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TITLE OF PROPOSED PROJECT **CREST Phase II: Center for Aquatic Chemistry and Environment (CAChE)**

REQUESTED AMOUNT	PROPOSED DURATION (1-60 MONTHS)	REQUESTED STARTING DATE	SHOW RELATED PRELIMINARY PROPOSAL NO. IF APPLICABLE
$ 5,000,000	60 months	08/01/21	

THIS PROPOSAL INCLUDES ANY OF THE ITEMS LISTED BELOW

- ☐ BEGINNING INVESTIGATOR
- ☐ DISCLOSURE OF LOBBYING ACTIVITIES
- ☐ PROPRIETARY & PRIVILEGED INFORMATION
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- ☐ VERTEBRATE ANIMALS IACUC App. Date ______
- PHS Animal Welfare Assurance Number ______
- ☒ TYPE OF PROPOSAL Research
- ☒ HUMAN SUBJECTS Human Subjects Assurance Number FWA00000060
- Exemption Subsection ______ or IRB App. Date Pending
- ☐ FUNDING OF INT'L BRANCH CAMPUS OF U.S IHE ☐ FUNDING OF FOREIGN ORG
- ☒ INTERNATIONAL ACTIVITIES: COUNTRY/COUNTRIES INVOLVED XX
- ☒ COLLABORATIVE STATUS A collaborative proposal from one organization (PAPPG II.D.3.a)

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PROJECT SUMMARY

Overview:

Novel environmental stressors ,such as microplastics, pharmaceuticals and other emerging contaminants, have caused significant environmental impacts and attracted worldwide attention. We initiated FIU's CREST Center for Aquatic Chemistry & Environment (CREST-CAChE) in 2016 to meet the need for enhanced research on the detection and impact of toxic substances in the environment, including pesticides, industrial chemicals, oil, pharmaceuticals, nutrients, and metals. Our Phase I research focused on the detection, fate and transport, and visualization of the impacts of contaminants on aquatic systems. In CREST-CAChE II, we will expand our detection capabilities to unknown and novel emerging contaminants, both natural and anthropogenic, as well as expand our ecosystems of study. While Phase I focused primarily on freshwater systems, such as the Everglades, and the near shore ecosystems, CREST-CAChE II will expand our study area to include aquatic systems along an urbanization gradient. By designing novel sensors and autonomous vessels that can explore difficult to reach urban areas, we will collect continuous and near real-time data on emerging contaminants. In Phase II, we will move from a focus on detection and transport of contaminants, to a mechanistic understanding of the impacts of these contaminants at the organismal, community, and population levels. Using an Understanding the Rules of Life (URoL, NSF 10 Big Ideas) conceptual approach, Phase II will unify across multiple scales, as well as integrate campus-wide programs in Chemistry, Biology, Earth Systems, Computer Science, Engineering, and Architecture.

In addition to advancing cutting-edge research, we will conduct a rigorous education program that builds from lessons learned in CREST-CAChE I. During the initial years of the CREST program, we discovered that students sought the skills to work collaboratively, especially for communicating across disciplines, and for using new emerging technologies. We also learned that undergraduates sought authentic research experiences beyond the Discovery courses that we developed, so will add an undergraduate research-fellows program to Phase II. Our formal and informal education programs will prepare students to communicate the results of their research and for the future STEM workforce. We will include training in core professional skills, as well as micro-credentials in tools necessary to understand complex environmental problems, including robotics, next generation sensor development, big-data analytics, virtual reality, and artificial intelligence.

Intellectual Merit:

CREST-CAChE II will increase participation of underrepresented groups in STEM while advancing cutting edge research in environmental chemistry, detection methodologies, molecular mechanisms and producing actionable science with government and private sector partners. Phase II will generate significant new knowledge regarding contaminants in aquatic environments, as well as produce innovative new methodologies for detecting unknown and emerging contaminants and their impacts in urban aquatic ecosystems. In CREST-CAChE II, we will expand our studies to look at the mechanisms leading to impacts of contaminants at the individual, community, population, and ecosystem levels.

Broader Impacts:

CREST-CAChE II will have three main components in its broader impacts program. a) We will recruit and retain underrepresented students in STEM using our near-peer team mentoring approach. We will build on the success of FIU's evidence-based transformation of STEM instructional practices to provide enhanced support for underrepresented students to successfully pursue STEM careers. b) We will provide students the core professional skills and micro-credentials needed to succeed in the workforce of the future. c) We will develop new technologies for improving water quality analysis and contaminant detection in a range of ecosystems, including freshwater, coastal, and urban environments, as well as provide data on the impacts of these contaminants on ecosystem function. Through partnerships with decision-makers and stakeholders, we will translate this data into actionable information for designing water quality solutions locally and globally.

FIU CREST Phase II: Center for Aquatic Chemistry and Environment (CREST-CAChE)

1. Rationale For CREST-CAChE Phase II

1.1 Background

Novel environmental stressors have caused significant environmental impacts and attracted worldwide attention. In our CREST Center for Aquatic Chemistry and Environment (CREST-CAChE I), we studied the identification, transport, fate and impacts of environmental contaminants on a variety of aquatic ecosystems. Coastal South Florida, with nearly 8 million people living close to biodiverse marine and freshwater ecosystems – from the inland Everglades to the near-shore coastal areas – is a living laboratory for studying potential conflicts between humans and nature as urbanization increases. In CREST-CAChE II, we will continue to develop innovative techniques and research methodologies that enable us to understand the nature, occurrence, distribution and impacts of novel environmental stressors, such as pesticides, industrial chemicals, oil, pharmaceuticals, nutrients and metals, and ***also add new methodologies to measure impacts on histone proteins and provide lipid biomarkers and metabolic profiling*** to directly measure exposure at the organism level. Using an 'Understanding the Rules of Life' and Biology Integration Institutes conceptual approach, [1,2] Phase II will unify across scales and integrate campus-wide programs in Biology, Chemistry, Computer Science, Engineering, Earth Systems and Architecture. During CREST-CAChE I, we developed a new innovations laboratory to add robotics, 3-D printing and visualization capabilities to our CREST Center, as well as technologies for novel environmental sensor development. We will use these new facilities to expand the scope of potential CREST-CAChE II projects and augment our educational platform with innovative, next generation skills to support multiple, cross-disciplinary career pathways.

During CREST-CAChE I, Research Focus Area 1 (RFA1) developed novel environmental chemistry tools, including Trapped Ion Mobility Spectrometry (TIMS-MS), that led to characterization of unknown chemical contaminants. In Phase II, we will continue to build unique analytic tools, while adding a focus on identifying and quantifying ***new emerging contaminants***. In CREST-CAChE I, RFA2 discovered that many responses to environmental stressors actually occur at the level of individual organisms. Therefore, in Phase II's RFA2, we will investigate multi-level ***organismal impacts*** and the mechanisms underlying responses (epigenetic, cellular, physiological, behavioral) to the contaminants identified in RFA1. In Phase II RFA3, we will quantify responses at the population, community and ***ecosystems levels*** in coastal, aquatic environments. These three RFAs will be integrated through our education and professional development programs, as well as our informal science and external engagement activities. We will engage students at multiple levels, with a focus on intentional strategies for recruitment of diversity as a necessary step towards retention of diversity in grad school and beyond. We also provide deep developmental support to enable students to understand the concepts being presented and developing their identity as scientists. We will provide professional development in core career skills, such as cross-disciplinary collaboration and science communication, as well as develop a suite of micro-credentialing modules for training in robotics, next generation sensor development, big-data analytics, virtual reality and artificial intelligence. These next generation skills will enable students to understand complex, environmental problems and prepare them for future STEM career pathways in industry, government and academia.

Intellectual Merit: ***CREST-CAChE II will advance transformative research and cutting-edge technological approaches to identify emerging contaminants and understand their multi-scalar impacts at the organismal, population, community and ecosystem level.*** We will integrate our research and education programs so that students are engaged in the process of discovery and innovation, while also gaining skills to work collaboratively, communicating across disciplines and use next-generation technologies. We will build on the strong partnerships we created in CREST-CAChE I with resource management agencies, municipalities and museums to translate the Center's findings into actionable information for designing water quality solutions locally and globally.

1.2 Building from the accomplishments of CREST-CAChE Phase I

The first 4 years of CREST-CAChE I were extremely successful, so we have designed Phase II to build off these accomplishments and use lessons learned to modify our research, training and engagement to expand our collective impact. Specifically, in CREST-CAChE I, we brought together a cross-institutional team of researchers from 14 departments and four colleges; trained 43 stipend-supported and 35 affiliated graduate students who became a tight knit, interdisciplinary cohort, which even during the pandemic, regularly participated in bi-weekly journal club zooms; ***graduated 22 PhD students, 95% who are***

employed in STEM careers; implemented a new Discovery Learning course that brought CACHE research experiences and career development to 130 undergraduates who had no prior experience in research, with 12 continuing on to conduct hands-on CREST research and 60% of these now enrolled in STEM graduate programs; submitted 13 collaborative grant proposals with CREST student and faculty teams, 69% of which were successfully awarded; piloted an undergraduate research fellows (URF) program for two summers (25 URFs) that resulted in an NSF-funded REU Site award; conducted cutting edge research on aquatic chemistry and the environment that resulted in 76 peer-reviewed publications and 118 presentations to scholarly and public audiences; and initiated an innovations track that led to the design and 3-D printing of a prototype low-cost, water quality sensor for citizen science engagement.

The inclusive diversity of our CREST team has been high – of our 15 postdoctoral scholars, eight were female and 73% from underrepresented backgrounds; of our 78 graduate students, 36 were female and 60% from underrepresented backgrounds; 78% of our Discovery students and 64% of our URF program were from underrepresented backgrounds, with over 60% of each represented by women. We also engaged a wide range of faculty from across the entire institution, many of whom were not even aware of each other's research and had never participated in large research and training programs before CREST.

Professional development was a major component of CREST-CAChE I. We used our biweekly student journal clubs to provide workshops on career skills, including science communication, data visualization, research presentations, resume preparation, fellowship applications and STEM career pathways. We also provided over $38K in travel for students to attend scientific conferences and training workshops. Of particular interest is the large percentage of our graduates who have chosen to pursue STEM jobs in non-academic institutions, including private industry and resource agencies. That observation, combined with knowledge of the future workforce needs[3] has led us to focus our CREST-CAChE II education platform around providing the next-generation job skills needed for multiple STEM career pathways (Section 5.1). In addition to core professional development workshops, we will offer an array of micro-credentialing modules focused on next generation STEM skills, such as artificial intelligence, robotics and fabrication, 3-D printing and sensor development.

We also used CREST-CAChE I funds to expand our research infrastructure. We designed and constructed three research buoys, critical to providing real-time information on water quality. The buoy platform was designed to work in both fresh- and saltwater to maximize use across the South Florida aquatic landscape. We have obtained a second TIMS-MS dedicated to use by the CREST students and faculty to extend our capacity to produce comprehensive molecular characterizations of new and unknown complex environmental mixtures. We also purchased over $21K in research equipment, such as hand-held water quality instruments, to facilitate student research. Finally, CREST funds were used to build a CREST-dedicated computer facility that provides computers, printers, data storage and data serving capabilities.

At the university level, CREST faculty worked with departmental administration to develop recruitment ads for three new faculty lines, essential to CREST-CACHE II. The university refurbished one research lab and provided three additional student meeting spaces with smart screens to maximize student cross-campus interactions, communications, and research collaboration development. The university also began construction of an innovations lab – the robotics and digital fabrication (RDF) facility – to enable new CREST-CAChE II research projects utilizing 3-D printing and other technologies for coastal sustainability solutions, as well as develop the education and training modules focused on next-generation jobs.

1.3 Goals of Phase II Center

CREST-CAChE II will build on the above accomplishments by expanding engagement of faculty and students, especially in the areas of genomics and informatics, sustainability and science communications, as well as enhancing our research and education infrastructure. We will extend our engagement with local stakeholders and citizens to ensure that CREST-CAChE II continues to produce actionable science to inform local coastal sustainability solutions to climate change and emerging urban contaminants. We will also provide the university a business model to fully capitalize on CREST research facilities, including a plan for revenue streams that will allow them to be maintained and even grow in the foreseeable future.

CREST-CAChE II and its activities will integrate undergraduate and graduate students into all research subprojects, emphasizing technological advances, enhancing analytical research infrastructure, authentic research experiences and near-peer mentoring. Through our partnerships with county, state, federal and Native American resource management agencies, and non-governmental organizations (NGOs), students will be provided opportunities to tie their research with local management and community-driven science, as well as provide pathways to permanent careers. Practical solutions to problems related to water quality in coastal and urban settings are critical components of the Center's mission. CREST-CAChE II will embed innovative educational opportunities within our science context in order to increase participation and retention of students from our highly diverse South Florida urban region and nationwide. Our current and future investments in faculty and student professional development are and will continue to create a sustainable environment for continuous development of future STEM professionals, especially as it advances FIU's institutional commitment for increasing Diversity, Equity and Inclusion (DEI). CREST-CAChE II will capitalize on the experience and expertise of faculty carrying out educational transformation at FIU, most notably through FIU's STEM Transformation Institute, as well as best practices nationally. We will also build upon FIU's experience as a public, urban, majority-minority research institution, with a commitment to inclusive excellence in all aspects of its education and research missions.

Specifically, the objectives of CREST-CAChE II are to:

1) Amplify the generation of new technologies and knowledge, building from CREST-CAChE I, to detect, identify and quantify water quality issues associated with emerging, novel contaminants in urbanizing coastal environments. Moreover, we will emphasize community engagement and socioeconomic and sociopolitical impact through community education and outreach activities enhanced through the use of the newest visualization methods to maximize stakeholder input, understanding and trust.

2) Increase student participation and retention in STEM education and careers related to water and environmental sciences, with an intentional plan for recruitment of students from traditionally underrepresented backgrounds. We will develop a series of workshops and modules to provide next-generation workforce skills and corresponding micro-credentials, such as robotics, artificial intelligence, 3-D printing, sensor development and visualization needed for future STEM positions in education, management and industry. We have strong partnerships with industry, government, resource agencies and NGOs to provide students with internship and career opportunities. Our professional development plans will help students identify career paths and develop the necessary skills to achieve them.

3) Build institutional capacity for research and education by making investments in faculty, courses and facilities, so that CREST-CAChE will be sustained long after this proposed Phase II. We will centralize our analytical chemistry capacity, our newly established Robotics facility, and our Sensor Development and Research Facility in an open, shared manner. We will build from lessons learned for achieving interdisciplinary collaboration to provide a sustainable mechanism for inclusive cooperation on complex environmental problems. CREST-CAChE II will provide immersive research and training opportunities to undergraduate and graduate students to increase access and participation of a highly diverse population, while also preparing students for next generation STEM jobs. CREST-CAChE II will formalize collaborations across the University and support faculty in seeking funding from diverse sources, thus further growing research and funding opportunities for students. These mechanisms will ensure sustainability of institutional transformations beyond the duration of the NSF CREST funding period.

Our objectives are perfectly aligned with the goals of NSF's CREST Program. A key CREST-CAChE II objective is to attract, retain and graduate underrepresented students into STEM careers. CREST-CAChE is committed to incorporating diversity at every level of the program, including teaching, research and engagement, so we will build new institutional capacity for recruiting and retaining diverse students and faculty, while leveraging existing inclusive excellence programs at FIU. We will also address the goal of pushing the frontiers of research excellence, so our CREST Center continues to produce advances in the actionable science and technologies needed for local and national coastal sustainability.

2. Research Objectives of the Center

In CREST-CAChE I, we established an interdisciplinary, university-wide team, while also creating an infrastructure that has significantly enhanced our ability to recruit and retain undergraduate, graduate and postdoctoral participants, especially from under-represented backgrounds. Our CREST Center has also significantly enhanced our success in placing students into multiple STEM career pathways, including graduate school, post-doctoral and faculty positions, as well as jobs in industry, environmental management and government. ***To date, 21 CREST-CAChE graduate student alumni are permanently employed in STEM-related jobs, including 5 females and 57% from underrepresented backgrounds.*** The current cohort of students will graduate within the next one to two years.

In CREST-CAChE II, we will continue to integrate undergraduate and graduate students, as well as post-doctoral fellows (supported by other projects) and faculty, into all research subprojects, especially emphasizing enhanced technological advances, analytical research infrastructure, authentic research experiences and actionable solutions. By expanding our partnerships with county, state, federal, and government resource management agencies, CREST-CAChE II will focus on communicating science to advance socially equitable solutions to water quality issues in coastal, urban settings.

2.1 Unifying Theme

In CREST-CAChE I, we pioneered approaches for measuring novel emergent contaminants by developing new technological methodologies, as well as by modifying existing analytic hardware.[4–6] We are just beginning to understand that even very low concentrations of new, emerging contaminants in coastal aquatic ecosystems result in impacts on individuals at the organismal level, including genetic and epigenetic changes that cause cellular and physiological responses. ***Our CREST-CAChE II unifying theme is to develop and use novel technologies to detect and characterize environmental stressors, such as emerging contaminants, urbanization and climate change, and then effectively communicate how those stressors have multi-level impacts on individual, community, population and ecosystem responses.*** We will generate new knowledge and technologies for detecting and sensing novel emerging contaminants (industrial chemicals, phthalates, petroleum-based pollutants, pharmaceuticals and microplastics) in fragile coastal, urban areas and their impacts on organisms, populations and ecosystems. Our educational-experiential approach depends on students learning new technologies for data collection, analysis and visualization, while applying those tools to quantify how novel, emerging contaminants and climate drivers are impacting coastal systems.

2.2. Research Focus Areas (RFAs)

Our research will focus on (1) advanced ***analytical methodologies*** for detecting low levels of emerging and novel chemical stressors; (2) multi-scale ***organismal responses*** (epigenetic, cellular, physiological, behavioral); and (3) using an integrated ecosystem assessment approach to quantify ***population, community and ecosystem responses***. RFA1 extends our advancements from CREST-CAChE I in detecting novel emerging contaminants and chemical stressors by including microplastics, phthalates and other emerging, relatively unknown stressors. RFA2 builds on the unexpected result that many organisms in our coastal ecosystems responded to novel stressors at multi-organismal scales, including epigenetic, cellular, physiological and behavioral. Thus, our RFA2 research follows the Understanding Rules of Life concept as defined by NSF's Big Ideas[1] to address impacts across biological scales. RFA3 will characterize the impacts of novel stressors and climate drivers at the population, community, and ecosystem levels through innovative, integrated assessment approaches, that will lead to model development and ecosystem intervention actions to enhance ecosystem services.

2.2.1. RFA1: Developing Detection Technologies for the Study of Environmental Stressors

In CREST-CAChE I, we assessed the presence, distribution and environmental processes controlling anthropogenic and natural stressors in aquatic ecosystems. The team implemented the use of novel technologies focused on alternative and innovative mass spectrometry tools. CREST-CAChE II will expand our ability to provide the high-resolution real-time data needed for meaningful actionable interventions,[7] through the use of tandem orthogonal separations (e.g., online-SPE-HRMS, speciation ICP-QqQ-MS, GCxGC-MS, GC-UHRMS and GC/LC-TIMS-UHRMS), development of low-cost in-situ sensing systems and sensor network integration. The goal of RFA1 is to generate the detection technologies and experimental workflows that can provide a comprehensive characterization of legacy, novel, emerging and soon-to-emerge environmental stressors, then use markers of organism exposure to detect their influence. ***This multidisciplinary team*** of biologists, chemists, engineers, and architects will ***develop:*** a) new detection technologies for the characterization of human-derived contaminants, new emerging environmental stressors (e.g., poly and perfluoroalkyl substances, PFAS) and markers for organism exposure at the individual level; and b) new in-situ sensor technologies for real-time, high-resolution monitoring of environmental stressors. The ultimate goal of RFA1 is to further develop and apply these novel technologies to better understand the dynamics and fate of environmental stressors, so we can improve risk assessment and rapid decision-making. Example projects are listed below.

Environmental stressor detection

The identity and nature of human-derived environmental contaminants have changed from traditional and legacy pollutants, such as nutrients, trace metals, DDT and PCBs, to other biologically active compounds, such as endocrine disruptors, antibiotics and a wide range of chemicals broadly classified as emergent chemicals of interest (e.g., PFAS and microplastics). Chemicals such as mercury, natural and synthetic estrogens, antibiotics, high use pharmaceuticals and even natural toxins, such as algal blooms, are now recognized as having significant effects – both directly on ecosystems and then indirectly on humans.

a) Human-derived stressors can be found in the ecosystem over a wide dynamic range. The use of advanced, high resolution MS, coupled with online solid phase extraction (SPE) and UHPLC or tandem mass spectrometry (MS-MS, TIMS-MS, TIMS-TIMS), has extended our analytical abilities from trace analysis of critical compounds to detailed analyses of metabolites,[8–10] organism markers of exposure,[11] and degradation products. This has enabled a more accurate depiction of the occurrence of emerging contaminants.[5,6,12] Automation enables high-throughput analysis of specific wastewater intrusions using recalcitrant tracers, such as sucralose,[13] combined with traditional nutrient and pathogen methods to determine the human-derived signature at low-enough costs for large scale projects.

b) Anthropogenic per- and polyfluoroalkyl substances (PFAS) and microplastics are manufactured chemicals with widespread commercial uses that are common products of human consumption. Despite their differences, they all share the one commonality of being designed to be persistent and inert.[14] The widespread use of PFAS has resulted in their ubiquitous presence in natural resources globally, including soils, sediments, drinking waters, surface and ground waters, and biological systems.[15–17] PFAS have been found to be the most prevalent contaminants in humans and wildlife throughout the world, presenting clear, potential hazards to human health even at low parts-per-trillion levels.[18–20] Our goal is to develop analytical strategies to comprehensively assess the spatial and seasonal trends of multiple PFAS and microplastics in surface and drinking water in a range of water bodies along an urbanization gradient.

c) In-situ sensor development for coastal, nearshore and urban ecosystem contaminants are needed to provide near real-time information on shallow freshwater ecosystems and near-shore marine environments. Buoys developed for CREST-CAChE I featured high-tech sensors to collect data on general water quality parameters (temperature, conductivity, dissolved oxygen, pH, turbidity, chlorophyll, directional flow speed). For CREST-CAChE II, we will add the capability to monitor nutrients responsible for eutrophication and algal blooms, including nitrate and phosphate. Most in-situ technologies are not affordable, sensitive or reliable in salt-water environments, so we will work with industry partners to assess available sensors, improve their performance and/or produce alternatives. The goal is to provide data needed to develop predictive 'early warning' models of nutrient-driven events, such as harmful algal blooms.

