are obtained by searching female tortoise burrows on state-owned land, as well as private lands.

Nest searches with this funding support have been conducted in Summers of 2019 and 2020. Nests finish incubating at SREL and hatchlings from each year's cohort are raised inside a controlled temperature greenhouse for approximately one year. At that time, they are released back at the same properties from which the eggs were obtained.

**Conclusions**

The project is on-going.

**Major Impact(s) of Research**

Determine benefits of headstarting to Gopher Tortoise conservation and population recovery.

**Other Project Personnel**

Amelia Russell, Graduate Student, SREL

**External Collaborators**

Lisa Lord, Longleaf Alliance

Andrew Grosse, Herpetologist, SCDNR

Craig Stockwell, private landowner

**Products**

Progress Report: Tuberville, T.D. and K.A. Buhlmann. SREL Status Update to Longleaf Alliance on Gopher Tortoise Head-starting Efforts, submitted to Longleaf Alliance. Nov 2020.

## **Support for Headstarting Gopher Tortoises**

### **Funding Entity**

Longleaf Alliance

### **Start Date and Funding Amount**

April 2020; \$2,500

### **SREL Collaborators**

Dr. Kurt A. Buhlmann, Senior Research Associate

Dr. Tracey D. Tuberville, Associate Research Scientist

### **Objectives**

The specific goals of this funding support are to fund travel to gopher tortoise sites to collect eggs for the headstarting process in 2020 and 2021. Funds will also be used to purchase field supplies.

### **Summary of Research Activities**

A total of 21 hatchling gopher tortoises has been successfully hatched from eggs collected in 2020. These individuals will be raised in SREL facilities during Fall 2020-Summer 2021.

### **Conclusions**

None to date.

### **Major Impact(s) of Research**

Determine benefits of headstarting to Gopher Tortoise conservation and population recovery.

### **Other Project Personnel**

Amelia Russell, Graduate Student, SREL

### **External Collaborators**

Lisa Lord, Longleaf Alliance

Andrew Grosse, Herpetologist, SCDNR

Craig Stockwell, private landowner

### **Products**

NA

**Monitoring Cadmium Levels in Avian Blood Samples from Sentinel Species Associated with the Savannah Harbor Expansion Project**

**Funding Entity**

U.S. Army Corps of Engineers

**Start Date and Funding Amount**

October 2019; \$83,929

**SREL Collaborators**

Olin E. Rhodes, Jr., Travis L. DeVault, A. Larry Bryan

**Objectives**

The overall goal of this research is to examine potential Cd risk to avifauna related to dredging activities during the Savannah Harbor Expansion Project.

**Summary of Research Activities**

The Savannah Harbor Expansion Project (SHEP) involves dredging the Savannah River and will eventually result in deposition of sediment containing potentially high concentrations of Cadmium (Cd) into dredged material containment areas (DMCAs) near Savannah, GA. Cadmium is a toxic metal with both natural and anthropogenic sources and no known nutritional value. Effects of toxic concentrations of Cd on birds range from organ damage to behavioral changes. The SHEP DMCAs contain upland and aquatic habitats and are bounded by salt marsh and riverine habitats and are attractive to a wide range of avifauna. Dredged material deposition may be a potential cadmium exposure route for birds attracted to these impoundments. As part of the monitoring program to determine potential environmental impacts associated with the SHEP, we examined Cd uptake by sentinel avian species associated with containment areas from 2014 – 2020, before dredging of the Cd-laden sediments began (scheduled to occur in late 2020). The primary approach to assess Cd risk was to analyze blood for Cd from the sentinel resident and migratory species because concentrations of Cd in blood are indicative of recent uptake in adult birds. During the 6th year of this project (2019-2020), we continued to capture and sample sentinel avian species and conduct chemical (primarily Cd) analyses. During this year's sampling, we found that only 52 of the samples (~62%) were above the minimum detection limit of 0.00134 ppm wet weight for Cd in blood, and the maximum blood Cd concentration was 0.0553 ppm wet wt. (in a Black-necked Stilt), far below the blood concentration (0.26 ppm wet wt.) associated with possible toxic effects for Cd.

**Conclusion**

Concentrations of Cd in blood of avian sentinel species from the Savannah Harbor DMCAs during pre-Cd deposition were generally low.

**Major Impact(s) of Research**

Determine whether the Savannah Harbor Expansion Project will introduce risk of toxic Cadmium uptake by native wildlife, as determined by sampling of avian sentinels.

**Other Project Personnel**

Matt Strassburg, Biological Sciences Technician, SREL

**External Collaborators**

Dr. Suzanne Wilde (UGA)

**Products**

O.E. Rhodes, Jr., T.L. DeVault, and A.L. Bryan. 2020. Monitoring potential cadmium levels in avian tissues associated with the Savannah Harbor Expansion Project: Year 6 of monitoring. Annual report to the U.S.A.C.E., Savannah District. University of Georgia's Savannah River Ecology Laboratory, Aiken, SC.

## **Adaptive and Disruptive Epigenome-by-environment Dynamics: Molecular Mechanisms to Ecological Impacts**

### **Funding Entity**

National Science Foundation

### **Start Date and Funding Amount**

August 2018; \$571,839

### **SREL Collaborators**

Dr. Ben Parrott

### **Objectives**

The overall goal of this research is to determine the origins of natural variation in the epigenome.

### **Summary of Research Activities**

The developmental environment can have profound impacts on an individual. For example, in many non-mammalian vertebrates including some fish, most turtles, and all crocodylians, environmental temperatures occurring during a specific period of incubation will determine if individuals develop as either a male or female. Previous findings have revealed a critical role for the endocrine system and epigenetics, heritable changes in gene function without changes in DNA sequence, in determining how the environment impacts traits. This project will determine the mechanisms by which environments and hormone signaling act on epigenetic processes to shape developmental trajectories and produce phenotypic diversity. The project focuses on species displaying temperature-dependent sex determination, to address how temperature induces and coordinates radically different phenotypic trajectories. The study will support the training and mentoring of two graduate students and two undergraduate researchers, with recruiting efforts aimed at under-represented groups in the sciences. The project will develop a primer focused on communicating common misperceptions about epigenetics. The findings will be presented to students at area schools as part of an established outreach program. Together, this work will advance the understanding of the mechanisms that determine how organisms interact with the environments and will provide training opportunities for the next generation of scientists.

The work will employ state of the art sequencing approaches to advance a developmental- and endocrine-based understanding of how adaptive epigenetic responses occur in nature, and how epigenetic responses are disrupted by environmental stressors for which a shared evolutionary history is absent. The Parrott Lab has previously observed widespread sexually dimorphic DNA methylation patterning across the gonadal genome. However, the developmental and molecular processes by which a bipotential genome acquires a sexually dimorphic epigenome is unclear. Developmental windows of environmental sensitivity will first be identified by resolving the temporal dynamics of the DNA methylome during temperature-dependent sex determination and reproductive development. Combinatorial treatments of temperature and hormones will then reveal the degree to which temperature and endocrine signals act on overlapping or distinct regions of the epigenome. Finally, the project will investigate the influence of environmental quality on the sexually dimorphic methylome. Given the inherent environmental sensitivity of temperature-dependent sex determination, it is hypothesized that environmental variables (other than temperature) also influence this process to affect the development and subsequent function of the reproductive system. Together, experiments will reveal how environmental and biological signals are integrated into developmental processes that result in phenotypic diversity. Findings from this work will be disseminated in peer-reviewed publications, presentations at national and international scientific, and more broadly via outreach activities in partnership with area schools.

### **Conclusions**

Work so far has modeled the influence of ecological and climatic factors on the thermal environment experienced by developing alligator embryos. When future climatic predications are integrated into the constructed model, sex ratios of alligator populations are predicted to be dramatically affected in the next 20 years. In the absence of significant compensatory behaviors in maternal nest site choice, skews in population sex ratios will likely negatively influence the ability of these populations to persist in the long-term.

