Analysis of Charles River (MA) Submerged Aquatic Vegetation (SAV) Using a Prototype - 2012MA318B

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Problem and Research Objectives:

This project utilizes robotic submersible technology (RUSS-2) to characterize submerged aquatic vegetation (SAV) blooms in the Charles River (MA) at the organismal, molecular and atomic levels. Data from this research has been useful in devising methodologies to control SAV contamination in the waterways of Massachusetts and other regions of the United States and its it territories; specifically the San Juan Estuary of Puerto Rico.

Seven converging interdisciplinary studies on the Estuary are enabled (^) or significantly enhanced (+) by RUSS-2:

1) Collection of [fresh] urban water algae that are promising candidates as biofuel sources^;
2) Genetic assessment and DNA data-basing of urban water algae and bacteria+;
3) Assessment of Charles River and San Juan Estuary bed mineral content^;
4) Determining contaminant indices in river and estuary aquatic plant root systems^;
5) Analysis of Charles River and San Juan Estuary snails as an environmental indicator species^;
6) Geology and topographical survey of the Charles River and San Jan Estuary bed^; and
7) Detection and quantification of coliforms in the Charles River and San Juan Estuary.

<u>Scientific Relevance of this Project</u>

This Project is scientifically aligned with the United States Environmental Protection Agency's (USEPA) Joint Initiative on Urban Sustainability (USEPA Report, 2011), West Coast Estuaries Initiative (USEPA Report, 2008), and Puerto Rico National Estuary Program (USEPA Report, 2007). Our Project is educationally aligned with the USEPA's "Science to Achieve Results (STAR)" Program and 'Environmental Education and Training Partnership (EETAP)" and other related public and private efforts to create this nation's future environmental scientists. It is in the interest of our nation and its territories to include urban waterways in environmental sustainability practices and to achieve the mutual goal of protecting all of the nation's natural resources. Urban community colleges are perfectly positioned to accomplish this goal. This is because 60 percent of all Americans and 80 percent of minorities begin their undergraduate careers at community colleges (Mullin and Phillipe, 2009). These large enrollments portend that a significant percentage of this nation's future scientists will begin their careers at these burgeoning institutions. Community colleges are central to President Obama's educational reform movement, which, relevant to this project, coincides with the President's national environmental initiatives. A national effort by community colleges to transform STEM curriculums through research-based learning that teaches science as a process began with MassBay's Biotechnology Program in 1993, spread nationally and gave rise to the PI's 2009 *Presidential Award for Excellence in Science, Mathematics and Engineering Mentoring* (awarded to him by President Obama in the Oval Office). This reform entails engaging community college scholars in research early in their collegiate careers and that is relevant to their community. Taken together, community colleges are ideal institutions to lead a national study of urban waterways.

Our interdisciplinary research entails an expansive and ongoing study of the Charles River and subsequently the San Juan Estuary, which were enabled or enhanced by RUSS-2. The collaborators that comprise our research team are well established in their respective disciplines. We utilize submersible technology that was developed at two Massachusetts' community colleges, MassBay and the Benjamin Franklin Institute of Technology (BFIT). Scientifically, RUSS-2 adds an analytical rigor to our research by allowing the study of aquatic regions inaccessible or dangerous to boats and/or research divers. RUSS-2's GPS and hovering capabilities allow precise regions of the Charles River and San Juan Estuary to be studied for extended periods. In temporal experiments, this capability allows water, sediment and biological samples to be collected from the same position with insignificant variation in the submersible's position, thus assuring experimental reproducibility.

The Charles River is a waterway that traverses several communities in Massachusetts and has significant historical, environmental and recreational importance. Likewise, the San Juan Estuary is a key ecological and economic resource for Puerto Rico and serves as a model for environmental studies of other urban waterways of America and its territories. The waterways contain extensive wetland forests, diverse biological communities, tidal basins, dunes and salt flats. The San Juan Estuary is also the breeding ground for numerous species that are on the Federal Endangered Species List, including the brown pelican, least tern, peregrine falcon, roseate tern and leatherneck sea turtle (USEPA Report, 2007). Both waterways possess valuable wetland and aquatic habitats, and are the gateways to commercial fishing areas and shipping lanes. Their urban locations, wide-ranging importance and fragile ecosystem validate our multidisciplinary research of the environmental issues affecting these urban waterways. Importantly, our research on the Charles River and San Juan Estuary is relevant to any other urban estuarial system in the world.