2.2.2. RFA2: Measuring Multi-Level Organismal Impacts and Responses

In CREST-CAChE I, we improved our understanding of pollutant source, transport and effects across urban-rural landscapes.[21–23] We have been able to combine the study of traditional ecological and toxicological endpoints (e.g., survival, growth, etc.) with the development of molecular 'omic' studies,[24,25] including genomics and epigenomics. By leveraging new knowledge and the next generation of technologies, we are better able to understand the impacts of multiple global change stressors (from subtle to lethal), as well as the mechanisms underlying their effects on multi-level organismal responses.

In CREST-CAChE II, we will expand stressor detection and study impacts at the organismal level (developed in CREST-CAChE I) to the molecular mechanistic basis. For that purpose, new innovative methods are incorporated including: genomics (genetic diversity), epigenomics (gene regulation capacity), transcriptomics (gene expression) and metabolomics (molecules involved in physiological responses). These 'omic' analyses comprise a very powerful toolkit to detect even the slightest impact of stressors on individuals and ultimately, populations. In combination with the study of the totality of exposures in organisms, i.e., the exposome,[26,27] this project will reliably and simultaneously measure both environmental exposures and genetic variation, providing a better understanding of the causal links among the genome, the environment and phenotypic response.

a) Population genomics and phylogenomics: Investigating genetic diversity, connectivity and relatedness. Genetic diversity is a key attribute of populations because it provides the foundation for evolutionary potential, including the ability to adapt to changing environmental pressures. CREST-CAChE II will use genetic diversity as a proxy to measure how individual impacts lead to population changes that ultimately impact communities and ecosystems. This measurement is intimately tied to an organism's ability to survive, with higher levels of genetic diversity often leading to higher survivorship and low genetic diversity resulting in severe consequences for populations.[8] Thus, determination of how genetic diversity is shared and exchanged within and across a system will help us to model and predict the recovery and resilience of a species. Such movement and distribution of genes within or between systems is described as ‘population connectivity’[28] and crucially relevant to species resilience.[29] By collectively assessing genetic diversity and connectivity, we will be able to inform resource management and conservation efforts.

b) Environmental epigenetics: Characterizing environmental-epigenetic-genetic linkages in adapted phenotypes. The diversity of living organisms mirrors not only their genetic variation, but also their ability to modulate genome function. Epigenetic modifications (e.g., DNA methylation and chromatin structure) constitute a ‘sensory’ mechanism altering how genetic instructions are interpreted, under different environmental conditions, without altering the DNA code itself.[30] While these modifications to organismal phenotype or function can be reversible, some of them are heritable and produce multiple outcomes from a single genome that affect ecological and evolutionary adaptation.[30,31] In CREST-CAChE II, we will expand on our prior work by studying the interconnections between environmental fluctuation and subsequent changes in both genetic and epigenetic states. By determining how these molecular changes drive physiological responses, we will be able to predict how population dynamics are influenced by epigenetically-modulated phenotypes. We will delineate fundamental links (Rules of Life) across scales from molecular to organismal to eco-evolutionary outcomes.

c) Physiological and metabolic responses: Phenotypic and eco-evolutionary consequences of environmental-energetic-epigenetic linkages. CREST-CAChE II will build on the identification of molecular signatures of environmental change identified in CREST-CAChE I to measure phenotypic responses at the organismal, community and population level. This project will study metabolic responses across different model organisms. Since increases in metabolic rates must be met with either increased energy intake or a reduction in growth or reproductive output, environmental stressors have the potential to cause changes in metabolic rates or require behavioral compensation.[32] We will assess the ability of different stressors to alter standard and maximum metabolic rates, as well as aerobic scope. We will also measure organismal responses to complex and often overlooked behavioral responses. For instance, we will investigate if metabolic responses to environmental stressors trigger compensatory alterations in aerobic metabolism, promoting changes in behavior, metabolic stress responses and potentially reducing fecundity. We will tie these responses to genetic and epigenetic changes, to understand how the modulation of dynamic energetic budgets causes changes in the epigenetic regulation of DNA function.

2.2.3. RFA 3: Innovating Integrated Ecosystem Assessment in an Urban and Urbanizing Coastal Environment that Maximizes Community Engagement and Communication

Urbanization is increasing globally, resulting in an increase in the types and levels of land-based contaminants and pollutants released into the environment, especially aquatic ecosystems. Many of these are unregulated and have largely unknown consequences on environmental health and human well-being. The intersection of anthropogenic activities, urbanization and climate change influence the sources, mobilization, transport and fate of contaminants across land-use boundaries.[33,34]

In CREST-CAChE I, we determined the hydrologic transport (flux) and fate (biogeochemical processes) of various contaminants across land-use boundaries. We focused on computational methods for amalgamating disparate environmental data sets for maximal analysis and interpretation. Building on those results and the increased public awareness and demand for science-based management of ecosystem services and environmental health, in CREST-CAChE II we will focus on **innovating novel integrated ecosystem assessment approaches** in our urban and urbanizing coastal region. Students will examine how urban coastal systems: (1) can adapt to changing climate and the stressor, (2) inform ecosystem-based management of urbanization pressures across the coastal ecosystems and seascapes to (3) reduce the impacts of climate and contaminant stressors that disrupt ecosystems and their services. We will use empirical and modeling approaches to quantify how mitigating urban toxins and pollutants can influence populations, communities, and ecosystem processes to enhance ecosystem services.

a) Integrate novel sensors into sensing platforms across spatial scales and in near-real time with experimental and observational approaches to quantify the interacting effects of coastal urbanization and climate change on ecosystem pressures, responses and services. In CREST-CAChE I, we designed and employed water quality buoys that provide real-time stressor and response data. We also initiated the design of a prototype **Autonomous Surface Vessel** (ASV) in partnership with SeaRobotics Corp., to quantify the distribution of nektonic (freely swimming) organisms,[35,36] while simultaneously examining substrate characteristics and bathymetry and water column properties. In CREST-CACHE II, we deploy an observing network, using the novel sensors developed in RFA1, and apply the integrated ecosystem services framework of Driver, Pressure, State, Impact, Response (DPSIR)[37] to evaluate the interaction of coastal urbanization and climate change. More specifically, we will examine relationships among: (1) variation in the quantity and location of contaminant loads and associated physio-chemical regimes using techniques and the sensing platform developed in RFA1 and (2) quantify effects on community and ecosystem processes, based on the behavioral impacts of epigenetic responses to contaminants from RFA2. We will also (3) disentangle the relative influence of these impacts with controlled experiments and (4) determine how climate drivers modulate those responses. We build on the organismal-scale impacts from RFA2 to quantify the cumulative direct and indirect effects on population dynamics, trophic linkages and coastal ecosystem structure and function.

b) Apply ecosystem pressure, response and services relationships to model and assess how regional hydrologic management, watershed land use and climate change influence contaminant loads to disrupt ecosystem services. We will employ the technologies and modeling developed in CREST-CACHE I and the other RFAs of CREST-CAChE II to synthesize and assess nutrient and emerging contaminant stressors across the inland-coastal–near-shore boundary. We will utilize the coupled stressor-ecosystem response data, obtained from our in-situ sensors developed in RFA1, to improve understanding of discrete and distributed contaminant sources (e.g., septic systems, stormwater outfalls, exfiltration trenches) of urban watershed fluxes into the coastal waterways. We will couple nutrient and emerging contaminant loads (RFA1) with integrated organismal (RFA2) and population, community and ecosystem responses to develop ecosystem models that elucidate stressor impacts.[38–40] We will use scenarios modeling to evaluate and predict the sensitivity, exposure, accumulative capacity and resilience of biotic systems within urbanizing ecosystems, and thus the influence of environmental stressors and watershed management on ecosystem services.

c) Develop and test ecosystem management interventions to reduce coastal urbanization impacts and enhance provision of ecosystem services. In CREST-CACHE I, we evaluated the impacts on key ecosystems, such as mangroves, coral reefs and aquatic animal populations. In CREST-CAChE II, we will use those findings to explore how conversion of natural to gray infrastructure in coastal environments results in excess nutrients, sediment and other pollutants. These challenges are exacerbated in coastal areas because of the integrated effects of urbanization, climate change and hydrogeological conditions (e.g., high water tables and low elevations), negatively affecting coastal water quality and ecosystems. Further, the provision of novel habitat (hard substrata) along sedimentary shores can have a number of ecological consequences, including altering local and regional biodiversity by modifying natural patterns of dispersal of species, or by facilitating the establishment and spread of exotic species.[41] Reversing this trend is an emerging area of research that cuts across disciplines of engineering, ecology, and hydrology, and requires integrated field experimentation, water quality monitoring, geospatial analyses and modeling. Building from RFA3 a) and b) above, complemented with field experiments, deployment of high-resolution sensing networks, and geospatial analyses of fine-scale infrastructure and land uses, we will evaluate how different forms of watershed land use (e.g., green infrastructure) and ecosystem management interventions modulate the transformation and retention of contaminants and carbon sequestration as well as prevent seagrass loss, algal blooms and unsuitable abiotic regimes for faunal species. In particular, we will leverage the characterization of seascape structure and function to identify the relative values of ecosystems at different states and potential management interventions.[42]

2.3 Interconnections between Research Focus Areas

In CREST-CAChE I, we established an internationally recognized facility who characterize and measure emerging, novel contaminants. We also discovered that those contaminants, even at very low concentrations, impacted organisms through epigenetic and other pathways that likely impact population, community and ecosystem responses. In CREST-CAChE II, our research, education, professional development and engagement are designed to fully integrate across the project (**Fig. 1**). RFA1 will provide new technical approaches to measuring emerging contaminants both in the laboratory and in-situ. RFA2 will leverage next generation technologies to understand the impacts of multiple global change stressors and mechanisms underlying their effects on multi-level organismal responses. Finally, RFA3 will deploy novel autonomous vehicle platforms that utilize sensors developed in RFA1 to quantify impacts of environmental stressors on population, community and ecosystem responses in heavily urbanized coastal areas. Science communication is an integrating thread throughout the proposal – through the RFAs we are detecting, understanding, and communicating the role of emerging contaminants, organismal impacts, and effects on ecosystem services – and how mitigating negative consequences of these is not only possible, but essential for coastal ecosystems. Taken in aggregate, CREST-CAChE I and II will significantly advance the field of environmental chemistry and provide the tools necessary to Understand the Rules of Life (URoL) governing coastal, urbanizing ecosystems. Utilizing these tools and convergent approaches, we will become a global leader in actionable, ecosystem science and applications to ensure coastal resilience.

3. Role of the Integrated CREST-CAChE Phase II to Mission of FIU

FIU is a majority-minority institution embedded in the multi-cultural social fabric of Miami that is dedicated to improving STEM education across the K20 spectrum and developing future STEM professionals, especially from historically underrepresented backgrounds. From its inception in the 1960s, FIU's mission has been to serve the community and provide relevant solutions to local and national challenges. The FIU STEM Transformation Institute is dedicated to discipline-based education research (DBER) that intentionally drives transformation of student courses and programs, as well as partnering with local K12 districts and teachers to support intensive professional development. These deep commitments to STEM education are driven by FIU's uniqueness as a large urban public research university that enrolled over 58,930 students in Fall 2020, of which 65% were Hispanic, 12% were African American/Black, and 57% were women. FIU has over 11,500 STEM majors, composed mostly of Hispanic students as well as First Generation (~20%) and Pell-eligible (~40%) students, and provides greater than 50% of the K12 teachers to the Miami area. FIU, like many institutions, has redoubled its commitment to promoting diversity, equity and inclusion, which CREST-CAChE II directly promotes both through its student-centered activities and faculty development.

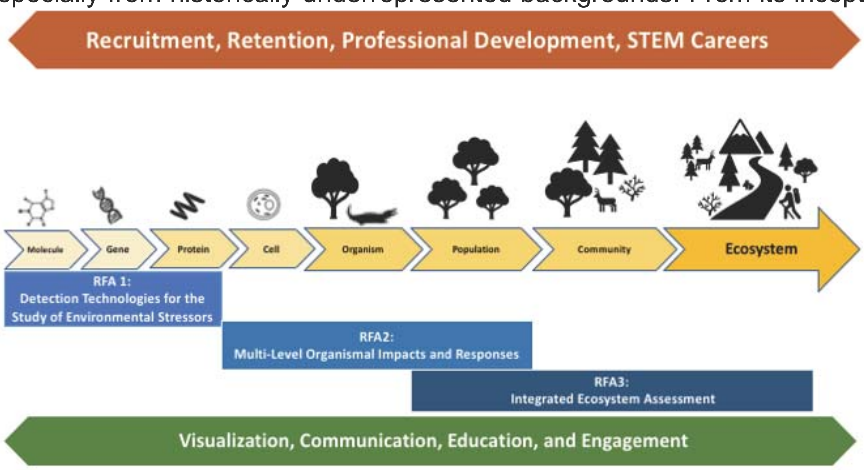

Figure 1: Integration across Research Focus Areas, Education, and Engagement

CREST-CAChE I used this collective knowledge of STEM best practices to design student programs that successfully recruited and retained both diverse graduate and undergraduate students (Section 1.2). CREST-CAChE I created linkages across 14 departments and four colleges through interdisciplinary research projects, which began an institutional transformation of both research and instructional practices at all educational levels. CREST-CAChE II will formalize the cross-campus, interdisciplinary collaborations that we established. In addition, CREST-CAChE I pioneered two Discovery-based undergraduate courses that engaged students in data analysis, hypotheses testing and research presentations, as well as career preparation. Evaluation of these courses indicated that while students valued the analytical skills they gained, they wanted authentic research experiences in the lab and field. Therefore, we will establish Course-based Undergraduate Research Experiences (CUREs) that integrate CREST-CAChE research, thus creating an institutionalized introductory lab replacement course that simultaneously promotes CAChE science and recruits future students. We will also offer an undergraduate research fellows (URF) program that builds off of the pilot from Phase I.

CREST-CAChE I pushed the research frontier – we quantified pollutants and contaminants across South Florida aquatic systems, built hydrologic models to follow those contaminants and pollutants across the landscape and, finally, determined the impacts of those stressors on aquatic habitats. CREST-CAChE II's will develop new cutting-edge technologies to advance beyond our prior discoveries and model potential scenarios for mitigating impacts on individuals, populations, and ecosystems. We initiated the CAChE Nutrient Lab, the Sensor Research and Development Lab, three CREST student conference rooms and a dedicated CREST computing facility. CREST-CAChE II will solidify these facilities as part of FIU's research infrastructure by establishing a university-wide business model with a cost structure to operate and maintain these facilities. The research, education and engagement components of CREST-CAChE will solidify FIU's recognition by the State of Florida as the University of Distinction in Environmental Resilience (Section 4).

4. Plan to Achieve and Sustain National Competitiveness

The successes of CREST-CAChE I was a major factor in FIU being recognized as 'The University of Distinction in Environmental Resilience' in the state of Florida. CREST directly contributes to the university's strategic plan for reaching a top 50 ranking in environmental science. CREST-CAChE I brought together faculty and students from 14 departments and four colleges to create an innovative program highly acclaimed by the university, the Florida Board of Governors, and nationally. CREST-CAChE II will further broaden participation across the university, as well as recruit top faculty and graduate students nationally to further enhance our reputation in coastal ecology and resilience. FIU is already recognized as the **9th ranked program in the world** for 'Positive Impacts on Life Under Water' by the Times Higher Education Impact Rankings. Through CREST-CAChE I and II, we will enhance this recognition by integrating a holistic ecosystems modeling approach to improve our ability to maintain coastal ecosystems integrity. While CREST students will continue to develop independent research projects, we have designed our research foci to bring together faculty, students and external partners to collaboratively produce cutting-edge research on coastal, urban ecology and resilience. A focus of CREST-CAChE II will be to produce publications in the most highly cited journals to enhance our visibility and recognition, as well as implement the actionable science we produce for coastal resilience solutions.

5. Education Plan

We will build on the successes of CREST-CAChE I, as noted in Section 1.2, as well as modify some of our approaches to increase their impact and reach. Our education plan is dedicated to recruiting and retaining diverse students in stem degrees and careers, so our objectives focus on that. We will: 1) deliver innovative environmental science modules which are research based, interdisciplinary and technology driven (CUREs), 2) promote scientific discovery and innovation by integrating students into our cutting-edge RFAs' research, 3) deliver training and access to innovative technologies to prepare our students to be competitive in the next generation STEM workforce (workshops, micro-credentials, URFs), 4) leverage advances in cyber-infrastructure to create immersive and interactive learning environments (Augmented Reality (AR), Virtual Reality (VR), emphasizing data visualization, and 5) provide context-based learning opportunities by that convey skills in critical thinking, creativity, collaboration, and communication (4C's).

5.1 Integrated Workshop Modules and Micro-credentialing

CREST-CAChE II will establish a set of micro-credentials, based on a series of integrated workshops that deliver sequenced, modular lessons to achieve comprehensive learning goals. Each workshop requires roughly 10 to 20-hours of effort. Micro-credentials have been embraced by universities to provide customized learning experiences in shorter time-blocks. RFA research teams will mentor students to identify workshops that are most relevant to their possible future careers, and complement their current expertise, so they gain the required hours to receive CREST micro-credentials.

Micro-credentialing and Badging: Research shows that micro-credentials lead to higher earning potential,[33,34] as well as enhanced job rewards.[43] Working with FIU's innovative micro-credentialing program and recent NSF C-ACCEL award (NSF-1937019), we have developed certification paths for both stipend-supported CREST fellows, as well as other FIU students who are interested in engaging as CREST affiliates. We have been working with, and will continue to consult our external partners, to design the specific micro-credentials that ensure our students gain the skills valued by prospective employers.

Content and Certification Paths: The content of the workshops will focus on training students for ***applications of emerging technologies in environmental sciences***. As NSF's 10 Big Ideas note, constantly evolving technologies are actively shaping the lives of workers. In this context, we are proposing four areas of concentration, each composed of several workshops (**Fig. 2**). These include: **1) *Science Communication*** with workshops in: Sustainable Water and Earth; Immersive Data Visualization; Storytelling with Data; **2) *Environmental Sensing and Mapping*** with workshops in: a) Computational Thinking, b) GIS and Geo-Spatial Data, c) Data Collection and Mapping with Drones, **3) *Data Visualization*** with workshops in: a) Computational Thinking, b) Immersive Data Visualization, c) Storytelling with Data, d) GIS and Geo-spatial Data, and **4) *Making Scientific Instruments*** with workshops in: a) Computational Thinking, b) Developing Sensing Kits, d) Precision Modeling and Robotics Fabrication. With successful completion of each individual workshop, students will receive a Badge demonstrating their competency in the workshop topic. The completion of all 3-4 workshops in a concentration area leads to a CREST Meta Badge. We have developed, implemented and issued Badges in two workshops through our NSF C-ACCEL funding and plan to continue our work in CREST-CAChE II.

Interactive and Inquiry-based Learning: All workshops are developed with inquiry-based learning methodology, which has shown to motivate students to develop an investigative attitude. Inquiry-based learning can promote opportunities for gaining knowledge in a conceptually consistent way and lead to development of important skills.[13,21] Because Inquiry-based learning is a form of active learning that starts by posing questions, problems or scenarios, it will allow us to frame the workshop assignments as interactive and immersive research questions. We will take advantage of immersive capabilities of VR and AR technologies, which can provide multi-sensory engagement with the workshop content when provided as interactive simulations. Simulations will allow students to manipulate environmental stressors to observe the impacts on water contamination and marine life, view the impact of sea level rise in urban settings, teleport on a drone which is collecting water samples to see the process, or interact with large quantities of data through different immersive visualizations. Thus, combining inquiry-based learning with interactive and immersive assignments will provide novel opportunities for fostering 4Cs learning.