### **Major Impact(s) of Research**

1. Establish fundamental insights into how environmental cues are sensed and integrated into developmental trajectories
2. Provide a basic understanding of how natural environmental conditions interact with contaminant exposures in biological systems

### **Other Project Personnel**

Samantha Bock, PhD Student, Odum School of Ecology

Jameel Moore, Undergraduate Researcher/Technician, Benedict College

Junsoo Baie, Undergraduate Researcher/Technician, Augusta University

### **External Collaborators**

N/A

### **Products**

- Bock, S.L., Hale, M.D., Leri, F.M., Wilkinson, P.M., Rainwater, T.R., Parrott, B.B. 2020. Post-transcriptional mechanisms respond rapidly to ecologically relevant thermal fluctuations during temperature-dependent sex determination. *Integrative Organismal Biology* [Published Early Online Access](#)
- Bock, S.L., Lowers, R.H., Rainwater, T.R., Stolen, E., Drake, J.M., Wilkinson, P.M., Weiss, S., Back, B., Guillette, L.J., Parrott, B.B. 2020. Ecological drivers of nest temperature variation in the American alligator: implications for future environmental change for a species with temperature-dependent sex determination. *Proceedings of the Royal Society B* 287:20200210
- Bae, J., Bertucci, E.M., Bock, S.L., Hale, M.D., Moore, J., Wilkinson, P.M., Rainwater, T.R., Bowden, J.A., Koal, T., PhamTuan, H., Parrott, B.B. Intrinsic and extrinsic factors interact to determine telomere length in hatchling alligators. *In Review*
- B.B. Parrott. Invited Seminar. February 25, 2020. Jekyll Island Authority Conservation and Georgia Sea Turtle Center Seminar Series. Jekyll Island, GA, USA
- B.B. Parrott. Invited Seminar. January 24, 2020. Department of Biology and Marine Biology Seminar Series. University of North Carolina — Wilmington. Wilmington, NC, USA
- Bock SL, Parrott BB. Epigenome-by-environment interactions underlying temperature-dependent sex determination in the American alligator. January 31, 2020. Oral Presentation. Odum School of Ecology Graduate Student Symposium, Athens, GA
- Bae J, Bertucci EM, Moore JA, Bock SL, Rainwater TR, Hale MD, Parrott BB. The effects of the developmental environment on telomere length in Alligator mississippiensis. January 4, 2020. Poster Presentation. Society for Integrative and Comparative Biology Annual Meeting. Austin, TX, USA
- Bock SL, Lowers RH, Rainwater TR, Hale MD, Leri FM, Parrott BB. Real-time responses to ecologically-relevant thermal fluctuations during temperature-dependent sex determination in the American alligator. January 4, 2020. Oral Presentation. Society for Integrative and Comparative Biology Annual Meeting. Austin, TX, USA
- Parrott, B.B. Environmental sex determination in the Anthropocene. 2019. Invited Seminar. Interdisciplinary Conservation Seminar Series. Athens, GA, USA
- Bock, S.L., Lowers, R.H., Rainwater, T.R., Stolen, E., Drake, J.M., Wilkinson, P.M., Weiss, S. Back, B., Guillette, L.J., Parrott, B.B. Ecological drivers of nest temperature variation in the American alligator: predicting the impact of future climatic scenarios. 2019. Oral Presentation, Palmetto Alligator Research & Management Symposium, Georgetown, SC, USA
- Bock, S.L., Lowers R.H., Rainwater, T.R., Hale, M.D., Parrott, B.B. Linking proximate mechanisms of developmental plasticity to environmental variation in nature. 2019. Oral Presentation, Odum School of Ecology Graduate Student Symposium, Athens, GA, USA
- Bock, S.L., Lowers, R.H., Rainwater, T.R., Hale, M.D., Parrott, B.B. Insights from the field: using a multi-year dataset of nest thermal profiles to investigate temperature-dependent sex determination in the American alligator. 2019. Oral Presentation, Annual meeting of the Society for Integrative and Comparative Biology.

Bock, S.L., Lowers, R.H., Rainwater, T.R., Hale, M.D., Parrott, B.B. Proximate and persistent effects of ecologically-relevant thermal fluctuations during temperature-dependent sex determination in the American alligator. 2019. Poster Presentation, North American Society for Comparative Endocrinology Biennial Meeting, Gainesville, FL, USA

Bock, S.L and Parrott, B.B. *Characterizing variation in nesting thermal dynamics of the American alligator and consequences for reproductive development*. 2-3 February, 2018, Oral Presentation, Odum School of Ecology Graduate Student Symposium, Athens, GA.

## **The Ecological Study of Birds in the Vicinity of Augusta Regional Airport at Bush Field**

### **Funding Entity**

City of Augusta, GA

### **Start Date and Funding Amount**

February 2020; \$64,789

### **SREL Collaborator**

Robert Kennamer

### **Objectives**

Conduct bird hazard research associated with the placement of a wastewater treatment wetland system adjacent to a commercial airport and provide wildlife hazard consultation to airport and wastewater treatment plant personnel.

### **Summary of Research Activities**

Since December 2001, we have been monitoring temporal and spatial activities of birds around Augusta Regional Airport. Within 2 years of the 2001 completion, the 360-acre wastewater treatment wetlands served as a nighttime roost for millions of migratory blackbirds that crossed the airfield daily at sunrise and sunset during fall/winter periods. We have been investigating the use of habitat alteration techniques to displace blackbirds, including use of airboats since 2008 to mechanically crush wetland vegetation in treatment wetlands each fall. Results have been highly significant, with long-term post-crush bird monitoring indicating that blackbird roosting within the wetlands became almost non-existent and blackbird activity around the airport was reduced. The ability of the wetland vegetation to process wastewater effluent was not negatively affected by vegetation alteration.

### **Conclusions**

1. Long-term monitoring showed a reduction in blackbirds around the airport by 2 orders of magnitude and this reduction has been maintained for seven years now.
2. Fall crushing of wastewater treatment wetlands vegetation has not harmed the vegetation and regrowth has been experienced each subsequent spring.

### **Major Impacts of Research**

1. The work demonstrated that with thoughtful wildlife hazard management, including the use of novel techniques, one can mitigate undesirable wildlife attraction associated with certain land-use activities.
2. Successful reduction of the bird-aircraft strike hazard was accomplished through non-lethal means.
3. Wastewater effluent concentrations of TSS, NH<sub>3</sub>-N, and BOD<sub>5</sub> were reduced (improved) as a result of the vegetation crushing (i.e., vegetation crushing contributed an added benefit).

### **Other Project Personnel**

Matthew Strassburg, Research Technician III - SREL

### **External Collaborators**

D. Allen Saxon, Jr. - Augusta, GA Utilities Department

Tim Weegar - Augusta Regional Airport

### **Products**

Kennamer, R. A., M.D. Strassburg, and I. L. Brisbin, Jr. 2019. Abundance, Distribution, and Movement Patterns of Avifauna in the Vicinity of Bush Field Airport: 2018–2019 Report. Final report submitted to Augusta Utilities Department, 174pp.

Kennamer, R. A. and M.D. Strassburg. Highlights from the 2018-19 report on bird activity around AGS and the Constructed Wetlands. Wildlife Hazard Management Group Meeting, Augusta Regional Airport, GA. November 14, 2019. (Oral Presentation).

Kennamer, R. A. and M.D. Strassburg. An update of SREL's recorded bird activity around Augusta Regional Airport. Wildlife Hazard Management Group Meeting, Augusta Regional Airport, GA. May 19, 2020. (Oral Presentation via ZOOM).

**Multi-year Mating Dynamics and Population Structure in a Coastal Population of Alligator mississippiensis at the Tom Yawkey Wildlife Center**

**Funding Entity**

MUSC, SC-DNR, The Yawkey Foundation

**Start Date and Funding Amount**

April 2012; \$10,000 in 2015, \$14,800 in 2016, \$74,020 2017-2019.

