

**Alabama Water Resources Research Institute
Annual Technical Report
FY 2012**

Introduction

The Alabama Water Resources Research Institute (AL-WRRI) was created in 1964 by the Alabama Legislature. In 2007 the AL-WRRI was combined with the newly created Auburn University Water Resources Center (AU-WRC), and in 2008 it was designated as part of the Auburn University Center of Excellence for Watershed Management by EPA. The AU-WRC and AL-WRRI function as a single university-based interdisciplinary, problem-oriented research and technology center under one Director with support from the federal government through the USGS that enables the programs to address broad national needs and relevant industrial technology.

The Alabama Water Resources Center and Research Institute coordinates research programs that contribute to the solutions of present and emerging water resources problems. In carrying out this mission, the Institute has developed a broadly based research, training, information transfer, and public service program involving personnel from many academic disciplines in the state's research universities

The Alabama Water Resources Center and Research Institute is one of 54 water resources institutes nationwide authorized by the federal Water Resources Research Act. The state-based Water Resources Research Institutes are located at land grant universities and function as a nation-wide network to promote research and information dissemination on the state's and nation's water resources problems.

Research Program Introduction

The essential ingredient for determining proper policies and practices is factual information. Often such information must be obtained by means of scientific research. The Institute conducts a program that stimulates, sponsors, and provides for research, investigation, and experimentation in the fields of water and of resources as they affect water, and encourages the training of scientists in the fields related to water.

Objectives of the AU-WRC and AL-WRRI are:

To plan, conduct and otherwise arrange for competent research that fosters (a) the entry of new research scientists into the water resources fields, (b) the training and education of future water scientists, engineers and technicians, (c) the preliminary exploration of new ideas that address water problems or expand understanding of water and water-related phenomena, and (d) the dissemination of research results to water managers and the public.

To identify major research needs and develop for Alabama and the Southeastern Region short- and long-term research priorities.

To encourage research applying to other environmental resources closely associated with water.

To maintain close consultation and collaboration with governmental agencies, public groups, and cooperate closely with other colleges and universities in the state that have demonstrated capabilities for research, information dissemination, and graduate training in order to develop a statewide program designed to resolve state and regional water and related land problems.

Forecasting toxic cyanobacterial blooms throughout the southeastern U.S.

Basic Information

Title:	Forecasting toxic cyanobacterial blooms throughout the southeastern U.S.
Project Number:	2011AL121G
Start Date:	9/1/2011
End Date:	8/31/2014
Funding Source:	104G
Congressional District:	3rd
Research Category:	Water Quality
Focus Category:	Models, Nutrients, Surface Water
Descriptors:	None
Principal Investigators:	Alan Elliott Wilson, Kevin Schrader, Russell Alan Wright

Publications

1. Wilson, Alan E.; Michael F. Chislock. In press. Ecological control of cyanobacterial blooms in freshwater ecosystems. in ed. Aloysio Ferrão-Filho, Cyanobacteria: Toxicity, ecology, and management. Hauppauge, New York, Nova Science Publishers, xx-xx.
2. Wilson, Alan E.; Michael F. Chislock. In press. Ecological control of cyanobacterial blooms in freshwater ecosystems. in ed. Aloysio Ferrão-Filho, Cyanobacteria: Toxicity, ecology, and management. Hauppauge, New York, Nova Science Publishers, xx-xx.

ANNUAL TECHNICAL REPORT SYNOPSIS

A. PROJECT TITLE:

USGS Project 2011AL121G – Forecasting toxic cyanobacterial blooms throughout the southeastern U.S.

Project website - http://wilsonlab.com/bloom_network/

B. PRIMARY PI(s): Name(s), Title(s) & Academic Rank(s)

Alan E. Wilson, Assistant Professor, Ph.D.

C. OTHER PI(s): Name(s), Title(s) & Academic Rank(s)

Russell A. Wright, Associate Professor, Ph.D.

Kevin Schrader, Microbiologist, Ph.D.

D. START DATE:

1 October 2011

E. END DATE:

30 September 2014

F. PROJECT OVERVIEW/SUMMARY: Provide a brief narrative overview or summary of the project.

Using a novel collaborative approach, we are collecting water quality samples and associated data from 400+ diverse freshwater systems, including lakes, reservoirs, ponds, and rivers, throughout much of the eastern U.S. These samples will be analyzed by the PIs for phycocyanin (cyanobacteria), cyanobacterial toxins, off-flavors, and phytoplankton enumeration. Data generated from these efforts will be used to refine and build models aimed at forecasting blooms of freshwater cyanobacterial blooms. Although the focus of the current project is on the Southeast, we have quickly expanded our efforts beyond this region. We hope to continue this expansion throughout the 3-year project.

G. PROJECT OBJECTIVE(s): Briefly explain the project objectives.

To enhance our network of water quality managers and scientists throughout the southeastern U.S. aimed at monitoring sites for toxic cyanobacterial blooms.

To test and refine current models that forecast toxic cyanobacterial blooms and off-flavor events in freshwater lakes, reservoirs, rivers, and ponds throughout the Southeast.

To train state and federal scientists, water quality managers, and aquaculturists on standard techniques to measure cyanobacterial toxin and phycocyanin concentrations and to identify and enumerate phytoplankton.

To train graduate and undergraduate students on field sampling and laboratory-based water quality analytical analyses.

To enhance our existing, user-friendly, interactive website where water quality managers and aquaculturists can determine the risk of their waterbodies for toxic cyanobacterial blooms and/or off-flavor events.

To create a model collaborative network that can be extended to other U.S. regions.

H. **METHODOLOGIES:** Briefly explain the research methodology used.

Sample sharing is central to the success of our project. We are also planning to share data among collaborators, but we are most excited about our approach for bringing together scientists in academia, agencies, and industry who all share a common concern – algal blooms. We are leveraging resources provided by our many colleagues throughout the eastern U.S. to collect and analyze water quality samples for us. In turn, we will analyze these samples for phytoplankton, cyanobacteria, and cyanobacterial toxins and off-flavors in order to build algal bloom forecasting models.

I. **PRINCIPAL FINDINGS/RESULTS:** Explain the results of findings of this research project.

Despite being in our first project year, we have observed a huge interest in our project by agency and academic scientists throughout the eastern U.S. We proposed to get samples and data from 200 sites per year. We will double that estimate in our first year! All of our sampling gear has been shipped to our colleagues (60+ individuals in 13 states and Puerto Rico). Our colleagues will return their samples to us this fall when we will begin our own analyses. We have held two water quality workshops this spring (Orlando and Auburn). Both were well attended (16-17 students each), and we received feedback showing that our students learned a lot about the project and our analytical and modelling approaches. We will be organizing similar workshops next spring. We have also given several presentations at regional and national conferences showcasing this project, and all have generated more excitement about our project and our analytical techniques (especially the phycocyanin analysis). One of Wilson’s students is in the process of running a laboratory experiment further validating the utility of our phycocyanin analyses, which we expect to submit for publication later this year. Given the feedback we have received from others, we expect these data to be of broad interest to scientists interested in quickly quantifying cyanobacterial abundance.

J. **NOTABLE AWARDS AND ACHIEVEMENTS.** List any awards or recognitions for this research
None

K. **PUBLICATIONS GENERATED:**

Number of Research Publications generated from this research project:	
Publication Category	Number
Articles in Refereed Journals	0
Book Chapters	1
Theses and Dissertations	0
Water Resources Institute Reports	0
Articles in Conference Proceedings	0
Other Publications	0

PROVIDE A CITATION FOR EACH PUBLICATION USING THE FOLLOWING FORMATS:

1. Articles in Refereed Scientific Journals Citation

Author (first author; last name, first name; all others; first name, last name), Year, Title, Name of Journal, Volume(Number), Page Numbers.

None

2. Book Chapter Citation

Author (first author; last name, first name; all others: first name, last name), Year, Title of chapter, "in" Name(s) of Editor "ed.", Title of Book, City, State, Publisher, Page Numbers.

Wilson, Alan E.; Michael F. Chislock. *In press*. Ecological control of cyanobacterial blooms in freshwater ecosystems. in ed. Aloysio Ferrão-Filho, *Cyanobacteria: Toxicity, ecology, and management*. Hauppauge, New York, Nova Science Publishers, xx-xx.

3. Dissertations Citation

Author (last name, first name), Year, Title, "MS (Ph.D.) Dissertation," Department, College, University, City, State, Number of Pages.

None

4. Water Resources Research Institute Reports Citation

Author (first author; last name, first name; all others: first name, last name), Year, Title, Name of WRRRI, University, City, State, Number of Pages.

None

5. Conference Proceedings Citation

Author (first author; last name, first name; all others: first name, last name), Year, Title of Presentation, "in" Title of Proceedings, Publisher, City, State, Page Numbers.

None

6. Other Publications Citation

Author (first author; last name, first name; all others: first name, last name), Year, Title, other information sufficient to locate publications, Page Numbers (if in publication) or Number of Pages (if monograph).

None

L. PRESENTATIONS MADE:

Presenter(s) (last name, first name; all others presentation authors: first name, last name), Year, Title, other information sufficient to identify the venue in which the presentation was made.

- Wilson, Alan E.; Russell A. Wright; Kevin. K. Schrader; Gina L. Curvin; Barry H. Rosen; Jennifer L. Graham, 2012, Creating cost-effective regional algal bloom monitoring networks: Extending beyond Alabama. Alabama Water Resources Conference, Orange Beach, Alabama.
- Wilson, Alan E.; Russell A. Wright; Kevin. K. Schrader; Gina L. Curvin; Barry H. Rosen; Jennifer L. Graham, 2012, Creating cost-effective regional algal bloom monitoring networks: The Southeast as a case study. 21st SE NALMS Southeastern Lake and Watershed Management Conference. Columbus, Georgia.
- Wilson, Alan E.; RajReni B. Kaul; Michael F. Chislock; Gina L. Curvin, 2012, Towards an improved understanding of the factors mediating toxic cyanobacterial blooms throughout the Southeast. Association of Southeastern Biologists, Athens, Georgia.
- Wilson, Alan E.; Russell A. Wright; Kevin. K. Schrader; Gina L. Curvin; Barry H. Rosen; Jennifer L. Graham, 2012, Creating cost-effective regional algal bloom monitoring networks. 8th National Monitoring Conference. Portland, Oregon.

M. STUDENTS SUPPORTED (Complete the following table)

Number of Students Supported, by Degree	
Type	Number of students funded through this research project:
Undergraduate	4
Masters	1
Ph.D.	0
Post Doc	0
Number of Theses and Dissertations Resulting from Student Support:	
Master's Theses	0
Ph.D. Dissertations	0

N. RESEARCH CATEGORIES: (In column 1 mark all that apply)

	Research Category
X	Biological Sciences
	Climate and Hydrological Processes
	Engineering
	Ground Water Flow and Transport
	Social Sciences
X	Water Quality
X	Other: Modelling

O. FOCUS CATEGORIES (mark all that apply with "X" in column 1):

	ACID DEPOSITION	ACD
	AGRICULTURE	AG
	CLIMATOLOGICAL PROCESSES	CP
X	CONSERVATION	COV
	DROUGHT	DROU
	ECOLOGY	ECL
	ECONOMICS	ECON
X	EDUCATION	EDU
	FLOODS	FL
	GEOMORPHOLOGICAL PROCESSES	GEOMOR
	GEOCHEMICAL PROCESSES	GEOCHE
	GROUNDWATER	GW
	HYDROGEOCHEMISTRY	HYDGEO
	HYDROLOGY	HYDROL
	INVASIVE SPECIES	INV
	IRRIGATION	IG
	LAW, INSTITUTIONS, & POLICY	LIP
X	MANAGEMENT & PLANNING	M&P
X	METHODS	MET
X	MODELS	MOD
X	NITRATE CONTAMINATION	NC
	NONPOINT POLLUTION	NPP
X	NUTRIENTS	NU
	RADIOACTIVE SUBSTANCES	RAD
	RECREATION	REC
	SEDIMENTS	SED
	SOLUTE TRANSPORT	ST
X	SURFACE WATER	SW
X	TOXIC SUBSTANCES	TS
	TREATMENT	TRT
	WASTEWATER	WW
X	WATER QUALITY	WQL
X	WATER QUANTITY	WQN
	WATER SUPPLY	WS

	WATER USE	WU
	WETLANDS	WL

P. DESCRIPTORS: (Enter keywords of your choice, descriptive of the work)

Algal blooms, cyanobacteria, off-flavor, toxin, microcystin, BMAA, cylindrospermopsin, saxitoxin, phytoplankton, modeling, forecasting, monitoring, network, collaboration

DEVELOPMENT OF AN IN-SITU CAPABLE METHOD FOR DETECTING PATHOGENIC BACTERIA IN THE ALABAMA WATER SUPPLIES – Phase 3

Basic Information

Title:	DEVELOPMENT OF AN IN-SITU CAPABLE METHOD FOR DETECTING PATHOGENIC BACTERIA IN THE ALABAMA WATER SUPPLIES – Phase 3
Project Number:	2012AL161B
Start Date:	3/1/2012
End Date:	2/28/2013
Funding Source:	104B
Congressional District:	3rd
Research Category:	Engineering
Focus Category:	Water Quality, Surface Water, Non Point Pollution
Descriptors:	Pathogenic bacteria, E. coli O157:H7, nanoparticle, DNA, in-situ detection, microfluidics, gene quantification
Principal Investigators:	Ahjeong Son

Publications

1. Mitchell, K.; Chua, B.; *Son, A. Development of first generation in-situ pathogen detection system (Gen1-IPDS) based on NanoGene assay for near real time E. coli O157:H7 detection. Environmental Science and Technology (Under review)
2. Mitchell, K. 2013, Development of the first generation in-situ pathogen detection system (Gen1-IPDS) based on NanoGene assay for near real-time E. coli O157:H7 detection. MS dissertation, Department of Civil Engineering, Auburn University, Auburn, Alabama, 55p.

Annual Technical Report
for
Water Resources Research Institute Program
under
Section 104, Water Resources Research Act of 1984
to the
Alabama Water Resources Research Institute

In support of the
Research Proposal

DEVELOPMENT OF AN IN-SITU CAPABLE METHOD FOR DETECTING PATHOGENIC
BACTERIA IN THE ALABAMA WATER SUPPLIES – *Phase 3*

March 2012 – February 2013

By

Ahjeong Son (Principal Investigator)
Assistant Professor
Department of Civil Engineering
College of Engineering
Auburn University
Telephone (334)-844-6260
Email: ason@auburn.edu

May 27, 2013

SYNOPSIS OF ANNUAL TECHNICAL REPORT

A. Title: DEVELOPMENT OF AN IN-SITU CAPABLE METHOD FOR DETECTING PATHOGENIC BACTERIA IN THE ALABAMA WATER SUPPLIES

B. Primary PI(s): Ahjeong Son, Assistant Professor in Department of Civil Engineering at Auburn University, AL.

C. OTHER PI(s): None.

D. START DATE: March 1, 2012

E. END DATE: February 28, 2013

F. PROJECT OVERVIEW/SUMMARY:

Proper stewardship of Alabama’s tremendous water resources relies on the amount of data and tools available to formulate and execute management strategies. In other words, the availability of a miniaturized in-situ pathogen detection system will have enormous impact on the way we manage the contamination of our water resources. It will open up numerous possibilities in terms of monitoring, tracing and rectifying the contamination source. However, the development of such an in-situ pathogen detection system is contingent on the availability of a rapid, accurate, and economic detection technology. For this reason, we developed a rapid, accurate, in-situ capable technique for the detection of pathogens (*E. coli* O157:H7) in water at levels as low as 100 organisms per mL.