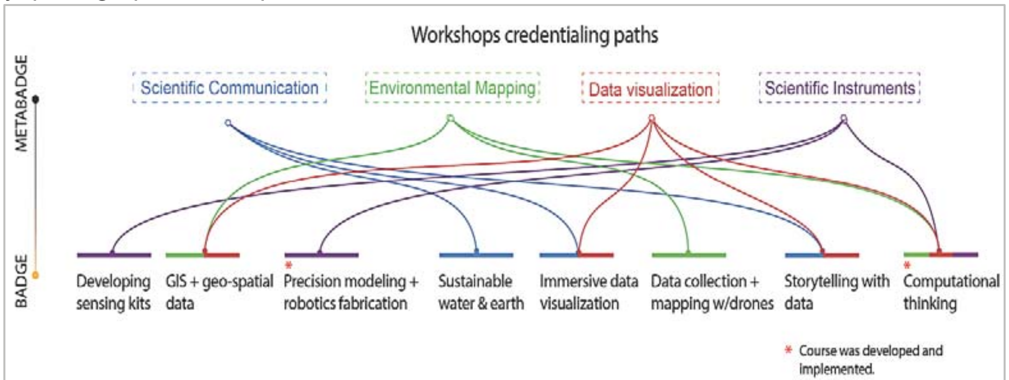

Figure 2: Micro-credentialing workshops on emerging technologies in environmental sciences

5.2. Graduate Experiences

Our graduate program integrates research, education, mentoring and career skills development.

Recruiting, Mentoring, and Retention: Through interaction with student organizations, diversity conferences, community colleges, secondary school and external collaborators, we will expand the diversity of our pool of applicants and accepted participants. We use the local ecological context and unique environmental challenges as the 'hook' to recruit interested students. We actively focus on student retention by employing the theory of action of near-peer mentoring,[44,45] which has co-benefits for both mentor and mentee. We also use our biweekly journal clubs to socialize students in their role as scientist and to integrate them into the overall, cross-institutional program. In addition, CREST faculty are provided training in recruiting and mentoring students from underrepresented backgrounds.

Orientation Boot Camp: We have added a new 2-day orientation boot camp before the start of each fall semester to socialize students into the broader context of CREST-CAChE and all of the resources available to them as a member of the program. This orientation will include new and current students, faculty, and external partners and will be co-organized with FIU's STEM Institute. In a series of discussion sessions with internal and external participants, students will be introduced to CREST-CAChE II, concepts, potential research projects, facilities and resources, and internship and career opportunities. Students will gain an understanding of the breadth and depth of the program, their role within the overall program's goals, as well as potential impacts of their results on providing actionable solutions.

Immersive Convergence Research: Graduate students will co-develop their individual research projects with faculty in each of our three convergent RFAs, using our extensive research facilities. CREST-CAChE student projects integrate across disciplines, methodologies, technologies, and vocabularies. Therefore, students will be trained to work across these boundaries both through their research, annual research symposia, and professional development experiences (Section 6).

Graduate Level Credentialing: We will provide guidance and mentoring for our graduate students to select from the menu of potential workshops (Section 5.1) to complement their existing core skills and career goals, by completing the 3-4 modules needed to receive a CREST Badge or a Meta Badge (**Fig. 2**).

Internship Opportunities: We will pair students with our network of partners to develop their professional skills and capability to work with people from different disciplines and backgrounds, as well as gain access to career paths. Our collaborators at local and national resource management agencies, local municipalities, museums and private industry partners (see Letters) all will offer internship opportunities.

5.3. Undergraduate Experiences

Mentoring Program: to ensure student engagement in various opportunities offered by CREST, we use a near-peer mentoring approach. Research teams of faculty, graduate students and senior undergraduates will mentor their junior peers. Mentoring will focus on interdisciplinary research, courses, credentialing and career pathways, as well as the challenges students face outside the classroom. Mentors will be trained using the model developed by FIU's STEM Transformation Institute and Provost's ADVANCE Institutional Transformation program that prepares mentors to navigate both the cognitive and non-cognitive domains, as many of our students face challenges as first generation, diverse and/or economically limited students.

Freshman Experience: The Discovery course developed in CREST-CAChE 1 was successful in piquing the interest of beginning undergraduates in environmental sciences and led to a subset of them pursuing CREST research projects. In order to institutionalize our Discovery approach, we will integrate the *Sustainable Water and Earth* workshop (**Fig. 2**) with FIU's *First Year Experience (FYE) course*. Our FYE option provide links to the local environment and how it impacts our community, directly increasing relevance to students and developing their scientist identity (and motivation to graduate). We will use full immersion experiences that enable discovery learning by creating engaging Virtual Reality (VR) simulations of our coastal ecosystems that promote 4C's through the lens of environmental and water-related issues. The FYE course is required of all Freshman (over 2,500 students per semester), so the other sections provide multiple comparators that we can use for education research (multivariate analyses) and provide the evidence needed to improve and expand our efforts.

Course-based Undergraduate Research Experiences (CUREs): We will develop a CREST-CAChE CURE, potentially the first CURE at a CREST Center, that can then be shared with other CRESTs. CUREs are replacing introductory confirmatory labs nationwide, since they provide increased relevance and engagement for students. Further, the adaptive, problem-solving nature of CUREs require students to draw on their experiences and collaborate with their partners, integrating culturally responsive instruction into the course. Our CUREs will serve as a replacement for other introductory labs, so can motivate students to pursue CAChE science in a credit-neutral way. Over the past three years, we have integrated CURE activities into large undergraduate classes using chemistry concepts to explain biological phenomena.[46] Evaluation of these activities found that students especially valued having the ability to connect their knowledge across different disciplines.[47] We will build upon this prior work and developmental framework to establish CUREs for students using data collected in the RFAs. We will work with CREST faculty to translate their results into activities for select undergraduate STEM courses and labs. Using a backward-design approach, we will outline the desired CREST learning objectives and match them with the appropriate course level for student understanding and multi-disciplinary connectedness. We will start by piloting a few sections, then collect evidence on student comprehension to modify and expand offerings, as we have done for prior course transformations.

Undergraduate Research Fellows (URFs): A subset of undergraduates will co-develop research projects with faculty mentors from the RFAs. Three to four research positions will be available each year with team mentorship provided by CREST graduate students, faculty, and senior undergraduates to emphasize near-peer mentoring and our collaborative approach, as well as provide valuable research experience. In CREST-CACHE I, we found that participating in research led to retention of students in CREST and STEM graduate programs. Of the 12 URFs supported in CREST-CAChE I, seven have entered graduate school.

Senior Projects: In the past two years, we have worked to integrate CREST activities with senior students' capstone projects. We have trained and mentored many seniors on interdisciplinary, water-related research projects facilitated by the RDF Lab. These projects have ranged from developing smart devices for flood level and water quality monitoring by citizen scientists, web and mobile water quality information visualizations, water salinity and conductivity sensor innovation, smart wearables for UV protection, affordable surface-drone development for improved water quality monitoring and 3-D printing of concrete to create artificial coral reefs. We plan to expand and institutionalize these efforts in CREST-CAChE II.

5.4 High School Experiences

MAST@FIU. We have partnered with Miami-Dade County school district and MAST@FIU (a STEM-focused Miami-Dade County high school), so will offer dual-enrollment credits to the CREST-CAChE CUREs and the suite of micro-credential, next generation skills workshops.

5.5. Research and Education Integration

CREST-CAChE II will provide students the opportunity to carry out cutting edge, convergent research, as well as develop their multidisciplinary, collaboration and communication skills to prepare them to solve future real-world problems and sustainability challenges. As shown in **Fig. 1**, the CREST RFAs and associated facilities integrate across the main theme and are central to the education and engagement plans. The RFAs provide the methodologies and data needed to understand the impacts of environmental stressors from the molecular to the ecosystem scale. Our education, professional development and engagement activities provide the skills needed to communicate, visualize, translate and provide the actionable science needed to inform stakeholder decision making.

6. Professional Development Experiences

Students: In addition to their research project, all students will attain ***core career skills***, including communicating science, convergence/interdisciplinarity, visualization, data analysis, innovation, and research proposal development, including the ***NSF Graduate Research Fellowship (GRFP).*** We will conduct workshops in our bi-weekly student journal club on each of these core skills. For the NSF GRFP, we offer a workshop on proposal preparation, but also have assembled a team of successful GRFP recipients on campus who serve as peer mentors and editors to the more junior applicants. We used a similar approach in CREST-CAChE I and the results showed that not only did the students gain the intended professional skills, but the biweekly sessions served to create a strong student cohort and even led to new, interdisciplinary project ideas. We will also offer an annual ***Career Pathways Panel*** with professionals from an array of STEM careers to discuss their positions and tips on successful career paths. We include early career and recent graduates on the panel since these near-peers are more relevant to graduating students. In addition to workshops on research presentation best practices, we also provide ***travel funds*** to CREST-supported fellows and affiliates to present at scientific conferences. This extends our ability to support additional students, both graduate and undergraduate, while also enhancing their professional skills.

Early career faculty: We will provide early career faculty with training on mentoring, with a focus on diversity, equity and inclusion; conducting interdisciplinary research; student recruitment and retention; career guidance and other challenges our population of students face. Mentors will be trained using the model developed by FIU's STEM Transformation Institute and Provost's ADVANCE Institutional Transformation program. In addition, we will partner with FIU's CASE Office of Research Development (led by Co-PI Teutonico) to provide trainings on writing competitive proposals, identifying funding sources, and developing an Independent Development plan (IDP), to maintain a tenure-track, career trajectory.

7. Collaborators

In CREST-CAChE I, we focused on federal environmental agencies to provide input into research topics and provide internships to CREST students. That approach did not provide nearly as many opportunities or synergies as envisioned. In CREST-CAChE II, we have partnered with more regional and local agencies, environmental NGOs and local museums, as well as specific county and city legislators (see Table). Each of these entities will provide a representative to sit on our External Partners Board (EPB) and be engaged in CREST activities from the outset. Indeed, many of these external collaborators have already provided input into this proposal. We will partner with these collaborators to link CREST students with these organizations to design and implement specific research and public engagement activities.

Institution/Organization	Category
Miami-Dade County Public Schools	Education
City of Coral Gables	Government – State/Local
Miami-Dade County Commission	Government – State/Local
Miami-Dade County Water & Sewer Department	Government – State/Local
Miami-Dade County Div. of Environmental Resources Management	Government – State/Local
South Florida Water Management District	Government – State/Local
National Oceanic & Atmospheric Administration	Government - Federal
Miccosukee Tribe's Fish and Wildlife Division	Government - Tribal
Coastal Ecosystems, LLC	Industry
SeaRobotics	Industry
Coral Gables Museum	Museum
Miami Waterkeeper	Non-Governmental Organization
Sachamama	Non-Governmental Organization
Everglades Foundation	Non-Governmental Organization
Ocean Conservancy	Non-Governmental Organization

8. Project Management Plan

The Center will be administered through FIU's Institute of Environment and directed by PI Crowl, who reports to the Dean of College of Arts, Sciences and Education. An internal management team made up of each RFA and education faculty lead, the STEM Institute Director, plus a graduate student representative from each RFA, will meet monthly with the Director and Associate Director, to determine overall program operations and decisions (**Fig. 3**). An External Advisory Committee (EAC), an External Partners Board (Section 7), and an External Evaluation team (Section 9), will provide at least annual feedback on CREST-CAChE's successes, challenges and opportunities.

Lead PI: Dr. Todd A. Crowl is Director of the Institute of Environment where CREST-CAChE is administered and will continue to serve as Lead PI. He has been the director of multiple large centers and institutes totaling more than $40M. He has trained 48 graduate and dozens of undergraduate students.

RFA/Subproject leads: Dr. Francisco Fernandez Lima, Co-PI, has pioneered the Trapped Ion Mobility Spectrometry field and will lead RFA1. Dr. Jose Eirin Lopez, Co-PI, is a leader in the field of epigenetics, recent recipient of NSF URoL award and will lead RFA2. Dr. Tiffany Troxler, Co-PI, is the Director of Science in the Sea Level Solutions Center, housed in the Institute of Environment, and will lead RFA3. Dr. Rita Teutonico, Co-PI, is Associate Dean of Research in the College of Arts, Sciences and Education and will serve Director of Education. All co-PIs have extensive experience mentoring graduate and undergraduate students, as well as large program management,

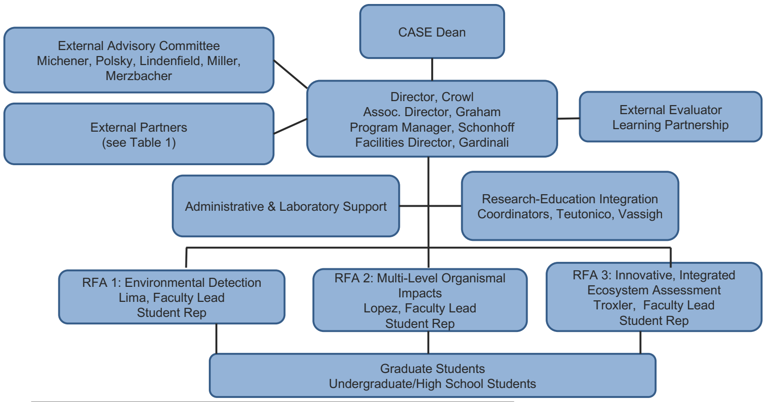

Figure 3: Project Management Structure

External Advisory Committee: The EAC is composed of leaders in science communication, community engagement, technology development, big data management, and CREST Center management who will advise the leadership team on program management and their individual areas of expertise.

External Partners Board: The EPB is composed of representatives from local and federal resource management agencies, NGOs, museums, and private industry. They will participate in career panels to advise students on STEM pathways; provide internship opportunities; and co-produce research, education, and outreach materials with students relevant to their research projects.

9. Evaluation Plan and Education Research

The Learning Partnership will conduct the independent formative and summative project evaluation. The evaluation will focus on the extent to which CREST-CAChE II achieves its expected outputs and outcomes related to (a) supporting faculty to initiate new lines of interdisciplinary research that impact local communities, (b) mentoring graduate students from underrepresented backgrounds to pursue interdisciplinary research while successfully completing their graduate education in a timely manner, and (c) inspiring undergraduate students from underrepresented backgrounds to pursue environmental science research careers by engaging them in authentic research experiences. The evaluation will be guided by the following evaluation questions: (EQ-1) Has the program successfully supported faculty to initiate new lines of interdisciplinary research that could impact local communities? (EQ-2) Has the program faculty successfully mentored graduate students from underrepresented groups to pursue interdisciplinary research while successfully completing their graduate education in a timely manner? (EQ-3) Has the program inspired undergraduate students from underrepresented groups to pursue environmental science research careers by engaging them in authentic research experiences.

The organization of CREST-CAChE I successfully supported faculty members in initiating new lines of interdisciplinary research. To address EQ-1, The Learning Partnership will conduct an analysis of the CREST-CAChE II publications to assess the interdisciplinarity of the research as represented by the disciplines of the co-authors and assess the contribution of the research to the field through the impact factor of the journals where research has been published and the citation factor of each article. The Learning Partnership is currently conducting these analyses for CREST-CAChE I, which will serve as a baseline for CREST-CAChE II. The Learning Partnership will use co-authorship as a proxy for a social network analysis to examine how the network evolves over time. The evaluation will pay particular attention to the integration of early career faculty. In order to examine the underlying supports provided by CREST-CAChE II, The Learning Partnership will conduct contrasting case studies of faculty who became integrated members of the network as well as those who remain isolated within the network.

To support graduate students, CREST-CAChE II will implement a variety of recruitment and initial socialization strategies, including boot camp, journal club, and micro-credentialing. To support the development of convergent dissertation research and support for degree completion, students will receive mentoring from faculty in each RFA. In addition, students will receive internship opportunities. To address EQ-2, The Learning Partnership will track the number and demographics of graduate students and the time to completion of key milestones. The Learning Partnership will conduct content analyses of students' presentations, publications, and dissertations to examine the interdisciplinarity of the research. In order to understand the role that mentoring plays in supporting degree completion, The Learning Partnership will also examine the nature of faculty mentoring of graduate students through longitudinal case studies of mentoring relationships over time. The results of these case studies will document successful mentoring practices and inform the design of the CREST-CAChE II faculty professional development program. The case study faculty and students will be selected in consultation with CREST-CAChE II leadership based on models of successful mentoring during CREST-CAChE I.

To inspire undergraduate students, CREST-CAChE II will provide a pathway of experiences from the Freshman experience, course-based research, and hands-on research. To address EQ-3, The Learning Partnership will track participation of undergraduates across the different CREST-CAChE II undergraduate experiences. At the end of each experience, the undergraduates will complete a survey on how participation in the research influenced their choices about majors and careers. The Learning Partnership will interview subsets of students who persist from one experience to the next to build case studies of the ways in which CREST-CAChE II inspires and supports undergraduate students to pursue a research pathway.

The Learning Partnership will provide formative reports on each evaluation question on at least an annual basis. The results will be presented at the annual advisory board meeting to inform the overall review of the center. Emergent results will be discussed with the CREST-CAChE II leadership team on a quarterly basis. A summative evaluation report will be provided at the end of the project.

10. Project Timeline

Timeline: Activities and Deliverables		YR 1	YR 2	YR 3	YR 4	YR 5
Project Office	Hire Outreach Coordinator					
	Bi-monthly PI Meetings					
	External Partners Meeting					
	Annual All-hands Meeting					
	Secure Permanent Center Funding					
Infrastructure	Design and Implement Field Sensors					
	Enhance CREST Computing Facility					
RFAs	Recruit PhD Cohort	8	10	10	10	10
	Recruit Undergraduates	3	4	4	4	
External Engagement	Miami River Cruise Outreach Event					
	Museum Exhibit					
Evaluation	EQ-1 Social Network Analysis					
	EQ-2 Track Demographic Milestones					
	EQ-3 Post-event Surveys					

Building from CREST-CAChE I, we will immediately recruit a new cohort of students, as well as hire an outreach coordinator as suggested by the pre-proposal panel (see Timeline Table). Evaluation and assessment activities will also begin immediately Major infrastructure development and external engagement activities are staged over the 5 years.

11. Broader Impacts

CREST-CAChE I successfully provided funding and opportunities to 195 undergraduate and graduate students, 60% from underrepresented background. To date, 21 graduate student alumni are permanently employed in STEM careers, 57% from underrepresented backgrounds. We will focus our CREST-CAChE II activities on transforming the university's institutional capacity to support research and training in the transdisciplinary focus areas of CREST-CAChE. The broader impacts of CREST-CAChE II will include a) We will recruit and retain underrepresented students in STEM using our near-peer team mentoring approach. We will build on the success of FIU's evidence-based transformation of STEM instructional practices to provide enhanced support for underrepresented students to successfully pursue STEM careers; b) We will provide students the core professional skills and micro-credentials needed to succeed in the workforce of the future; c) We will develop new technologies for improving water quality analysis and contaminant detection in a range of ecosystems, including freshwater, coastal, and urban environments, as well as provide data on the impacts of these contaminants on ecosystem function; and d) Faculty, postdocs and graduate students will be engaged in developing undergraduates and high school students into future scientists, thus providing an authentic mechanism to develop their diversity, equity and inclusion advocacy. Through expanded partnerships with decisionmakers and stakeholders, we will translate this data into actionable information for designing local and global water quality solutions.

FIU (a minority serving institution) hereby certifies that we comply with the NSF CREST program's conditions for PI, institutional eligibility and organizational limits as detailed in the Eligibility Information section of the NSF CREST solicitation.

12. Results from Prior NSF support:

Crowl is PI of a) HRD-1547798, $5,000,000, 4/01/2016-3/31/21; b) CREST: Center for Aquatic Chemistry and the Environment; c) **Intellectual Merit:** The theme of CREST is contaminant detection, transport and ecological impacts, **Broader Impacts:** CREST provides research, training and professional development experiences to students from underrepresented groups and has supported 78 graduate students, 15 postdoctoral scholars and 155 undergraduate students; 73% from underrepresented groups. Twenty-one CREST students are currently employed in STEM positions; 95% of our CREST graduates. CREST-CAChE I engaged over 50 faculty from 14 departments and 4 colleges; 60% from underrepresented backgrounds; d) Students and faculty have produced 63 peer-reviewed publications;[35,48] and e) As publications, datasets and other research products are produced, they are made available on the CREST website; f) This proposal builds on the success of our current CREST award by expanding our contaminant detection abilities, as well as enhancing our focus on genomic and metabolomic impacts of novel, emerging contaminants.

Eirin-Lopez is PI of a) 1921402, $629,339, 09/01/19 - 08/31/24; b) URoL Epigenetics 2: Predicting phenotypic and eco-evolutionary consequences of environmental-energetic-epigenetic linkages c) **Intellectual Merit:** This project examines how nutrient metabolism in the mitochondria generates cofactors and energy that will instruct the epigenetic machinery in the cell nucleus to modulate genome function to appropriately respond to environmental conditions. The major pioneering outcome of this work will be delineating fundamental links (Rules of Life) between ubiquitous organismal energetic processes, epigenetics, and eco-evolutionary outcomes. **Broader Impacts:** This award is training 2 graduate students, 1 postdoc, and 1 REU. The Broader Impacts activities parallel the project's integrative approach, linking insights from Environment x Energetics x Epigenetics x Ecology for Education into an E5 platform. The E5 platform will provide i) early career STEM training, ii) local and global community education, and iii) educational resources for open science, quantitative approaches, and research reproducibility. d) **Publications and Products**[49] e) Data corresponding to this work are deposited in the E5coral.org repository and available on GitHub.

Teutonico is PI of a) DBI-1852123, $360,000, 4/01/2019-3/31/22; b) REU Site: Understanding Coastal Ecosystems c) **Intellectual Merit:** The theme of the REU Site is the ecology of coastal ecosystems across gradients of human impact, **Broader Impacts:** The REU provides research and professional development experiences to students from underrepresented groups and to those with less access to research resources. The first 9 students were 77% external, 89% from MSIs, 45% URMs, 67% female; d) Five of the 9 students have already disseminated their results in 2 peer-reviewed publications[49,50] and 3 presentations at scientific conferences; and e) Data and publications will be made available on the REU site website.