**SREL Collaborators**

Dr. Stacey L. Lance

Dr. Ben Parrott

Joshua Zajdel

**Objectives**

The overall objectives in this project are 1) identify parentage for clutches of alligators from the Yawkey Wildlife Center (YWC) collected from 2011-2015, 2) characterize the breeding population with respect to animal size, range, and multiple paternity and 3) quantify the genetic diversity of alligators at YWC and compare to populations from other parts of South Carolina and Florida.

**Summary of Research Activities**

We have completed the objectives of identifying parentage and characterizing the breeding population at YWC. In total we examined 151 nests and were able to assign a mother to 78 nests and at least one father to 38. The majority of maternity assignments matched the female that was caught at the nest (80%). We have completed an initial analysis of single nucleotide polymorphisms across twenty four individuals but had significant issues when scaling up to an 8-population study. We have now received sequence data for new libraries and are analyzing the data.

**Conclusions**

1. A few large males are achieving most of the male reproductive success.
2. Females appear to pay a fitness cost by mating multiply.
3. Data are still being collected for the population genetics objective.

**Major Impact(s) of Research**

1. Mating systems of alligators appear to be driven by sexual conflict between males and females.
2. Multiple mating may overall reduce alligator fertility and decrease genetic diversity.
3. Our population genetics data will impact management practices moving forward as pressure increases to expand the hunting of alligators.

**Other Project Personnel**

NA

**External Collaborators**

Dr. Thomas Rainwater, Clemson University, Georgetown, SC

Phil Wilkinson, SC DNR, Georgetown, SC

Jamie Dozier, SC DNR, YWC, Georgetown, SC

Todd Pierson, Kennesaw State University, Kennesaw, GA

**Products**

Zajdel, J., S. L. Lance, T. R. Rainwater, P. M. Wilkinson, M. D. Hale, and B. B. Parrott. Mating dynamics and multiple paternity in a long-lived vertebrate. *Ecology and Evolution* 9:10109-10121.

Zajdel, J., S.Lance, T.Rainwater, P.Wilkinson and B.Parrott. Multiple paternity within American alligator nests at the Tom Yawkey Wildlife Center. 2019. Palmetto Alligator Research and Management Symposium. Georgetown, SC. (Platform presentation).

Zajdel, J., S.Lance, T.Rainwater, P.Wilkinson and B.Parrott. Multiple paternity within American alligator nests at the Tom Yawkey Wildlife Center. 2018 Annual meeting of the Southeastern Partners in Amphibian and Reptile Conservation in Helen, GA. (Platform presentation).

**Halidon Hill Wetland Restoration: a Case Study on the Impacts of Tree Removal Strategies on Amphibian Communities**

**Funding Entity**

SREL

**Start Date and Funding Amount**

July 2019; NA

**SREL Collaborators**

Dr. Stacey L. Lance

**Objectives**

The overall goal of this research is to experimentally examine how different wetland restoration techniques compare in terms of speed of recovery of plant and animal communities, cost, and labor. Our objectives are to 1) establish three treatments of restoration and apply them to 12-15 wetlands, 2) determine how amphibian communities respond to restoration treatments and 3) assess which treatment is the most appropriate (based on success, cost, and labor) for future restoration of longleaf ecosystem wetlands.

**Summary of Research Activities**

We have conducted site visits to categorize thirty wetlands, collect eDNA from eight wetlands, and dipnet for amphibians at ten wetlands. In conjunction with land owners, environmental consultant and Army Corp of Engineers we have now defined our three treatment conditions and are finalizing the wetlands that we will apply the treatments to.

**Conclusions**

The data are still being collected.

**Major Impact(s) of Research**

The data are still being collected.

**Other Project Personnel**

Dr. Jason O'Bryhim, Research Professional, SREL

Adam McFall, MS student, SREL

Kiersten Nelson, PhD student, SREL

Tucker Stonecypher, MS student, SREL

**External Collaborators**

Lisa Lord, The Longleaf Alliance

Richard Coen Sr., Halidon Hill

Richard Coen Jr., Halidon Hill

Travis Folk, Folk Land Management Inc.

**Products**

No publications, presentations, or reports have been prepared yet.

**External (non-SRS) Funding Received in FY20**

***The Ecological Study of Birds in the Vicinity of Augusta Regional Airport at Bush Field***

**Funding Entity**

City of Augusta, GA

**Start Date and Funding Amount**

February 2020; \$64,789

**SREL Investigators and Roles**

R. A. Kennamer (PI)

**External Collaborators**

D. A. Saxon, Jr. Augusta, GA Utilities Department

***Conservation and management of gopher frogs in South Carolina***

**Funding Entity**

Longleaf Alliance via USFWS Coastal Programs

**Start Date and Funding Amount**

August 1, 2020; \$107,000

**SREL Investigators and Roles**

S. Lance (PI)

**Co-Investigators, Roles, and Affiliations**

Lisa Lord (Co-PI) Longleaf Alliance

***Environmental and ecological determinants of epigenetic aging***

**Funding Entity**

National Science Foundation

**Start Date and Funding Amount**

September 2020; \$853,295

**SREL Collaborators**

N/A

**External Collaborators and Affiliations**

Dr. Kristen Navara, UGA

***Adaptive and disruptive epigenome-by-environmental dynamics: molecular mechanisms to ecological impacts***

**Funding Entity**

National Science Foundation

**Start Date and Funding Amount**

August 2018; \$571,839

**SREL Collaborators**

N/A

**External Collaborators and Affiliations**

N/A

***Monitoring Cadmium Levels in Avian Blood Samples from Sentinel Species Associated with the Savannah Harbor Expansion Project***

**Funding Entity**

U.S. Army Corps of Engineers

**Start Date and Funding Amount**

October 1, 2019; \$83,928.71

**SREL Collaborators**

Olin E. Rhodes, Jr., Travis L. DeVault, A. Larry Bryan

**External Collaborators and Affiliations**

Dr. Suzanne Wilde (UGA)

***Canopy complexity, physiological function, and ecosystem resilience of longleaf pine***

**Funding Entity**

Tall Timbers Research Inc.

**Start Date and Funding Amount**

08/01/2018; \$23,188

**SREL Investigators and Roles**

D. Aubrey (PI)

**Co-Investigators, Roles, and Affiliations**

None

***Comparative hydrologic budgets and water use efficiencies of developing bioenergy plantation***

**Funding Entity**

USDA National Institute of Food and Agriculture

**Start Date and Funding Amount**

09/01/2019; \$498,663

**SREL Investigators and Roles**

D. Aubrey (PI)

**Co-Investigators, Roles, and Affiliations**

R. Jackson (CoPI), University of Georgia

***Managing forests for ecological services and environmental sustainability***

**Funding Entity**

USDA Forest Service

**Start Date and Funding Amount**

05/13/2019; \$479,480

**SREL Investigators and Roles**

D. Aubrey (PI)

**Co-Investigators, Roles, and Affiliations**

None.

**Characterizing multiscale feedbacks between forest structure, fire behavior and effects: Integrating measurements and mechanistic modeling for improved understanding of pattern and process**

**Funding Entity**

US Department of Defense, Strategic Environmental Research and Development Program

**Start Date and Funding Amount**

01/23/2020; \$329,320

**SREL Investigators and Roles**

D. Aubrey (PI)

**Co-Investigators, Roles, and Affiliations**

None

**MWMF Forest Growth**

**Funding Entity**

USDA Forest Service

**Start Date and Funding Amount**

05/20/2020; \$290,000

**SREL Investigators and Roles**

D. Aubrey (PI)

**Co-Investigators, Roles, and Affiliations**

None

**Characterizing dose-dependent fire effects of vegetative thermal environment on tree stems**

**Funding Entity**

USDA Forest Service

**Start Date and Funding Amount**

08/01/2020; \$22,280

**SREL Investigators and Roles**

D. Aubrey (PI)

**Co-Investigators, Roles, and Affiliations**

None

**Spatial and temporal patterns and ecological implications of leaf litter dispersal**

**Funding Entity**

The Jones Center at Ichauway

**Start Date and Funding Amount**

08/01/2020; \$24,024

**SREL Investigators and Roles**

D. Aubrey (PI)

**Co-Investigators, Roles, and Affiliations**

None

**Managing forest ecosystem processes through physiology**

**Funding Entity**

USDA National Institute of Food and Agriculture McIntire Stennis

**Start Date and Funding Amount**

08/01/2020; \$350,561

**SREL Investigators and Roles**

D. Aubrey (PI)

**Co-Investigators, Roles, and Affiliations**

D. Johnson (co-PI), Warnell School of Forestry and Natural Resources, University of Georgia

**Evaluation of crop damage by wild pigs**

**Funding Entity**

USDA-APHIS – Wildlife Services – National Wildlife Research Center

**Start Date and Funding Amount**

February 20, 2017; \$58,883

**SREL Collaborators**

Dr. James C. Beasley (PI), Chris Boyce (student)