In light of the need for the *in-situ* pathogen detection system and the limitations of the currently available methods, we envisioned the development of an in-situ capable pathogen detection method as outlined below. The development of the in-situ capable pathogen system consists of three phases; however, the research scope described in this report **only included PHASE 3**. The scope and objectives of all three phases can be seen below in Figure 1.

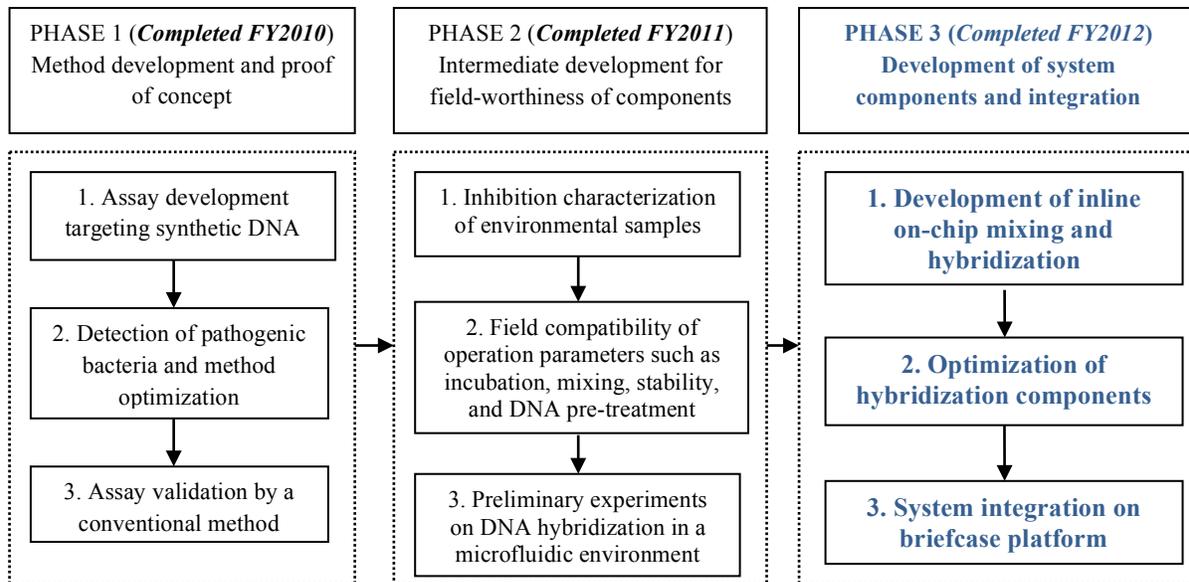


Figure 1. Scope and objectives of each research phase

Phase 1 (FY2010) was a proof-of-concept study in a laboratory setup to develop a novel, in-situ capable technique for rapid, accurate, and sensitive pathogen detection in water environments. The methodology is based on the specific DNA hybridization using our custom configured multi-functional nanoparticle labels. Our unique hybridization method was investigated for its ability to quantitatively detect pathogens in the forms of synthetic linear DNA (Task 1) and genomic DNA from bacterial culture (Task 2). Both tasks determined the quantitative parameters for pathogen DNA detection such as linearity (correlation coefficient and range of quantification), assay sensitivity (detection limits), specificity (mismatches), and rapidity of assay (reaction kinetics) in the laboratory. In Task 3, conventional plate counting method was used to verify our proof-of-concept as well as to mitigate the possibility of false positive results. As a result of the research in Phase 1, two scientific papers have been published in the major journals of analytical chemistry and bioengineering (Kim & Son, 2010a; Kim & Son, 2010b).

Phase 2 (FY 2011) consisted of the intermediate development of the technique and the investigation of the compatibility of the developed assay for non-laboratory environment usage. In particular the inhibition effects of environmental samples were identified and we have found that our technology has shown the resistance ability to a number of inhibitors such as humic acids (Task 1). We have identified the key parameters of the techniques that were not field-ready and to perform further investigative studies as well as modifications to enable them for in-situ operation (Task 2). In Task 3, we transformed the format of the method from a microplate format into a microfluidic platform in order to examine the feasibility of in-situ capability of the developed method by testing several operation parameters. Parameters made known from Phase 1 were used as preliminary data for Phase 2 research. The research at this phase is critical for the further development (Phase 3) of the proposed assay into a well-designed, in-situ capable engineered system. As a result of the research in Phase 2, two scientific papers have been published in the major journals of environmental engineering and science (Kim et al., 2011a; Kim et al., 2011b).

Phase 3 of the research (this report, FY2012) consists of the first inline fluidic components development and characterization as well as the first integration effort on a *briefcase platform* for the in-situ pathogen detection system. It will be the complete research scope for this report. Phase 3 is essentially the embodiment of the envisioned **In-situ Pathogen Detection System (IPDS)** on a briefcase platform. Our long term vision is to further miniaturize the briefcase platform implementation of the IPDS and to commercialize the handheld version of the IPDS as shown in Figures 2 and 3.

Finally, the results of this research will lead to a technology that can be patented and commercially developed for *public good*. For regular pathogen monitoring in water, the in-situ pathogen detection system will enable faster response time to trace the source of contamination in the event of an outbreak. For example, park rangers and water officials will be able to perform routine monitoring without the time-consuming need to collect samples and send them to laboratories for analysis. Minimal laboratory expertise will be required to operate the developed in-situ system. With continuous improvement to the system driven by future funding from NIH, NSF, AWWA, WERF or EPA, it will eventually be autonomous without the need of a human operator. Multiple units of the autonomous system can be positioned in the field for ubiquitous monitoring of water pathogens via sensor networks. A real-time, spatially and temporally distributed water quality map will be an invaluable resource to both prevent and control pathogenic outbreaks and their costly aftermath in terms of human lives and resources.

G. PROJECT OBJECTIVE(S):

The objectives of this study were to implement and characterize the developed assay on a *briefcase platform* and to identify technical risks as well as solutions. In particular, we constructed a briefcase platform (IPDS) for hybridization and magnetic separation of the NanoGene assay (Task 1). We then used the IPDS to optimize hybridization components of the assay such as hybridization buffer composition,

hybridization flow rate, hybridization time, and hybridization temperature (Task 2). Finally, we implemented the parameters determined in Task 2 to quantify *E. coli* O157:H7 (both linearity/sensitivity and specificity) and correlate the results with the laboratory method (Task 3).

The proposed research scope only covers *Phase 3* and the research objectives will be referred to as Task 1 through 3.

The specific research objectives are:

- 1) Constructing a briefcase platform (IPDS) for hybridization and magnetic separation of the NanoGene assay
- 2) Optimizing hybridization components of the assay
- 3) Implementing parameters to quantify and correlate results

H. METHODOLOGIES:

Phase 3. Inline mixing/hybridization on microfluidic chip with magnetic trap and its integration on briefcase platform.

Mixing/hybridization and magnetic separation are two critical steps in our assay. To enable in-situ field portability, these steps have been brought out from the laboratory and implemented using miniaturized fluidic systems.

Task 1: Constructing a briefcase platform (IPDS) for hybridization and magnetic separation of the NanoGene assay

Materials and apparatus. Single-stranded DNA oligonucleotides were commercially synthesized in accordance with the sequence of *Escherichia coli* O157:H7 gene (Integrated DNA Technologies, Coralville, IA). The DNA sequences can be seen in Table 1. Aminated magnetic beads (MB, Invitrogen, Carlsbad, CA) were coated with carboxyl quantum dots (QD₅₆₅, Invitrogen) and the signaling DNA was labeled with carboxyl quantum dots (QD₆₅₅, Invitrogen). Complexes were stored in centrifuge tubes which had been pretreated with a 0.5% bovine serum albumin (BSA, New England BioLabs, Ipswich, MA) in phosphate buffer saline (0.1 M, pH 7.4) solution to prevent nonspecific binding [20, 21]. Tris-EDTA buffer, ethylcarbodiimide hydrochloride (EDC), *N*-Hydroxysulfosuccinimide (NHS), monobasic sodium phosphate (H₂NaO₄P·2H₂O), and dibasic sodium phosphate (Na₂HPO₄) were purchased from Sigma-Aldrich (St. Louis, MO). Sodium borohydride (NaBH₄) and sodium dodecyl sulfate (SDS) were purchased from MP Biomedicals, LLC (Solon, OH). Saline-sodium citrate (SSC, 20×) was purchased from Fisher Scientific (Fair Lawn, NJ). DIG easy hybridization buffer (Roche Diagnostic, Basel, Switzerland) and a hybridization oven (UVP, HB-500 Minidizer Hybridization Oven) were used for DNA hybridization in the laboratory NanoGene assay. A magnet was used for the magnetic separation and washing of MB-QD-DNA complexes (Invitrogen, DynaMag™-2). A water bath (Fisher Scientific, ISOTEMP 202S) was used for the passivation of and a centrifuge was used to wash the DNA-QD conjugations (Eppendorf, Centrifuge 5418).

Fluidic, electromechanical, and electronic components of the Gen1-IPDS were purchased from various retailers and assembled. Fluidic components include microfluidic chips, tubing, and connectors. Electromechanical components include a magnet positioner (in the form of a linear actuator) and miniature peristaltic pumps. Electronic components include a microcontroller, switches, wires, liquid crystal display (LCD), and breadboards. Other components include grade N52 neodymium magnets (K&J Magnetics, Jamison, PA) [22] and laser cut acrylic plates (Pololu Laser Cutting, Las Vegas, NV). One hundred twenty μL poly-methyl methacrylate (PMMA) rhombic chamber microfluidic chips, male mini luer fluid connectors, male mini luer plugs, and a microfluidic support kit including silicon tubing,

forceps, and syringes were purchased from the Microfluidic ChipShop (Jena, Germany). Miniature peristaltic pumps, a peristaltic pump tubing pack, polytetra-fluoroethylene (PTFE) tubing (1/16 inch outer diameter, 0.5 mm inner diameter), and a PTFE tube cutter were purchased from Dolomite (Royston, UK). The magnet positioner used for positioning the magnet as well as alternating waste and sample collection was purchased from Firgelli (British Columbia, Canada). The microcontroller used is an Arduino Uno which is based on ATmega328 and has 14 digital input/output pins as well as 6 analog input pins (Italy).

NanoGene Assay. The laboratory version of the NanoGene assay suitable for quantification and detection of *E. coli* O157:H7 has been previously developed [18]. The NanoGene assay employs magnetic beads encapsulated with QD₅₆₅ and tethered with probe DNA and signaling DNA labeled with QD₆₅₅. These particles are then hybridized with target DNA. The target DNA is captured during hybridization. Fluorescence measurements can allow for the quantification and detection of the target.

Preparation of MB-QD particle complexes and DNA-QD conjugation. A suspension of MBs (2×10^7 MB) and QD₅₆₅ (16 moles) were added to a BSA treated centrifuge tube and thoroughly mixed. A solution of EDC-NHS (1:1 molar ratio) was prepared immediately prior to use and 10 μ L was added to the MB-QD solution to promote covalent bonding. The tube was then placed in a mix plate (Eppendorf, MixMate), protected from photobleaching, and incubated at 1500 rpm for 2 hours at ambient temperature. The complexes were then washed 3 times with phosphate buffer saline (0.1 M, pH 7.4) in conjunction with magnetic separation and supernatant disposal. 500 picomoles of aminated probe DNA was added to the MB-QD complexes along with 10 μ L of EDC-NHS solution. The tubes were incubated in accordance with the previously described procedure. Following incubation, the complexes were washed 3 times with phosphate buffer (0.1 M, pH 7.4) using magnetic separation and supernatant disposal. The complexes were stored in PB. A suspension of QD₆₅₅ (16 moles), 160 picomoles of signaling DNA, and 10 μ L of EDC-NHS solution were added to a BSA treated centrifuge tube and thoroughly mixed. The tube was incubated in accordance with the previously described procedure. Following incubation, the complexes were passivated to prevent non-specific binding by inactivating the remaining functional groups. A passivation solution was prepared using 10 mL 20 \times SSC, 0.5 mL 10% SDS, and 90 mL autoclaved deionized water. Immediately prior to passivation, 5 mg NaBH₄ was dissolved in 1 mL of the passivation solution; 100 μ L of the NaBH₄-passivation solution was added to the QD₆₅₅ labeled signaling probe DNA complexes. The complexes were passivated in a 42 $^{\circ}$ C water bath for 20 minutes. The signaling probe DNA labeled with QD₆₅₅ were washed twice using 1 \times SSC and 0.2 \times SSC in conjunction with centrifugal separation and supernatant disposal. The complexes were stored in phosphate buffer.

Quantification of E. coli using MB-QD particle complexes based on DNA hybridization. In order to quantify the ssDNA target gene using the laboratory method, 10 μ L of the prepared MB-QD-DNA complexes, 1.6 μ L of the prepared signaling DNA complexes, and target DNA was suspended in 400 μ L DIG easy hybridization buffer. The complexes were placed in the hybridization oven on a slow rotation at 37 $^{\circ}$ C for 8 hours. After DNA hybridization, a magnet was used to hold the tethered particles while the untethered particles were removed. The tethered particles were released from the magnet and washed with PB; this process was repeated three times. The sample was transferred to a 96-well plate and the fluorescent intensity was measured.

Fluorescence measurement. To quantify the target DNA post-hybridization, the fluorescent intensity of the internal standard of QD₅₆₅ and the labeled probe of QD₆₅₅ were measured using a bench-top spectrofluorometer (Molecular Devices, SpectraMax M2, Sunnyvale, CA). An excitation of 360 nanometers (nm) was used for both signals, while an emission of 560 nm was measured for QD₅₆₅ and 650 nm for QD₆₅₅. The intensity of the signaling probe was normalized (QD₆₅₅/QD₅₆₅) to adjust for the possibility of varying amounts of MB-QD complexes.

Design and construction of the Gen1-IPDS. The Gen1-IPDS was designed to implement the three earlier mentioned key steps using miniature components instead of laboratory bench top equipment. Sample and reagent introduction was performed via a miniature peristaltic pump (also referred to as injection pump) instead of manual pipetting. Hybridization was performed in a microfluidic chip instead of a vial. A second miniature peristaltic pump (also referred to as recirculation pump) was used to provide agitation during hybridization instead of a rotator in the hybridization oven. It was also used to control retention time during hybridization. Finally, a magnet positioner and magnet replaced a handheld magnet during magnetic separation.