Troxler was co-PI of a) DEB-1801244, $178,159, 12/14/2017-12/13/2019; b) RAPID: Hurricane Irma: How do ecosystem perturbations interact to influence long-term resilience mechanisms? c) **Intellectual Merit:** This research addressed whether the extent and immediate impacts of a hurricane disturbance influenced short- and long-term ecosystem dynamics to determine resilience capacity (i.e., recovery duration and rate) of communities across the Everglades landscape. **Broader Impacts:** The research provided state and federal agencies critical information to maximize guidance for decisions about water management to improve resilience and supported K-12 classroom activities through our FCE Schoolyard Program. d) The work resulted in 1 publication[51] and 1 presentation at a scientific conference; and e) Data and publications are accessible through the FCE LTER website.

Fernando-Lima is PI of a) CHE-1654274, $600,000, 02/01/17 - 01/31/22; b) CAREER: Development of gas-phase, post-ionization structural tools for the study of DNA-protein complexes. c) ***Intellectual Merit:*** The proposed platform will transform the investigation of biological molecules to one capable of providing detailed, structural information on co-existing states in the conformational ensemble (e.g., equilibrium and kinetic intermediates). These tools will provide new structural information, permit mobility-selected conformation interrogation, enable the study of conformational changes from solution to the gas-phase, and provide information on the energy barriers for conformational rearrangement and their time scales. ***Broader Impacts:*** To enable comprehensive outreach to students, faculty, and the community that will increase the number of minority students exposed to modern MS techniques, the PI has planned for a multi-level approach integrating seminar series, undergraduate and graduate courses, AMSF open-house events, hands-on MS practices, development of MS visual experiments and summer internships; d) one patent and 30 publications have resulted to date.[5,52] e) Data is stored in Dr. Fernandez-Lima's laboratory data storage and redundant locations (FFL_VAULT, AMSF_DRIVE and a centralized server at FIU).

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Facilities, Equipment and Other Resources

Florida International University

The CREST Program will benefit from the infrastructural support of resources currently in-place at FIU, namely: the STEM Transformation Institute, Environmental Molecular Genetics and Epigenomics Laboratory, the shared analytical resources of the Institute of Environmental Field Operations Center (FOC) and the computational support of the School of Computing and Informational Sciences. This section provides a summary of existing resources that are relevant to this solicitation and that could be available to support the objectives of CREST. The facilities described in this section are fully available for this project.

Institute of Environment facilitates linkages between academic scientists and local, state, and federal agencies, providing an organizational structure for regional environmental research initiatives and interdisciplinary environmental investigations. Institute of Environment research continues to be involved in many of the most pressing issues confronting our environment and society including: work instrumental in developing Federal limits to excess phosphorus in water discharged into the Everglades, evaluation of the effects of the Deep-Water Horizon oil spill, and the social and environmental threats of extreme weather and rising sea level. Examples of some significant Institute of Environment projects/programs include the "Numerical Interpretation of the Class III Narrative Nutrient Water Criteria for Everglades Wetlands" that was conducted 1995 – 2005 and largely provided the Total Phosphorus limits for water being discharged into the Everglades under the Everglades Forever Act. The ability to conduct large-scale, collaborative, interdisciplinary, environmental science was recognized in 2001, when Institute of Environment received National Science Foundation funding to establish a Long-Term Ecological Research program in the Florida Coastal Everglades (FCE-LTER).

The shared analytical facilities of the combined Institute of Environment laboratories have a long history of supporting research that has generated world-class environmental information. Institute of Environment personnel have engaged in a broad dissemination of results and have recently (as of December, 2014) surpassed 700 contributions to the peer-reviewed literature (since 1993). Much of the data included in these papers were supported by the Institute of Environment Labs. In keeping with the mission of FIU, as the nation's largest Hispanic Serving Institution, this CREST Program and all associated FIU Institute of Environment labs and facilities have continually integrated opportunities for undergraduate, graduate, and post-doctoral education, training, and involvement in research. Many of these people are from traditionally underrepresented groups in the science fields. The Institute of Environment analytical facilities provide an opportunity for students to learn important analytical techniques, chemical and biogeochemical ecology, and other research approaches. All shared analytical facilities are focused Institute of Environment on involving students, either through direct analysis of their samples and/or by including them in the analysis process. The Institute of Environment analytical laboratories are user-based, shared facilities where students, when possible, learn to analyze their samples. When "hands-on" student involvement is not possible Research Assistants or, more commonly, staff technicians run the samples thus allowing the completion of student/faculty Projects. The Institute of Environment labs support the activities of Institute of Environment/FIU faculty and their students. These activities support numerous undergraduate and graduate students and post-doctoral associates. For instance, in 2013-2014 Institute of Environment research grants supported 54 Ph.D. students, 27 M.S. students, 15 post-docs, and many undergraduates.

Institute of Environment-NSF Center of Excellence in Aquatic Chemistry and Environment (CREST)

To address water contamination in South Florida, the NSF (National Science Foundation) CREST (Center of Research Excellence in Science and Technology) Center for Aquatic Chemistry and Environment (CAChE) at Florida International University has deployed three cutting-edge research buoys, specially-designed to be utilized in both shallow freshwater ecosystems and near-shore marine environments. Developed by Xylem Inc., our customized system features the EMM150 Research Buoy platform equipped with YSI's EXO2 water quality sonde and a Doppler Current Sensor by Aanderaa. The buoys measure the following parameters: temperature, conductivity (salinity), dissolved oxygen, pH, turbidity, chlorophyll, depth, and directional water flow rates. All sensors are maintained automatically by a self-cleaning wiper system that prevents biofouling and allows for long-term deployment. Additionally, the buoys are equipped with a set of solar panels, which recharge the central battery and can extend sampling periods to several months. Data collected are automatically transmitted via cellular uplink every 15 minutes, making the information available in real time to our CREST CAChE team and the general public. The Center also has two additional water quality sondes available with handheld readers, for in-person field sampling or deployment in locations not suitable for a research buoy, such as in groundwater wells.

The integration of these sensors into a relatively-compact buoy platform represents a novel application for water quality monitoring; the buoys are able to be deployed in a range of aquatic ecosystems of varying depths, and our researchers can receive live data on water conditions and potential issues remotely. Furthermore, the water current data provide useful information for tracking contaminants' pathways, indicating the general direction of the source and its trajectory. These buoys are placed in diverse locations around southeast Florida to monitor water quality and collect baseline data in our near shore ecosystems and urban waterways. Our first buoy deployment in October 2018 was in response to a red tide outbreak on the southeast coast of Florida. Since then we have maintained a buoy at this site in Haulover Inlet, located in the northern area of Biscayne Bay, to continue monitoring this unique environment which receives a mix of freshwater inputs and tidal exchanges. Next, we deployed a buoy in a Coral Gables canal to assess and compare conditions in an urban environment. Our third buoy was temporarily deployed within a harbor in the Florida Keys—where harmful algae and fish kills had been reported—and then a mangrove forest in Biscayne Bay. This buoy was later deployed for long-term monitoring in the Miami River, arguably one of the most polluted waterways in south Florida. More recently in summer 2020, two of these buoys were moved to strategic spots within Biscayne Bay, in response to exceedingly low oxygen levels that led to fish kills in the area. Each of these locations represents a unique environment, and the use of these research buoys provides valuable information on the conditions within our aquatic ecosystems, which our team can then share with public audiences and decision makers.

Institute of Environment-Analytical Capacity and Infrastructure.

Laboratories are individually supervised by faculty scientists specializing in their respective analysis with administrative organization through FIU Institute of Environment. Our laboratories are located on FIU's Modesto A. Maidique Campus (11200 SW 8th Street, Miami, FL 33199) and Biscayne Bay Campus (3000 NE 151 Street, North Miami, FL 33181).

The following laboratories are available to assist in CREST research.

- Ecotoxicology Laboratory (EL)
- Environmental Analysis Research Laboratory (EARL)
- Environmental Geochemistry Laboratory (EGL)
- Environmental Bioinorganic Chemistry Laboratory (EBCL)
- Hydrogeochemistry Laboratory (HGCL)
- Nutrient Analysis Laboratory (NAL)
- Soil/Sediment Biogeochemistry Laboratory (SBL)
- Periphyton Analysis Laboratory (PAL)
- Stable Isotope Laboratory (SIL)
- Terrestrial Ecosystem Laboratory (TEL)
- Population and Community Ecology Lab
- Aquatic Ecology Lab
- Wetlands Ecosystem Research Laboratory (WERL)

Detailed descriptions of laboratories, capabilities, and equipment currently in place and potentially involved with CREST include:

Ecotoxicology Laboratory (EL) **-** is a state-of-the-art NELAC (E76988) accredited facility that conducts ecotoxicity studies with organic and inorganic chemicals and a multitude of exposure types (e.g., single-slug, intermittent and continuous) with indigenous, exotic and standard test species. These facilities provide unique capabilities in aquatic and sediment toxicity to conduct exposures in waters ranging from fully freshwater to full-strength seawater. Experimental systems are designed to evaluate direct and indirect effects of chemical stressors in sediment-water systems on the structure of different trophic groups, species interactions, and other functional aspects of these systems.

Environmental Analysis Research Laboratory (EARL) - is NELAC certified (E761019) and equipped to conduct specialized trace organic analyses in a wide range of environmental samples (waters, sediments/soils, biological tissues, etc.), with methods aimed to achieve detection limits in consonance with the low-level concentrations of pollutants generally present in the biotic and abiotic compartments in the South Florida Ecosystems.

Environmental Geochemistry Laboratory (EGL) **-** is focused on research activities primarily related to studying the biogeochemistry of organic matter in aquatic environments. This includes, soil/sedimentary OM as well as dissolved organic matter (DOM). The research group has focused mainly on investigations on organic matter dynamics in wetlands, riverine ecosystems and estuarine environments, but has recently also started marine systems work. Our work also includes paleoenvironmental assessments and issues related to soil formation and sustainability. The lab is equipped with several GC/MS systems including pyrolysis-GC/MS, HPLC with dual UV-Vis/Fluorescence detection, UV-Visible spectroscopy and 3-D Fluorescence spectroscopy, among others.

Environmental Bioinorganic Chemistry Laboratory (EBCL) **-** is NELAC (E76960) certified the determination of trace metals, metalloids, and organometallics in a variety of environmental and biological samples using several state-of -art instruments. The laboratory is equipped to carry-out speciation research for toxic chemical contaminants; one of the emerging areas in environmental chemistry. The laboratory currently has one hydride generation-atomic fluorescence spectrometry (HG-AFS) system for arsenic and selenium analysis and speciation; two gas chromatography- atomic fluorescence spectrometry (GC-AFS) system for mercury speciation, one gas chromatography atomic emission spectrometry (GC-AES) for simultaneous determination and speciation of organometallics; one graphite furnace atomic absorption spectrometry (GFAAS); two high performance liquid chromatography (HPLC), which can be coupled to AFS for speciation of metals and metalloids; and one microwave digestion system for digestion of environmental and biological samples. Additionally, several ICP-MS and ICP-high resolution/MS systems are available through the Department of Chemistry.

Hydrogeochemistry Laboratory (HGCL) **-** includes analytic and field capabilities with the following laboratory equipment: Los Gatos DTL-100 Liquid Water Isotope Analyzer and PAL autosampler for determinations of the stable oxygen and hydrogen isotopic composition of water samples; Dionex DX-120 Ion Chromatograph and Dionex Autosampler for determinations of major cations (Ca2+, Mg2+, Na+, K+, NH4+) and anions (Cl-, SO4 2-, F-, NO3 -) in water samples; Metohm Auto Titrator system with autosampler designed to measure the total alkalinity of water samples; Thermo Scientific™ Barnstead™ Water Nanopure Dionized Water System to produce 18 mhom water.

Nutrient Analysis Laboratory (NAL) - is a NELAC certified (E76930-17) lab for non-potable water - General Chemistry (dissolved and total nutrients in fresh and salt waters), Chlorophyll *a* and Total P, C, and N in solids and tissues. Additional, non-certified analysis includes: salinity, pH, temperature, turbidity, etc. Equipment includes: Bausch and Lomb and Shimadzu spectrophotometers, Gilford Fluoro-IV scanning fluorometer, Shimazdu scanning fluorometer, RC-5 refrigerated centrifuges, Antek 9000N total nitrogen analyzer, Shimadzu 5000 TOC analyzers, 3 Shimadzu TOC-V with TN option, Alpkem rapid flow nutrient analyzers, Amicon Model 200 ultrafiltration units, Beckman LS3801 scintillation counters, and Carlo Erba elemental analyzers, autoclaves, water baths, incubators, balances, drying ovens, and muffle furnaces. The facility also includes all the equipment necessary to support these analytical laboratories (glass washing facilities, ice machines, electron microscopes, ultra-low temperature freezers, radioisotope and autoradiography rooms, etc.).

Soil/Sediment Biogeochemistry Laboratory (SBL) **-** provides a range of nutrient and chemical analysis on environmental water and soil samples including a wide variety of extractable nutrients, gas samples (CO_2, CH_4,N_2O, etc.), enzyme activities, and other physicochemical properties. The SBL specializes in the extraction and analysis of chemical components from soil/sediments and soil/sediment porewater. In addition, the SBL is capable of analysis of surface water, and plant tissue. The facility includes state of the art equipment for research, service, and training in soil/sediment sample collection and nutrient and gas analyses. An abbreviated analytical equipment list includes: a Cytofluor 4000 multi-well plate reader, Fisher Scientific (FS) Accu 224 Analytical Balance, FS Accu 3102T triple range top-loading balance, FS Accumet AR50 dual channel pH/ion/Conductivity meter, Hewlett Packard 5890 Gas Chromatograph with a 7694 headspace sampler and equipped with flame ionization and electron capture detectors and a Shimadzu MTN-1 methanizer, a Technicon 3-channel Automated colorimeter, and a Perkin Elmer 2400 CHNS/O analyzer. Field sampling equipment includes coring apparatus capable of collecting soil/sediments from uplands, wetlands, and lakes (< 35 m), a Licor LI-1400 light meter with air and underwater sensors, a YSI DO meter with 5905 BOD probe and a YSI 600QS probe for profiling physicochemical conditions of water columns.

Periphyton Analysis Laboratory (PAL) **-** is a recharge center that provides support for the quantification, identification and enumeration of algae from lakes, rivers and wetlands throughout the world. Activities in the center include guidance on sampling and collections management protocols, assistance and training in algal identification for grants, contracts and workshops, and processing of algal samples to quantify abundance and composition. The lab uses the following equipment in their analyses: Zeiss Axioscope with 10x, 63x, and 100x objectives and Nikon 400E and 600E microscopes with 10x, 60x, and 100x objectives for diatom and soft algae analyses.

Stable Isotope Laboratory (SIL) **-** is a full-service stable isotope facility capable of analyzing stable isotopes of C, N, O, and H in inorganic and organic solids (soils, tissues) and water samples. Equipment includes: Thermo Delta C EA-IRMS, Thermo Delta Plus GC-IRMS w/ EA option, Thermo Delta V GC-IRMS w/GasBench & TC/EA options and a Picarro CRDS coupled with an OI TOC system.

Terrestrial Ecosystem Laboratory (TEL) **–** takes a field-oriented, multi-disciplinary approach to the study of the wetland ecosystems of southern Florida, from the Lower Keys to Lake Okeechobee, and from flooded herbaceous wetlands to forested uplands in Everglades National Park and Big Cypress National Preserve. The lab conducts investigations on spatial distribution of wetland vegetation and interaction between vegetation, hydrology and soils. The lab has a full suite of equipment for measuring topography, vegetation and soils, and personnel trained to operate airboats and direct helicopter crews to access field sites throughout South Florida.

Macrophyte Laboratory **–** working with FIU's GIS/RS Center, the lab uses remote sensing techniques to map Everglades vegetation and estimate their hydrologic requirements; conducts study on biology of wetland plant species, especially members of Everglades ridge and slough vegetation and biology of Florida invasive species. The lab has a full suite of equipment for monitoring vegetation and plant growth, as well as mesocosm and greenhouse facilities for experimental studies.

Population and Community Ecology Lab – population dynamics of wetland animals (e.g. fish); role of life history patterns both within populations and communities; spatial patterns of fish (e.g. movement across the Everglades). The lab has a full suite of equipment for conducting fish population dynamic studies in wetlands and personnel trained to operate airboats and direct helicopter crews to access field sites throughout South Florida.

Aquatic Ecology Lab **–** studies include behavioral, population and community ecology, species interactions, predator-prey dynamics, anthropogenic disturbance, ecology of biological invasions, hydrologic disturbance, fish ecology, aquatic and wetland ecology. The lab has a full suite of equipment for conducting fish population dynamic studies in wetlands and coastal regions, and personnel trained to operate airboats and small marine boats to access field sites throughout South Florida.

Wetlands Ecosystem Research Laboratory (WERL) **-** work integrates hydrology, plant ecology and biogeochemistry to understand ecosystem-level structure, process and change in the Everglades. The lab has a full suite of equipment for conducting wetland studies and personnel trained to operate airboats to access field sites throughout South Florida.

Analytical Capacity, Quality Assurance.
All laboratories will be scheduled to provide support as needed and to analyze samples as scheduled. Technical task plan and project management plans will be prepared to ensure coordinated sampling and analysis. Analytical procedures used will be tailored to the specific chemical environment of the CREST projects. The Field and Laboratory Operators are familiar with requirements for a Quality Assurance and Performance Plan (QAPP) and have experience in developing robust QAPP for specific projects. Standard methods will be followed wherever applicable throughout a project. Several associated labs are compliant with the requirements of FAC rule 64E-1 and NELAC Standards.

Field Operations Center.
FIU maintains the Field Operations Center (FOC), which is part of Institute of Environment, a core facility responsible for maintaining and providing a fleet of vehicles and vessels to facilitate environmental field work. Currently the FOC has 11 vehicles, most of which are large 4x4's capable of towing and rough terrain use, 5 airboats and 12 small- and large-size boats that are used for transportation in wetlands and coastal waters. In addition, FOC maintains a full inventory of field and safety equipment.

Aquarius Reef Base
The Medina Aquarius Program at FIU is dedicated to the study and preservation of marine ecosystems worldwide. As part of the FIU Institute of Environment, the Medina Aquarius Program is enhancing the scope and impact of FIU on teaching, research, educational outreach, technology development, and professional training. At the heart of the program is the one-of-a-kind Aquarius Reef Base, the world's only undersea research laboratory.

Deployed 60 feet beneath the surface in the Florida Keys National Marine Sanctuary, Aquarius is a globally significant asset that provides unparalleled means to study the ocean, test and develop state-of-the-art undersea technology, train specialized divers and astronauts and engage the imagination of people all around the world. At Aquarius, scientists are at the forefront of research on coral reefs, ocean acidification, climate change, fisheries, and the overall health of the ocean.

Since its deployment in 1993, aquanauts and their support teams have used Aquarius to answer critical challenges facing scientists and policymakers. Universities, government agencies, and private industry have conducted more than 130 missions to discover, preserve, train, and innovate. More than 600 scientific research papers have been published based on Aquarius science.

STEM Transformation Institute.
Florida International University includes over 4,800,000 square feet of assignable space in 94 buildings on its Modesto Maidique and Engineering Center campuses. There are a wide range of classrooms available, the majority with multimedia equipment and Internet access. Active learning STEM courses have access to newly built classrooms designed for active learning. These include one 96-student classroom in AHC V as well as 4 additional classrooms in PG6 that open in Fall 2015 (capacity: 174, 96, 50, and 50). The standard design of these active learning classrooms are flexible table and chair configurations, complete media equipped, and space for learning assistants (LA) and faculty to access

and engage all students. All the active learning classrooms, general multimedia classrooms and the following university resources will be available to the project.

Physics Learning Center - The Physics Learning Center includes two studio classrooms, conference room, lounge, and offices for the Physics Education Research Group's staff and graduate students. The classroom is used to support innovative implementations of introductory physics courses where students learn science as scientists in a lecture-free environment. The roughly 4,000 sq ft space supports the informal physics learning community as students have access to the classrooms, lounge, and conference room around the clock. The space will evolve into a STEM Learning Space during the project, expanding capacity to serve all STEM degree programs and faculty.

STEM Transformation Institute - The STEM Transformation Institute is housed in the AHC IV building, third floor to support the Institute's education research activities. The Institute currently occupies 9 offices to support the 6 faculty 5 post-doctoral researchers, and one administrative assistant leading the Institute, as well as a research space that hosts space for the dozen graduate students doing STEM education research. The Institute also has a data analysis and storage room plus interview room located near the Physics Learning Center.

Institute of Environment Environmental Molecular Genetics and Epigenomics Laboratory.
This facility studies the chromatin structure, genomics, and transcriptional responses to environmental stressors: anthropogenic pollution (i.e., oil spills, pesticides) to understand the molecular mechanisms that impact the ecosystem. We approach this objective by using state of the art molecular biological tools such as gene microarrays, ChIP-sequencing, and bioinformatics tools to identify changes in gene expression and epigenomic marks (DNA methylation, histone modifications, chromatin accessibility) relevant to environmental exposures.

Florida International University - School of Computing and Information Sciences
The School of Computing and Information Sciences (SCIS) maintains a data center, research and instructional labs, and computer classroom facilities. These facilities are housed in the Engineering and Computer Science Building (ECS) on the Modesto A. Maidique Campus located in Miami, Fl. The facility is maintained by a dedicated professional IT support staff as noted in the staffing section below.