Our Specific Research Aims are to:

1. Utilize a prototype submersible "Robotic Underwater Sampling and Surveillance (denoted RUSS-2)" vehicle to measure the levels of SAV, specifically the blue green algae, *Microcystis*, along the 80-mile (129 km) stretch of the Charles River of Massachusetts. During this funding period we expanded our research area to include the San Juan Estuary, Puerto Rico because of its similarity to the Charles River Basin with regards to its egress to Boston Harbor;

2. Employ RUSS's specialized "multibeam" sonar transducer system to measure and define the acoustic characteristics of SAV and map SAV blooms in the Charles River and San Juan Estuary;

3. Utilize RUSS's water and riverbed collection capabilities to measure the relative phosphorus and microstatin levels in water and riverbed soil in regions of heavy, moderate and light SAV in both waterways;

4. To determine if bloom-specific and/or region-specific polymorphisms occur in the hypervariable regions (HVr) I and II of *Microcystis* mitochondrial (mt) DNA. If so, we will create a mtDNA profile database of *Microcystis* collected from the Charles River and San Juan Estuary by RUSS-2 for our use and for other researchers conducting similar studies;

5. Involve 25 nontraditional community college and nontraditional high school (HS) scholars for environmental science and submersible technology through our long-time educational partners, "Eco-Academy" and Boston Green High School Academy by their direct involvement in the research proposed in this project;

Analysis of SAV, water and riverbed soil samples from the Charles River and San Juan Estuary was conducted in the state-of-the-art Biotechnology laboratories at MassBay. This project revealed identifying characteristics of SAV blooms at the macro- and molecular levels. Information from this project enhanced our understanding of the persistence of SAV and elucidate more effective processes for its control. Genomic and mitochondrial DNA sequencing of algae and prokaryotes from the Charles River and San Juan Estuary are in progress.

Methodology:

The projects which have been proposed under this Award have been advanced except the geological survey of the Charles River and San Juan Estuary floors. These will be completed after the modification of RUSS-2 as described below.

Biological Sample Collection: Because Microcystis thrives at permissive temperatures in the shallows of slow moving or calm waters containing high levels of phosphorus and nitrogen, samples were collected from water and riverbed samples at SAV sites of low, moderate and heavy bloom in the Charles River and San Juan Estuary. Collected water and riverbed samples are assayed for phosphorus and microcystin in MassBay's BT laboratories using Gas Chromatography (GC) and ELISA, respectively.

Genetic Analysis: Algae and prokaryotic DNA were extracted and purified using the Epicentre™ method. PCR templates were prepared as described by Kondo *et al.* (1999). Polymerase chain reaction (PCR) amplifications are performed using a single set of primers for each of the hypervariable (HV) 1 and HV2 regions of Algae mtDNA using the thermo-cycling conditions described by Wilson *et al* (2010).

After amplification, all algae samples plus controls are typed by direct DNA sequencing across the HV1 and HV2 regions. In order to sequence the PCR product, fragments are then cycle sequenced in the forward and reverse directions using the same PCR primers with the BigDye Terminator V1.1 chemistry kit (Applied Biosystems (ABI), Foster City, CA). After cycle sequencing, samples are ethanol precipitated and nucleotide base sequences determined in an ABI PRISM 377 DNA Sequencer. Editing and alignment of the sequences are performed using Sequencher 4.7 Forensic Edition software (Gene Codes Corp, Ann Arbor, MI) and a similar sequence alignment protocol developed and used by MassBay's Biotechnology Program.

Species origin and phylogeny were assigned by DNA sequencing of the hypervariable (HV) region I and II of the D-loop and determining the polymorphisms against a reference sequence. With regard to Charles River Microcystis this technology is not without limitations due to the small size of algae DNA databases, undefined mutation rates in various species of algae and an absence of uniform algae sampling methodologies for genetic analysis. We are interested in whether Microcystis blooms in the Charles River differ in HV1 and HV2 haplotype depending on their location and/or to the levels of phosphorus to which they are exposed.