The components were mounted on a laser cut acrylic platform (Figure 2). Holes were laser cut to hold the vials and the miniature peristaltic pumps were held down by a washer and screw. Similarly, the microfluidic chip was secured to the platform via screws and washers to allow easy attachment and adjustment. The microfluidic chip contained two chambers and each chamber has two inlets and two outlets. The chamber and channels have a depth of 500 μm and each chamber is capable of holding a volume of 120 μL . Two separate acrylic pieces were custom cut, joined with epoxy, and attached to the magnet positioner in order to hold the magnet. The magnet positioner moved the magnet onto and off of the microfluidic chip in accordance with the programmed sequence. The Arduino Uno microcontroller board was wired to both a 16 \times 2 LCD screen as well as to the electromechanical components via two breadboards. A toggle switch controlled the power supply to the microcontroller from a 9 V battery. Pictures of the assumed Gen1-IPDS are shown in Figure 3a to 3c. The assembled Gen1-IPDS was sufficiently small and fit inside a briefcase (with dimensions of 37 \times 20 \times 12 cm and weight of 2.25 kilograms) as shown in Figures 3a and 3c.

After the microcontroller was powered up and the start button was pressed, the injection pump drew the sample and reagents from its vial into the fluidic chamber at a programmed flow rate. The injection pump was turned off and the recirculation pump was turned on to initiate the hybridization cycle at a programmed flow rate and retention time. At the end of the hybridization cycle, the recirculation pump was turned off. The inlet tubing was manually connected to the washing buffer vial and the outlet tubing was manually directed to the waste vial. Magnetic separation and washing were performed by turning on the injection pump. Finally, the magnet positioner was retracted and the outlet tubing was manually directed to the sample collection vial to collect the washed MB-QD-DNA complexes. Figures 4a to 4d show the 4-step sequence schematic of the Gen1-IPDS performing the three key steps and the sample collection with washing.

Software control of peristaltic pumps and magnet positioner. The Arduino Uno microcontroller was programmed with the provided Arduino 1.0.3 software. Pulse-width modulation (PWM) was used to control the magnet positioner extension and the flow rates of the pumps. PWM is a method of delivering electrical power in pulses instead of a continuous analog signal [23]. PWM provides a series of on-off analog patterns, using analog signals to simulate signals between full on and full off [24]. The percentage of time the pulse is “on” is referred to as the duty cycle. The PWM settings for the Arduino Uno microcontroller output pins ranged from 0 to 255, with 255 representing a 100% duty cycle and an output voltage of 5 V. For example, a PWM setting for the Arduino Uno of 127 (or a 50% duty cycle) would provide 2.5 V at the designated output pin. This feature was used to control the voltage supplied to the pumps which in turned controlled the flow rate.

The magnet positioner has a built-in position feedback feature that uses PWM to determine its position. A 100% duty cycle will give a full extension of the magnetic positioner at 50 mm. At full retraction, the magnet was approximately 30 mm away from the center of the fluidic chamber. To determine the appropriate PWM setting for the magnet positioner in order to trap the MB-QD complex effectively, PWM settings ranging from 125 to 155 (in increments of 5) were investigated. These values represent the range of settings that placed the magnet over the microfluidic mixer chip. Values outside of this range

placed the magnet off of the microfluidic chip. For each data point, the magnet positioner was extended in accordance with a PWM setting. One hundred μL of MB-QD-DNA complexes were flushed through the microfluidic chip and the waste was collected. Since the complexes were fluorescently labeled, the fluorescence of the complexes held could be correlated to the percent of complexes held by the magnet. The magnet positioner was retracted and the microfluidic chip was washed with 200 μL of phosphate buffer to collect the complexes held by the magnet. The fluorescence intensity of the sample was measured. Duplicate samples were used for each setting and the average fluorescence intensity was calculated. The highest average fluorescence intensity was assumed to correspond to one-hundred percent of complexes held by the magnet. All other samples were normalized to this data point.

Table 1. Sequences and modifications of ssDNA nucleotides. The boldface basepairs represent complementary sequences while the underlined basepairs represent mismatched sequences.

	Sequence (5' → 3') and modification
Probe DNA	NH_2 - CGGAT AAGAC TTCCG CTAAA
Signaling DNA	CTTAT ACCGC GACGG TGA AA - NH_2
Target ssDNA	ACCGT CGCGG TATAA GTAAT GGTAT CGGCG TTATC CGCTT TAGCC GAAGT CTTAT
1 bp mismatched target DNA	ACCGT CGCGG TATAA GTAAT GGTAT CGGCG TTATC CGCTT TAGCC GAACT CTTAT
2 bp mismatched target DNA	ACCGT CGCGG TATAA GTAAT GGTAT CGGCG TTATC CGCTT TA <u>CC</u> GAACT CTTAT
Non-matched target DNA	ATAAG ACTTC GGCTA AAGCG GATA <u>A</u> CG <u>CC</u> G ATACC ATTAC TTATA CCGCG ACGT

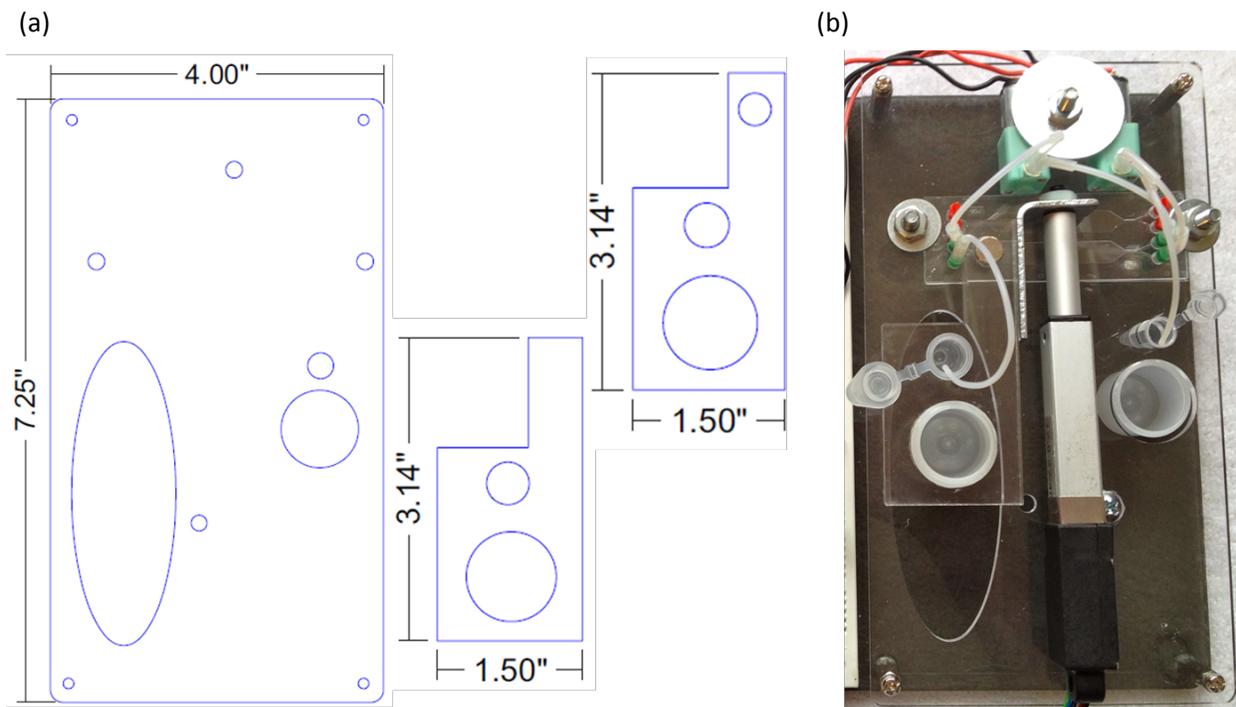


Figure 2. Acrylic cut pieces for the Gen1-IPDS. (a) The computer aided drawings for the acrylic platform and magnet/effluent vial holder; (b) Assembled acrylic pieces in the Gen1-IPDS.

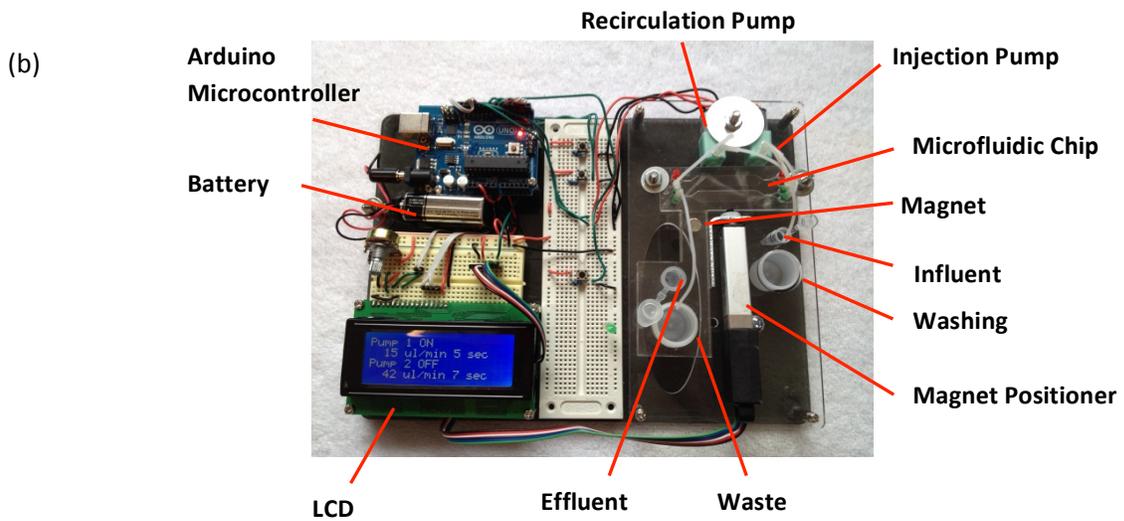
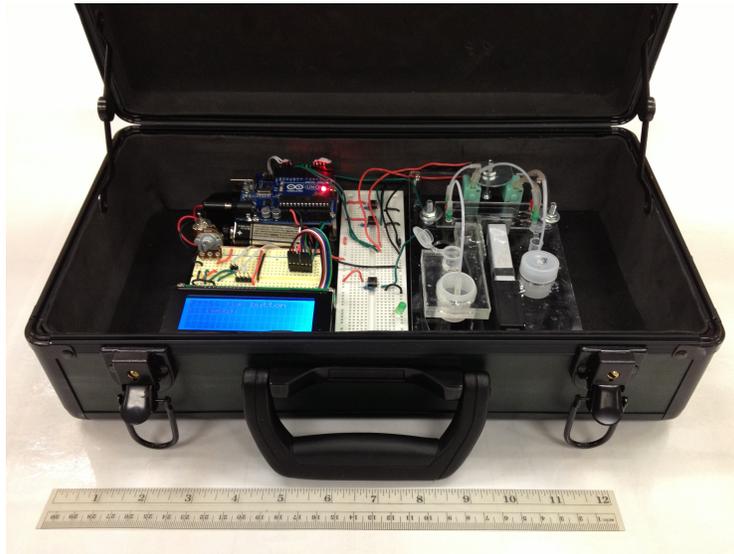


Figure 3. Pictures of the Gen1-IPDS showing: (a) a size comparison of Gen1-IPDS, (b) the labeled hardware of the Gen1-IPDS, and (c) Gen1-IPDS in its portable briefcase.

Experimental Investigation of Stormwater Runoff Quality Originating from Urban Vegetated Roofs

Basic Information

Title:	Experimental Investigation of Stormwater Runoff Quality Originating from Urban Vegetated Roofs
Project Number:	2012AL162B
Start Date:	3/1/2012
End Date:	2/28/2013
Funding Source:	104B
Congressional District:	7
Research Category:	Engineering
Focus Category:	Water Quality, Surface Water, Non Point Pollution
Descriptors:	• Stormwater Quality, Urban Runoff
Principal Investigators:	Jason T. Kirby

Publications

1. Price, J.G., A.N. Wright, J.T. Kirby, R.W. Peters, and S.A. Watts. 2013. Irrigation Affects Plant Community Structure on Southeastern Green Roofs. *Ecological Engineering* (under review).
2. Price, J.G., A.N. Wright, J.T. Kirby, R.W. Peters, and S.A. Watts. 2013. Influence of Water Availability and Season on Photosynthetic Gas Exchange of Three Green Roof Plant Species. *Oecologia* (under review).
3. Peters, Robert W., Ronald D. Sherrod, and Matt Winslett, 2013. “Energy Savings Resulting from Installation of an Extensive Vegetated Roof Systems on a Campus Building in the Southeastern United States”, Chapter 2, pages 21–49 in *New Developments in Renewable Energy*, InTech Publishers, Rijeka, Croatia.
4. Price, Julie. 2012. Plant selection, community dynamics, ecophysiology, and stormwater mitigation on green roofs in the southeastern U.S. “Ph.D. Dissertation”, Biology Department, College of Arts and Sciences, University of Alabama at Birmingham, Birmingham, Alabama.
5. Kirby, Jason T., Ronald Sherrod, Matthew Winslett, and Robert W. Peters, 2012. “Variations in Temperature in Mini-Roof Structures Employing Different Roofing Materials”, Proc. 2012 AIChE Annual Meeting, Pittsburgh, PA, (October 28–November 2).
6. Pettway, Yasminye D., Robert W. Peters, Jason T. Kirby, Stephen A. Watts, and Julie G. Price, 2012. “Final Report: The Effects of Green Roofs on Nitrogen in Stormwater Runoff”, Report submitted to STEP-UP High School Program, National Institute for Diabetes and Digestive and Kidney Diseases (NIDDK), U.S. Department of Health and Human Services, Bethesda, MD, (August 12 – 14).
7. Sherrod, Braxton E., Robert W. Peters, Julie G. Price, Jason T. Kirby, and Stephen A. Watts. 2012. “Final Report: The Effect of Vegetated Roofs on Phosphorus Leaching and Stormwater Quality”, Report submitted to STEP-UP High School Program, National Institute for Diabetes and Digestive and Kidney Diseases (NIDDK), U.S. Department of Health and Human Services, Bethesda, MD, (August 12 – 14).

AWRRI ANNUAL TECHNICAL REPORT SYNOPSIS

- A. PROJECT TITLE: Experimental Investigation of Stormwater Runoff Quality Originating from Urban Vegetated Roofs
- B. PRIMARY PI(s): Jason T. Kirby, UAB Associate Professor
- C. OTHER PI(s): Robert W. Peters, UAB Professor; Stephen A. Watts, UAB Professor; and Matthew Winslett, UAB Energy Manager
- D. START DATE: March 1, 2012
- E. END DATE: February 28, 2013
- F. PROJECT OVERVIEW/SUMMARY:

This research effort supported the continuing investigation of runoff water quality originating from rainfall events on vegetated roofs planted with different vegetation types (including sedum plants, bunchgrass and phlox plants along with combined plant systems, and soil only systems) grown under irrigated and non-irrigated conditions.

The study responded to surface water (quantity and quality) [Area 3] described in the solicitation, addressing water quality identification and characterization from non-point sources. Additionally, the study ties in with Area 5 related to hydrology, climatology and hydraulics, addressing water use by plants. This project was a collaborative effort involving the Department of Civil, Construction, and Environmental Engineering and the Department of Biology at the University of Alabama at Birmingham, along with collaboration from UAB's Facilities Management Department.