**External Collaborators**

Dr. Kurt VerCauteren, USDA – Wildlife Services – National Wildlife Research Center  
Steve Smith, USDA – Wildlife Services

**Distribution and relative abundance of wildlife in Fukushima along a gradient of contamination and human land-use intensity, and spatial ecology of invasive civets**

**Funding Entity**

University of Georgia, Office of International Partnerships, Office of the Vice President for Research

**Start Date and Funding Amount**

June 1, 2016; \$15,350

**SREL Collaborators**

Dr. James C. Beasley (PI), Phillip Lyons (student)

**External Collaborators**

Dr. Thomas Hinton, Fukushima University, Japan  
Dr. Kei Okuda, Fukushima University, Japan

***<sup>137</sup>Cs Activity Levels, Movement Behavior, and Efficiency of Vertebrate Scavengers in the Fukushima Exclusion Zone***

**Funding Entity**

University of Georgia, Office of International Education

**Start Date and Funding Amount**

May 1, 2018; \$5,000

**SREL Collaborators**

Dr. James C. Beasley (PI), Hannah Gerke (student)

**External Collaborators**

Dr. Thomas Hinton, Fukushima University  
Dr. Kei Okuda, Fukushima University

***Effects of Chronic Radiation Exposure on the Health of Wild Boar in Fukushima Prefecture, Japan***

**Funding Entity**

Fukushima University, Institute of Environmental Radioactivity; University of Georgia, Office of International Education; University of Georgia, Graduate School; Environmental Radioactivity Research Network Center

**Start Date and Funding Amount**

May 1, 2018; \$17,936

**SREL Collaborators**

Dr. James C. Beasley (PI), Sarah Chinn (student)

**External Collaborators**

Dr. Thomas Hinton, Fukushima University  
Dr. Kei Okuda, Fukushima University  
Ms. Aryn Bordman, Colorado State University  
Dr. Thomas Johnson, Colorado State University  
Dr. Sami Pederson, Colorado State University  
Ms. Maggie Roberts, Colorado State University  
Dr. Rao Veeramachaneni, Colorado State University  
Dr. Jodi Flaws, University of Illinois  
Dr. Yui Nemoto, Fukushima Prefecture  
Dr. Diana Lafferty, Northern Michigan University

***Spatial Ecology, Reproduction and Social Structure of Invasive Wild Pigs***

**Funding Entity**

USDA-APHIS – Wildlife Services – National Wildlife Research Center

**Start Date and Funding Amount**

September 5, 2018; \$338,960

**SREL Collaborators**

Dr. James C. Beasley (PI), Lindsay Clontz (student), Allison Rakowski (Research Professional)

**External Collaborators**

Dr. Kim Pepin, USDA – Wildlife Services – National Wildlife Research Center  
Dr. Nathan Snow, USDA – Wildlife Services – National Wildlife Research Center  
Dr. Kurt VerCauteren, USDA – Wildlife Services – National Wildlife Research Center

***Wild pig management in Sumter and Francis Marion National Forest***

**Funding Entity**

USDA-USFS

**Start Date and Funding Amount**

May 21, 2018; \$43,759

**SREL Collaborators**

Dr. James C. Beasley (PI)

**External Collaborators**

Mr. Donny Ray, USFS

***Feral swine field studies***

**Funding Entity**

USDA-APHIS – Wildlife Services – National Wildlife Research Center

**Start Date and Funding Amount**

September 5, 2019; \$200,000

**SREL Collaborators**

Dr. James C. Beasley (PI), Caitlin Kupferman (Research Professional)

**External Collaborators**

Dr. Kim Pepin, USDA – Wildlife Services – National Wildlife Research Center  
Dr. Nathan Snow, USDA – Wildlife Services – National Wildlife Research Center  
Dr. Kurt VerCauteren, USDA – Wildlife Services – National Wildlife Research Center  
Mr. Mike Lavelle, USDA – Wildlife Services – National Wildlife Research Center

**Assessment of Changes in Population Size,  
Agricultural Damage, and Environmental  
Impacts of Wild Pigs in Response to Control**

**Funding Entity**

NWRC, SCDNR, Newberry Soil and Water  
Conservation District

**Start Date and Funding Amount**

September 5, 2019; \$220,000

**SREL Collaborators**

Dr. James C. Beasley (PI), Joseph Treichler  
(student)

**External Collaborators**

Dr. Kurt VerCauteren, USDA – Wildlife  
Services – National Wildlife Research Center  
Mr. Noel Myers, USDA – Wildlife Services

**Evaluation of head-starting as a recovery tool  
for the Mojave desert tortoise**

**Funding Entity**

National Park Service

**Start Date and Funding Amount**

June 2013; \$450,000

**SREL Investigators and Roles**

Dr. Tracey Tuberville (PI), Dr. Kurt  
Buhlmann (co-PI), Pearson McGovern (student),  
Carmen Candal (student)

**Co-Investigators, Roles, and Affiliations**

Dr. Brian Todd, co-PI, University of California-  
Davis

Mr. Collin Richter, Research Technician,  
University of California-Davis

Ms. Susanna Mann, Research Technician,  
University of California-Davis

**Technical Expertise Requests in FY20**

**SREL Investigator**

R. A. Kennamer

**Date of Request**

FY2020

**Requesting Entity**

Augusta Regional Airport at Bush Field

**Nature of Request**

Wildlife hazard consultant for Augusta Regional Airport at Bush Field. Member of Augusta Regional Airport Wildlife Hazard Management Group

**SREL Investigator**

Dr. S. Lance

**Date of Request**

FY2020

**Requesting Entity**

South Carolina landowners

**Nature of Request**

Provide management recommendations for wetland restoration

**SREL Investigator**

Dr. S. Lance

**Date of Request**

FY2020

**Requesting Entity**

Halidon Hill and Pecan Hill Plantations

**Nature of Request**

Survey lands for likelihood of supporting at-risk amphibian populations

**SREL Investigator**

Dr. James C. Beasley

**Date of Request**

January 2020

**Requesting Entity**

International Atomic Energy Association

**Nature of Request**

Consult with Fukushima Prefecture, Japan

**SREL Investigator**

D.E. Scott

**Date of Request**

FY2020

**Requesting Entity**

USFS

**Nature of Request**

Consulted on bay restoration for gopher frogs and wetlands burning plans for SRS

**SREL Investigator**

D.E. Scott

**Date of Request**

FY2020

**Requesting Entity**

USGS

**Nature of Request**

Consulted on habitat suitability and population models for gopher frogs

**SREL Investigator**

D.E. Scott

**Date of Request**

September 2019

**Requesting Entity**

Amphibian Foundation, Atlanta

**Nature of Request**

Consulted on approach to breeding flatwoods salamanders and gopher frogs

**SREL Investigator**

Dr. Tracey Tuberville

**Date of Request**

FY2020 (ongoing)

**Requesting Entity**

USFWS

**Nature of Request**

Serve on Herpetofauna Repatriation Working Group

**SREL Investigator**

Dr. Tracey Tuberville and Dr. Kurt Buhlmann

**Date of Request**

FY2020 (ongoing)

**Requesting Entity**

American Zoo and Aquarium Association

**Nature of Request**

Consult on Turtle SAFE Program

**SREL Investigator**

Dr. Kurt Buhlmann

**Date of Request**

October 2019

**Requesting Entity**

Eglin AFB

**Nature of Request**

Consultation on gopher tortoise reintroduction methodology and habitat restoration needs