The School provides computing services such as file, compute, web, email, messaging, backup, print, and other computing services. Our networking services include a 10 Gigabit Ethernet core network that interconnects rack mounted switches and servers. All school desktop systems are connected by 1 Gigabit switched ports. Our network is highly redundant with multiple fiber and copper paths and is designed with routing fail-over capacity. We provide automated monitoring of our network and servers 24x7. The building subscribes to the university 802.11 WiFi network and SCIS maintains a legacy research WiFi network. Our network interconnects at 10GBs to the campus backbone, which provides a 10GBs connection to the NAP of the Americas to provide for connections to Internet, Internet2, Florida and National Lambda Rail, and CLARA (South American Research) networks.

Our systems feature a variety of open source, commercial development and scientific software products from numerous vendors including IBM, Microsoft, ESRI, MathWorks, etc. We provide middleware technologies to support web services. Our environment takes advantage of hundreds of open source software solutions including Apache with full mods, PHP, Perl, and many others. Many of our shared infrastructures provide virtualization services.

Notable Shared Computing Infrastructure

- File Server 1 (faculty, grad students, researchers): Silicon Mechanics Storform 2x Opteron 6320 proc, 128GB ram, Intel X520 10Gb NIC, 24x4TB 7.2k hdd (80TB after RAID), Linux ZFS system.
- File Server 2 (undergrad students): Silicon Mechanics Storform 2x Opteron 6320 proc, 128GB ram, Intel X520 10Gb NIC, 36TB (30TB after RAID), Linux ZFS system.

- Mail Server: Silicon Mechanics 1U server, 128gb ram, 2x Intel Xeon E5-2620 proc, 2x 600gb hdd, 384gb SATA Solid State Disk, gigabit nic,
- Active Directory Domain Controllers: (2) Dell Poweredge R210, 8gb ram, Intel Xeon X3430 2.4ghz proc, raid 1, gigabit nic, 2x 250gb 7.2k hdd
- Computing Server: (1) Dell Poweredge R210, 8gb ram, Intel Xeon X3430 2.4ghz proc, raid 1, gigabit nic, 2x 250gb 7.2k hdd
- Webserver and Learning Management System:
    - Silicon Mechanics Storform, 96gb ram, 2x Intel Xeon E5-2407 2.93ghz proc, raid 15, gigabit nic, 2x 2TB 15k hdd, 2x 480GB SSD
    - Dell Poweredge 2900 III, 32gb ram, 2x Intel Xeon 5410 proc, 4x 300gb hdd, gigabit nic.
    - Dell Poweredge R310, 16GB ram, 2x Intel Xeon X3470, 2TB mechanical storage.
- Instructional Clusters:
    - Dedicated 8+ node research and instructional Linux Beowulf cluster.

Dedicated Shared Research and Instructional Infrastructure
- Virtual Machine Servers: (3) Dell Poweredge R410, 64gb ram, 2x Intel Xeon E5620 2.4ghz proc, gigabit nic, 1.8TB raid 5 storage, (3) Dell Poweredge R420, 96gb ram, 2x Intel Xeon E5-2450L @ 1.8GHz, gigabit nic, 1TB raid 5 storage
- General Compute Servers:
    - (2) Dell Poweredge 29xx, 64gb+ ram, Intel Xeon proc,
    - (3) Dell Poweredge R410, 64gb ram, 2x Intel Xeon X5650, gigabit nic, 3x 600gb hdd
    - (2) Dell Poweredge R710, 72gb ram, 2x Intel Xeon X5570, gigabit nic, 8x 300gb hdd
    - (2) Dell Poweredge R815, 512gb ram, 4x AMD Opteron 6380 @ 2.5GHz, 10gb nic, 1.5TB disk

Business Continuity Infrastructure
- General Compute Server: Dell Poweredge R410, 64gb ram, 2x Intel Xeon E5620 2.4ghz proc, raid 5, gigabit nic, 4x 600gb 15k hdd
- (2) 80TB Online Backup server for File Server 1 (one off-site, one in ECS Building): Silicon Mechanics Storform 2x Opteron 6320 proc, 128gb ram, Intel X520 10Gb NIC, 24x4TB 7.2k hdd (80TB after RAID). Linux ZFS system.
- (2) 80TB Online Backup server for File Server 2 (one off-site, one in ECS Building). Silicon Mechanics Storform 2x Opteron 6320 proc, 128GB ram, Intel X520 10Gb NIC, 36TB (30TB after RAID), Linux ZFS system.
- (2) 80TB Online Backup server for General Backup and Standalone systems (one off-site, one in ECS Building): Silicon Mechanics Storform 2x Opteron 6320 proc, 128gb ram, Intel X520 10Gb NIC, 24x4TB 7.2k hdd (80TB after RAID). Linux ZFS system.

I-CAVE™
Established in 2015 as in instructional and research visualization laboratory for faculty and students at Florida International University. Located on the MMC Campus in Miami, Florida. Our integrated-CAVE or I-CAVE™ will allow for interactive visualization for student and instructor projects. The facility along with computer support will be available for use by students and instructors in all departments from anthropology and astronomy to visual arts and zoology. The system will be provided as a resource for students and instructors at the university to use. We provide standard software information for basic 2-D and 3-D visualization including animations, videos, graphics software and 3D modeling.

Visualization Lab
The Visualization Lab in the Engineering and Computer Science building, room 232a, houses two Sharp PN-L802B 80" Class AQUOS BOARD LED Displays which are multi-touch capable. Each display is driven by a Dell Workstation containing Intel i7 processors with 16 gbs of RAM and an ATI 7 series graphic processing units for high load applications and 3d rendering. The workstations are running Windows 8 and support Surface gesturing. Metro Applications can be built that provide native gesture

support for touch input. Other lab software includes AutoDesk 3D Studio Max for industrial quality 3D design and animation capabilities.

Student Laboratories

SCIS operates seven instructional laboratories for use by undergraduates and graduate students in support of our computer science and information technology degree programs. Our instructional labs offer students access to Windows 7/XP, CentOS Linux, and Mac OS X which run a variety of software development tools, libraries, databases, and have the capacity to host virtual machines. The specific lab equipment is listed in sections below. The School has dedicated servers for student files and/or computing services and a printer in each lab. Each student receives at least 25GB of backed up file storage space. Students can login remotely into several Linux and Solaris file and compute servers. In addition to workstations provided in the open labs, students may connect their laptops to the SCIS network via WiFi. Students may also utilize their laptops with 40” LCD displays to collaborate on programming assignments or other joint projects. The school maintains a computer training classroom also noted in the specifications below.

ECS 241 - John C. Comfort Collabrium

- (48) Dell Precision T1700, 8 Core i7-3770 3.4ghz, 16gb ram, 256gb ssd hdd, nVidia Quardo 600, 1gb to desktop, 16x dvd rw single 24in display

ECS 237 - Advanced Undergraduate Lab

- (11) Apple iMac 27, Quad Core i5 3.2ghz, 16gb ram, 256gb ssd hd, NVIDIA GeForce GT 755M
- (2) Apple iMac 27, Quad Core i5 2.9ghz, 16gb ram, 256gb ssd, NVIDIA GeForce GTX 600M
- (11) Dell Precision T1700, QuadCore i7-4770 3.4ghz, 16gb ram, 256gb ssd hdd, nVidia Quardo 600, 1gb to desktop, 16x dvd rw single 24in display or dual 22in display
- (2) Samsung 40in displays for group collaboration
- (2) Standing, height adjustable desks with single 24" displays for collaboration

ECS 235: Active Learning Lab

- This multimedia enabled lecture room provides flexible seating and workstations for student groups to use to solve in class assignments. The room contains lecture recording and streaming capabilities via Sonic Foundry MediaSite.

ECS 237a: Tutorial Lab

- (4) Apple iMac 27, Quad Core i5 2.9ghz, 16gb ram, 256gb ssd, NVIDIA GeForce GTX 600M
- This room also contains data projection and flexible seating for instructors and students to hold ad-hoc tutoring.

ECS 256 - Networking and Hardware Lab

- 42 Pentium class machines configured into clusters to provide multiple simulated wide area networks.
- Cisco routers, switches and KVMs necessary to manage WANs.

ECS 252 - Graduate Lab

- (12) Dell Precision T1650 8 core 3.4ghz, Intel Core i7-3770, 16GB memory, 256GB SSD, NVIDIA Quadro 600, DVD RW Drive, dual 22" displays.
- (1) Apple iMac 27 Quad Core i7 2.8ghz, ATI Radeon HD 4850, 4gb, 1tb hdd, 8x Superdrive
- (1) 42” Display LCD Monitor for group projects.

ECS 141 - Instructional Lab (ILab)

- (48) Dell Precision T1600, Dual Core i3-2100 (2nd gen) 3.1 ghz, 1gb nVidia Quadro 600, 8gb, 250gb hdd, 16x dvd rw, P2210 display
- Lecture recording/streaming via Sonic Foundry MediaSite.

SCIS Support Staffing
The school maintains all its computing facilities (total research and instruction: 26 labs, 350+ desktops, 100+ servers, layer 2 and 3 networking) via a dedicated Technology Group. The SCIS Technology Group consists of 5 FTE of permanent professional staff assigned to all of the school's research and instructional laboratories management. In addition, there are at least 2 FTE of temporary students specifically assigned to laboratory assistance. The SCIS Technology Group staff is organized into three groups: Engineering Services, including Networking, Systems, Desktop, and Help Desk Support, and Business Services including Technology Procurement, Asset Management, and Budget/Contract Management, and a Marketing Technology group that promotes the school via digital and social media outlets.

Additional Key FIU Faculty and Staff:
In addition to the budgeted PI, co-PIs, and Senior Personnel, the following FIU faculty and staff may support CREST-CaChE II through mentorship, advising and research. The personnel will be selected based on availability, number of graduate students and research interests of students.
RFA1 Faculty: Elizabeth Anderson, John Berry, Henry Briceno, Yong Cai, William McDowell, Paulo Olivas, Vladimir Pozdin, Gregory Reis, Ryan Smith
RFA2 Faculty: Alastair Harbonrne, Jeremy Kiszka, Yannis Papastamatiou
RFA3 Faculty: Leonardo Bobadilla, Mark Butler, Edward Castaneda, Marta D'Elia, Ali Ebrahimian, James Fourqurean, Evelyn Gaiser, Emel Ganapati, Khandker Ishtiaq, John Kominoski, Arturo Leon, Jayantha Obeysekera, Randall Parkinson, Rene Price, Michael Ross, Rolando Santos, Leonardo Scinto

The Learning Partnership
General Equipment: The Learning Partnership is equipped with computing resources and software for conducting research. All offices are equipped with the basic office furniture and high-speed internet connection as well as a scanner, laser printer, and automated software backup system. The Learning Partnership also has a corporate account for Zoom video conferencing to communicate with institutions in United States and other countries. The Learning Partnership has a corporate account for Dropbox cloud storage and backup.

Other resources:
The following are unfunded collaborators that will participate to our External Partners Board (EPB) in support of the Center's activities. The EPB is composed of representatives from local and federal resource management agencies, NGOs, museums, and private industry. They will participate in career panels to advise students on STEM pathways; provide internship opportunities; and co-produce research, education, and outreach materials with students relevant to their research projects.

Institution/Organization	Category
Miami-Dade County Public Schools	Education
City of Coral Gables	Government – State/Local
Miami-Dade County Commission	Government – State/Local
Miami-Dade County Water & Sewer Department	Government – State/Local
Miami-Dade County Div. of Environmental Resources Management	Government – State/Local
South Florida Water Management District	Government – State/Local
National Oceanic & Atmospheric Administration	Government - Federal
Miccosukee Tribe's Fish and Wildlife Division	Government - Tribal
Coastal Ecosystems, LLC	Industry
SeaRobotics	Industry
Coral Gables Museum	Museum
Miami Waterkeeper	Non-Governmental Organization
Sachamama	Non-Governmental Organization
Everglades Foundation	Non-Governmental Organization
Ocean Conservancy	Non-Governmental Organization

Data Management Plan

I. Types of data

Student/Project Success Metric Data – In CREST I, we increased the participation of underrepresented minorities (URM) in STEM professions and unified many of our existing individual programs in environmental chemistry/ecotoxicology/hydrology/ecology and computer-intensive data and visualization to provide a sustainable infrastructure to enhance FIU's research and education competitiveness in this evolving area of STEM. We also conducted annual formative evaluations using data from participating faculty, students, and other team members, including questionnaires, interviews, journals, and students' performances and other qualitative data. Using our cumulative data to date, we also conducted an summative evaluation to inform our objectives, activities and organization of CREST Phase II.

These same data will be used to evaluate the program progress towards meeting its goals (see Evaluation section), and to formatively improve the program by continuously adjusting its various components in CREST II. In addition, the project team will collect and showcase project successes in various venues including CREST's symposia and website, as well as NSF reports and highlights. All data will be protected from access to identifiable information and will be kept in a secure locked repository accessible by the project PI (see Section III below) and shared with the Learning Partnership, our external educational assessment company, responsible for project evaluation.

Aquatic Chemistry, Molecular Biology, Hydrologic and Ecological Data – In CREST I, we created a data repository for all raw data sets as well as a data repository for data products including model outputs, supplementary data for publications as well as the publications themselves. We also created a Metadata portal using EML standards, consistent with our existing Long-Term Ecological Research Program so that all data and products are fully discoverable and usable.

CREST II will continue to support the full data life cycle – with a focus on curting and sharing quality controlled data and developing tools, repositories and/or web services that assist CREST researchers, and students, as well as external data consumers, in discovering, visualizing, and accessing data. Development of the required repositories, protocols and methods for enabling shared data access is part of the intellectual merit of this project. Where possible, we will use existing web services (e.g., CUAHSI-HIS, http://his.cuahsi.org/) or develop data services that interface with existing, Internet-based systems (e.g., the USGS Seamless Data Server for the National Land Cover Dataset or National Elevation Datasets).

CREST II data will will be combined with existing datasets from CREST I, various collaborating agency data (see letters) as well as new field and laboratory measurements. Types of data to be collected, stored, and shared include: a) time series of observations from in situ sensors along three transitional transects (e.g., flow and water quality gages, weather stations, atmospheric flux sites, etc.) as well as observations derived from field measurements and water quality samples; b) geospatial datasets and other landscape/geographic features describing the S. Florida coastal water system; c) environmental data including observations of contaminants, pollutants and other natural stressors; d) hydrologic data including surface and groundwater withdrawals and deliveries, wastewater discharge, water conservation, as well as past and current climatological information; and e) molecular data, including gene and protein sequences and transcriptome data. Finally, project investigators will develop derived datasets from analysis or modeling exercises using combinations of the above data types.

II. Standards

CREST II will continue to make full use of existing and emerging standards for sharing environmental and molecular data. In addition to storing all CREST data in our CREST Computer Center, data such as time series data collected by this project will be stored using the CUAHSI-HIS Observations Data Model (ODM) and published in Water Markup Language (WaterML) format using CUAHSI-HIS WaterOneFlow web services. For sharing geospatial datasets, we will use existing Open Geospatial Consortium (OGC) standard interfaces such as Web Map Services, Web Feature Services, and Web Coverage Services. We will also provide standard HTTP and FTP access to download datasets, but will ensure that published data are accompanied by appropriate metadata descriptions, which will conform to appropriate ISO, EML, and/or FGDC metadata specifications. The design of the microarray experiment will comply with the requirements of the Minimum Information About Microarray Experiment (MIAME). Gene and protein sequences will be annotated and subsequently submitted to the general database GenBank.

Transcriptomic datasets will be submitted to the Gene Expression Omnibus functional genomics data repository (GEO). By using these accepted standards for data interfaces and format encodings, we will ensure that CREST data are interoperable with existing data repositories. Dr. William Michener, PI of the NSF funded DataONE project is a member of our External Advisory Committee and will provide invaluable input regarding data publication and interoperability.

III. Policies for access, sharing, and provisions for appropriate protection/privacy

As in CREST I, CREST II project data will be collected and handled according to FIU's IRB and NSF's policies. All CREST participants will be guided through the informed consent process and invited to participate by signing the consent form. Only the project evaluation team will be involved in personal data collection and analysis. All identifiable information linking participants' information and performance to the database will be removed and the project evaluation team will work with codified information only. The release forms and other data will be kept in a locked cabinet accessible only by the PI, per the approved IRB protocol.

IV. Policies and provisions for re-use and re-distribution

While IRB policies are strictly followed during data collection and analysis, and all identifiable information to individuals are removed, the project team will share significant results with the research community via journal publications, conferences, workshops, and seminars. Collected data can be made available upon request without access to individual identification. An open source license will apply to software tools developed by this project, which will allow further non-commercial development. A licensing agreement will be required for commercial use of source code or its derivatives.

Data collection, quality assurance/quality control, and annotation with metadata will be the responsibility of CREST II researchers for all project data, and will be conducted in accordance with protocols developed in CREST I. All of the primary datasets collected as part of this project will be made freely and publicly available using the data and metadata standards described above. Finalized datasets and research products will be published within one year or as soon as they are certified as publication ready. Up to the point of publication, access to primary datasets may be limited to CREST I and II researchers and data managers. All data and research products published by CREST I and II will include appropriate attribute and citation information, and accessing published data and research products may include agreement to an access/use agreement specified by the authors. This will ensure that intellectual rights of CREST I and II investigators/data publishers are protected while granting redistribution rights to CREST and its archiving collaborators for purposes of long-term data sharing.

Groups of researchers may wish to share derived data, model input packages, or simulation results within their group before they are published externally. To promote collaboration among CREST I researchers, we developed a private collaboration spaces accessible only to CREST I project participants with full access to our CREST Computer Storage and Computation and GIS Facility. All CREST I and II participants will be afforded access to the contents of private research group working areas at the discretion of the members of the group to protect the intellectual property that they create.

As a general policy, all source code developed by this project will be open source and will be distributed under an open source license to be agreed upon by project members. We will use open source code repositories for our software development, which will enable us to coordinate our activities across multiple collaborators and to engage developers and contributors from outside of the immediate project.

V. Plans for archiving and preservation of access

Research data will be kept in a repository developed at FIU. A copy of the stored data will be maintained periodically on external hard drive, which will be secured in the offices of the principal investigators. The principal investigators will hold the intellectual property rights to the data but will grant redistribution rights to other users for research and education purposes. The FIU Office of Intellectual Property Management and the project team will both maintain a list of open source software initiatives developed by CREST. We will also publish this open source via a public portal like Github or Sourceforge. These sites will mirror the source code repositories. Data are backed up on the Institute of Environment server that is backed up nightly to onsite storage and then replicated to the data center at FSU in Tallahassee.

There is no post-doctoral support requested in this proposal. A separate undergraduate and graduate mentoring plan is described in the Other Supplementary Docs section.

FIU CREST Phase II: Center for Aquatic Chemistry and Environment (CREST-CAChE)

Subproject 1: Developing Detection Technologies for the Study of Environmental Stressors and Organism Exposure

Project Summary
Novel environmental stressors, such as microplastics, pharmaceuticals and other emerging contaminants, have caused significant environmental impacts and attracted worldwide attention. *We initiated FIU's CREST Center for Aquatic Chemistry & Environment (CREST-CAChE) in 2016 to meet the need for enhanced research on the detection and impact of toxic substances in the environment,* including pesticides, industrial chemicals, oil, pharmaceuticals, nutrients, and metals. *Our Phase I research focused on the detection, fate and transport, and visualization of the impacts of contaminants on aquatic systems.* In CREST-CAChE II, we will expand our detection capabilities to unknown and novel emerging contaminants, both natural and anthropogenic, as well as expand our ecosystems of study. While Phase I focused primarily on freshwater systems, such as the Everglades, and the near shore ecosystems, CREST-CAChE II will expand our study area to include aquatic systems along an urbanization gradient. By designing novel sensors and autonomous vessels that can explore difficult to reach urban areas, we will collect continuous and near real-time data on emerging contaminants. In Phase II, we will move from a focus on detection and transport of contaminants, to a mechanistic understanding of the impacts of these contaminants at the organismal, community, and population levels. Using an Understanding the Rules of Life (URoL, NSF 10 Big Ideas) conceptual approach, Phase II will unify across multiple scales, as well as integrate campus-wide programs in Chemistry, Biology, Earth Systems, Computer Science, Engineering, and Architecture.

In addition to advancing cutting-edge research, we will conduct a rigorous education program that builds from lessons learned in CREST-CAChE I. During the initial years of the CREST program, we discovered that students sought the skills to work collaboratively, especially for communicating across disciplines, and for using new emerging technologies. We also learned that undergraduates sought authentic research experiences beyond the Discovery courses that we developed, so will add an undergraduate research-fellows program to Phase II. Our formal and informal education programs will prepare students to communicate the results of their research and for the future STEM workforce. We will include training in core professional skills, as well as micro-credentials in tools necessary to understand complex environmental problems, including robotics, next generation sensor development, big-data analytics, virtual reality, and artificial intelligence.