- G. PROJECT OBJECTIVE(s): Briefly explain the project objectives.

Extensive green roofs will comprise the majority of roof retrofits over the next twenty years; however, the scientific community does not yet understand the factors that affect the qualitative characteristics of runoff from these systems, particularly in terms of nutrients and heavy metals that ultimately affect stormwater runoff characteristics. Factors influencing runoff dynamics from vegetated roofs include soil characteristics (which contain varying percentages of compost), season, rainfall characteristics, age of the green roof, and vegetation (Berndtsson, 2010). In this research effort, continued efforts among engineers, biologists,

facilities management, and community partners were utilized to help obtain fundamental information and knowledge for establishing green roofs in the southeastern U.S.

The objectives of the research proposal were:

1. To compare runoff water quality during rainfall events among roofs planted with different vegetation types, grown under irrigated and non-irrigated conditions.
2. To compare runoff water quality from new installations and established vegetated roofs.
3. To investigate runoff water quality with respect to first flush effects.

H. METHODOLOGIES: Briefly explain the research methodology used.

To address the above objectives, the following research tasks were performed:

- Task 1. Retrofit existing UAB Greenroofs for Water Quality Analysis
- Task 2. Soil Substrate Assessment
- Task 3. Water Quality Analysis

Task 1. Retrofit existing UAB Greenroofs for Water Quality Analysis

The vegetative roof systems adapted to this research are located on the Business and Engineering Complex (BEC) on the University of Alabama at Birmingham campus (see Figure 1). These experimental structures (Thirty roofs @ 48 ft², circa 2010) were originally instrumented to evaluate surface and subsurface water flow rate, soil temperature, moisture content, along with providing irrigation. Additional environmental data was collected via an on-site weather station capable of measuring ambient temperature, rainfall duration and intensity, wind speed, dew point, barometric pressure, relative humidity, wind chill, wind direction, heat index, and solar intensity.

The roof of each “building” is fitted with standard materials required for an extensive vegetated roof on a commercial building, including: waterproofing, insulation, drainage layers, filter fabric, and four inches of green roof soil. Six mini-roofs were planted with each of the following scenarios (see Figure 2):

1. Monoculture *Bouteloua curtipendula*, a bunchgrass;
2. Monoculture *Phlox bifida*, a forb;
3. Monoculture *Sedum album*, a succulent plant;
4. A mix of the 3 species above, planted in a randomized arrangements



Figure 1: Construction of UAB BEC Mini-roofs.

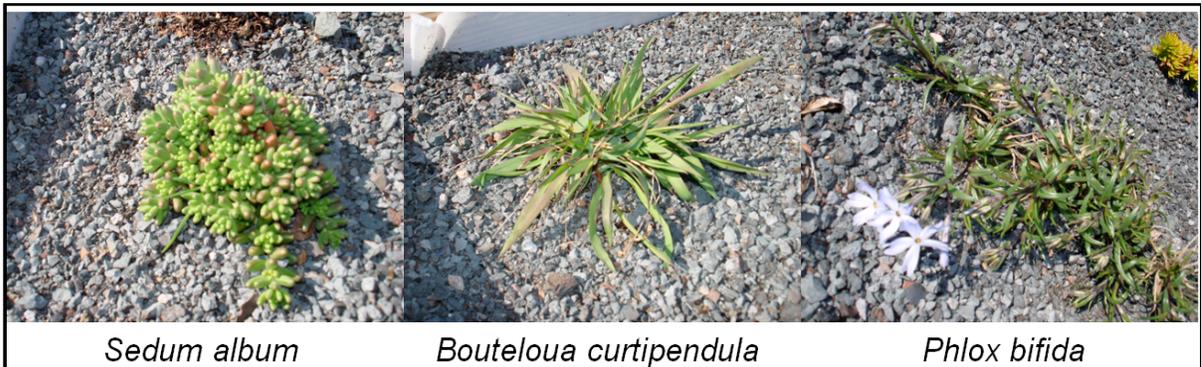


Figure 2: Experimental Vegetation.

Each mini-roof has a two percent slope so that stormwater flow may be captured via two separate drains: a floor drain (under the soil) utilized to capture percolating water, and a surface drain (flush with the soil surface) for sheet/overland flow (surface runoff). Each drain leads to a tipping bucket rain gauge (TE-525, Campbell Scientific, Logan, UT) connected to a data logger (CR-800, Campbell Scientific, Logan, UT) that measures runoff quantity during natural rainfall events (see Figure 3).



Figure 3: Roof Drains and Tipping Buckets (Campbell TE-525).

These drainage systems were effectively modified (see Figure 4) so that manual composite, grab and first flush samples could be collected via the existing drains enabling both surface and subsurface flows to be analyzed with regards to water quantity and quality.

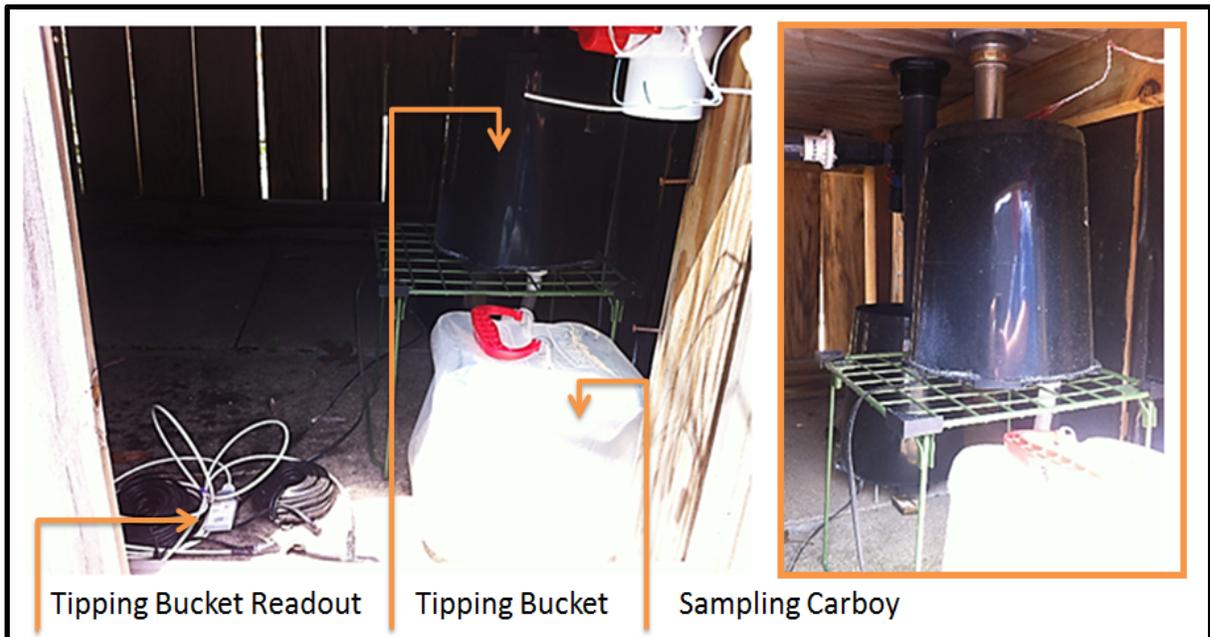


Figure 4: 2012-2013 Modifications to Capture Surface and Subsurface Flow for AWRRI Water Quality Analysis.

Task 2. Soil Substrate Assessment

Soils used in the UAB green roof experimental plots were produced by ITSaul Natural (Dahlonega, GA) and is composed of 80% recycled expanded slate fines and 20% worm castings. Substrate samples from fresh and aged substrate were analyzed for mineral nutrients, including Ca, Mg, K, P, Cu, Fe, Mn, Zn, B, Al, Cd, Cr, Pb, Na, and Ni using saturated extract at the Auburn Soil Testing Laboratory. Substrate samples were collected (n = 5 combined samples from each miniroof; 2 years old at purposed experiment execution) from existing plots:

1. Irrigated plots planted with *Sedum album*, *Bouteloua curtipendula*, and *Phlox bifida*
2. Non-irrigated plots planted with *Sedum album*, *Bouteloua curtipendula*, and *Phlox bifida*
3. Irrigated plots with no plants (substrate only)
4. Non-irrigated plots with no plants (substrate only)

Additional samples of fresh (stored since original purchase) soil from the same substrate batch as existing roofs were also analyzed.

Task3. Water Quality Analysis

Numerous water quality parameters were assessed from the captured vegetated roof runoff in an effort to characterize potential adverse impacts to the receiving watershed. Storm events with a recurrence interval of less than 5 years were prioritized as these are most valuable to continuing quantity experiments and represent the most frequent rainfall events. Larger storms were analyzed when encountered. Water quality samples were analyzed with respect to Standard Methods for the Examination of Water and Wastewater (21st edition) or relevant Hach testing protocols.

I. PRINCIPAL FINDINGS/RESULTS: Explain the results of findings of this research project.

The main intent of this research effort was to obtain fundamental information and knowledge for establishing green roofs in the southeastern U.S., with particular emphasis on determining their impact on stormwater runoff quality.

Based on the water quality data collected to date, the green roofs appear (additional storm samples are needed to provide conclusive statistical analysis) to have a beneficial impact on a range of water quality parameters. Temperature and dissolved oxygen fluctuations are static among treatments. Conductivity and Turbidity levels, post treatment, are consistent with spring water (< 300 μ S/cm and 5 NTU respectively). Current analysis of nutrient levels (nitrogen and phosphorus) indicate that while green roof subsurface flow does elevate nutrient concentrations slightly, it is not statistically significant (+ 0.10 level). Most notable was a universal observation in the green roofs ability to intercept acidic rainwater (4.10 to 5.43 pH) and buffer subsurface flow to an average pH of 7.45 (a value more consistent with natural ecosystems).

Additional experimentation on the underlying green roof soils (see Table 1) indicated that all soils (irrigated and non-irrigated, aged 2yr, 6 month, and new) were statistically indistinguishable with regards to the parameters of interest. That is to say, the active use of these soils in an urban green roof environment / application does not significantly alter their nature and presents no additional threat to the urban environment.

Green roof research relevant to both water runoff quality and quantity are ongoing at UAB. UAB researchers have taken findings from the aforementioned efforts and used them to write an updated NSF grant entitled "Green Engineering to Mitigate Urban Stormwater". This grant while not funded in 2011-12, was strongly encouraged to be resubmitted. It is envisioned that coupled with data collected from the above AWRRI research, UAB will transition to become nationally competitive in this emerging research area. Furthermore as the researchers feel this is an important topic with regards to the future of water resources in Alabama, we look forward to sharing our latest findings at the 27th Annual Alabama Water Resources Conference, September 2013.

Table 1.: Student t-Test (2 Tail, 0.05)

	Significance Level (+/-)	Roof Bias
Calcium (%)	0.5	+
Potassium (%)	0.1	=
Magnesium (%)	0.8	=
Phosphorus (%)	0.6	=
Aluminum (ppm)	0.5	=
Arsenic (ppm)	BDL	=
Boron (ppm)	0.4	+
Barium (ppm)	0.9	+
Cadmium (ppm)	BDL	=
Chromium (ppm)	0.2	+
Copper (ppm)	0.2	=
Iron (ppm)	0.3	=
Manganese (ppm)	0.1	+
Molybdenum (ppm)	0.4	=
Sodium (ppm)	0.1	-
Nickel (ppm)	0.1	+
Lead (ppm)	BDL	=
Zinc(ppm)	0.7	=

*BDL: Analysis of Compound Found to be Below Detectable Limit

*Bias indicates an active green roof soil compared to levels found in new soil (ex. + indicates more presence than in new soil; yet not statistically significant; = similar presence ; - less presence).

PROBLEMS EXPERIENCED DURING AY 2012-2013:

An unfortunate side effect of real-world experimentation, especially in environments as harsh as urban rooftops, is the potential for experimental damage. Such was the case in 2012, where several of our non-irrigated treatments (see Figure 5) were unable to sustain adequate coverage. While care was taken to preserve plant life, other long-standing experiments (i.e. plant selection) demanded non-interference. Similar cessation of coverage was experienced in sedum, bunchgrass and mixed plots, though not as severe as observed below.



Figure 5. 2012 Phlox Treatments (Left: Non-Irrigated; Right: Irrigated).

The tipping buckets purchased to collect runoff (quality and quantity) have a maximum capacity to receive flow (~ 1.95 gal/hr). Prior to purchase of the gauges, we calculated anticipated runoff volumes and rates based on the surface area of the mini-roofs and determined that the selected tipping buckets would be sufficient. However, for larger, more intense rainfall events (> 2.5 cm/hr), the rate of runoff coming from the mini-roofs exceeded the maximum tipping rate of the buckets. Excess runoff would overflow from the bucket result in total runoff values that cannot be calculated or estimated. Additionally, this apparatus shortcoming has hindered first flush chemical analysis to date.

The data loggers for the tipping buckets failed with increasing frequency as the 2012-2013 experiment season progressed. The loggers would either fail to communicate with the data shuttle when offloading, or would have inaccurate time stamps such that values from one mini-roof to the next could not be compared. These issues were evaluated by the logger vendor. The vendor did not have any solutions for recovering data with bad time stamps, but suggested using a computer to relaunch loggers that would not communicate with the data shuttle. Loggers connected to a computer would often fail to relaunch or following a successful relaunch would fail again at the next offload with the data shuttle. Housed in transparent plastic cases, these loggers were launched and offloaded using an optical

interface that we learned later, via user blogs and verification from the manufacturer, can be corrupted in high light intensities, that is, when opening outdoors on a sunny day. In addition, though the loggers were carefully stowed under the housing of the mini-roofs, there were no doors on the openings to the mini-roof instrumentation area. The angle of the early morning and late afternoon sun, combined with the high reflectivity of the pavers on the roof, inevitably led to UV degradation of the plastic containers over the course of the experimental period. We believe this led to loss of the moisture seal and subsequent moisture seepage into some loggers, despite repeated removal and replacement of desiccant packs inside the loggers. Some loggers had circuit boards with visible moisture damage.

Limited data collected during the study period indicated that, over time, and for a particular season, for certain storm sizes/intensity, or for different storm frequencies, the planting combinations mitigated stormwater differently. High failure rates of the instrumentation precluded statistical assessment of multiple rain events and treatment effects to date. Water Quality Results obtained to date are summarized in Table 2. Water quality analysis is integrated into several outstanding undergraduate and graduate research initiatives and will continue beyond the present AWRRI funding period.