**SREL Investigator**

Dr. Tracey Tuberville and Dr. Kurt Buhlmann

**Date of Request**

February 2020

**Requesting Entity**

USFWS

**Nature of Request**

Participated in Structured Decision Making Workshop on Bog Turtles

**SREL Investigator**

Dr. Kurt Buhlmann

**Date of Request**

November 2019

**Requesting Entity**

National Parks Service

**Nature of Request**

Assisted with monitoring surveys of Texas tortoises

**SREL Investigator**

Dr. Tracey Tuberville

**Date of Request**

FY2020

**Requesting Entity**

USFWS

**Nature of Request**

Serve on gopher tortoise expert panel for the Species Status Assessment review process

**SREL Investigator**

Dr. Kurt Buhlmann

**Date of Request**

January 2020

**Requesting Entity**

WV DNR

**Nature of Request**

Consulted on wood turtle restitution assistance

**SREL Investigator**

Dr. Tracey Tuberville

**Date of Request**

FY2020

**Requesting Entity**

SCDNR

**Nature of Request**

Participate in guidance and development of monitoring plan associated with acquisition of confiscated box turtles transferred to SREL for repatriation on the SRS

**SREL Investigator**

Dr. Kurt Buhlmann

**Date of Request**

March 2020

**Requesting Entity**

US NAVY

**Nature of Request**

Consulted on Flatwoods Salamander management

**SREL Investigator**

Dr. Kurt Buhlmann

**Date of Request**

March 2020

**Requesting Entity**

US ARMY

**Nature of Request**

Assisted with establishment of emergency captive rearing facilities for flatwoods salamander larvae

**SREL Investigator**

Dr. Kurt Buhlmann

**Date of Request**

September 2020

**Requesting Entity**

New Jersey DEP

**Nature of Request**

Consulted on wood turtle conservation strategy

**SREL Investigator**

Dr. Kurt Buhlmann

**Date of Request**

June 2020

**Requesting Entity**

USFWS

**Nature of Request**

Served on At-Risk species gopher frog Working Group

**SREL Investigator**

Dr. Kurt Buhlmann

**Date of Request**

May 2020

**Requesting Entity**

Georgia DNR

**Nature of Request**

Provided records for Natural Heritage database

**SREL Investigator**

Dr. Kurt Buhlmann

**Date of Request**

August 2020

**Requesting Entity**

Conecuh National Forest, Alabama

**Nature of Request**

Consulted on gopher frog pond management

TASK 9. SREL scientists will work closely with SRS personnel to assist DOE and other SRS contractors in making wise and informed decisions concerning land and facilities management. SREL will continue to publish its scientific findings in peer-reviewed scientific journals to aid the public and to assist DOE in making policy decisions by providing a basis of independent, verifiable science

Please see SECTION VIII of this report for a list of SREL publications in FY20. Below we provide examples of specific activities that SREL personnel have conducted in FY20 to assist DOE and other SRS tenants with ongoing missions and to leverage federal funding provided to SREL to attract non-federal funding to conduct research activities on the SRS.

#### **Department of Energy – EM**

- SREL personnel participated in site visits with USDA personnel to evaluate potential research on wild pig control technologies on the SRS
- SREL personnel participated in site visits with USDA personnel to evaluate potential research on rabies elimination in the Southeastern US
- SREL leveraged DOE funding against UGA funding to conduct research on the development of pilot projects in proteomics/metabolomics at the SREL low dose facility to examine consequences of low dose exposures to aquatic species on the SRS
- SREL leveraged DOE funding and SRS site assets to obtain ~ 1.6 million dollars in new external funding during the FY20 fiscal year
- SREL personnel hosted multiple DOE personnel to tour SREL's analytical capabilities that might be used in support of SRS missions

#### **Department of Energy – NNSA**

- SREL personnel leveraged funding from NNSA to conduct research on biogeochemical cycling and efficiency of metal treatment of the HO2 wetland associated with the regulatory requirements of tritium production on the SRS
- SREL personnel leveraged funding from NNSA to conduct ecotoxicological research on amphibians utilizing the HO2 metal treatment wetlands to elucidate the biological effects of copper and other metals associated with tritium production on the SRS
- SREL personnel leveraged funding from NNSA to conduct studies of ecosystem health associated with the HO2 metal treatment wetlands
- SREL personnel leveraged funding from the NNSA to conduct research focused on the impacts of the former MOX construction site on the viability of upper three runs creek
- SREL outreach personnel conducted extensive community outreach and education programs for NNSA and SRS

#### **Savannah River Remediation**

- SREL provided a support to SRR on technical aspects of saltstone weathering and radionuclide release over time
- SREL personnel completed their technical support to SRR on addressing wildlife contamination issues

#### **Savannah River Nuclear Solutions**

- SREL personnel leveraged funding from ACP to conduct radioecology research programs involving environmental characterization, contaminants in wildlife, soil and water amendments, and tritium mitigation activities at the Mixed Waste Facilities on SRS
- SREL provided tours and presentations as requested to provide support to ACP during visits by regulators to the SRS

- SREL provided support to SRNS Corporate Communications by providing programs for numerous public tours to the general public or site visitors
- SREL provided several Wildlife Safety Talks to SRS site personnel

#### **Savannah River National Laboratory**

- SREL collaborated with Dr. Larry Lowe to provide research opportunities on the SRS in support of the SRNL's Minority Serving Institution Initiative
- SREL faculty collaborated with various SRNL scientists to accomplish a variety of research projects focused on environmental remediation and monitoring

#### **US Forest Service**

- SREL personnel continue to inform USFS of habitat conditions at the Gopher Tortoise (*Gopherus polyphemus*) reintroduction site (Compartment 24)
- SREL personnel met multiple times with USFS personnel to discuss potential research initiatives to establish the SRS as a center for development of feral swine control methods
- SREL personnel worked with USFS personnel to plan and implement habitat management objectives for various Set-Aside areas on the SRS to facilitate environmental stewardship objectives of the site

TASK 10. SREL will provide stipend support to college undergraduates, graduate students, and visiting faculty to conduct research on the Savannah River Site in association with ongoing environmental research studies. The objective of the program will be to provide participants, including minority students and Historically Black Colleges and Universities, with an opportunity to pursue ecological research and training under the direction and supervision of SREL scientific staff members

The 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 members 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 sixty 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 FY20, SREL faculty and staff mentored and supervised over 108 graduate students (Table 10.1) from universities across the country. 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.

During the FY20 fiscal year, an emphasis has been placed on finding creative ways to cost share graduate students by leveraging UGA funding with federal funding acquired through SREL's cooperative agreement with the Department of Energy. In addition, new emphases have been placed on enhancing participation of SREL Research Faculty in both graduate and undergraduate instruction. To these ends, SREL accomplished the following in FY20.