Intellectual Merit
CREST-CAChE II will increase participation of underrepresented groups in STEM while advancing cutting edge research in environmental chemistry, detection methodologies, molecular mechanisms and producing actionable science with government and private sector partners. Phase II will generate significant new knowledge regarding contaminants in aquatic environments, as well as produce innovative new methodologies for detecting unknown and emerging contaminants and their impacts in urban aquatic ecosystems. *In CREST-CAChE II, we will expand our studies to look at the mechanisms leading to impacts of contaminants at the individual, community, population, and ecosystem levels.*

Broader Impacts
CREST-CAChE II will have three main components in its broader impacts program. a) We will recruit and retain underrepresented students in STEM using our near-peer team mentoring approach. We will build on the success of FIU's evidence-based transformation of STEM instructional practices to provide enhanced support for underrepresented students to successfully pursue STEM careers. b) We will provide students the core professional skills and micro-credentials needed to succeed in the workforce of the future. c) We will develop new technologies for improving water quality analysis and contaminant detection in a range of ecosystems, including freshwater, coastal, and urban environments, as well as provide data on the impacts of these contaminants on ecosystem function. Through partnerships with decisionmakers and stakeholders, we will translate this data into actionable information for designing water quality solutions locally and globally.

Investigator Team

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RFA 1: Developing Detection Technologies for the Study of Environmental Stressors and Organism Exposure

SUBPROJECT RELEVANCE STATEMENT

In CREST-CAChE I, we pioneered approaches for measuring contemporary and legacy contaminants, at extremely low concentrations, by developing new technological methodologies, as well as by modifying and adapting existing analytic hardware. Recent studies have shown that even very low concentrations of new, emerging contaminants in aquatic ecosystems result in impacts on individuals at the organismal level, including genetic and epigenetic changes that cause cellular and physiological responses. Our Phase II unifying theme is to develop and implement novel workflows to detect and characterize emerging contaminants and to understand how those stressors have multi-level impacts on individual, community, population, and ecosystem responses.

RFA1 broadens our advancements from CREST-CAChE I in timely detection of novel emerging contaminants and chemical stressors. RFA2 builds on the unexpected result that many organisms in our aquatic ecosystems responded to novel stressors at multi-organismal scales, including epigenetic, cellular, physiological and behavioral. RFA3 will characterize the impacts of novel stressors at the population, community and ecosystem levels through innovative, integrated sensor approaches, with a focus on urban and highly managed environments. The Table below shows how RFA1 research will inform RFAs 2 and 3.

Links between CAChE subprojects/RFAs	**Subproject/RFA2: Multi-Level Organismal Impacts and Responses**	**Subproject/RFA3: Integrated Ecosystem Assessment**
Subproject/RFA1: Detection Technologies for the Study of Environmental Stressors	• Provide robust chemistry data on contaminants and exposure • Provide data on contaminant impacts on histone proteins • Provide lipid biomarkers • Provide metabolic profiling for exposure	• Detect, quatify and trace contaminants • Provide analytic methods to quantify novel anthropogenic contaminants • Provide in-situ sensors to detect and measure contaminant and climate stressors

RFA 1: Developing Detection Technologies for the Study of Environmental Stressors and Organism Exposure

PROJECT DESCRIPTION

In CREST-CAChE I, RFA1 aimed to assess the presence, distribution and environmental processes controlling anthropogenic and natural stressors in aquatic ecosystems. The team implemented the use of novel technologies focused on alternative and innovative mass spectrometry tools. CREST-CAChE II will expand our ability to provide the high-resolution real-time data needed for meaningful actionable interventions, through the use of tandem orthogonal separation systems (e.g., online-SPE-HRMS, speciation ICP-QqQ-MS, GCxGC-MS, GC-UHRMS and GC/LC-TIMS-UHRMS), development of low-cost in-situ sensing systems for high-value environmental data and sensor network integration.

The **goal of RFA1** is to generate the detection technologies and experimental workflows that can provide a comprehensive characterization of legacy, novel, emerging and soon-to-emerge environmental stressors and use markers of organism exposure to detect their influence. This multidisciplinary team will develop: i) new detection technologies for the characterization of human-derived contaminants (e.g., wastewater source and tracking indicators), new emerging environmental stressors (e.g., poly and perfluoroalkyl substances) and markers for organism exposure at the individual level; and ii) new in-situ sensor technologies for real-time, high-resolution monitoring of high-value environmental data on a wide range of environmental stressors.

The **ultimate goal** of RFA1 is to apply, further develop and transfer these novel technologies to better understand the dynamics and fate of environmental stressors and their impacts on ecosystems, so we can provide the timely data and interpretative tools needed to improve risk assessment and rapid decision-making.

a) Environmental detection: from atomic to molecular to system level

The identity and nature of human-derived environmental contaminants have changed from traditional and legacy pollutants, such as nutrients, trace metals, DDT and PCBs, common pesticides, to other biologically active compounds such as endocrine disruptors, antibiotics and, more recently, a wide range of chemicals broadly classified as emergent chemicals of interest (e.g., PFAS and microplastics). This trend requires de defiance of conventional chemical analysis from specific target molecules to a more diffuse list of non-target analytes. Chemicals such as mercury, arsenic and lead, natural and synthetic estrogens, antibiotics, high use pharmaceuticals and even natural toxins, such as algal blooms, are now recognized as having significant effects – both directly on ecosystems and then indirectly on humans. Human-derived stressors can be both chemically diverse and found in the ecosystem over a wide dynamic range. The use of advanced analytical tool comprising online solid phase extraction (SPE), gas chromatography (GC) and liquid chromatography (LC) in tandem with trapped ion mobility (TIMS) and ultra-high resolution mass spectrometry (FT-ICR MS), has extended our analytical abilities from trace analysis of critical compounds to detailed analyses of metabolites, organism markers of exposure and degradation products. This has enabled a more accurate depiction of the occurrence of emerging contaminants. Automation enables high-throughput analysis of specific wastewater intrusions using recalcitrant tracers, such as sucralose, that combined with traditional water measurements such as optical properties, nutrient and pathogen methods, allow the determination of source specific signatures at low-enough costs for large scale projects.

Subproject a.1: *Metals (Hg) speciation and aquatic cycling.*

Metals, especially Hg, pose severe risks to wildlife in the Florida Everglades.[1] Our goal is to identify the importance, mechanism, and diversity of Hg biogeochemical cycling and bioaccumulation in aquatic environments and the key drivers of Hg transformation and transport, such as dissolved organic matter (DOM), and to elucidate the pathways through which DOM and other drivers influences Hg aquatic chemical processes and bioaccumulation. Our research under the funded CREST I program revealed that DOM and exudates leached from aquatic planktonic assemblages play a critical role in interacting with mercury and subsequently regulating its speciation and transformation. We have used an array of analytical techniques to characterize the DOM percolated from different types of periphyton in the Everglades at the molecular level, and explored its interaction with Hg.[2] The Everglades is abundant in periphyton which could produce and release a significant amount of fresh DOM into the water, resulting in a profound effect on aquatic mercury cycling. We propose here further studies to systematically investigate the role of periphyton DOM in Hg biogeochemical cycling, by analyzing Hg speciation (e.g., Hg-DOM complexes) and elucidate the involvement of these complexed Hg species in Hg transformation including oxidation/reduction and methylation/demethylation.

Subproject a.2: *Dissolved Organic Matter structural characterization using MS based tools.*

Critical environmental and ecological processes are strongly influenced by Dissolved Organic Matter (DOM),[3,4] one of the most studied natural complex mixtures. Thus, a thorough knowledge of DOM chemical composition and structure at the molecular level is essential for the understanding of its role in the aquatic environments. Although the molecular features of DOM has been the focus of a multitude of studies over the last decades,[4-6] the elucidation of its chemical structure and a clear view of DOM isomeric complexity, persist as one of the most challenging analytical problems.[7-10] Analytical approaches integrating ultra-high resolution mass spectrometry, gas/liquid pre-separation techniques and tandem mass spectrometry strategies have provided much of the existing information on the chemical diversity of DOM.[6,11-18] Novel workflows that combine both LC and ion mobility spectrometry (IMS) have been explored to assess the DOM complexity at the level of single isomer.[19-21] With the advent of high-resolution ion mobility analyzers (R>80), several groups have work on their integration to ultra-high resolution mass analyzers. [22-31] Our team has pioneered the integration of trapped IMS (TIMS) with FT-ICR MS since 2015 for the characterization of isomeric species in complex mixtures.[32] The unique advantages of TIMS-FT-ICR MS for the characterization of the isomeric content in complex mixtures has been shown (e.g., endocrine disruptors, glycans, water accommodated fractions of crude oils, DOM, etc.).[33-38] We have recently developed a TIMS-q-FT-ICR MS/MS technology capable of DOM structural elucidation at the nominal mass and at the chemical formula level.[9,39] In this project we will continue to develop high resolution mobility and mass strategies for the DOM structural characterization.

Figure 1. Simplified schematics of the TIMS-FT-ICR MS developed at FIU

Subproject a.3: *Detection of environmentally induced epigenetic changes at the histone level.*

Increasing evidence shows that environmental factors (e.g., arsenic,[40] black carbon,[41] benzene,[42] PAHs,[43-45] and POPs[46]) may influence epigenetic mechanisms, such as DNA methylation, histone modifications and microRNA expression.[47] Initially, all genetic information was believed stored in the DNA sequences and changed via their mutation. While that pathway remains central to evolution, we now know that same sequences can lead to critically distinct gene functions. The pertinent information must then reside epigenetically - beyond DNA sequence.[48] One key mechanism is apparently modification of histone proteins - the spools holding DNA in nucleosomes.[49-52] Biological materials are normally characterized by MS, particularly tandem MS where mass selected ions are broken apart and the stoichiometry of fragments elucidates the precursor structure.[53] The PTM sites in individual proteins (including histones) are most effectively determined by ETD - a direct process that severs peptide bonds while retaining PTM links.[54] Continuous improvement of the MS resolving power and mass accuracy, especially supplied by Orbitrap platforms over the last decade, has drastically enhanced proteomic investigations.[55] Still, most endogenous

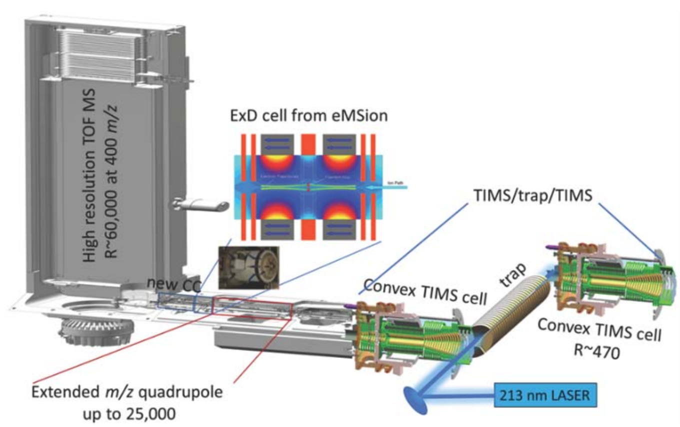

Figure 2. Simplified schematics of the TIMS/UVPD/TIMS-q-ExD/CC-TOF MS

samples including histones are complex enough to call for extensive prior separations, traditionally performed by LC or capillary electrophoresis (CE). Those approaches could resolve the histone forms with distinct total PTM content (e.g., number of ac groups), but not the variants differing in PTM localization.[50,56] A rising alternative or complement to LC or CE is ion mobility spectrometry (IMS), where ion species are separated while driven through gas by an electric field. Since the introduction of Trapped Ion Mobility Spectrometry in 2011,[57,58] the ion dynamics,[59] fundamentals[60-64] and applications for a wide variety of analytes have been explored mainly using TOF-MS analyzers.[65-86] Our project focuses on the integration of multidimensional, post-ionization separations based in IMS and MS/MS. In particular, we are integrating non-linear and linear IMS separations prior to MS/MS analysis. The orthogonality between FAIMS and TIMS separations was demonstrated from independent analyses of variant histone tails using FAIMS and TIMS.[87] The high-resolution TIMS can broadly separate peptide variants with ~50 residues fully or into binary mixtures quantifiable by tandem MS.[87] In addition, non-ergodic fragmentation of intact histones will be implemented by inserting an ExD cell adding a tandem TIMS/UVPD/TIMS stage (Figure 2).

Subproject a.4: *Development of LC-IMS-MS/MS tools for metabolic profiling.*
Environmental biomarker discovery has significantly shifted towards lipid analysis from biological samples due to the role of lipids in many environmental processes. From the analytical standpoint, lipid analysis is challenging due to the complexity and diversity of the lipid structures.[88-93] Lipid characterization to different levels (e.g., lipid class/subclass, length of acyl chains, position of the acyl chains and number double bonds and cis/trans orientation) has been made accessible mostly through mass spectrometric techniques.[94-102] The combination of ion mobility spectrometry (IMS) with mass spectrometry for lipid analysis,[103-119] with or without LC pre-separation, allows for the separation of isomeric content based on differing mobilities.[103,105,108,111,120-130] Whereas LC may be capable of separating lipid classes and isomers, typical LC-MS workflows require tens of minutes while similar results were obtained by direct infusion IMS-MS analyses with IMS separations performed in the millisecond-timescale.[109,131] We have recently shown the advantages of direct analysis at ambient pressure using LESA when combined with ultrahigh resolution mass spectrometry (LESA-FT-ICR MS/MS).[132] As the improved MS peak capacity has ameliorated spectral congestion and thus the need to separate isobars of different stoichiometry, isomer resolution has come to the forefront of lipidomic analysis. Our project focuses on the integration of orthogonal separation techniques based on liquid chromatography, ion mobility spectrometry and tandem mass spectrometry (LC-IMS-MS/MS), in order to increase the analytical throughput and coverage, and to improve the limits of detection of lipid environmental biomarkers. Noteworthy, our workflow utilizes a trapped ion mobility spectrometry, that can provide mobility resolution in excess of 400 (5-10x better than existing commercial instruments), in tandem with high-resolution MS (i.e., TOF MS/MS) and ultra-high-resolution MS (i.e., FT-ICR MS/MS) instruments. In addition, our project includes the utilization of several tandem MS fragmentation strategies for a better lipid assignment and structural coverage (i.e., CID, UVPD, and OzID). Utilizing recently developed statistical and *in silico* library searching engines, the proposed assay will allow the separation, identification and quantification of environmental lipid biomarkers.

Subproject a.5: *Detections of Metabolic Pathways associated with environmental exposure.*
Our research group focuses on the study of metabolic pathways associated with environmental exposure, ranging from naturally occurring biotoxins (e.g., algal toxins, mycotoxins)[133-135] to anthropogenic pollutants (e.g., persistent organic pollutants)[136] using has MS- and NMR-based techniques. A major component of this research has been the development and application of high-resolution magic angle spinning (HRMAS) NMR as a means of metabolic profiling of model animal systems in relation to environmental stressors. When coupled to reference libraries of NMR data for compounds, as well as the considerable structural information afforded by NMR, and to multivariate statistical analyses, this method effectively enables identification and quantitation of metabolites. Quantitative analyses by NMR and complementary MS-based techniques reveal highly reproducible patterns of metabolic organismal alterations in response to xenobiotics that, in turn, facilitate identification of molecular transformations and de-novo structures that serve as biomarkers of exposure and/or effect of these contaminants. Such biomarkers provide a potentially transformative applied tool for not only monitoring exposure of target species to environmental stressors (i.e., ecotoxicology), but also assessment of human exposure to waterborne contaminants as it relates to food (e.g., seafood) and water safety, environmental health and regulatory efforts. When coupled to a suitable model (i.e., organism, cellular) system, it has been shown that HRMAS NMR, particularly when complemented by MS-based techniques, can elucidate metabolic pathways associated with stressors. Our

projects focus on i) fundamental studies of metabolic pathways associated with exposure, and subsequent effects, of toxicants and other relevant environmental stressors at the system-level; and ii) identification of robust biomarkers of pollutants in relation to environmental stressors. We will expand this integrated metabolomic strategy to a number of environmentally relevant target organisms including, in particular, commercially and ecologically relevant species of fish and shellfish and a far broader range of environmental stressors and anthropogenic pollutants.

Subproject a.6: *Detection of Anthropogenic per- and polyfluoroalkyl substances.*
Poly and perfluoroalkyl substances (PFAS), epitomized by perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA), are widespread environmental contaminants used in a range of commercial and consumer products, including surfactants, water repellents, lubricants, adhesives, additives and coatings, and in firefighting foams.[137] Having been manufactured for over 60 years, their ubiquitous presence in the environment is of great concern. The extremely high bonding between carbon and fluorine atoms that makes these perfluoroalkyl substances (PFAS) highly commercially attractive, also enables them to persist in the environment by resisting chemical, microbial, and photolytic degradation, being referred as “forever chemicals”.[138,139] They are, thus, regarded as highly toxic, extraordinarily persistent chemicals with the potential to bioaccumulate and biomagnify through the food chain.[140,141] The ubiquitous presence and high concentrations of PFAS in the environment poses a huge concern to wildlife exposure. Although limited studies are available in Florida, specially South Florida, PFAS have been reported in dolphins inhabiting Florida’s Indian River Lagoon, Saratoga Bay and the coastal waters of Florida including the Gulf of Mexico; in sea turtles found in offshore waters of northeastern Florida, Florida Bay and Cape Canaveral; in alligators from different locations in Florida.[142-144] Our group performs chemical analyses of PFAS to assess the water quality, and the presence, concentrations, and trends of PFAS in South Florida environments, including Miami River and canals and protected areas of the Everglades and Biscayne National Park (Figure 3). The list of target compounds is selected based on their frequent detection in U.S. surface and drinking waters, and are comprised of the most studied PFAS (PFOS, PFOA, PFOSA, PFHxS, PFHxA, PFHpA, PFDS, PFDA, PFDoA, PFNA, PFTA, PFTrDA, and PFUnA) and their replacements (PFBS, GenX and ADONA). A targeted LC-MS/MS approach using authentic and isotope-labeled standards will be used for the determination of a wide range of PFAS, including long-chain legacy and short-chain emerging PFAS. Although analytical methods for targeted PFAS are well defined in the literature, simple and easy-to-use non-targeted or suspect screening methods for unknown PFAS are still underdeveloped14. With the increasing availability of high-resolution mass spectrometry (HRMS) systems, non-targeted analysis (NTA) has emerged as a powerful method for identification of unknown compounds in a range of matrices. A non-targeted analysis (NTA) approach will be established to identify unknown PFAS degradation and transformation products in environmental samples. Our ultimate goal is to provide a comprehensive description of the distribution, levels and fate of PFAS in the South Florida environments.

Figure 3. Map showing selected sampling

b) FIU CRESTcNear Coastal Observatory System

Buoys developed for CREST-CAChE I featured high-tech sensors to collect data on general water quality parameters (temperature, conductivity, dissolved oxygen, pH, turbidity, chlorophyll, directional flow speed). A large success of the CREST buoys is both the density rich data they create and the instant availability of it through a open access web portal. Much of the information gathered from a recent fish kill event in Biscayne Bay was produced from these sensing platforms that yielded critical information on a 24/7 regime.

Many of the processes that may have contributed to the even are related to diurnal cycles that can't be sampled by people, boats and discrete sampling of the water column. For CREST-CAChE II, we will add the capability to monitor two key parameters for which technologies are emerging or could now be integrated to stand-alone observation platforms. The CREST 2 IBIS (Integrated Buoy Information Systems) will add doppler current profilers (ADCP) to calculate magnitude and direction of the underwater flows over the entire water column, a radiometer to assess the light penetration under natural environmental conditions and commercial sensors (modified to allow for enhanced sensitivity) for nutrients responsible for eutrophication and algal blooms, including nitrate and phosphate. Most in-situ technologies are not affordable, sensitive, or reliable enough in salt-water environments, so we plan to work with partners to assess available sensors, improve their performance or produce alternatives. The goal is to provide the data needed to build predictive models for 'early warning' of environmental changes and extreme events. We now know the conditions of large portions of Biscayne Bay that are conducive for hypoxia evens to the extent that decisions on water releases from the managed tributaries are more carefully evaluated before implemented. Before CREST CAChE1 that was not an option.

Subproject b.1: *Development of low cost, miniaturized, and animal-deployable sensing platform.*
Miniaturization trends in electronics and the development of wearable electronics has led to the recruitment of animals (birds, cockroaches, mice, etc.) to help with environmental sensing or mapping. Animals are ideal candidates to investigate the environment that they inhabit and to measure the impact of environmental pollutants on their behavior and health. A low-cost, miniaturized, and animal-deployable sensing platform with environmental and health monitoring sensors would provide a combined data stream of environmental stressors and the effects on the organism. Large scale and dense sensing for existing and emerging pollutants is needed to map the pollutant pathways; traditional fixed-position sensors (such as buoys) provide limited spatial data and fail to simultaneously capture the biological effects of the changing environment. The sensing modalities of active buoys will be integrated into a miniaturized sensing platform for deployment on animals to improve the density and scale of data collection (see Example in Fig 4 of wearable sensor modalities).[145,146] Additional sensors will be incorporated to evaluate the animal's health and activity to capture direct and immediate responses to changing environments and stressors. [147] Optical detection will be the primary sensing modality for the sensing platform, as it allows high sensitivity and sensing from a distance, which is less susceptible to biofouling. Dissolved oxygen detection can be implemented with fluorescence quenching sensing. Surface plasmon resonance (SPR) techniques have been demonstrated to achieve high sensitivity to environmental pollutants[148] and work on miniaturization strategies. Fusion of data streams from environmental sensing and organism response through the proposed sensing platform will provide new insight into the effects of environmental pollutants. The added value of using animals is the ability to immediately correlate environmental stressors to the organism's response with the fusion of data streams. With high quality data from non-stationary sensors, environmental research will be able to identify transport pathways and sources of pollutants.