Table 2: Current UAB Green Roof Water Quality Data (October 2012-April 2013)

		Temp (oC)	pH	DO	Conductivity (μ S/cm)	Turbidity (NTU)	Total N (mg/L)	Total P (mg/L)
Rainwater		18.2 (1.3)	5.8 (1.4)	7.9 (0.2)	41.8 (23.5)	1.8 (1.7)	0.6 (0.2)	0.2 (0.0)
Soil Only	Irr.	18.2 (2.6)	7.6 (0.3)	7.9 (0.6)	131.0 (39.1)	2.2 (1.0)	1.1 (0.1)	0.4 (0.2)
	Non-Irr.	16.9 (1.2)	6.7 (1.5)	8.1 (0.1)	129.7 (20.2)	2.8 (0.6)	1.5 (0.2)	0.4 (0.2)
Mixed Plants	Irr.	17.8 (2.5)	7.4 (0.5)	7.8 (0.7)	175.7 (70.8)	2.0 (0.7)	0.8 (0.5)	0.2 (0.1)
	Non-Irr.	18.0 (2.7)	7.6 (0.2)	7.8 (0.6)	116.6 (21.8)	2.3 (1.0)	1.6 (0.6)	0.5 (0.2)
Phlox	Irr.	17.5 (2.8)	7.0 (1.0)	7.9 (0.7)	112.0 (75.5)	2.2 (0.7)	0.9 (0.3)	0.3 (0.1)
	Non-Irr.	Dead	Dead	Dead	Dead	Dead	Dead	Dead
Sedum	Irr.	18.2 (2.6)	7.8 (0.2)	8.0 (0.6)	136.8 (46.8)	2.8 (0.8)	0.6 (0.4)	0.5 (0.5)
	Non-Irr.	18.2 (2.0)	7.7 (0.2)	8.0 (0.4)	129.7 (20.2)	2.8 (0.6)	0.4 (0.6)	0.8 (0.4)
Bunchgrass	Irr.	18.0 (2.8)	7.6 (0.2)	7.9 (0.7)	175.6 (51.2)	2.3 (1.0)	0.7 (0.4)	0.3 (0.2)
	Non-Irr.	17.8 (2.4)	7.7 (0.2)	8.0 (0.4)	114.3 (23.1)	2.0 (0.6)	1.0 (0.4)	0.4 (0.2)

*Data format: average (standard deviation)

RELEVANT LITERATURE REVIEW:

Wang *et al.* [2010] note that surface water quality is negatively influenced by urbanization processes causing increased pollutant loads and increases in runoff peak flows and volumes. Storm water best management practices (BMPs) have been shown to reduce stormwater quantity while improving stormwater quality [Holloway *et al.*, 2009]. Green roofs have advantages of mitigating air pollution and carbon dioxide emissions, carbon sequestration, longevity of roofing membranes resulting in fewer roofing materials being disposed of in landfills, reduction of noise pollution, and improved water quality of storm water runoff [Rowe, 2011]. Emerging low impact development approaches have advantages of reducing urban runoff, restoring more naturalized hydrographs, delaying costly replacements of aging roofing infrastructure [Drake *et al.*, 2010], promoting green construction, mitigating urban heat island effects, and thermal reduction and moderate temperature variations around buildings [Chen, 2013]. Green roofs provide an option to improve storm water runoff

[Hathaway *et al.*, 2008]. A number of research investigations addressing runoff water quality emanating from vegetated roofs have been reported in the technical literature. A number of these investigations are described below.

Increasing urbanization and rapidly growing populations are placing a strain on the world's potable water supply [Nicholson *et al.*, 2010]. Chen [2013] notes that without additional maintenance, green roofs can contribute to nonpoint source pollution in wet and hot weather zones due to high runoff and associated mass loading. Beck *et al.* [2011] note that with increasing use of vegetated roofs in urban centers, designing a green roof soil is essential to reducing the amount of nutrients in the storm water runoff. This was also confirmed in a study by Moran and Hunt [2005]. Alexander [2004] notes that a good green roof media should have good drainage and aeration, good water holding capacity, good nutrient holding capacity (cation exchange capacity), and permanent, light-weight, sturdy, and stable media. Vijayaraghavan *et al.* [2012] note that the impact of green roofs on stormwater quality is a major topic of concern for city planners and environmental policy makers. They studied whether green roofs act as a source or as a sink for various metals, including: sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), aluminum (Al), iron (Fe), cadmium (Cd), lead (Pb), zinc (Zn), manganese (Mn), lithium (Li), and cobalt (Co), along with inorganic anions [nitrate (NO_3^-), nitrite (NO_2^-), phosphate (PO_4^{3-}), sulfate (SO_4^{2-}), chloride (Cl^-), fluoride (F^-), and bromide (Br^-)]. Using several different experimental green roof systems, four rainfall events and several artificial rain events were investigated. Their results indicated that concentrations of most of the chemical components in the runoff were highest at the beginning of the rainfall event (i.e., the "first-flush" effect) and subsided in subsequent rain events. Notable contaminants present in the runoff included: Na, K, Ca, Mg, Li, Fe, Al, Cu, NO_3^- , PO_4^{3-} , and SO_4^{2-} . They further note that the concentrations of these contaminants are dependent on the nature of the substrate used in the green roof and the volume of rainfall [Vijayaraghavan *et al.*, 2012]. They concluded that the water quality emanating from green roofs is good, except that the runoff can contain significant amounts of NO_3^- and PO_4^{3-} .

Wang *et al.* [2010] studied first-flush effects on stormwater runoff from a blacktop driveway, a concrete roof, and a vegetated roof. They investigated first-flush effects of

chemical oxygen demand (COD), ammonia nitrogen, nitrate nitrogen, and total phosphorus for these three surfaces. They observed that 80% of the pollution load was delivered during the first 40% of the whole runoff period. By retaining the initial runoff, runoff pollution can be significantly reduced.

Alsup *et al.* [2013] examined heavy metal concentrations in runoff from simulated green roof systems after 22 to 32 months in the field. They observed that green roofs were not significant sources of heavy metals,

In a review paper, Berndtsson [2010] addressed the role of green roofs in urban drainage in terms of water quantity and quality. Factors influencing green roof performance included: green roof type, its geometrical properties (e.g., slope), soil moisture characteristics, weather and rainfall characteristics, age of the green roof, and its vegetation. Berndtsson's study showed that there is a significant need for research involving green roof performance. Additionally, the difference in water quality results between new and aged vegetated roof systems indicate a long-term need for monitoring of green roof system.

Berndtsson *et al.* [2009] note that although pollutant removal within vegetated roofs is often expected, it is not commonly a design feature. The researchers investigated the influence on runoff water quality from two full-scale vegetated roofs (using an intensive vegetated roof in Japan and an extensive vegetated roof in Sweden). Their results indicated that both intensive and extensive vegetated roofs act as a sink for nitrate nitrogen and ammonia nitrogen with similar performance. The intensive green roof also was a sink for total nitrogen, in contrast to the performance exhibited by the extensive green roof. Phosphorus release was observed from the extensive green roof, but not from the intensive green roof. Release of dissolved organic carbon and potassium was observed from both roof systems. The pH increased slightly during rainfall passage through the intensive vegetated roof, indicating rapid neutralization of the acid depositions [Berndtsson *et al.*, 2009].

Teemusk and Mander [2011] investigated runoff water quality of light weight aggregates (LWA)-based extensive green roofs and sod roofs in Estonia. Samples were collected from

August 2004 to April 2009 from ten different green roofs to determine the resultant water quality. Their results indicated that green roofs strongly influenced the resultant water quality. Runoff water of LWA-based green roofs generally had higher values of pH, BOD₇, total phosphorus, and PO₄-P than that from sod roofs, while COD, total nitrogen, SO₄⁼, and Ca-Mg salt were higher in sod roofs than in green roofs. The results for NH₄-N and NO₃-N were similar for both roof types. The character of the runoff and the contents in the substrate layer when the runoff samples were collected, affected runoff quality more than the age and location of the vegetated roof. The use of NPK-nutrients in the substrate or in the soil caused significantly higher values of COD and concentrations of total phosphorus, total nitrogen, PO₄-P, NH₄-N, and NO₃-N in runoff water than on non-fertilized green roofs. Samples taken from the LWA-green roofs each spring (when the snow had almost melted) from 2005 through 2009 indicated a gradual decrease in the various compounds.

Dr. van Seters *et al.* [2009] conducted a 3-year study to investigate the quality and quantity of runoff from an extensive green roof on a multi-story building in Toronto. Laboratory physical, chemical, and leachate analyses of 11 commercially available vegetated roof growing media were performed to identify the influence that growing media had on runoff water quality. Continuous runoff and precipitation data were collected over an 18-month period. Results indicated that runoff from green roofs averaged 42% less than that from conventional roofs during the period of April through November, and during the winter, the runoff from green roofs averaged 63% less than that from conventional roofs. During the summer month, runoff was 93% less for green roofs compared to conventional roofs. Water samples were collected from both roof types during 21 rain events in 2003 and 2004, and analyzed for general chemistry (pH, total suspended solids), metals, nutrients, bacteria, and polycyclic aromatic hydrocarbons. Loads for most chemical variables were generally lower for the green roof compared to the conventional roof. Exceptions to this generalization involved calcium, magnesium, and total phosphorus, which were either naturally present or were added to promote plant growth. Total phosphorus concentrations were significantly higher in the green roof than for the conventional roof at a statistical level of significance $\alpha = 0.01$. Phosphorus concentrations dropped significantly after the first year of monitoring ($\alpha = 0.001$), suggesting the nutrient was being leached from the media. Leachate concentrations

from several media exceeded receiving water standards for phosphorus, aluminum, copper, iron, and vanadium.

The green roof field study involving 28 ground level built-in-place vegetated roof models, four modular units, four roof decks, was studied by Morgan *et al.* [2013]. Storm water runoff quantity and quality was monitored from September 2005 through June 2008. Results indicated that vegetated roof systems significantly reduced storm water runoff and that system design, growth media depth, and presence of plants impacted green roof performance. In a second study on a building roof, two modular systems were used to evaluate water loss through evapotranspiration. Water loss in both systems was significant, and was influenced by system design, presence of plants, and the depth of the growth media. Runoff water quality from the ground-level field study and from laboratory pot studies, indicated that nitrate concentration, total suspended solids, and turbidity were generally low following a first-flush effect. The researchers also noted that runoff water quality was also influenced by system design, presence of plants, and the depth of the growth media.

Storm-water runoff from three full-scale roof surfaces (an asphalt roof, a stone-ballasted roof, and a vegetated roof) was investigated by Carpenter and Kaluvakolanu [2011]. Both the vegetated roof and the stone-ballasted roof were effective in reducing storm water runoff and attenuating peak runoff discharge, with the green roof more efficient for rainfall events less than 1.0 inch. Overall, the green roof retained 68.25% of rainfall volume and reduced peak discharge by an average of 88.86%. The water quality results were inconclusive, but generally showed that green roof systems could reduce nutrient loadings.

Runoff water quantity and quality from a 248 m² extensive green roof and a control were compared in a paired-watershed study [Gregoire and Clausen, 2011]. Weekly and individual rainstorm samples were collected and analyzed for total Kjeldahl nitrogen (TKN), NO₃ + NO₂ – N, total phosphorus, PO₄ – P, and total and dissolved copper (Cu), lead (Pb), zinc (Zn), cadmium (Cd), chromium (Cr), and mercury (Hg). The green roof watershed retained 51.4% of the rainfall during the study period. Overall, the green roof retained 34% more precipitation than predicted by the paired watershed calibration equation. Mean

concentrations of total phosphorus and phosphate phosphorus were higher in the green roof runoff than the precipitation, but were lower than in runoff from the control. The vegetated roof was a sink for ammonia-nitrogen, zinc, and lead, but not for total phosphorus, phosphate-phosphorus, and total copper. Vegetated roofs reduced the transport of total nitrogen, total Kjeldahl nitrogen, $\text{NO}_3 + \text{NO}_2 - \text{N}$, mercury, and dissolved copper, which was attributed to a reduction in stormwater runoff. More than 90% of the Cu, Hg, and Zn concentrations observed in the green roof runoff were in the dissolved form. The authors concluded that green roofs are effective in reducing stormwater runoff and overall pollutant loading for most contaminants.

Mentens *et al.* [2006] analyzed original measurements reported in 18 publications studying 628 data records. They studied the surface runoff from various roof types when roof characteristics and annual or seasonal precipitation data were given. The annual rainfall-runoff relationship for green roofs was strongly affected by the depth of the substrate layer. The retention of rainwater on green roofs was lower in winter months than in summer months. Their study indicated that if 10% of the roofs in Brussels were converted to green roofs, it would result in a runoff reduction of 2.7% in the region and a 54% reduction for individual buildings. They concluded that green roofs are a very useful tool for reducing urban rainfall runoff and pollutant loadings. Nicholson *et al.* [2009] concluded that certain roofing materials may be a pollution source. They studied several conventional roofs and an extensive green roof. During the first year, significant releases of zinc and copper (with potential toxicity concerns) originated from uncoated galvanized roofs and from two treated woods, and could cause significant buildup in the receiving soils. They further showed that traditional roofing materials such as uncoated galvanized metal and treated wood are more likely to leach heavy metals, nitrates and ammonia than other materials such as green roofs and coated metal roofs [Nicholson *et al.*, 2010].

Scholz [2004] investigated the water treatment potential of a storm water pond system for 15 months of operation. The system used a combined silt trap, attenuation pond, and a vegetated infiltration basin. Treatment of rainwater runoff from roofs was largely

unnecessary for recycling (e.g., irrigation of plants). The author noted seasonal variation in biochemical oxygen demand (BOD), dissolved oxygen, and pH.

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ACKNOWLEDGEMENTS:

Funding for this research-demonstration project was provided by the United States Geological Survey through the Water Resources Research Institute Program, under Section 104, Water Resources Research Act of 1984 to the Alabama Water Resources Research Institute. UAB researchers are grateful for the opportunity to utilize these funds to extend knowledge regarding the effectiveness of green roofs in the Southeastern United States.

J. NOTABLE AWARDS AND ACHIEVEMENTS.

- Yasminye Pettway (Research Intern) awarded 2012 US Navy Award and Stockholm Water Prize (to represent Alabama at National High School Science Fair Competition).
 - Construction of catchment systems and analysis of green roof runoff water quality.
- Julie Price awarded Outstanding Doctoral Graduate Student in UAB Biology 2012.

K. PUBLICATIONS GENERATED:

Number of Research Publications generated from this research project:	
Publication Category	Number
Articles in Refereed Journals	2 (under review)
Book Chapters	1
Theses and Dissertations	1
Water Resources Institute Reports	0
Articles in Conference Proceedings	1
Other Publications	2

1. Articles in Refereed Scientific Journals Citation

- Price, J.G., A.N. Wright, J.T. Kirby, R.W. Peters, and S.A. Watts. 2013. Irrigation Affects Plant Community Structure on Southeastern Green Roofs. *Ecological Engineering* (under review).
- Price, J.G., A.N. Wright, J.T. Kirby, R.W. Peters, and S.A. Watts. 2013. Influence of Water Availability and Season on Photosynthetic Gas Exchange of Three Green Roof Plant Species. *Oecologia* (under review).

2. Book Chapter Citation

- Peters, Robert W., Ronald D. Sherrod, and Matt Winslett, 2013. “Energy Savings Resulting from Installation of an Extensive Vegetated Roof Systems on a Campus Building in the Southeastern United States”, Chapter 2, pages 21–49 in *New Developments in Renewable Energy*, InTech Publishers, Rijeka, Croatia.