- SREL leveraged SRS site assets to acquire external resources to conduct UGA Maymester courses in wildlife ecology in May 2020
- SREL leveraged UGA funding against project specific funding from DOE and other sources to cost share over 40 graduate students, all of whom have projects which will contribute to the knowledge base and needs of the SRS
- SREL leveraged DOE dollars to obtain salary support for 6 faculty members to provide instructional support to UGA departments as a means to maintain critical environmental expertise on the SRS

Table 10.1. SREL Graduate Student Program Participants, FY20

Student	Degree	University	SREL Faculty	Role
Elizabeth Felt	M.N.R.	University of Georgia	Abrams	Advisor
Samantha Siragusa	M.N.R.	University of Georgia	Abrams	Advisor
Amanda Komasinski	M.S.	University of Georgia	Abrams	Advisor
Destin Kee	M.S.	University of Georgia	Abrams	Advisor
James Chapin	M.S.	University of Georgia	Abrams	Advisor
Behnoosh Abbasnezhad	Ph.D.	University of Georgia	Abrams	Advisor
Chase Cook	Ph.D.	University of Georgia	Abrams	Advisor
Abbie Judice	M.S.	University of Georgia	Abrams	Committee
Tara Denley	M.S.	University of Georgia	Abrams	Committee
Akanksha Sharma	Ph.D.	University of Georgia	Abrams	Committee
Alexander Nelson	Ph.D.	University of Georgia	Abrams	Committee
Carzon Gutierrez	Ph.D.	University of Georgia	Abrams	Committee
Maureen Essen	Ph.D.	University of Idaho	Abrams	Committee
Nicole Bernsen	Ph.D.	University of Maine	Abrams	Committee
Elliot Lewis	M.F.R.	University of Georgia	Aubrey	Advisor
Laura Fowler	M.S.	University of Georgia	Aubrey	Advisor
Ream Thomas	M.S.	University of Georgia	Aubrey	Advisor
Suzanne Henderson	M.S.	University of Georgia	Aubrey	Advisor
Jennifer McDaniel	Ph.D.	University of Georgia	Aubrey	Advisor
Scott Oswald	Ph.D.	University of Georgia	Aubrey	Advisor
Seth Younger	Ph.D.	University of Georgia	Aubrey	Co-Advisor
Luke Wilson	M.S.	University of Georgia	Aubrey	Committee
Callie Oldfield	Ph.D.	University of Georgia	Aubrey	Committee
Emilee Poole	Ph.D.	University of Georgia	Aubrey	Committee
Karuna Paudel	Ph.D.	University of Georgia	Aubrey	Committee
Monica Harmon	Ph.D.	University of Georgia	Aubrey	Committee
Chelsea Titus	M.S.	University of Georgia	Beasley	Advisor
Hannah Gerke	M.S.	University of Georgia	Beasley	Advisor
Helen Bontrager	M.S.	University of Georgia	Beasley	Advisor
Joe Treichler	M.S.	University of Georgia	Beasley	Advisor
Lindsay Clontz	M.S.	University of Georgia	Beasley	Advisor
Miranda Butler-Valverde	M.S.	University of Georgia	Beasley	Advisor
Chris Leaphart	Ph.D.	University of Georgia	Beasley	Advisor
Sara Chinn	Ph.D.	University of Georgia	Beasley	Advisor
Sara Webster	Ph.D.	University of Georgia	Beasley	Advisor

Cody Tisdale	M.S.	University of Georgia	Beasley	Co-Advisor
Ashlyn Halseth	M.S.	University of Georgia	Beasley	Committee
Kyle Lunsford	M.S.	University of Georgia	Beasley	Committee
Darren Fraser	Ph.D.	University of Georgia	Beasley	Committee
John Grinder	Ph.D.	University of Georgia	Beasley	Committee
Juan Sebastian Ortiz	Ph.D.	University of Georgia	Beasley	Committee
Rebecca McKee	M.S.	University of Georgia	Buhlmann	Committee
Christine Fallon	M.S.	University of Georgia	Capps	Advisor
E. Madison Monroe	M.S.	University of Georgia	Capps	Advisor
Denzell Cross	Ph.D.	University of Georgia	Capps	Advisor
Keysa Rosas-Rodriguez	Ph.D.	University of Georgia	Capps	Advisor
Emily Martin	M.S.	University of Georgia	Capps	Co-Advisor
Kyle Connelly	M.S.	University of Georgia	Capps	Co-Advisor
Greg Jacobs	Ph.D.	University of Georgia	Capps	Committee
Jeffery Beauvais	Ph.D.	University of Georgia	Capps	Committee
Lynda Bradley	Ph.D.	Emory University	Capps	Committee
Sophie Racey	Ph.D.	University of Georgia	Capps	Committee
Suneel Kumar	Ph.D.	University of Georgia	Capps	Committee
Carson Pakula	M.S.	University of Georgia	DeVault	Co-Advisor
Shane Guenin	M.S.	University of Georgia	DeVault	Co-Advisor
David Bernesconi	M.S.	University of Georgia	Dharmarajan	Co-Advisor
Eric Neff	M.S.	University of Georgia	Dharmarajan	Co-Advisor
Wesley Dixon	M.S.	University of Georgia	Dharmarajan	Co-Advisor
Pooja Gupta	Ph.D.	University of Georgia	Dharmarajan	Co-Advisor
Alec Thompson	Ph.D.	University of Georgia	Dharmarajan	Committee
Julia Fredrick	Ph.D.	University of Georgia	Dharmarajan	Committee
Adam McFall	M.S.	University of Georgia	Lance	Advisor
Corinne Sweeney	M.S.	University of Georgia	Lance	Advisor
Tucker Stonecypher	M.S.	University of Georgia	Lance	Advisor
Cara Love	Ph.D.	University of Georgia	Lance	Advisor
Kiersten Nelson	Ph.D.	University of Georgia	Lance	Advisor
Elizabeth Shadle	M.S.	Virginia Tech	Lance	Committee
Matthew Tatz	M.S.	University of Georgia	Lance	Committee
Jordan Youngmann	Ph.D.	University of Georgia	Lance	Committee
Andrew Ward	M.S.	University of Georgia	Martin	Advisor
Bradley Kubecka	M.S.	University of Georgia	Martin	Advisor
Emily Prosser	M.S.	University of Georgia	Martin	Advisor
Justin Rectenwald	M.S.	University of Georgia	Martin	Advisor
Michael Hazelbaker	M.S.	University of Georgia	Martin	Advisor

Rachel Gardner	M.S.	University of Georgia	Martin	Advisor
Shelby Simons	M.S.	University of Georgia	Martin	Advisor
Craig Marshall	Ph.D.	University of Georgia	Martin	Advisor
Justin Hill	Ph.D.	University of Georgia	Martin	Advisor
Heather Levy	M.S.	University of Georgia	Martin	Committee
Kelsey McClearn	M.S.	University of Georgia	Martin	Committee
Seth Wyckoff	M.S.	University of Georgia	Martin	Committee
Tori Mezebish	M.S.	University of Georgia	Martin	Committee
Angela Burrow	Ph.D.	University of Georgia	Martin	Committee
Melanie Kunkel	Ph.D.	University of Georgia	Martin	Committee
Patrick Wrightman	Ph.D.	University of Georgia	Martin	Committee
Thomas Prebyl	Ph.D.	University of Georgia	Martin	Committee
Chris Smaga	M.S.	University of Georgia	Parrott	Advisor
Josiah Johnson	M.S.	University of Georgia	Parrott	Advisor
Kristen Zemaitis	M.S.	University of Georgia	Parrott	Advisor
Laura Kojima	M.S.	University of Georgia	Parrott	Advisor
Emily Bertucci	Ph.D.	University of Georgia	Parrott	Advisor
Samantha Bock	Ph.D.	University of Georgia	Parrott	Advisor
Ashley LaVere	M.S.	University of Georgia	Parrott	Committee
Olivia Delgado	Ph.D.	University of Georgia	Parrott	Committee
Natalia Gelvez	Ph.D.	University of Georgia	Parrott	Committee
James Helton	M.S.	University of Georgia	Rhodes	Advisor
Natalia Perez-Gomez	Ph.D.	University of Georgia	Rhodes	Committee
Emily Dorward	M.S.	University of Georgia	Seaman	Advisor
Jarad Cochran	M.S.	University of Georgia	Seaman	Advisor
Liyun Zhang	Ph.D.	University of Georgia	Seaman	Advisor
Amelia Russell	M.S.	University of Georgia	Tuberville	Co-Advisor
Emma Browning	M.S.	University of Georgia	Tuberville	Co-Advisor
Kyle Brown	M.S.	University of Georgia	Tuberville	Co-Advisor
Pearson McGovern	M.S.	University of Georgia	Tuberville	Co-Advisor
David Lee Haskins	Ph.D.	University of Georgia	Tuberville	Co-Advisor
Meghan Kelley	Ph.D.	Auburn University	Tuberville	Committee
Brittany Jensen	M.S.	University of Georgia	Xu	Co-Advisor

TASK 11. The participant will operate and maintain the SREL facilities on the SRS to efficiently and successfully perform the research, education, and outreach programs described in this project description (Appendix A of the Cooperative Agreement)

### **Facilities Maintenance**

SREL is the custodian of twelve DOE-owned buildings; the largest of these is the 45,000 square foot main laboratory and office complex. SREL also maintains a 4,000 square foot radioecology laboratory located near Par Pond, four animal holding facilities, a greenhouse complex, two office buildings, and an assortment of utility buildings (maintenance shops, receiving building, and storage sheds). SREL is also the custodian of building 772-25B in B-Area, an 8000 square foot building that contains 12 laboratories in various states of functionality and renovation.