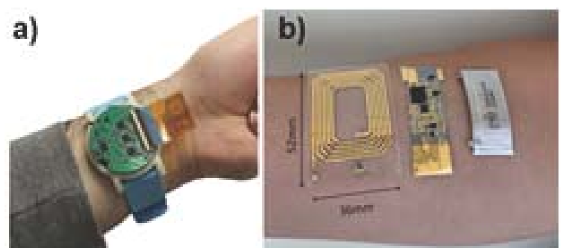

Figure 4: Wearable health monitoring system. a) System with pH, temperature, lactate, and glucose measurements. b) System for dissolved oxygen, temperature, and pulse oximetry measurements.

Subproject b.2: *Development of an autonomous observation platform-"the flying IBIS".*
Continued development and advances in remote and autonomous technology has led to significant advantages for safely and efficiently collecting data across broad regions which can be repeatedly sampled across relevant temporal scales. These platforms range from instrumented stationary floats/buoys that archive records of ambient physicochemical conditions and underwater soundscapes and animal movement patterns to fully autonomous robotic platforms that execute preplanned transects and collect a myriad of environmental, hydrographic, and biological data and telemeter status and data summaries in real-time. In some cases, these autonomous platforms can be configured to compute metrics in real-time to adaptively respond to detected gradients among measured parameters for fine-scale assessment of a particular area. Developing a series of fixed and mobile observation platforms will permit broad temporal

and spatial coverage and serve as an effective 'early alert system' for detecting changes and transmitting that data to managers and end users. At FIU there are several autonomous sensing platforms that will be used to evaluate ecosystem dynamics. In addition to the stationary buoys, we will implement a series of autonomous vehicles that are programmed to collect an array of data types to inform questions on role of environmental and habitat gradients for structuring biological communities. Specifically, we will use the 4m autonomous surface vehicle (ASV) configured with a series of environmental and acoustic sampling instruments to simultaneously integrate physicochemical, biological, and bathymetric data. The main benefits of this remote platform are the ability to autonomously and non-invasively access shallow habitats (~0.5 m); provide real-time display of spatially-referenced on-board data within ~2-km range and accommodate a broad range of instruments. Currently the ASV is outfitted with a suite of underwater acoustic devices (broadband echosounders, imaging sonar, multibeam sonar) to quantify nekton and characterize the substrate, in addition to a top-side camera to provide user with vessel perspective as well as a multiparameter sonde. The ASV can be used as a non-invasive platform with configurable payloads to accommodate diverse science needs and efficiently operate in remote or challenging environments where traditional vessels would be limited. Coupling a series of buoy sensors with the ASV platform we will extend the spatial and temporal resolutions of the physical measurements. The ASV can be equipped with a current profiler (RDI 1200 kHz ADCP), to measure velocity and direction of the current along the water column, with a wide-swath multibeam sonar (Mesotech M3 multimode sonar) for bathymetric data, a multiparametric sonde (YSI EXO3) for physical-chemical variables (salinity, temperature, conductivity, turbidity, dissolved oxygen, fluorescence), and a SeaBird Cycle-P phosphate analyzer to obtain nutrient levels.[149,150] The ASV will be pre-programmed to sample within each waterbody along a series of transects creating a repeatable ground truthing dataset to integrated into the time series from moored station data. Transects will target areas along which exchange of water and suspended materials occurs. The mooring data and the survey data will be then combined for extrapolation of exchange rate of water, salt, and substances. These data will be ultimately used to model the temporal and spatial variability of currents speed, flux measurements and water column stratification and to refine existing near-shore wave models used to predict storm surges and wave inundation. Transects will target areas of the coast along which exchange of water and suspended materials occurs. The mooring data and the survey data will then be combined to extrapolate the exchange rate of water, salt, and substances. These data will be ultimately used to model the temporal and spatial variability of current speed, flux measurements and water column stratification and to refine existing near-shore wave models used to predict storm surges and wave inundation.

BROADER IMPACTS

RFA1 will support the overall broader impacts of CREST-CAChE II. Specifically, students will co-develop research projects with RFA1 faculty and be guided by a near-peer mentoring team of postdocs and senior graduate and undergraduate students. They will develop innovative detection technologies and learn their application to monitoring new and emerging contaminants in aquatic systems. They will also help design the sensors for the CREST near coastal observatory buoy system. In addition, RFA1 research will inform the micro-credentialing workshops offered to graduate and undergraduate students. Through our partnerships network, students will gain the skills to communicate and translate their data into actionable information for decisionmakers and stakeholders

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FIU CREST Phase II: Center for Aquatic Chemistry and Environment (CREST-CAChE)

Subproject 2: Multi-Level Organismal Impacts

Project Summary

Novel environmental stressors, such as microplastics, pharmaceuticals and other emerging contaminants, have caused significant environmental impacts and attracted worldwide attention. *We initiated FIU's CREST Center for Aquatic Chemistry & Environment (CREST-CAChE) in 2016 to meet the need for enhanced research on the detection and impact of toxic substances in the environment,* including pesticides, industrial chemicals, oil, pharmaceuticals, nutrients, and metals. *Our Phase I research focused on the detection, fate and transport, and visualization of the impacts of contaminants on aquatic systems.* In CREST-CAChE II, we will expand our detection capabilities to unknown and novel emerging contaminants, both natural and anthropogenic, as well as expand our ecosystems of study. While Phase I focused primarily on freshwater systems, such as the Everglades, and the near shore ecosystems, CREST-CAChE II will expand our study area to include aquatic systems along an urbanization gradient. By designing novel sensors and autonomous vessels that can explore difficult to reach urban areas, we will collect continuous and near real-time data on emerging contaminants. In Phase II, we will move from a focus on detection and transport of contaminants, to a mechanistic understanding of the impacts of these contaminants at the organismal, community, and population levels. Using an Understanding the Rules of Life (URoL, NSF 10 Big Ideas) conceptual approach, Phase II will unify across multiple scales, as well as integrate campus-wide programs in Chemistry, Biology, Earth Systems, Computer Science, Engineering, and Architecture.

In addition to advancing cutting-edge research, we will conduct a rigorous education program that builds from lessons learned in CREST-CAChE I. During the initial years of the CREST program, we discovered that students sought the skills to work collaboratively, especially for communicating across disciplines, and for using new emerging technologies. We also learned that undergraduates sought authentic research experiences beyond the Discovery courses that we developed, so will add an undergraduate research-fellows program to Phase II. Our formal and informal education programs will prepare students to communicate the results of their research and for the future STEM workforce. We will include training in core professional skills, as well as micro-credentials in tools necessary to understand complex environmental problems, including robotics, next generation sensor development, big-data analytics, virtual reality, and artificial intelligence.

Intellectual Merit

CREST-CAChE II will increase participation of underrepresented groups in STEM while advancing cutting edge research in environmental chemistry, detection methodologies, molecular mechanisms and producing actionable science with government and private sector partners. Phase II will generate significant new knowledge regarding contaminants in aquatic environments, as well as produce innovative new methodologies for detecting unknown and emerging contaminants and their impacts in urban aquatic ecosystems. *In CREST-CAChE II, we will expand our studies to look at the mechanisms leading to impacts of contaminants at the individual, community, population, and ecosystem levels.*

Broader Impacts

CREST-CAChE II will have three main components in its broader impacts program. a) We will recruit and retain underrepresented students in STEM using our near-peer team mentoring approach. We will build on the success of FIU's evidence-based transformation of STEM instructional practices to provide enhanced support for underrepresented students to successfully pursue STEM careers. b) We will provide students the core professional skills and micro-credentials needed to succeed in the workforce of the future. c) We will develop new technologies for improving water quality analysis and contaminant detection in a range of ecosystems, including freshwater, coastal, and urban environments, as well as provide data on the impacts of these contaminants on ecosystem function. Through partnerships with decisionmakers and stakeholders, we will translate this data into actionable information for designing water quality solutions locally and globally.

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RFA 2: Multi-Level Organismal Impacts

SUBPROJECT RELEVANCY STATEMENT
Understanding coastal urban and urbanizing ecosystems requires the ability to measure novel, emerging contaminants, their impacts on cellular, metabolomic, and genomic impacts on organisms and how those impacts structure populations, communities and ecosystem.[1,2,3] CREST-CAChE I focused on improving our understanding of pollutant source, transport, and effects across a range of diverse ecosystems and human populations [e.g.,[4–7]]. We developed new technologies in the fields of environmental chemistry and biology to characterize different types of stressors (both biotic and abiotic). We also discovered that many responses to environmental stressors actually occur at the physiological, behavioral and epigenetic level of individual organisms. This finding underscores the importance of the mechanisms underlying phenotypic responses to the genome. We have just started to combine the study of traditional ecological and toxicological endpoints with the development of molecular "omic" studies,[8–10] including genomics, epigenomics and metabolomics. Along with the characterization of traditional endpoints (e.g., survival, growth, etc.), the resulting models will be much more powerful to assess not just impacts (from subtle to lethal), but also to understand the mechanisms linking the effects of stressors to multi-level organismal responses, and their consequences on ecosystem function.

In CREST-CAChE II, we will build a multi-level organismal impact framework, based on the integrative study from molecules to organisms to ecosystems within urban marine seascapes. RFA1 will continue to build unique analytic tools, with a focus on identifying and quantifying new emerging contaminants. ***In RFA2, we will investigate multi-level organismal impacts and responses to the contaminants identified in RFA1, to determine the molecular mechanistic basis of responses****.* Therefore, new innovative methods are incorporated including: genomics (genetic diversity), epigenomics (gene regulation capacity), transcriptomics (gene expression), and metabolomics (molecules involved in physiological responses). Collectively, these "omic" analyses comprise a very powerful toolkit to detect even the slightest impact of stressors on populations. Yet, in order to fully understand such effects, we must gather accurate information about the totality of exposures in organisms, simultaneously identifying, characterizing, and quantifying the exogenous and endogenous exposures and modifiable risk factors that predispose to and predict phenotypic responses throughout their life span. In other words, this proposal aims to combine molecular "omic" approaches with the study of the exposome.[11,12] With the aim of better understanding the causal links among the genome, the environment, and phenotypic responses, unraveling the exposome allows us to reliably and simultaneously measure both environmental exposures and genetic variation. The Table below shows how RFA2 research will inform RFAs 1 and 3.

Links between CAChE subprojects/RFAs	**Subproject/RFA1: Detection Technologies for the Study of Environmental Stressors**	**Subproject/RFA3: Integrated Ecosystem Assessment**
Subproject/RFA2: Multi-Level Organismal Impacts and Responses	• Prioritize epigenetic and metabolic markers for further analyses based on multi-level organismal responses • Refine species-specific detection methodologies • Refine development of tools needed to test specific contaminant and stressor impacts at the organism scale	• Predict population-level responses for assessment based on organismal responses • Identify species most vulnerable to contaminant impacts for community and ecosystem services assessment • Identify interactions between climate stressors and organismal response capacity for ecoystem models

RFA 2: Multi-Level Organismal Impacts

PROJECT DESCRIPTION

For this purpose, we will **expand research developed in the previous CREST by leveraging on resources developed within FIU's CREST-CAChE Center** (e.g., Nutrient laboratory, Genetic facilities, Bioinformatic resources, etc.) as well as on additional funding awarded to CREST participants including: NSF's Rules of Life, Division of Integrative Organismal Systems, Division of Environmental Biology, and state and local grants, increasing the scope of the project and providing additional opportunities for undergraduates, graduate students and postdoctoral scholars.

a) Measuring genomic diversity and connectivity to investigate health and resilience from populations to communities

Rationale. Genetic diversity is a key attribute of populations because it provides the foundation for evolutionary potential, including the ability to adapt to changing environmental pressures. **This evolutionary potential is particularly relevant for systems exposed to natural and anthropogenic threats. Here, we propose to measure genetic diversity across diverse species to infer the health and recovery potential of Biscayne Bay and surrounding regions**. This parameter is intimately tied to an organism's ability to survive, with higher levels of genetic diversity often leading to higher survivorship and low genetic diversity resulting in severe consequences within populations including reduced biological fitness.[13] Genetic diversity can be reduced by rapid declines in population sizes (e.g., due to anthropogenic events) resulting in genetic bottlenecks, with further erosion caused by increased inbreeding within the smaller populations and subsequent impacts of genetic drift. Low genetic diversity can reduce individual fitness and viability of populations by increasing extinction risks and reducing recovery rates.[13] As such, assessing genetic diversity is a critical measurement in evaluating the health of the community.

Genetic connectivity is an equally important metric to consider alongside genetic diversity, and can be defined as the movement of genes within or between systems.[14] Understanding how genetic diversity is shared and exchanged within and across an ecosystem informs the recovery and resilience of populations to communities. Population connectivity can be characterized as inter-population gene flow or migration, or the historical demography of populations, such as recent separation or re-mixing of distinct populations and/or changes to population size.[14] The ecological implications of these population dynamics are crucial to species resilience: following a localized perturbation event, gene flow between geographically separated populations can provide a functional genetic reservoir outside the disturbed area.[15] Data collected from this project will allow us to determine the ability for local populations to be "genetically rescued" from neighboring localities. **Collectively, assessing genetic diversity and connectivity are fundamental first steps that can inform management and conservation efforts and will be developed as part of this proposal.**

Subproject a.1: *Evaluating population to community health using measurements of genetic diversity across local and regional scales*. The goal of this subproject is to establish current baselines of genetic diversity in Biscayne Bay and surrounding waters for species that play a critical role in the health of our systems, from seagrass to crustaceans and fish. Using this approach, we will be able to characterize short and long-term changes in genetic diversity for indicator species. This diversity is particularly relevant for the management and health of Biscayne Bay as the system suffers from increased nutrient loading, metal and antibiotic contamination, hypoxia, among other anthropogenic threats. Investigation into genetic diversity will also allow for the identification of new and cryptic species, which can have economic and ecological implications. In northern Biscayne Bay, a recent example of hidden diversity was found within the economically important pink shrimp genus, *Farfantepenaeus*. This group represents one of the most profitable fisheries in the United States, bringing in $213 million dollars in 2015.[16] In this study, cryptic diversity was found within the species, *F. brasiliensis*, with enough genetic variation to suggest a potentially new species within Biscayne Bay. The discovery of a new species has huge implications for management and conservation across the distributional range,[16] especially in Brazil where this species supports the pink shrimp fishery.[17] In this subproject, pink shrimp populations, along with other species (from seagrass to fish) will be subsampled from Oleta River to Coral Gables, to assess genetic diversity measurements of populations from across this range. Localities across this region will be sampled quarterly over a five-year period to account for seasonal changes in genetic diversity. Data collected as part of this project will aid in baselines estimates and launch a long-term monitoring in the face of future threats. This will allow our team to infer if estimates of genetic diversity have signatures of recent declines due to massive hypoxia events and seagrass die off. Life history characters will be considered alongside genetic diversity estimates to infer how development, behavior, dispersal ability might contribute to genetic diversity over time.

Subproject a.2: *Investigation of genetic connectivity across indicator species to evaluate how diversity is structured across environmental gradients and the potential impact of future threats.*
In this subproject we will investigate the genetic connectivity of indicator species (same targeted species from a.1) to determine which species are under the greatest threat of continued anthropogenic disturbances. For example, information collected as part of this subproject will help identify how genetic diversity is distributed across Biscayne Bay and how this diversity is connected to the larger Atlantic Ocean. This will allow us to answer if the Bay is a genetically opened or closed system, and if targeted species have different patterns of isolation. This information, combined with genetic diversity estimates, can be very powerful indicators as to which populations are under the greatest threat to anthropogenic disturbances. Sampling extensively within the species' distributional range of target species will allow us to identify the source and sink origins of diversity and how genetic linkages are made across regions. Lastly, the exchange of individuals among populations might introduce foreign alleles that can spread adaptive change. This process is particularly relevant in the context of large-scale disturbances such as the recent fish kill or constant influx of pollutants. There may be positive selection for alleles conferring resistance to pollutants, which can then be shared with other populations, increasing their resilience to future events.

Experimental Approach. Taxon Sampling: To estimate levels of genetic diversity and connectivity, we will sample at least 30 individuals per population. Targeted species include seagrass species such as the Turtle grass *Thalassia testudinum*, the mangrove flattree oyster *Isognomon alatus*, the pink shrimps, *Farfantepenaeus* spp., the mangrove crab *Aratus pisonii*, the mud crab *Eurypanopeus depressus,* and the bicolor damselfish *Stegastes partitus*, that is an abundant reef fish that is commonly used for genetic and connectivity work.[18,19] Localities from within Biscayne Bay, from the Oleta River to Coral Gables, will be sampled quarterly each year for the duration of the project. Metadata related to environmental and ecological conditions (temperature, pH, oxygen, light attenuation, nutrients, contaminants) will be monitored continuously for the sites.

Experimental Analysis: Double digest Restriction-site Associated DNA sequencing (ddRADseq) is the current gold-standard to estimate genomic diversity and connectivity by identifying large sets of genome-spanning genetic markers. This method's reproducibility allows for comparable reduced representations of genomes to be generated across many individuals within a species, while simultaneously reducing sequencing costs. Perhaps most importantly, this method can be applied to non-model organisms that lack annotated genomes, many of which will be targeted for this work. DNA extraction and library preparation will follow the protocol as described in.[20,21] Quality filtering and data assembly will be done using STACKS v1.45[22]on the FIU High Performance Computing Cluster (HPCC). Genetic diversity indices will be calculated in GENODIVE v2.0b23,[23] including: observed heterozygosity (Ho), the inbreeding coefficient (Gis), and expected heterozygosity (He, which was calculated from the Ho and Gis values). To estimate historical population size for each species, we will execute Extended Bayesian Skyline Plot (EBSP) analyses in BEAST2,[24] Genetic distances due to population differentiation (F_{ST}) will be calculated in GenoDive v2.0b23. Genetic variance within and between regions will be assessed through Analyses of Molecular Variance (AMOVA).[23] To test for population structure, *K*-means clustering will be conducted in the Bayesian program STRUCTURE v2.3.4.[25] STRUCTURE results will be collated in STRUCTURE HARVESTER v0.6.94[26] and final distruct plots will be generated and edited using STRUCTURE PLOT v2.0.[26,27] Non-model-based methods for inferring and visualizing population structure will also be used: multi-dimensional scaling (MDS) plots using the R package MASS.[28] Finally, we will utilize affinity propagation[29] to infer the number of clusters found within each data set.

Expected Outcomes: This project will allow us to monitor genetic diversity over spatial and temporal scales and assess how that diversity is connected within Biscayne Bay and adjacent waters. The metrics of genetic diversity and connectivity will inform the health and potential for recovery and resilience across ecologically and economically important species. It is hypothesized that we will see evidence of genetic bottlenecks over the next few years due to the increasing anthropogenic threats and recent seagrass die-offs. It is also hypothesized that species with different life histories (i.e. dispersal) and behaviors will exhibit varied patterns and levels of connectivity within and outside of the bay. With this knowledge species-to-ecosystem management and conservation efforts can be applied across the system.

b) Environmental epigenetics: Characterizing environmental-epigenetic-genetic linkages promoting adapted phenotypes.

Rationale. The diversity of living organisms mirrors their gene and genomic diversity. However, such diversity will not be possible without a "sensory" mechanism by which environmental changes can reach the information on the DNA. Epigenetic modifications constitute such a mechanism, altering the way in which genetic instructions are interpreted without altering the DNA code itself.[30] By doing so, **epigenetic regulation provides a basis for acclimatory and evolutionary adaptive responses.** Formally, epigenetics is defined as "the study of molecules and mechanisms that can perpetuate alternative gene activity states in the context of the same DNA sequence".[31] In combination with traditional ecological approaches, **epigenetics provides a framework for understanding the enigmatic crosstalk between heterogeneous environmental signals and genome function, generating phenotypic plasticity**. While these modifications to organismal phenotype or function can be reversible, some of them may be inherited by offspring, potentially producing multiple, heritable outcomes from a single genome and affecting ecological and evolutionary outcomes.[30,32]

Characterizing epigenetic triggers, mechanisms, and their role in organismal acclimatization and adaptation is therefore critical to understanding ecosystem function. **However, given the early onset and dynamic nature of epigenetic modifications, these marks constitute increasingly used tools to assess the impact of environmental stressors on diverse ecosystems.** In the present project, we aim to expand the work initiated in our previous CREST grant (i.e., epigenetic characterization in model native organisms of south Florida) by incorporating an integrative focus including links with genomic and ecological studies improving our understanding of the interconnections between environmental fluctuation and the subsequent chances in genetic, and epigenetic states. **By determining how these molecular changes drive physiological responses, this project will facilitate predicting how population and community dynamics are influenced by changes in the environment through epigenetically-modulated phenotypes.** This work will advance biological knowledge by delineating fundamental links (Rules of Life) between ubiquitous organismal energetic processes, epigenetics, and eco-evolutionary outcomes encompassing applications for ecosystem assessment.

Subproject b.1: *Characterization of epigenetic determinants of phenotypic plasticity in response to global change stressors.* In the particular case of marine ecosystems, global change impact is best illustrated by alterations in "cradles of diversity" such as coral reefs. Indeed, there is a growing literature describing changes at different levels, from molecules to ecosystems, regarding key species ranging from reef-building corals to reef fish [e.g.[33–35]]. For that reason, the present objective will focus on the use of coral reefs ecosystems as model systems, including organisms across different taxa and with different lifestyles such as reef-building corals and echinoderms.