3. Dissertations Citation

- Price, Julie. 2012, Plant selection, community dynamics, ecophysiology, and stormwater mitigation on green roofs in the southeastern U.S. “Ph.D. Dissertation”, Biology Department, College of Arts and Sciences, University of Alabama at Birmingham, Birmingham, Alabama.

4. Water Resources Research Institute Reports Citation

None to Date.

5. Conference Proceedings Citation

- Kirby, Jason T., Ronald Sherrod, Matthew Winslett, and Robert W. Peters, 2012. “Variations in Temperature in Mini-Roof Structures Employing Different Roofing Materials”, *Proc. 2012 AIChE Annual Meeting*, Pittsburgh, PA, (October 28–November 2).

6. Other Publications Citation

- Pettway, Yasminye D., Robert W. Peters, Jason T. Kirby, Stephen A. Watts, and Julie G. Price, 2012. “Final Report: The Effects of Green Roofs on Nitrogen in Stormwater Runoff”, Report submitted to STEP-UP High School Program, National Institute for Diabetes and Digestive and Kidney Diseases (NIDDK), U.S. Department of Health and Human Services, Bethesda, MD, (August 12 – 14).
- Sherrod, Braxton E., Robert W. Peters, Julie G. Price, Jason T. Kirby, and Stephen A. Watts. 2012. “Final Report: The Effect of Vegetated Roofs on Phosphorus Leaching and Stormwater Quality”, Report submitted to STEP-UP High School Program, National Institute for Diabetes and Digestive and Kidney Diseases (NIDDK), U.S. Department of Health and Human Services, Bethesda, MD, (August 12 – 14).

L. PRESENTATIONS MADE:

- Glassie, Heidi, 2013, Experimental Investigation of Stormwater Runoff Quality Originating from Urban Vegetated Roofs, UAB Science and Technology Honors Program Defence, UAB Campbell Hall, Birmingham AL.
- Manzella, Ashlyn, and Katie Rigney, 2013. “Greenroofs and their Effects on Energy Costs”, Class project report and class project presentation for CE 431/531 (Energy Resources) class, University of Alabama at Birmingham, Birmingham, AL.
- Peters, R.W., Price, J.G., and J.T. Kirby. 2012. Tour of Hulsey Center pilot green roof and discussion of green roof principles for Little Garden Club (Birmingham Chapter), UAB Campus, Birmingham, AL, (April 4).
- Pettway, Yasminye D., 2012. “The Effects of Green Roofs on Nitrogen in Stormwater Runoff”, Oral Presentation at the STEP-UP High School Program Conference, National Institute for Diabetes and Digestive and Kidney Diseases (NIDDK), U.S. Department of Health and Human Services, Bethesda, MD, (August 12 – 14).
- Sherrod, Braxton E., “The Effect of Vegetated Roofs on Phosphorus Leaching and Stormwater Quality”, Oral Presentation at the STEP-UP High School Program Conference, National Institute for Diabetes and Digestive and Kidney Diseases (NIDDK), U.S. Department of Health and Human Services, Bethesda, MD, (August 12 – 14).

- Kirby, J., K. Sheibley, J. Price, M. Winslett, S. Watts, and R. Peters. 2012. Runoff Mitigation from Urban Vegetated Roofs in the Southeastern US. Proc. Alabama Water Resources Research Conference, Orange Beach, AL (September). (oral presentation with abstract)
- Price, J.G., A.N. Wright, S.A. Watts, J.T. Kirby, and S.A. Watts. 2012. Photosynthetic Gas Exchange and Transpiration of Three Diverse Species in Response to Water Deprivation and Recovery on a Southeastern Green Roof. Proc. Alabama Water Resources Research Conference, Orange Beach, AL (September). (poster presentation with abstract)
- Price, J.G., A.N. Wright, S.A. Watts, J.T. Kirby, and R.W. Peters. 2012. Photosynthetic Rates of Two Southeastern Green Roof Species in Response to Drought and Recovery. HortScience in press. (oral presentation)
- Price, J.G., A.N. Wright, J.T. Kirby, R.W. Peters, and S.A. Watts. 2012. Photosynthetic Gas Exchange of Phlox bifida and Sedum album in Response to Water Deprivation and Recovery on a Southeastern Green Roof. J. Ala. Acad. Sci. in press. (oral presentation with abstract).

M. STUDENTS SUPPORTED (Complete the following table)

Number of Students Supported, by Degree	
Type	Number of students funded through this research project:
Undergraduate	5 - High School Honors Program Interns 2 - UAB Science and Technology Honors Program
Masters	0
Ph.D.	1 - UAB (Biology)
Post Doc	0
Number of Theses and Dissertations Resulting from Student Support:	
Master's Theses	0
Ph.D. Dissertations	1 - UAB (Biology)

N. RESEARCH CATEGORIES: (In column 1 mark all that apply)

	Research Category
X	Biological Sciences
	Climate and Hydrological Processes
X	Engineering
	Ground Water Flow and Transport
	Social Sciences
X	Water Quality
	Other: Explain

O. FOCUS CATEGORIES (mark all that apply with “X” in column 1):

	ACID DEPOSITION	ACD
	AGRICULTURE	AG
	CLIMATOLOGICAL PROCESSES	CP
	CONSERVATION	COV
	DROUGHT	DROU
	ECOLOGY	ECL
	ECONOMICS	ECON
	EDUCATION	EDU
	FLOODS	FL
	GEOMORPHOLOGICAL PROCESSES	GEOMOR
	GEOCHEMICAL PROCESSES	GEOCHE
	GROUNDWATER	GW
	HYDROGEOCHEMISTRY	HYDGEO
X	HYDROLOGY	HYDROL
	INVASIVE SPECIES	INV
	IRRIGATION	IG
	LAW, INSTITUTIONS, & POLICY	LIP
	MANAGEMENT & PLANNING	M&P
	METHODS	MET
	MODELS	MOD
	NITRATE CONTAMINATION	NC
X	NONPOINT POLLUTION	NPP
	NUTRIENTS	NU
	RADIOACTIVE SUBSTANCES	RAD

	RECREATION	REC
	SEDIMENTS	SED
	SOLUTE TRANSPORT	ST
	SURFACE WATER	SW
	TOXIC SUBSTANCES	TS
	TREATMENT	TRT
	WASTEWATER	WW
X	WATER QUALITY	WQL
	WATER QUANTITY	WQN
	WATER SUPPLY	WS
	WATER USE	WU
	WETLANDS	WL

P. DESCRIPTORS: (Enter keywords of your choice, descriptive of the work)

- Stormwater Quality, Urban Runoff.

USGS Project 2011AL121G – Forecasting toxic cyanobacterial blooms throughout the southeastern U.S.

Basic Information

Title:	USGS Project 2011AL121G – Forecasting toxic cyanobacterial blooms throughout the southeastern U.S.
Project Number:	2012AL163G
Start Date:	10/1/2011
End Date:	9/30/2014
Funding Source:	104G
Congressional District:	3rd
Research Category:	Biological Sciences
Focus Category:	Toxic Substances, Water Quality, Nutrients
Descriptors:	Algal blooms, cyanobacteria, off-flavor, toxin, microcystin, BMAA, cylindrospermopsin, saxitoxin, phytoplankton, modeling, forecasting, monitoring, network, collaboration
Principal Investigators:	Alan Elliott Wilson

Publications

1. Doster, Enrique; Chislock, Michael F.; Roberts, John; Kottwitz, Jack; and Wilson, Alan E. Recognition of an important water quality issue at zoos: prevalence and potential threat of toxic cyanobacteria. Accepted pending revisions at Journal of Zoo and Wildlife Medicine.
2. Chislock, Michael F.; Doster, Enrique; Zitomer, Rachel A.; and Wilson, Alan E. 2013. Eutrophication: Causes, consequences, and controls in aquatic ecosystems. Nature Education Knowledge 4(4):10.
3. Wilson, Alan E. and Chislock, Michael F. 2013. Ecological control of cyanobacterial blooms in freshwater ecosystems. Invited book chapter in Cyanobacteria: Toxicity, ecology, and management. Editor: A. Ferrão-Filho. Nova Science Publishers, Inc., New York.
4. Fowler, Samuel; Deutsch, William; Wilson, Alan, E.; and Reutebuch, E. 2012. Tallapoosa River basin numerical nutrient criteria for wadeable streams. Final Report for the Alabama Department of Environment Management, Agreement ADEM-C00594051

ANNUAL TECHNICAL REPORT SYNOPSIS

The Terms and Conditions of the grants awarded under the Water Resources Research Act state that each institute shall prepare an Annual Program Report summarizing its activities during the reporting period under its base grant, and National Competitive Grant Program awards. The reporting period is March 1, through February 28. All Annual Reports must be submitted by 5:00 PM, Eastern Daylight Time, June 1, and must be submitted electronically. In order to do this we need your assistance by providing the following information about your current or recent WRRI-funded research project:

- A. PROJECT TITLE:
USGS Project 2011AL121G – Forecasting toxic cyanobacterial blooms throughout the southeastern U.S.
Project website - http://wilsonlab.com/bloom_network/
- B. PRIMARY PI(s): Name(s), Title(s) & Academic Rank(s)
Alan E. Wilson, Assistant Professor, Ph.D.
- C. OTHER PI(s): Name(s), Title(s) & Academic Rank(s)
Russell A. Wright, Associate Professor, Ph.D.
Kevin Schrader, Microbiologist, Ph.D.
- D. START DATE:
1 October 2011
- E. END DATE:
30 September 2014
- F. PROJECT OVERVIEW/SUMMARY: Provide a brief narrative overview or summary of the project.
Using a novel collaborative approach, we are collecting water quality samples and associated data from 400+ diverse freshwater systems, including lakes, reservoirs, ponds, and rivers, throughout much of the eastern U.S. These samples will be analyzed by the PIs for phycocyanin (cyanobacteria), cyanobacterial toxins, off-flavors, and phytoplankton enumeration. Data generated from these efforts will be used to refine and build models aimed at forecasting blooms of freshwater cyanobacterial blooms. Although the focus of the current project is on the Southeast, we have quickly expanded our efforts beyond this region. We hope to continue this expansion throughout the 3-year project.
- G. PROJECT OBJECTIVE(s): Briefly explain the project objectives.
 - 1) To enhance our network of water quality managers and scientists throughout the southeastern U.S. aimed at monitoring sites for toxic cyanobacterial blooms.
 - 2) To test and refine current models that forecast toxic cyanobacterial blooms and off-flavor events in freshwater lakes, reservoirs, rivers, and ponds throughout the Southeast.
 - 3) To train state and federal scientists, water quality managers, and aquaculturists on standard techniques to measure cyanobacterial toxin and phycocyanin concentrations and to identify and enumerate phytoplankton.

- 4) To train graduate and undergraduate students on field sampling and laboratory-based water quality analytical analyses.
- 5) To enhance our existing, user-friendly, interactive website where water quality managers and aquaculturists can determine the risk of their waterbodies for toxic cyanobacterial blooms and/or off-flavor events.

To create a model collaborative network that can be extended to other U.S. regions.

H. **METHODOLOGIES:** Briefly explain the research methodology used.

Sample sharing is central to the success of our project. We are also planning to share data among collaborators, but we are most excited about our approach for bringing together scientists in academia, agencies, and industry who all share a common concern – algal blooms. We are leveraging resources provided by our many colleagues throughout the eastern U.S. to collect and analyze water quality samples for us. In turn, we will analyze these samples for phytoplankton, cyanobacteria, and cyanobacterial toxins and off-flavors in order to build algal bloom forecasting models.

I. **PRINCIPAL FINDINGS/RESULTS:** Explain the results of findings of this research project.

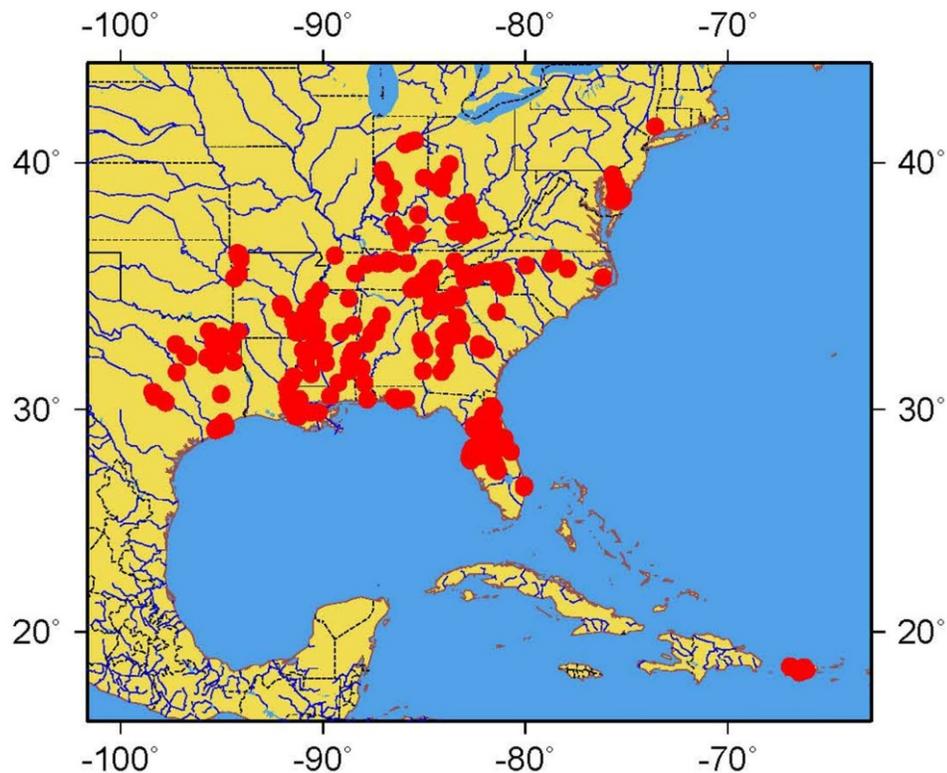
Our first full project year has been remarkable. We have received significant interest in our project from dozens and dozens of agency and academic scientists throughout the eastern U.S. (13 states and Puerto Rico, see map of 2012 study sites below). Much of this interest was created through a large number of conference presentations (n = 11) that Wilson and his students gave about this project at diverse venues around the county. Although we proposed to get samples and data from 200 sites per year, we processed samples from almost double this number in 2012 (toxins = 389 waterbodies, phycocyanin = 363 waterbodies, off-flavors = 100 waterbodies). Wilson planned to begin counting phytoplankton samples from the summer of 2012 during the spring of 2013, but he was awarded a highly competitive one semester fellowship at the University of North Carolina Global Research Institute where he was able to interact with other water resource scientists from around the world (<http://gri.unc.edu/people/alan-wilson>). In addition to conducting research, Wilson also helped organize a new course at UNC-CH, called Water in Our World. Wilson will count phytoplankton samples from 2012 during the summer of 2013.