SREL operates its own maintenance staff consisting of three fulltime technicians and three part-time temporary workers. This group is responsible for grounds maintenance, routine infrastructure repair, and preventive maintenance duties for over 120 infrastructure assets. Our maintenance group also undertakes a limited number of fabrication projects in support of our research efforts. Our maintenance crew has expertise in welding-fabrication, vehicle repair, construction-renovation, electrical-HVAC, and equipment operation. This capability allows us to address facilities issues in an efficient and cost-effective manner.

SREL made a significant operation change this year with regard to custodial maintenance. Our most recent fulltime custodian retired early in FY20. We ultimately decided to enter a contract with a professional custodial company to clean our facilities. This arrangement is working well and gives us greater cleaning capability among our facilities. The price differential between maintaining permanent custodial staff and hiring a contractor only slightly increases our operational costs, which are offset by an increased cleaning capability.

As a partner on the Savannah River Site, SREL strives to maintain its facilities to comply with all the DOE guidelines for property use and safety standards. We have also worked to develop safe, attractive facilities that provide our scientists with the best possible environment within which to conduct their research. To that end, we have set aside significant parts of our overhead budget and dedicated many personnel hours to the maintenance and renovation of our facilities.

Our major facilities improvements this year were centered around the total renovation of 5 of our laboratories. Three of those labs are located in our main 737-A building and the other two are located at or 772-25B facility. In each lab, all of the existing furniture, casework, fume hoods, and flooring were removed. Two of the labs contained excess equipment, furniture, and chemicals which had to be excessed or properly disposed of. The labs were then repainted, the suspended ceilings were replaced, and new LVT vinyl flooring was installed. The electrical, HVAC, ventilation, lighting, and plumbing systems were reworked. Lastly, a contractor was hired to install the new lab furniture. These renovations mark the 28<sup>th</sup> lab renovation we have completed during the last six years.

Over the last year we also completed a number of other significant renovations to our facilities. Some of these significant projects are summarized below.

**Replacement of architectural plate glass windows:** Our main building complex, 737-A, was designed and constructed such that most of its exterior walls in the office section are double-paned, vacuum-sealed architectural glass. These panels are typically four-foot-wide by eight-foot-high and serve as the exterior walls for most of our offices. We have experienced a number of seal failures with these glass units, which led to occluded windows that cannot be cleaned, water intrusions, and air leaks. During the past year we replaced eight of these glass panels.

**Continued renovation of faculty and staff offices:** This year we completely renovated four offices. This included re-carpeting, painting, furnishing, and making other necessary repairs. These renovations marked our 40th office renovation over the last five years. At present, 80% of our offices have been completely renovated.

**Improvements to air handling infrastructure (HVAC):** This year we replaced three of our HVAC distribution boxes with a new electronically controlled air damper distribution system. This new system allows for better control of specific room temperatures and is more efficient. This system is replacing 40-year-old manual air dampers that were performing poorly.

**Installation of LED lighting:** SREL maintenance continued the phased replacement of the fluorescent lighting throughout our facility with new LED fixtures. To date, approximately 65% of the old fixtures have been replaced with LEDs and the new fixtures have dramatically improved interior lighting throughout the facility. Our goal is to eventually replace all the lighting in our main facility with efficient LED lighting.

We also continued our emphasis on cleaning and organization this year. Our property coordinator leads our efforts to clean laboratories and storage facilities by disposing of any unneeded supplies and excessing surplus equipment. We made significant progress in this area and will continue to work to improve our facilities in terms of proper organization and housecleaning. To that end we added two new storage sheds to our existing inventory to provide new faculty with an appropriate space to store equipment and supplies. Currently, every SREL faculty and most research professionals have a designated storage shed.

While much has been achieved this past year, SREL remains committed to aggressively pursuing our goal of developing facilities that comply with DOE guidelines, as well as reflect positively on our staff and research efforts. We will continue to use our in-house maintenance staff and available funding to cost-effectively maintain the DOE-owned facilities we occupy.

### **Environmental Health and Safety (EH&S) Program**

SREL continues to operate successfully under safety and environmental requirements and standards established by UGA, the SREL Safety Manual, and the SRS Policy Manual established by DOE. 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 maintained a commitment of two fulltime safety support positions (SREL EH&S Manager and Environmental Safety Coordinator) in FY20. The SREL EH&S Manager serves as the manager of SREL's safety and environmental compliance programs. The SREL Environmental Safety Coordinator provides focused laboratory research-related safety and hazardous waste management support to SREL researchers. The SREL EH&S Manager interfaces with UGA's safety programs, DOE-SR's Safety and Compliance oversight programs, and SRS Contractor Environmental Health and Safety Programs, Committees, and Professionals to implement the safety and environmental compliance programs for SREL.

The SREL EH&S Manager functions as an interface with other SRS organizations in receiving and distributing applicable safety and environmental related 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 12 targeted lessons learned and safety notices in FY20 to specific worker groups at SREL. Additionally, more than 100 SRS operational safety and environmental related announcements and notices were communicated 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 2 work related, recordable injury/illness during FY20.

The SREL EH&S Program continues to place an emphasis on safety and environmental training of SREL personnel. All new SREL personnel receive an initial, 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 general SREL safety training and job-specific safety training provided by their SREL supervisor. Approximately 28 SREL personnel received this required training during FY20. Additionally, SREL personnel received EH&S related training during FY20 in the following functional areas as their job tasks required:

- Radiological Training – Radiological Worker Training, Advanced 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 that utilize hazardous chemicals in the work place.
- Resource Conservation Recovery Act (RCRA) training for employees involved in the management, handling, or manipulation of hazardous or universal wastes.

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 continue to be incorporated into experimental protocols, reducing the generation of chemical wastes while supporting the SRS's pollution prevention efforts. SREL generated approximately 382 pounds of hazardous wastes in FY20. One hundred percent of the hazardous wastes generated was from disposal of laboratory research process generated wastes. As part of SREL waste minimization efforts and to ensure that chemical hazards are addressed prior to purchasing chemicals, the SREL Environmental Safety Coordinator reviewed and approved separate chemical purchase orders made by SREL personnel.

SREL received no Notices of Violation in FY20 as the result of external or internal reviews, inspections, or assessments. During FY20, SREL's assigned DOE Facility Representative (FR) conducted periodic walk-down inspections of SREL operated SRS facilities during which minor safety issues were identified and promptly corrected. Additionally, 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.

### **Analytical Services**

The SREL Analytical Services was established to assist SREL researchers with sample preparation and to provide in-house analysis of metals/metalloids and mercury from environmental samples. Fee for Service analysis by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) provides researchers with data on metals/metalloids. Total mercury analysis employing a Milestone DMA-80 Direct Mercury Analyzers is

also available with a fee for service. Multiple research programs at SREL rely on metals and mercury analysis for a wide range of research projects through multiple funding agencies.

Six laboratories have dedicated workspace for all tasks associated with sample prep through analysis. One full-time position (SREL Analytical Services Manager) is dedicated to maintain these lab spaces and to operate and maintain the equipment. The manager maintains Standard Operating Procedures, Project Safety Appraisal Forms, and provides and documents Job Specific Training in sample preparation and equipment operations. In addition, the manager performs chemical coordinator duties and maintains all safety related information including chemical inventories and safety data sheets. The manager coordinates equipment operation schedules and maintenance. These services allow students and other researchers to receive hands-on experience in sample preparation and equipment operation. In FY20, 16 people received training to use the analytical services labs.