Because mtDNA persists in dead organisms for considerable periods of time we are preparing to perform nucleotide sequence analysis of the HVr1 and II regions of Mircocystis cells recovered from low, moderate and high bloom sites. We will analyze and compare mtDNA sequences to determine if bloom-specific sequence variations can be identified. If identified we will establish a public mtDNA database of Charles River *Microcystis* mtDNA for research purposes.

A significant research effort established under MWRA support by participating MassBay Biotechnology Scholar, Carolyn Lanzkron (See "Student Support", below), was entitled *Experimental Model for Separation of the West Nile Virus from its Insect Vector*. RUSS-2 will be a major collection vehicle of the mosquito larvae utilized in this study. Of importance, MS. Lanzkron was awarded the 2013 Barry M. Goldwater Scholarship for this study (See "Student Support").

Briefly, insect-borne diseases have had a significant impact on humans and human evolution. Insects such as flies, fleas, mosquitoes, and lice serve as prolific vectors of diseases, transmitting their pathogens to humans through their blood meals. The insects transmitting these diseases commonly begin their life cycles in waterways similar to the Charles River and San Juan Estuary. Ms. Lanzkron's research establishes a molecular model for abating the virulence of one such disease, West Nile Virus (WNV), through the creation of a genetic barrier that separates the causative pathogen from its insect host. This is achieved by 1) determining and characterizing the co-evolutionary factors that govern pathogen-insect host selectivity at the DNA level; and 2) developing, in collaboration with Olaf Pharmaceuticals, Inc., a Massachusetts biotechnology company, a panel of drugs that target and disrupt the obligate molecular linkage(s) between pathogen and host.

Current mosquito vector control measures focus on habitat control (draining rivers, removal of local pools of stagnant water, etc.), insecticides, larvicides, and the introduction of infertile male mosquitoes. This research seeks to establish a molecular alternative.

Submersible Engineering and Design:

WRIP support allowed us to test our submersible engineering concepts and the impact on our research projects to which this technology is linked. Given the multiple uncertainties associated with underwater studies of urban waterways our efforts were to determine if our designs would perform in the difficult environs typical of shallow urban waterways.

Several resolvable problems occurred with RUSS-2 under this Award. Several design changes of the submersible occurred and addressed these problems. RUSS-2 possesses the same basic design as its [prototype] predecessors but with these additions:

RUSS-2 was fitted with a larger propulsion system that uses 7 thrusters that are directly driven brushless electric motors. The major reason that we replaced the propulsion system used on RUSS-2 is to significantly enhance the maneuverability of RUSS-2. The primary purpose of thrusters on a submersible is to surmount the resistive hydrodynamic forces placed on the vehicle, in particular in descents and sudden collision avoidance maneuvers (a common event in urban waterways). These forces are magnified in urban waterways where pervasive submerged natural and manmade objects further impede the movement of the submersible. The speed and maneuverability provided by direct drive is greatly superior to that produced by small thrusters fitted fore and aft of vehicles, as is the case with RUSS-2. Another reason for the change in propulsion systems is power conservation. Smaller thrusters are a considerable draw on the lithium batteries that were the original power source. This draw on power is increased when thrusters must be throttled up in order to propel the vehicle through submerged vegetation such as milfoil. The new thruster systems will make the RUSS-2 extremely maneuverable. Two main thrusters will control forward and backward movement. We will add two angled thrusters, one each on the port and starboard sides of the submersible, giving increased capability side-to-side movements, and ascents and descents. The new thrusters system will allow RUSS-2 the ability to travel underwater at a speed of 9 to 13 knots in any direction.