In addition to many conference presentations, this project has already been productive, including 2 journal articles, 1 book chapter, and 1 agency report that discuss the dangers and controls of eutrophication, water quality in moats, and reservoir nutrient criteria development. The articles and book chapters include student co-authors. We also have two manuscripts in preparation to be submitted to appropriate journals this summer. One of these manuscripts describes general water quality models for the southeastern U.S. The other manuscript validates the utility of our phycocyanin protocol for quickly quantifying cyanobacterial abundance in waterbodies of varying trophic state. We expect our project collaborators to find these publications useful for their research and water resource management.

Our outreach activities continue to be wildly successful. We held two water quality workshops during the spring of 2012 (Orlando and Auburn) and another set of workshops in the spring of

2013 (Orlando and Chapel Hill). All workshops were well-attended (16-22 students each), and we received feedback showing that our students learned a lot about the project and our analytical and modeling approaches. We will be organizing similar workshops next spring. In addition to our workshops, Wilson also trained a collaborator from the Florida Fish and Wildlife Conservation Commission in phytoplankton enumeration during an intensive one-week training program. Wilson was also invited to give a seminar about algal blooms and silt issues in Jackson Lake (Georgia) to the Jackson Lake Homeowners Association. More than 200 attendees were present for the seminar, which also received some press (<http://www.covnews.com/multimedia/archives/493/>). Wilson was also invited to record a podcast about algal blooms for GreenSense (<http://greensenseshow.com/Ways-To-Listen/Show.aspx?ShowNum=135>). In 2012, we continued our outreach and research activities at the Montgomery Zoo where we were interested in evaluating moat water quality. Lastly, Wilson continues to lead outreach activities at daycares and prisons to educate unique audiences about water quality. Wilson was recently awarded an Auburn University outreach grant (\$20,000) to extend our prison science seminar series for the next academic year. This project has engaged 19 Auburn University faculty with prison populations. Wilson gave a lecture about eutrophication during his visit.

2012 sample site locations



J. NOTABLE AWARDS AND ACHIEVEMENTS. List any awards or recognitions for this research
PI HONORS AND AWARDS

Alan Wilson Semester fellowship at UNC-CH Global Research Institute, \$35,000
 Alan Wilson Auburn University outreach grant, \$20,000

STUDENT HONORS AND AWARDS

Enrique Doster Auburn University Research Week 3rd place STEM poster
 Jo-Marie Kasinak Auburn University Outstanding Oral Presentation at Graduate Forum
 Enrique Doster Auburn University Undergraduate Research Fellow award, \$6,000

K. PUBLICATIONS GENERATED:

Number of Research Publications generated from this research project:	
Publication Category	Number
Articles in Refereed Journals	2
Book Chapters	1
Theses and Dissertations	0
Water Resources Institute Reports	0
Articles in Conference Proceedings	0
Other Publications	1

PROVIDE A CITATION FOR EACH PUBLICATION USING THE FOLLOWING FORMATS:

1. Articles in Refereed Scientific Journals Citation

Author (first author; last name, first name; all others; fist name, last name), Year, Title, Name of Journal, Volume(Number), Page Numbers.

- 1) Doster, Enrique; Chislock, Michael F.; Roberts, John; Kottwitz, Jack; and Wilson, Alan E. Recognition of an important water quality issue at zoos: prevalence and potential threat of toxic cyanobacteria. *Accepted pending revisions* at Journal of Zoo and Wildlife Medicine.
- 2) Chislock, Michael F.; Doster, Enrique; Zitomer, Rachel A.; and Wilson, Alan E. 2013. Eutrophication: Causes, consequences, and controls in aquatic ecosystems. *Nature Education Knowledge* 4(4):10.
<http://www.nature.com/scitable/knowledge/library/eutrophication-causes-consequences-and-controls-in-aquatic-102364466>

2. Book Chapter Citation

Author (first author; last name, first name; all others: first name, last name), Year, Title of chapter, "in" Name(s) of Editor "ed.", Title of Book, City, State, Publisher, Page Numbers.

- 1) Wilson, Alan E. and Chislock, Michael F. 2013. Ecological control of cyanobacterial blooms in freshwater ecosystems. Invited book chapter in *Cyanobacteria: Toxicity, ecology, and management*. Editor: A. Ferrão-Filho. Nova Science Publishers, Inc., New York.

https://www.novapublishers.com/catalog/product_info.php?products_id=39768&osCsid=4cbf40b3b6af7b278a7aadd48558f395

3. Dissertations Citation

Author (last name, first name), Year, Title, "MS (Ph.D.) Dissertation," Department, College, University, City, State, Number of Pages.

None

4. Water Resources Research Institute Reports Citation

Author (first author; last name, first name; all others: first name, last name), Year, Title, Name of WRRI, University, City, State, Number of Pages.

None

5. Conference Proceedings Citation

Author (first author; last name, first name; all others: first name, last name), Year, Title of Presentation, "in" Title of Proceedings, Publisher, City, State, Page Numbers.

None

6. Other Publications Citation

Author (first author; last name, first name; all others: first name, last name), Year, Title, other information sufficient to locate publications, Page Numbers (if in publication) or Number of Pages (if monograph).

- 1) Fowler, Samuel; Deutsch, William; Wilson, Alan, E.; and Reutebuch, E. 2012. Tallapoosa River basin numerical nutrient criteria for wadeable streams. Final Report for the Alabama Department of Environment Management, Agreement ADEM-C00594051

PRESENTATIONS MADE:

Presenter(s) (last name, first name; all others presentation authors: first name, last name), Year, Title, other information sufficient to identify the venue in which the presentation was made.

- 1) Doster, Enrique; Chislock, Michael F.; Kottwitz, Jack; and Wilson, Alan, E. 2013. A survey of moat water quality at the Montgomery Zoo (Alabama). 27th National Conference on Undergraduate Research (NCUR), University of Wisconsin-La Crosse, Wisconsin.
- 2) Doster, Enrique; Chislock, Michael F.; Kottwitz, Jack; and Wilson, Alan, E. 2013. A survey of moat water quality at the Montgomery Zoo (Alabama). Auburn University Research Week, Auburn University, Auburn, Alabama. (poster) **(3rd place STEM poster presentation)**
- 3) Kasinak, Jo-Marie.; Holt, Brittany; and Wilson, Alan E. 2013. Phycocyanin fluorometric analysis: a new method for estimating cyanobacterial abundance. Auburn University Research Week, Auburn University, Auburn, Alabama.

- 4) Kasinak, Jo-Marie.; Holt, Brittany; and Wilson, Alan E. 2013. Phycocyanin fluorometric analysis: a new method for estimating cyanobacterial abundance. Auburn University Graduate Student Council Forum, Auburn University, Auburn, Alabama. **(Outstanding oral presentation award)**
- 5) Holt, B.; Kasinak#, and Wilson, Alan E. 2012. Developing a new method using phycocyanin to quickly and reliably estimate cyanobacterial biomass. Annual Biomedical Research Conference for Minority Students, San Jose, California. (poster)
- 6) Wilson, Alan E.; Wright, Russell, A.; Schrader, Kevin, K.; Curvin, Gina, L.; Rosen, Barry; and Graham, Jennifer, L. 2012. Creating cost-effective regional algal bloom monitoring networks: Extending from the Southeast to the Midwest. 37th Annual Great Plains Limnology Conference, University of Arkansas, Fayetteville, Arkansas.
- 7) Wilson, Alan E.; Wright, Russell, A.; Schrader, Kevin, K.; Curvin, Gina, L.; Rosen, Barry; and Graham, Jennifer, L. 2012. Creating cost-effective regional algal bloom monitoring networks: The Southeast as a case study. University of North Carolina at Chapel Hill Water and Health Symposium, Chapel Hill, North Carolina.
- 8) Wilson, Alan E.; Wright, Russell, A.; Schrader, Kevin, K.; Curvin, Gina, L.; Rosen, Barry; and Graham, Jennifer, L. 2012. Creating cost-effective regional algal bloom monitoring networks: Extending beyond Alabama. Alabama Water Resources Conference, Orange Beach, Alabama.
- 9) Wilson, Alan E.; Wright, Russell, A.; Schrader, Kevin, K.; Curvin, Gina, L.; Rosen, Barry; and Graham, Jennifer, L. 2012. Creating cost-effective regional algal bloom monitoring networks: The Southeast as a case study. 21st SE NALMS Southeastern Lake and Watershed Management Conference. Columbus, Georgia.
- 10) Wilson, Alan. E.; Kaul, RajReni B.; Chislock, Michael F.; and Curvin, Gina L.. 2012. Towards an improved understanding of the factors mediating toxic cyanobacterial blooms throughout the Southeast. Association of Southeastern Biologists, Athens, Georgia.
- 11) Wilson, Alan E.; Wright, Russell, A.; Schrader, Kevin, K.; Curvin, Gina, L.; Rosen, Barry; and Graham, Jennifer, L. 2012. Creating cost-effective regional algal bloom monitoring networks. 8th National Monitoring Conference. Portland, Oregon, 2012.

L. STUDENTS SUPPORTED (Complete the following table)

Number of Students Supported, by Degree	
Type	Number of students funded through this research project:
Undergraduate	6
Masters	2
Ph.D.	1
Post Doc	0
Number of Theses and Dissertations Resulting from Student Support:	
Master's Theses	0
Ph.D. Dissertations	0

M. RESEARCH CATEGORIES: (In column 1 mark all that apply)

	Research Category
X	Biological Sciences
	Climate and Hydrological Processes
	Engineering
	Ground Water Flow and Transport
	Social Sciences
X	Water Quality
X	Other: Modelling

N. FOCUS CATEGORIES (mark all that apply with "X" in column 1):

	ACID DEPOSITION	ACD
	AGRICULTURE	AG
	CLIMATOLOGICAL PROCESSES	CP
X	CONSERVATION	COV
	DROUGHT	DROU
	ECOLOGY	ECL
	ECONOMICS	ECON
X	EDUCATION	EDU
	FLOODS	FL
	GEOMORPHOLOGICAL PROCESSES	GEOMOR
	GEOCHEMICAL PROCESSES	GEOCHE
	GROUNDWATER	GW
	HYDROGEOCHEMISTRY	HYDGEO
	HYDROLOGY	HYDROL
	INVASIVE SPECIES	INV
	IRRIGATION	IG
	LAW, INSTITUTIONS, & POLICY	LIP
X	MANAGEMENT & PLANNING	M&P
X	METHODS	MET
X	MODELS	MOD
X	NITRATE CONTAMINATION	NC
	NONPOINT POLLUTION	NPP
X	NUTRIENTS	NU
	RADIOACTIVE SUBSTANCES	RAD
	RECREATION	REC

	SEDIMENTS	SED
	SOLUTE TRANSPORT	ST
X	SURFACE WATER	SW
X	TOXIC SUBSTANCES	TS
	TREATMENT	TRT
	WASTEWATER	WW
X	WATER QUALITY	WQL
	WATER QUANTITY	WQN
	WATER SUPPLY	WS
	WATER USE	WU
	WETLANDS	WL

- O. DESCRIPTORS: (Enter keywords of your choice, descriptive of the work)
 Algal blooms, cyanobacteria, off-flavor, toxin, microcystin, BMAA, cylindrospermopsin, saxitoxin,
 phytoplankton, modeling, forecasting, monitoring, network, collaboration

Quantitative PCR-based assays for detection of wildlife and pet fecal pollution in water

Basic Information

Title:	Quantitative PCR-based assays for detection of wildlife and pet fecal pollution in water
Project Number:	2012AL164B
Start Date:	3/1/2012
End Date:	2/28/2013
Funding Source:	104B
Congressional District:	3rd
Research Category:	Biological Sciences
Focus Category:	Non Point Pollution, Water Quality, Surface Water
Descriptors:	Bacteria, water quality, biotechnology, pollution control, water quality management, watershed management
Principal Investigators:	, Yucheng Feng

Publications

1. Wijesinghe, R. U. and Feng, Y. (2012) Identification of fecal contamination sources in Parkerson Mill Creek at Auburn, Alabama. The 2012 Annual Meeting of the Alabama, Georgia, and Florida Chapters of the Soil and Water Conservation Society. Eufaula, AL.
2. Xue, J., Wijesinghe, R. U., Feng, Y., Wood, C. (2012) Determination of fecal pollution sources at an Alabama beach. The 26th Annual Alabama Water Resources Conference. Orange Beach, AL.
3. Wijesinghe, R. U. and Feng, Y. (2012). Evaluation of human and cattle host specific genetic markers for bacterial source tracking in a small urban watershed. Bacterial Source Tracking State of the Science Conference Proceedings. Texas Water Resources Institute.

Project Report
For
Alabama Water Resource Research Institute/USGS
May 29, 2013

- A. PROJECT TITLE:** Quantitative PCR-based assays for detection of wildlife and pet fecal pollution in water
- B. PRIMARY PI:** Yucheng Feng, Professor
- C. OTHER PI:** Eve F. Brantley, Extension Specialist and Assistant Professor
- D. START DATE:** March 1, 2012
- E. END DATE:** February 28, 2013, no-cost extension to April 30, 2013

The water quality of many waterways in our state and nation is deteriorating due to point and nonpoint source pollution from human and animal wastes. Accurate identification of contamination sources is essential for developing cost-effective pollution control strategies. Direct detection of host-specific genetic markers by polymerase chain reactions (PCR) has been widely used in identifying sources of fecal contamination in environmental waters. In this study, we conducted experiments to validate three genetic markers associated with wildlife and pets for fecal source identification in Alabama. More than 430 end-point PCR were performed on 10 raw sewage samples and 133 fecal samples from nine animal species. Our results show that the avian marker, GFD, and the deer/elk marker had acceptable specificity and sensitivity and thus can be used for bacterial source tracking studies in Alabama. The dog marker, however, was found in only 12 of the 22 dog fecal samples and showed cross reactivities with horse and sewage samples. To further validate the host-specific markers in environmental water, we collected 12 water samples over a 22-day period from Parkerson Mill Creek, an impaired creek on Alabama's 303(d) list. Human, avian, and dog markers were detected in several water samples. No cattle and deer/elk markers were detected. A very high concentration of the human marker was found by qPCR after a significant rainfall event. The presence of the GFD marker indicates that Canada geese and other avian species may contribute to fecal contamination in the creek as well. We are still in the process of testing the qPCR protocol for the GFD marker.

G. PROJECT OBJECTIVES

The specific objectives of this study were to

1. Validate three genetic markers associated with wildlife and pets for fecal source identification in Alabama;
2. Develop quantitative PCR assays for enumeration of host-specific genetic markers;
3. Determine the performance of host-specific qPCR assays in environmental water samples.

H. METHODOLOGIES

Fecal and water sample collection. A total of 133 fecal samples and 10 raw sewage samples were collected around Auburn, Opelika, and Montgomery, Alabama. Fecal specimens represented nine different animal species, including dogs (22), deer (26), Canada geese (26), cats (12), cattle (11), chickens (11), ducks (8), horses (14), and goats (3). Raw sewage samples were concentrated by centrifugation. Fecal samples were stored at -80°C until use. Stream water samples were collected weekly from three sites in the Parkerson Mill Creek watershed for a 22-day period. Grab samples were collected in sterile 1-liter bottles; 500 ml of each sample was vacuum filtered through 0.45 µm membrane filters to collect bacterial cells. Membrane filters were then stored at -20°C until use.