Instrumentation, services, and sample preparatory equipment include:

**ICP-MS Analysis:** The Perkin-Elmer NexION 300X is a fee for service ICP-MS. The Analytical Services manager maintains this instrument, performs calibrations, quality control checks, and analyzes samples for a fee. In FY20, we analyzed 3,197 samples that provided data for 10 research groups at SREL including graduate students, post docs, and principle investigators.

**Mercury Analysis:** A Milestone DMA-80 measures total mercury following EPA Method 7473. This is a fee for service instrument with charges per sample for analysis. The manager is responsible for maintenance, calibration, quality control checks, and data output. The manager provides training to students and researchers for operation of these instruments. In FY20, 7 researchers, including graduate students, post-docs, technicians, and principle investigators, performed total mercury analysis on 803 samples. The Brooks Rand MERX Methyl mercury analyzer is currently used by one research group. In FY20, approximately 61 samples were analyzed for methyl mercury.

**Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES):** The Perkin Elmer Optima 4300DV analyzes samples for trace and major elements. This instrument is available on a supply reimbursement basis with a small fee imposed for argon use. In FY20, 67 samples were analyzed for two research groups.

**Sample Preparation for Metals and Mercury:** Four laboratories house three chemical fume hoods, two laminar flow clean hoods, and two CEM microwave digestion ovens. These are used for acid digestions for metals analysis and for sample and calibration standards preparation. Four freeze dryers with a total capacity of 50 ports, 4 drying ovens, sample-grinding equipment, and an analytical balance are available for use for sample preparation. Consumables are supplied by either the researchers or are provided on a supply reimbursement basis in lieu of fees for lab use. Multiple research programs benefit from these dedicated lab spaces that allow for streamlined sample processing in a well-equipped setting while following SREL lab safety guidelines.

A new CEM microwave digestion oven was installed in FY20. This unit replaces one that has been re-located to a newly renovated additional lab space. The oldest Milestone DMA-80 was taken out of service due to age and unavailability of parts and service by the manufacturer. A new purchase of a Milestone DMA is being considered for the upcoming FY21 to relieve the burden on the DMA currently under heavy use.

FY20 saw a new challenge to the Analytical Services labs due to COVID-19 precautions. Sample preparation and analysis was slowed with reduced numbers of working days to process samples. Shared lab space was carefully coordinated to allow staff to socially distance.

Analytical Services FY20 Summary

Personnel Trained in FY20	Number of Individuals
Graduate Students	3
Faculty	1
Post Docs	2
Technicians/Research Professionals	10
Total	16

Equipment Description	Number of Samples Analyzed	Number of Users/Research Groups	Number of Days/Times Used
ICP-MS	3,197	Samples from 10 Groups	93 days
DMA-80 Mercury Analyzer	803	7 users, 7 groups	50
Methyl Mercury Analyzer	61	1 user/group	N/A
ICP-OES	67	2 users, 2 groups	2
CEM Microwaves (3)	N/A	5 users, 4 groups	33
Freeze Dryers (4)	N/A	20 users, 9 groups	261

TASK 12. UGARF will be responsible for management and engineering services for the planning, design, and construction of approved projects as may be required to repair, modify, or upgrade existing facilities or construct new facilities, not to include line item projects, necessary to support the UGARF scope of work, as approved by the Contracting Officer and appropriate DOE program personnel. Funding for major repairs and new construction will be provided by DOE

No new construction was undertaken in FY20. See Task 11 (above) for summary of facilities upgrades.

### **SECTION III. Cost Status Report**

Provided to DOE-SR budget office monthly and final FY20 report was submitted on time.

### **SECTION IV. Schedule Status Report**

No significant changes in the schedule of deliverables or achievement of milestones were experienced by SREL in FY20.

### **SECTION V. Changes in Approach or Goals**

In FY20 SREL continued to implement a number of cost sharing initiatives with main campus units at UGA designed to improve accountability, facilitate the conduct of business, and focus resources and procedures within those areas deemed most critical to carrying out the mission of the laboratory. These initiatives include:

- Cost sharing of 6 tenure track faculty lines with UGA main campus units (3 housed at SREL and 3 housed at UGA)
- Cost sharing graduate student stipends with UGA main campus units to leverage additional graduate students working on research issues on the SRS
- Leveraging research funding with UGA main campus faculty and with external funding agencies to increase SREL-based research activities on the SRS in mission critical areas such as radioecology and human-wildlife conflict resolution
- Cost sharing support personnel salaries such as the Outreach Program and equipment costs with the Office of the Vice President for Research at UGA to increase the quality of SREL programs

In addition, the director of SREL has challenged the research scientists and staff at the laboratory to increase the proportion of total funding received by the laboratory from sources external to the SRS in an effort to both diversify funding streams for the laboratory and effectively leverage federal dollars to attract external funding to the SRS. In FY20, external funding (non-SRS or UGA dollars) totaled 23% of the laboratories externally funded budget.

## **SECTION VI. Actual or Anticipated Problems, Delays and Remedial Actions**

Savannah River Nuclear Solutions has withdrawn their support for SREL participation in public tours on the SRS. As a result, the SREL director chose to redirect DOE-SR funding to cover the deficit and continue to provide SREL support for the SRNS program. In the latest Facilities Service Agreement with SRNS, SREL has indicated that it will continue to try to provide support for public tours as long as it (SREL) has the funding to support these activities.

SREL continues to work with SRNS to achieve a balance in Site Services that meets the needs of the laboratory as it increases in size and work scope to meet the needs of the SRS site tenants. Delays have occurred in delivery of services to SREL for a variety of activities despite the availability of funding. The inability to get these issues resolved has resulted in delays in research activities as well as unexpected costs to SREL's operating budget to prepare facilities for renovation or repairs. The SRS Interface Management Team has been helpful in resolving a number of these issues and with their help, there have been some success stories in FY20 despite these delays.

## **SECTION VII. Absence or Changes in Key, Non-temporary Personnel or Team Arrangement**

### **Administrative**

No Change

### **Support Staff**

No Change

### **Tenure-track Faculty**

No Change

### **Research Faculty**

Hired – Dr. Raven Bier

### **Postdoctoral Researchers**

Hired – Dr. Jacob Hill

Hired – Dr. Zeinah Baddar

Hired – Dr. Chongyang Qin

### **Research Professionals**

Hired – Caitlin Kupferman

Separated – Matt Baker

### **Safety**

No Change

### **Outreach Personnel**

Separated – Megan Winzeler

Separated – Natalie Herrington

### **Research Technicians**

Separated – Morgan Shapiro

Separated – Katherine McCallie

Separated – Kaitlin Wilms

Separated – Abby Riggs

**SECTION VIII. Products or Technology Transfer Accomplished: Publications, Websites, Collaborations, Technologies, Inventions/patents, other Products**

**SREL faculty and staff added 78 new publications to the SREL reprint list in FY20**

- 3524 Webster, S. and J.C. Beasley (2019). "Influence of Lure Choice and Survey Duration on Scent Stations for Carnivore Surveys." *Wildlife Society Bulletin* 43(4): 661-668.
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## **SECTION IX. Special Accomplishments by Laboratory Personnel**

- Olin Rhodes was named a UGA Athletic Association Professor of Applied Ecology
- James Beasley won the University of Georgia's Warnell Alumni Association Faculty Award for Research
- James Beasley was awarded The Wildlife Society's Publication Award in the Edited Book category for Ecology and Management of Terrestrial Vertebrate Invasive Species in the United States
- James Beasley was awarded Special Recognition by the USDA National Wildlife Research Center for his co-edited book Ecology and Management of Terrestrial Vertebrate Invasive Species in the United States
- Krista Capps won a CAREER Award from the National Science Foundation
- SREL graduate students won more than 15 competitive scholarships or received awards for presentations at regional, national or international meetings
- SREL research was highlighted in print, TV, and web-based media hundreds of times
- Numerous SREL faculty were asked to serve as peer reviewers on national funding panels for NSF, USDA, and other entities
- SREL Faculty were asked to give over 40 invited presentations to professional audiences in FY20