RUSS is connected to a cabin cruiser (“chase boat”) by a tether that contains guidance, power and communications cables. RUSS-1’s tether is problematic to its mission for several reasons: 1) as the length of the tether increases a significant proportional drag is placed on the submersible as it moves through the water. This drag decreases the submersibles maneuverability especially in tight turns or ascents made to avoid submerged obstacles. Deployment and recovery of the submersible is also problematic because entanglement is common due to the substantial natural and manmade debris submerged in urban waterways. A narrow but accurate analogy to this type of entanglement is that experienced by a person fishing for Bass in a pond with dense milfoil and/or lily pad growth; 2) the tether length required for RUSS-1’s activities in urban waterways is only 300 feet. However, the power cable component of the tether is aluminum wire. This is because aluminum has a significantly higher conductivity to weight ratio than copper, a factor that is important in signal transmission. However, aluminum wire requires a larger gauge than copper to carry the same current, which increases tether weight and rigidity. Also, aluminum wire is subject to a phenomenon known as "cold creep" in which the metal expands proportionately with increases in temperature and contracts as temperatures decrease (Jenkins and Willard, 1966). This effect is most prominent when the tether is exposed to seasonally changing temperatures and/or temperature gradients in a body of water. “Cold Creep” causes the tether to lose its flexibility and degrades submersible’s maneuverability, deployment and recovery over time. Further, marine grade aluminum is exceedingly expensive. Its use in the hull of RUSS-1 and RUSS-2 is imperative. However, its use in a key cable component of the tether is problematic. RUSS-2 is now soft tethered and will have two operation systems that can be operated by a single pilot. The first will be a remote computerized control system (CCS) in which operational signals are relayed to RUSS-2 via a single small gauge wire that will not affect any aspect of the submersible’s movements. The second is a programmable guidance system (PGS) that requires no connection to the surface and renders the vehicle under complete computerized control. Both the CCS and PGS systems will utilize a standard 486 computer as the brain and a subsystem control interface. A 486 computer is ideal for RUSS-2’s underwater functions because its software, though older, was designed more efficiently and maintains the simplicity, reliability and serviceability of the submersible. The CCS will manage the control input from the pilot at the surface into the submersible’s actions under the water. All data required by the pilot on the surface to know RUSS-2’s precise position under the water will be collected by GPS sensors on its foredeck and continuously transmitted back to the pilot’s control console.

Principal Findings and Significance:

Scientific Relevance of this Project:

The academic and industry collaborators that comprise our estuary research team are well established in their respective disciplines. We utilize submersible technology that was developed at 2 Boston community colleges, MassBay and the BFIT.

Scientifically, RUSS-2 adds an analytical rigor to our research by allowing the study of estuarial regions inaccessible or dangerous to boats and/or research divers. RUSS-2’s GPS and hovering capabilities allow precise regions of the estuary to be studied for extended periods. In temporal experiments, this capability allows water, sediment and biological samples to be collected from the same position with insignificant variation in the submersible’s position.

Of all the tasks RUSS-2 will be capable of, its usefulness lies in the ability to provide a platform for researchers and scholars to make personal observations in previously inaccessible urban waterways. Our ability to directly observe the environment of the Charles River and San Juan Estuary is beneficial to the multiple STEM disciplines operating in our study. We made operation of the submersible [generationally] intuitive by using as its control system the same joystick technology familiar to anyone born after 1980. Our submersible-based research, though still evolving, reveals scientific wonders of underwater ecosystems to nontraditional scholar-explorers as much as it does seasoned field scientists.

The Charles River and San Juan Estuary are key ecological and economic resources for Massachusetts and Puerto Rico, respectively and serve as a model for environmental studies of other urban waterways of America and its territories. The San Juan Estuary is the breeding ground for numerous species that are on the Federal Endangered Species List, including the brown pelican, least tern, peregrine falcon, roseate tern and leatherneck sea turtle (USEPA Report, 2007). Both waterways possess valuable wetlands and aquatic habitats, and are the gateway to commercial fishing areas and shipping lanes. Their urban locations, wide-ranging importance and fragile ecosystem validate our multidisciplinary research of urban waterways and the environmental issues affecting them. Importantly, our research is relevant to any other urban system in the world.

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Student Support

<u>Educational Relevance of this MWRA-funded Project</u>
Educationally, the project develops a replicable model based on project-based learning, which engages scholars who are underrepresented in science with authentic and relevant research that stimulates their interest in environmental science careers. In addition, the proposed project has mechanisms to disseminate the technology, data and educational model to a national community of scientists and faculty.