DNA extraction. DNA from fecal and water samples were extracted using the MoBio PowerSoil DNA extraction kit following the manufacturer's instructions. About 0.15 to 0.25 g of fecal samples and 300 µl of concentrated sewage were used to extract DNA. DNA from water samples was extracted from bacteria adhering to the surface of membrane filters that were cut into small pieces prior to DNA extraction. DNA concentrations were quantified with a NanoDrop ND-1000 UV spectrophotometer.

Enumeration of *E. coli* in water samples. *E. coli* concentrations in the water samples were determined by membrane filtration, followed by cultivation on modified mTEC agar (USEPA 2002). The mTEC agar plates were incubated at 35°C for 2 hours and then at 44.5°C for 22-24 hours. Plates having 20 to 80 *E. coli* colonies were counted.

End-point and quantitative PCR assays. Validation of published host-specific genetic markers was performed to verify the discriminating power of these markers among animal host groups. End-point PCR assays were performed to assess the sensitivity and specificity of bird-, deer/elk-, and dog-specific markers. We evaluated two genetic markers (GFC and GFD) for birds and one marker each for deer and dogs. Table 1 shows PCR primers used for each assay. More than 430 end-point PCR were performed using these host-specific markers. Quantitative PCR (qPCR) assays were performed using general *Bacteroidales* markers and human- and cattle-specific markers (Table 1).

Table 1. Regular and quantitative PCR assays used in this study

Assay	Target	Primer sequence (5'-3')	Product size (bp)	Annealing Temp (°C)	Reference
AllBac296F	General	GAGAGGAAGGTCCCCAC	106	60	Layton et al. 2006
AllBac412R	<i>Bacteroidales</i>	CGCTACTTGGCTGGTTCAG			
HF183F	Human	ATCATGAGTTCACATGTCCG	82	60	Bernhard & Field 2000
HF265R		TACCCCGCCTACTATCTAATG			
CowM3F	Cattle	CCTCTAATGGAAAATGGATGGTATCT	122	60	Shanks et al. 2008
CowM3R		CCATACTTCGCCTGCTAATACCTT			
CowM2F	Cattle	CGGCCAAATACTCCTGATCGT	92	63	
CowM2R		GCTTGTTGCGTTCCTTGAGATAAT			
GFD F	Bird	TCGGCTGAGCACTCTAGGG	123	57	Green et al. 2012
GFD R		GCGTCTCTTTGTACATCCCA			
GFC F	Bird	CCCTTGTCGTTAGTTGCCATCATTC	162	69	
GFC R		GCCCTCGCGAGTTCGCTGC			
EF447F	Deer/Elk	AATAACACCATCTACGTGTAGA	663	62	Dick et al. 2005
EF990R		GCCTGTCCAGTGCAATTTAA			
DF475F	Dog	CGCTTGATGTACCGGTACG	251	62	
Bac708R		CAATCGGAGTTCTTCGTG			

I. PRINCIPAL FINDINGS/RESULTS

Validation of host-specific genetic markers

Performance of host-specific genetic markers must be evaluated to ensure data quality and proper use of methods. Specificity and sensitivity are two criteria that are typically used in the evaluation of host-specific genetic markers in bacterial source tracking studies. Specificity is the probability to detect a source when it is not present and sensitivity is the probability to detect a source when it is present. The following formulas are used for calculations:

Specificity= [No. of True Negatives/(No. of True Negatives + No. of False Positives)] x 100%

Sensitivity= [No. of True Positives/(No. of True Positives + No. of False Negatives)] x 100%

Genetic markers for birds. Two avian genetic markers, GFC and GFD, targeting gulls, Canada geese, ducks, and chicken (Green et al. 2012) were evaluated in this study. The GFC marker was detected in 84.6% of Canada goose fecal samples; however, it also cross-reacted with all seven sewage samples tested. Because of the cross reaction with sewage samples, we did not further evaluate the GFC marker using fecal samples from other animal species. The GFD marker was detected in 84.6% and 27.3% of Canada goose and chicken samples, respectively, but in none of the duck samples (Table 2). There were no false positive reactions observed for this marker.

Table 2. Summary of host-specific marker evaluation by end-point PCR

Fecal source	Percentage of samples amplified (Positive/Total)		
	GFD	Deer/Elk	Dog
Canada Geese	85 (22/26)	0 (0/26)	0 (0/26)
Chicken	27 (3/11)	0 (0/11)	0 (0/11)
Duck	0 (0/8)	0 (0/8)	0 (0/8)
Deer	0 (0/26)	100 (26/26)	0 (0/26)
Dog	0 (0/22)	0 (0/22)	54 (12/22)
Horse	0 (0/14)	0 (0/14)	100 (14/14)
Cat	0 (0/12)	0 (0/12)	0 (0/12)
Goat	0 (0/3)	100 (3/3)	0 (0/3)
Cattle	0 (0/11)	27 (3/11)	0 (0/11)
Sewage	0 (0/10)	0 (0/10)	100 (10/10)

Genetic marker for deer/elk. Evaluation of the genetic marker developed for deer/elk (Dick et al. 2005) showed that it was present in 100% of the deer fecal samples and 27.3% of the cattle samples (Table 2). The overall specificity and sensitivity of the deer/elk marker were 94.9% and 100%, respectively.

Genetic marker for dog. The dog marker was detected in 12 of the 22 dog fecal samples, all 10 sewage samples, and all 14 horse samples (Table 2). Therefore, the dog marker had the lowest specificity (80.2%) and sensitivity (54.5%) among the three markers evaluated. Currently, there are only two genetic markers reported for dogs and both suffer from low specificity and sensitivity.

Testing of host-specific markers in environmental water samples

Twelve environmental water samples were collected weekly from three locations along Parkerson Mill Creek from April 12 to May 3, 2013. Site I was located on West Thach Ave. near the Rugby field, Site B is located at the intersection of Samford Ave. and Biggio Dr., and Site Q is near Shug Jordan Parkway next to the AU Beef Teaching Unit. *E. coli* were found in all water samples, ranging from 225 CFU/100 ml on April 19 at site B to 5200 CFU/100 ml on April 26 at site I (Table 3). The highest geometric mean (GM) *E. coli* concentration was found at Site I and the lowest at site B, all which exceeded the EPA statistical threshold value of 410 CFU/100ml water for primary contact recreation (USEPA 2012).

Table 3. *E. coli* concentrations (CFU/100 ml) at Parkerson Mill Creek from April 12 to May 3, 2013

Site	12-Apr	19-Apr	26-Apr	3-May	GM
Site I	1633±141	3150±71	5200±566	385±49	1791
Site B	2300±849	225±71	1220±141	867±94	860
Site Q	2267±47	1433±94	540±170	665±78	1039

A total of 84 end-point PCR were performed on environmental water samples using seven genetic markers. The AllBac marker targeting the general *Bacteroidales* was detected in all 12 water samples (Table 4). The human marker, HF183, was detected in six of the 12 water samples, four of which from Site B and two from Site Q. The avian marker, GFD, was detected in 7 out of 12 water samples (58.3%), including all four samples from Site B. The dog assay showed positive results in 4 out of 12 water samples, three of which also contained the human marker. No cattle and deer/elk markers were detected in any of the water samples by end-point PCR.

A total of 48 qPCR was performed using AllBac, HF183, and CowM3 markers. The qPCR assay performance characteristics can be found in Table 5. The AllBac marker was detected in all 12 water samples and gene copy numbers ranged from 16,576 copies/100 ml to 597,895 copies/100 ml (Table 4). Site B had the highest average concentration for the AllBac marker, which was 234,543 copies/100 ml, whereas Site I had the lowest average concentration of 32,442 copies/100 ml. The human marker, HF183, was quantifiable on three occasions: twice at Site B and once at Site Q (Table 4). The highest concentration was observed at Site B on April 12 after a 1.13' rainfall event the night before. Although both end-point and quantitative PCR gave consistent results, the human marker was not quantifiable in three cases. This may be caused by the presence inhibitors, which are common in environmental samples. qPCR assays did not detect the cattle marker, CowM3, in any water samples. We are still in the process of testing the qPCR protocol for the GFD marker.

Table 4. Detection of genetic markers in water samples collected from Parkerson Mill Creek from April 12 to May 3, 2013 (The unit for qPCR is gene copies/100 ml water)

Date	Site	AllBac		Human		CowM3		CowM2	Bird	Deer	Dog
		PCR	qPCR	PCR	qPCR	PCR	qPCR	PCR	PCR	PCR	PCR
12-Apr	I	+	30636	-	ND	-	ND	-	+	-	+
	B	+	175248	+	1.8E+09	-	ND	-	+	-	+
	Q	+	50264	+	94	-	ND	-	+	-	+
19-Apr	I	+	16576	-	ND	-	ND	-	-	-	-
	B	+	52990	+	<LOD	-	ND	-	+	-	-
	Q	+	30126	-	<LOD	-	ND	-	-	-	-
26-Apr	I	+	39138	-	ND	-	ND	-	-	-	-
	B	+	597895	+	24375	-	ND	-	+	-	+
	Q	+	64696	+	<LOD	-	ND	-	+	-	-
3-May	I	+	43417	-	ND	-	ND	-	-	-	-
	B	+	112040	+	<LOD	-	ND	-	+	-	-
	Q	+	38674	-	ND	-	ND	-	-	-	-

ND: not detected; <LOD: below limit of detection.

Table 5. Performance characteristics of quantitative PCR assays

Assay	Chemistry	Regression equation For standard curves	Amplification efficiency (%)	R ²	LOD (Gene copies/reaction)
AllBac	SYBR	y=34.137-3.1877X	99.214	0.9999	1.2
HF183	SYBR	y = 33.745-3.293X	97.756	0.9998	4.4
CowM3	SYBR	y=33.916-3.38X	97.624	0.9993	1.7

References

- Bernhard, A. E., K. G. Field. 2000. A PCR assay to discriminate human and ruminant feces on the basis of host differences in *Bacteroides-Prevotella* genes encoding 16S rRNA. *Appl. Environ. Microbiol.* 66:4571-4574.
- Dick, L. K., M. T. Simonich, K. G. Field. 2005. Microplate subtractive hybridization to enrich for *Bacteroidales* genetic markers for fecal source identification. *Appl. Environ. Microbiol.* 71: 3179-3183.
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- Shanks, O. C., E. Atikovic, A. D. Blackwood, J. Lu, R. T. Noble, J. Santo Domingo, S. Seifring, M. Sivaganesan, R. A. Haugland. 2008. Quantitative PCR for detection and enumeration of genetic markers of bovine fecal pollution. *Appl. Environ. Microbiol.* 74:745-752.
- Green, H. C., L. K. Dick, B. Gilpin, M. Samadpour, K. G. Field. 2012. Genetic markers for rapid PCR-based identification of gull, Canada goose, duck, and chicken fecal contamination in water. *Appl. Environ. Microbiol.* 78:503-510.
- USEPA. 2002. Method 1603: *E. coli* in water by membrane filtration using modified membrane-thermotolerant *Escherichia coli* Agar (modified mTEC). EPA-R-02-023. US Environmental Protection Agency, Washington, DC.
- USEPA. 2012. Recreational water quality criteria. Office of water 820-F-12-058. US Environmental Protection Agency, Washington, DC.

J. NOTABLE AWARDS AND ACHIEVEMENTS

The poster by Wijesinghe and Feng (2012) won the first place poster award at the 2012 Annual Meeting of the Alabama, Georgia, and Florida Chapters of the Soil and Water Conservation Society.

K. PUBLICATIONS

Number of Research Publications generated from this research project:	
Publication Category	Number
Articles in Refereed Journals	
Book Chapters	
Theses and Dissertations	
Water Resources Institute Reports	
Articles in Conference Proceedings	1
Other Publications (conference abstracts)	2

Abstracts

Wijesinghe, R. U. and Feng, Y. (2012) Identification of fecal contamination sources in Parkerson Mill Creek at Auburn, Alabama. *The 2012 Annual Meeting of the Alabama, Georgia, and Florida Chapters of the Soil and Water Conservation Society*. Eufaula, AL.

Xue, J., Wijesinghe, R. U., Feng, Y., Wood, C. (2012) Determination of fecal pollution sources at an Alabama beach. *The 26th Annual Alabama Water Resources Conference*. Orange Beach, AL.

Conference Proceedings Citation

Wijesinghe, R. U. and Feng, Y. (2012). Evaluation of human and cattle host specific genetic markers for bacterial source tracking in a small urban watershed. *Bacterial Source Tracking State of the Science Conference Proceedings*. Texas Water Resources Institute.

L. PRESENTATIONS MADE

Feng, Y. (2012) Assessment of nonpoint sources of fecal pollution in water. The 2012 Annual Meeting of the Alabama, Georgia, and Florida Chapters of the Soil and Water Conservation Society, Eufaula, AL.

Feng, Y. (2012) From soil to water: All about microbes. Invited seminar in the Department of Agronomy and Soils, Auburn University, Auburn, AL.

M. STUDENTS SUPPORTED

Number of Students Supported, by Degree	
Type	Number of students funded through this research project:
Undergraduate	1
Masters	
Ph.D.	1
Post Doc	
Number of Theses and Dissertations Resulting from Student Support:	
Master's Theses	
Ph.D. Dissertations	

N. RESEARCH CATEGORIES: (In column 1 mark all that apply)

	Research Category
X	Biological Sciences
	Climate and Hydrological Processes
	Engineering
	Ground Water Flow and Transport
	Social Sciences
X	Water Quality
	Other: Explain

O. FOCUS CATEGORIES (mark all that apply with "X" in column 1):

	ACID DEPOSITION	ACD
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	MODELS	MOD
	NITRATE CONTAMINATION	NC
X	NONPOINT POLLUTION	NPP
	NUTRIENTS	NU
	RADIOACTIVE SUBSTANCES	RAD
	RECREATION	REC
	SEDIMENTS	SED
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	TREATMENT	TRT
	WASTEWATER	WW
X	WATER QUALITY	WQL
	WATER QUANTITY	WQN
	WATER SUPPLY	WS
	WATER USE	WU
	WETLANDS	WL

P. DESCRIPTORS: (Enter keywords of your choice, descriptive of the work)

Bacteria, water quality, biotechnology, pollution control, water quality management, watershed management

Information Transfer Program Introduction

None.

USGS Summer Intern Program

None.

Student Support					
Category	Section 104 Base Grant	Section 104 NCGP Award	NIWR-USGS Internship	Supplemental Awards	Total
Undergraduate	9	6	0	0	15
Masters	1	2	0	0	3
Ph.D.	2	1	0	0	3
Post-Doc.	0	0	0	0	0
Total	12	9	0	0	21

Notable Awards and Achievements

Enrique Doster Auburn University Research Week 3rd place STEM poster Jo-Marie Kasinak Auburn University Outstanding Oral Presentation at Graduate Forum Enrique Doster Auburn University Undergraduate Research Fellow award, \$6,000

Yasminye Pettway (Research Intern) awarded 2012 US Navy Award and Stockholm Water Prize (to represent Alabama at National High School Science Fair Competition).

Julie Price awarded Outstanding Doctoral Graduate Student in UAB Biology 2012.