The educational problem this project addressed is the paucity of underrepresented groups enrolled in field science and marine engineering degree programs and hence engaged in careers in these disciplines. To help remedy this situation MassBay, BFIT and Sagrado utilized this project to link their long-standing, research-based undergraduate degree programs. These degree programs immerse nontraditional scholars in extensive, sophisticated and relevant scientific investigations for the entirety of their undergraduate careers. MassBay's Biotechnology Program, created by the PI in 1993, has produced an unprecedented, for two-year colleges, 19 Goldwater Scholars (America's highest undergraduate science award). Under the mentoring of Co-PI James Giumara, BFIT scholars devise elaborate and effective engineering solutions that enable the research of scientists across multiple disciplines. RUSS-1 demonstrates BFIT's prowess in scientific capacity building through engineering. Since 1993, Co-PI Mayra Rolon has excited scores of Puerto Rican undergraduate scholars from Sagrado for science careers through their participation in her pioneering work on the San Juan Estuary.

This project creates a replicable, national model for interdisciplinary education that stimulates a passion for STEM careers among nontraditional undergraduate scholars at community colleges and minority serving institutions. It also has well-developed dissemination mechanisms reaching a national community of STEM faculty from these institutions. Our MWRA-funded project engages nontraditional scholars in interdisciplinary research that will be useful in studying, monitoring, restoring and sustaining an estuarial ecosystem that is critical to Puerto Rico and pertinent to other urban waterways worldwide. Importantly, our submersible-based research immerses our scholars in early and extensive research experiences at the scientific realms where the biological sciences and marine engineering intersect. The integration of submersible technology into our ongoing research on the Charles River and San Juan Estuary significantly enhances the scientific education of participating scholars in both depth and breadth. The MassBay scholars supported under this Award were:

1. Elias **Gilkes** (Major: Biotechnology). Mr. Gilkes conducted the culturing of algae collected from the Charles River and San Juan Estuary. He also designed a self-contained *in vitro* system for the large scale culturing of algae in semi-dry conditions. Mr. Gilkes will attend Brandeis University in the Fall as a Biology major;

2. Carolyn **Lankron** (Major: Biotechnology, Forensic DNA Science). Ms. Lanzkron's research contributions and scholastic recognition is described above In Section IV," Methodology", and her scholastic recognition stemming from this project below in Section VII, "Notable Achievements and Awards". She will attend MIT as a Biology major in Fall 2013;

3. Geoffrey **Reimann** (Major: Engineering). Mr. Reimann oversaw the design changes described above for RUSS-2;

4. Kimberly **Ramos** (Major: Biotechnology), Ms. Ramos is involved in the large-scale production of algae;

5- Alberto **Velez** (Graduate: Biotechnology, 2001). Mr. Velez designed the RUSS prototype and played a prominent role in the redesign of RUSS-2. He is a 2002 graduate of MassBay's Biotechnology Program and was selected as a recipient of the Barry M. Goldwater Scholarship in the same year. Mr. Velez is Facilities Manager at SBH Scientific in Natick, MA. Like many alumnae of the Biotechnology Program Mr. Velez plays an active role in its research efforts and the mentoring of its students.

Notable Achievements and Awards

1-On March 30, 2013 the Goldwater Foundation released its 2013 Awardees of the Barry M. Goldwater Scholarship, this country's highest undergraduate science award.
See "Massachusetts" at: http://www.act.org/goldwater/sch-2013.html

Carolyn Lanzkron, Biotechnology (Forensic DNA Science) whose project on the genetic basis of water-borne insect host/pathogen interaction was supported, in part, by the WRIP grant was one of the winners. Of importance, Carolyn was the only Goldwater Awardee among the national cohort recipients from a community college. Carolyn is the 19th Goldwater Awardee from MassBay's Biotechnology Program since its inception in 1993.

A newspaper article on Carolyn sums up the prestige she has bought to this MWRA award and program in general.

http://www.metrowestdailynews.com/news/education/x1431009363/MassBay-Biotech-student-wins-Goldwater-Scholarship

Carolyn will be attending MIT in Fall 2013.

Follow-on Funding

Funding Agency: National Science Foundation
Grant Program: "The MassBay Scholarship for Science, Technology, Engineering and Math (MSTEM) Program,"
Grant Title: MassBay Science Scholars Program
Institution: MassBay Community College
Award Period: 6/1/2012-5/31/17
Award Amount: $347,000
Cognizant Officer: Dr. Joyce B. Evans (jevans@nsf.gov)

This 5-year Award allows us to provide full scholarships to 10, Second-year MassBay Community College STEM Scholars who have terminal degree goals in the basic sciences.