<u>Approach:</u> Coral model: Corals display high levels of plasticity in response to environmental variation. However, the extent to which epigenetic modifications facilitate coral phenotypic responses is still yet to be determined. The present work leverages ongoing experiments funded by RAPID and Rules of Life awards to characterize epigenetic responses in corals subject to different stressors, notably nutrient pollution. The present work will use the samples collected during these projects to develop epigenetic and demographic analyses providing insight into the temporal dynamics of epigenetic modifications in response to changes in environmental conditions. For that purpose, samples from coral nurseries before and after reciprocal transplants will be analyzed for DNA methylation patterns.

Echinoderm model: Among reef organisms, sea urchins provide a broader insight into how epigenetic mechanisms govern responses in more complex and vagile organisms (i.e., as opposed to corals). The ecological role of these herbivores on coral reefs is critical, clearing space and facilitating the settlement of new coral recruits by feeding on macroalgae.[36,37] This is best exemplified by the sea urchin *Diadema* *antillarum* which prevents macroalgal overgrowth in threatened Caribbean coral reefs, and disease-induced reductions in their densities has fundamentally altered the ecology of Caribbean reefs.[38] The present work builds on the strong collaborations between FIU and the Univ. of Puerto Rico, and current fieldwork developed by a NSF postdoctoral fellow at FIU, to investigate the role of epigenetic mechanisms as "sensors" able to translate environmental signals into regulatory modifications of genome function and modulating phenotypic plasticity under different thermal conditions. For that purpose, sea urchin samples will be collected at four different locations displaying heterogeneous thermal regimes along Biscayne Bay and subsequently analyzed for epigenetic modifications and phenotypic responses.

This proposal will use coral and sea urchin samples collected at small-scale nurseries set at four reef locations differing in temperature and nutrient concentrations **as part of our ongoing research on Biscayne Bay and the upper Florida Keys, as well as on the island of Culebra (Puerto Rico)**. Coral samples collected after a 5-month growing period at each site (n=40 per site, 4 different genotypes), followed by a reciprocal transplant for additional 5 months, will be analyzed for epigenetic modifications. The same approach will be used in the case of sea urchins (*D. antillarum*, n=50 per site). In all cases, a modified MSAP protocol[39] will be used to generate DNA methylation epigenetic restriction enzyme profiles that will be analyzed with GeneMapper (Applied Biosystems) and the R package, *msap.*[40] Principal coordinate analysis and an analysis of molecular variance will be used to test for genetic and epigenetic variation between sites.

Expected Outcomes: This Objective will provide critical information linking environmental change to phenotypic plasticity through the knowledge of the epigenetic mechanisms regulating such responses. For that purpose, epigenetic results will be compared with phenotypic performance in each case (demographic performance, coral symbiont densities, urchin righting responses). We will take advantage of recent analytical tools developed at FIU CREST to study epigenetic changes by measuring post-translational modifications (PTMs) at the histone level. The histone analysis will provide a comprehensive characterization of the type and position of the PTM at the single organism level and can be complemented with state-of-the-art metabolomics (e.g. lipidomics) and proteomics studies. Overall, the knowledge of the epigenetic underpinnings of environmental responses will facilitate the assessment of coral reef ecosystems, providing a framework for management and conservation efforts.

Subproject b.2: *Application of environmental epigenetic tools for population profiling and assessment.* Epigenetic modifications contribute to the rapid onset of acclimatized phenotypes under global change.[41] In addition to acclimatization under persistent exposures to environmental stressors, organisms also have a capacity for "environmental memory" where enhanced stress tolerance of an individual is maintained either after a return to non-stressful conditions or upon a new or more intense stress exposure. Given the rapid pace of global climate change and its critical impact on marine environments, the characterization of the role played by epigenetic mechanisms during acclimatization, adaptation and stress memory will help develop better population assessment and management strategies.[42]

Approach: *Coral model:* Anthropogenic nutrient pollution constitutes one the major drivers of coral decline, causing bleaching, disease, reduced growth rates, and impaired reproduction.[8] Overall, the effects of nutrient pollution will work synergistically with other stressors (particularly thermal stress) increasing coral mortality.[43] Although the potential ways in which nutrient and thermal stress can affect corals are well studied, the identity and the precise role of the epigenetic mechanisms linked to acclimatory and adaptive responses to these stressors remain unknown. In order to fill that gap, the present project will leverage on ongoing projects in the Florida Keys and Puerto Rico to perform coral exposures to different nutrient stress regimes, analyzing subsequent epigenetic changes and their role driving phenotypic responses.

Marine mammal model: As apex and long-lived marine predators, marine mammals can bioaccumulate significant levels of contaminants in their tissues, including organic and inorganic compounds.[44] Toxic contaminants such as persistent organic pollutants (POPs) or heavy metals (e.g. mercury) are known to affect the immune and endocrine systems of marine organisms, particularly marine mammals.[45,46] Bottlenose dolphins (*Tursiops truncatus*) are one of the most abundant predators in the coastal waters of South Florida, including in the Florida coastal Everglades[47] and in Biscayne Bay[48]. In Biscayne Bay, monitoring of the abundance, home range, and social structure of bottlenose dolphins since 1994 has shown that two social clusters occur: one in northern and the other in southern Biscayne Bay, and movements between these two areas are limited.[48] Previous work has also shown some significant spatial variations in POP levels in coastal bottlenose dolphins for which sighting histories were known, with individuals from northern Biscayne Bay exhibiting higher POP levels than their counterparts from southern Biscayne Bay.[48]

Similar to the previous objective, coral samples will be exposed to nutrients using a common garden experiment, collecting samples at small-scale nurseries set at four reef locations differing in temperature and nutrient concentrations as part of our ongoing research on the Florida Keys and on the island of Culebra (Puerto Rico). Two types of epigenetic mechanisms will be studied, including histone modifications [histone H2A.X phosphorylation also known as gamma-H2A.X, a histone modification involved in DNA repair and[39] a universal marker of DNA damage[49] and DNA methylation using a modified MSAP protocol.[39] In the case of marine mammals, a long-term dataset on the individual movements, home range, and distribution patterns of bottlenose dolphins in Biscayne Bay (since 1994) will be combined with existing data on mercury (Hg), POPs levels[48] and stable isotopes (N and C) in bottlenose dolphins from Biscayne Bay We will investigate how the spatial variation in POPs, Hg concentrations, and relative trophic level and foraging habitats (inferred from stable carbon and nitrogen isotopes, respectively) impact the epigenome of these organisms using MSAP and pyrosequencing approaches.

<u>Expected Outcomes</u>*:* It is hypothesized that nutrient enrichment will accelerate the growth of the symbiont population within the holobiont, resulting in a higher production of ROS which will in turn cause DNA damage, triggering an increase in gamma-H2A.X (associated to DNA repair activation) and changes in DNA methylation. It is also hypothesized that gamma-H2A.X formation will be impaired in corals exposed only to N enrichment due to the P limitation caused by proliferation of symbionts in the absence of a P supply.

c) Physiological and metabolic responses: Phenotypic and eco-evolutionary consequences of environmental-energetic-(epi)genetic linkages.

Rationale. The range of stressors affecting tropical marine habitats (e.g. coral reefs, mangroves, and seagrass beds) include global climate change, novel pathogens, overfishing, and coastal development (e.g.[50]). The threats from many of these stressors have been studied in detail, with a large literature documenting, for example, the impacts of coral bleaching,[51] coral disease,[52] loss of fishes,[53] and destruction of mangroves.[54] **However, the direct impacts of many changes in water quality are poorly understood for many taxa, but are likely to be critically important.** For example, building on studies that highlight the impact of terrigenous sediment on corals,[55] there is now an increasing recognition that sediment can also impact fish habitat choice, foraging, predator avoidance, and larval development of fishes.[34,56–58] However, this work is still limited to a few species in a few locations, and to a few potential pollutants. **There are significant gaps in our knowledge regarding how many of the pollutants studied by NSF's CREST-CAChE center at FIU and RFA1 of this proposal affect even the most common species in tropical seascapes.** Furthermore, there are few studies that examine the synergistic effects of these pollutants with other threats to marine ecosystems, despite these synergies being potentially critical for ecosystem functioning.[59]

Organismal responses to environmental change can be quantified at many different levels.[9,60] **This proposed range of projects aims to build on the wealth of results produced during our CREST-CAChE project in order to bridge molecular signatures of environmental change with organismal phenotypic responses.** These responses can be varied and multifaceted, but we will focus on two: behavioral and physiological. Accordingly, this project will incorporate a strong physiological component, studying metabolic responses across different model organisms. Since increases in metabolic rates must be met with increased energy intake or a reduction in growth or reproductive output, stressors such as temperature and turbidity have the potential to cause changes in metabolic rates or require behavioral compensation (e.g. reduced activity) to prevent these changes.[61] A **key hypothesis in this proposal is that a variety of stressors cause alterations in standard and/or routine metabolic rates, maximum metabolic rates, and aerobic scope, leading to predictions that these impacts significantly affect the fitness of organisms.** This project will also consider organismal responses through complex and often overlooked behavioral responses. For instance, we know little about how tropical organisms change their behavior when affected by environmental stress. Clearly behavioral and metabolic changes do not occur in isolation, and both abiotic (e.g., turbidity, temperature) as well as biotic (e.g., predation risk, invasive species) can drive myriad changes in behavior, metabolic stress responses, and potentially change population demographics. This project will also tie these responses to epigenetics, and investigate how stressors are responsible for changes in the epigenetic regulation of DNA function.[62]

Subproject c.1. *Reef-fish species.* In this project we will examine the effects of decreasing water quality on a range of small-bodied reef fishes that are common in south Florida and amenable to laboratory studies. These species will include the bicolor damselfish, *Stegastes partitus*, the bluehead wrasse, *Thalassoma bifasciatum*, and juvenile striped parrotfish, *Scarus iseri*. All of these species are relatively well studied, easy to catch, and can be kept in modestly sized aquaria. Furthermore, these species represent a range of key trophic categories on reefs: planktivore, invertivore, and herbivore. These species will provide us with a range of behavioral metrics, and insights that can potentially be extended to a wide range of reef species. In addition, we will also investigate the behavioral and metabolic responses of the regal demoiselle, *Neopomacentrus cyanomos*. The regal demoiselle is a relatively recent invasive species that has established in the Gulf of Mexico, and is spreading towards south Florida.[63] Likely introduced by the movement of offshore petroleum platforms, the regal demoiselle represents a potential new threat to native species, but little is known about its biology.

<u>Objective 1. The effect of pollutants on fish behavior and physiology</u>. The first project component will investigate the effect of existing and emerging pollutants, as identified in NSF's CREST-CAChE center at FIU and through RFA1, on reef fishes. The effects of pollutants on the early stage development of reef fish will be evaluated by isolating embryos/larvae of target species in microfluidic devices designed to precisely control the exposure of a single embryo to prescribed environmental conditions (temperature, pH, salinity, turbidity, pollutant concentration/composition (micro/nanoplastics, metals etc.) and raise them through the larval stage of 14 days post hatch (dph). Sensors embedded in the chamber can measure O2 and TAN outputs in real time as critical indicators of metabolic rates. Since many species are transparent at these early larval stages, this data can be correlated with continuous visual measurement of organ and morphological development and uptake of particulate pollutants. The described microfluidic platform is currently being developed to examine the effects of nanoplastic exposure on mahi-mahi in an NSF funded collaboration with the University of Rhode Island. A key advantage of the microfluidic system is the precise control of the environmental which enables quantitative measurement and comparative studies of synergistic effects of multiple stressors

In addition, we illustrate an approach that can be used for any pollutant by considering increased turbidity, which is an important issue on the reefs of south Florida because of land use changes and dredging.[64] Fishes from each target species (wrasse, damselfish, parrotfish, demoiselle) will be collected and then acclimated in aquaria. Individual fish will then be randomly assigned to treatments (seawater, low, medium or high turbidity). Turbidity will be controlled by varying the amount of commercially available clay, following the protocol of [34]. After further acclimation, feeding rates and distance from protective shelter will be assessed during feeding periods. These observations will provide the phenotypic signal of environmental change. Key physiological metrics (e.g. resting metabolic rate, maximum metabolic rate, aerobic scope, and critical oxygen level) will be measured using standard respirometry techniques.[65,66]

To examine the synergistic effects of different pollutants, treatments of fish exposure (to, for example, increased turbidity) will be crossed with low dissolved oxygen (representing eutrophic conditions that have recently occurred in Biscayne Bay), elevated temperatures (representing the potential impacts of climate change), predation risk (adding the odor of a predatory fish), and the availability of habitat structure. Elevated temperatures are known to have significant impacts on many reef fishes,[67] but the synergistic effects with pollutants are poorly known. Similarly, there is an increased predation risk for many small-bodied reef fishes because of meso-predator release following overfishing of large-bodied predators[68] and introduction of invasive predatory species, such as lionfish,[68,69] but how this risk is mediated by pollutants is virtually unstudied. Finally, many coral reefs are becoming flatter because of loss of coral structure and affecting fishes by removing refuges from predators,[70] which may exacerbate the negative impacts of pollutants. Following each experiment, the epigenetic signal of the stressor(s) will be investigated by

quantifying histone modifications and DNA methylation in different fish tissues, thus integrating this project component with Section (b).
Expected Outcomes. This project will provide new insights into which species towards the base of tropical marine food webs are most affected by a range of acute and chronic water quality metrics, and if this will be made worse by climate change, overfishing, and loss of habitat complexity. The connection between stressors, behavior, physiology, and epigenetic effects has not previously been studied in reef fishes, and represents potentially exciting new insights into how decreasing water quality will impact fishes through multiple pathways. Furthermore, students working on these projects will receive a uniquely broad training in reef ecology, fish husbandry, physiology, fish behavior, and epigenetics.

Subproject c.2. *The effect of pollutants on energy budgets.* This project will extend the work done in Objective 1 for one or two model species and examine how pollutants such as turbidity affect the energy budget of small-bodied reef fishes.

Expected Outcomes. Defining energy budgets for a species provides a powerful tool to examine the impacts of pollutant stressors, but is rarely done for reef fishes. This project will provide one of the first energy budgets for a reef fish and provide new insights into how pollutants will impact key demographic processes such as somatic growth and reproductive potential. Students involved in this work will receive broad training in ecology and physiology, and will lead high-profile scientific papers.

Subproject c.3. *Influence of disturbance and habitat modifications on the behavior of coastal bottlenose dolphins.* Investigating the influence of habitat variability on the distribution and abundance of mobile and elusive coastal marine predators such as coastal cetaceans can be challenging. As a result, the synergetic influence of abiotic conditions (e.g. turbidity, temperature, salinity), prey dynamics, and disturbances due to vessel traffic and habitat modifications are poorly understood.[71–73] The risk disturbance hypothesis suggests that animals respond to human disturbance in a similar way they reduce predation risk,[74] and can therefore result in tradeoff foraging opportunities for safer habitats. Coastal bottlenose dolphins in Biscayne Bay experience a low level of natural predation, but disturbance from vessel traffic is very high and could have significant effects on their movement and foraging decisions.[75] Here, we propose to investigate fine-scale distribution, habitat selection, and the foraging behavior of coastal bottlenose dolphins in Biscayne Bay, where this species has been monitored since 1994. In Biscayne Bay, vessel traffic varies significantly, both in space and time. However, prey availability and abiotic conditions do not vary significantly temporally, which provides an opportunity to test the influence of vessel traffic on the movement and foraging decisions of dolphins. We hypothesize that in areas with intense vessel traffic or when vessel traffic is high, bottlenose dolphins will avoid risky habitats, and will tradeoff foraging opportunities to avoid disturbance and reduce risk of boat collision.

Expected Outcomes. This project will identify the factors affecting the movement and foraging decisions of bottlenose dolphins in a highly disturbed subtropical lagoon, and assess whether marine predators such as coastal bottlenose dolphins experience tradeoffs in foraging opportunities to reduce risk from vessel traffic disturbance.

BROADER IMPACTS
RFA2 will support the overall broader impacts of CREST-CAChE II. Specifically, students will co-develop research projects with RFA2 faculty and be guided by a near-peer mentoring team of postdocs and senior graduate and undergraduate students. They will learn innovative ‘omics technologies, including genomics (genetic diversity), epigenomics (gene regulation capacity), transcriptomics (gene expression), and metabolomics (molecules involved in physiological responses). They will also learn methods for measuring behavioral responses to emerging contaminants and climate change. In addition, RFA2 research will inform the micro-credentialing workshops offered to graduate and undergraduate students. Through our partnerships network, students will gain the skills to communicate and translate their data into actionable information for decisionmakers and stakeholders.

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FIU CREST Phase II: Center for Aquatic Chemistry and Environment (CREST-CAChE)

Subproject 3: Innovating Integrated Ecosystem Assessment in an Urbanizing Coastal Environment

Project Summary

Novel environmental stressors, such as microplastics, pharmaceuticals and other emerging contaminants, have caused significant environmental impacts and attracted worldwide attention. *We initiated FIU's CREST Center for Aquatic Chemistry & Environment (CREST-CAChE) in 2016 to meet the need for enhanced research on the detection and impact of toxic substances in the environment,* including pesticides, industrial chemicals, oil, pharmaceuticals, nutrients, and metals. *Our Phase I research focused on the detection, fate and transport, and visualization of the impacts of contaminants on aquatic systems.* In CREST-CAChE II, we will expand our detection capabilities to unknown and novel emerging contaminants, both natural and anthropogenic, as well as expand our ecosystems of study. While Phase I focused primarily on freshwater systems, such as the Everglades, and the near shore ecosystems, CREST-CAChE II will expand our study area to include aquatic systems along an urbanization gradient. By designing novel sensors and autonomous vessels that can explore difficult to reach urban areas, we will collect continuous and near real-time data on emerging contaminants. In Phase II, we will move from a focus on detection and transport of contaminants, to a mechanistic understanding of the impacts of these contaminants at the organismal, community, and population levels. Using an Understanding the Rules of Life (URoL, NSF 10 Big Ideas) conceptual approach, Phase II will unify across multiple scales, as well as integrate campus-wide programs in Chemistry, Biology, Earth Systems, Computer Science, Engineering, and Architecture.

In addition to advancing cutting-edge research, we will conduct a rigorous education program that builds from lessons learned in CREST-CAChE I. During the initial years of the CREST program, we discovered that students sought the skills to work collaboratively, especially for communicating across disciplines, and for using new emerging technologies. We also learned that undergraduates sought authentic research experiences beyond the Discovery courses that we developed, so will add an undergraduate research-fellows program to Phase II. Our formal and informal education programs will prepare students to communicate the results of their research and for the future STEM workforce. We will include training in core professional skills, as well as micro-credentials in tools necessary to understand complex environmental problems, including robotics, next generation sensor development, big-data analytics, virtual reality, and artificial intelligence.

Intellectual Merit

CREST-CAChE II will increase participation of underrepresented groups in STEM while advancing cutting edge research in environmental chemistry, detection methodologies, molecular mechanisms and producing actionable science with government and private sector partners. Phase II will generate significant new knowledge regarding contaminants in aquatic environments, as well as produce innovative new methodologies for detecting unknown and emerging contaminants and their impacts in urban aquatic ecosystems. *In CREST-CAChE II, we will expand our studies to look at the mechanisms leading to impacts of contaminants at the individual, community, population, and ecosystem levels.*

Broader Impacts

CREST-CAChE II will have three main components in its broader impacts program. a) We will recruit and retain underrepresented students in STEM using our near-peer team mentoring approach. We will build on the success of FIU's evidence-based transformation of STEM instructional practices to provide enhanced support for underrepresented students to successfully pursue STEM careers. b) We will provide students the core professional skills and micro-credentials needed to succeed in the workforce of the future. c) We will develop new technologies for improving water quality analysis and contaminant detection in a range of ecosystems, including freshwater, coastal, and urban environments, as well as provide data on the impacts of these contaminants on ecosystem function. Through partnerships with decisionmakers and stakeholders, we will translate this data into actionable information for designing water quality solutions locally and globally.

Investigator Team
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RFA 3: Innovating Integrated Ecosystem Assessment in an Urbanizing Coastal Environment

SUBPROJECT RELEVANCY STATEMENT
In CREST-CAChE I, we determined the hydrologic transport (flux) and fate (biogeochemical processes) of various contaminants across land-use boundaries in coastal South Florida. We also focused on computational methods for coalescing disparate environmental data sets for synthetic analysis and interpretation. In CREST-CAChE II we will build on these results, as well as increased public awareness and demand for science-based management of ecosystem services and environmental health. We will focus on innovating ***novel integrated ecosystem assessment approaches*** and maximizing community engagement and communication in our continuously urbanizing coastal region.

Urbanization is expanding both globally and locally, resulting in an increase in the types and levels of land-based contaminants released into the environment through changes in land use, regional hydrologic management, and loss of watershed habitat.[1] Coastal south Florida, with nearly 8 million people living close to biodiverse marine and freshwater ecosystems, is a valuable natural laboratory to study potential conflicts between humans and nature as urbanization and associated stressors increase, and serves as a model for how to remediate impacts and manage solutions elsewhere.

RFA3 will study how urban coastal systems: (1) can adapt to changing climate and extremes, through (2) ecosystem-based management of urbanization pressures across the Biscayne Bay watershed and seascapes to (3) reduce the impacts of contaminant loads that disrupt ecosystems and their services. We will use Miami-Dade County and the Biscayne Bay watershed as the platform for our CREST-CAChE II laboratory - from the highly urbanized northern and eastern reaches of the Biscayne Bay watershed to rural and suburban landscapes along the southern reaches of Biscayne National Park and south to the edge of protected wetlands of Everglades National Park. This approach bridges ***cutting-edge sensing technologies (RFA1) and discoveries in epigenetics (RFA2)*** with foundational estuarine and marine urban ecology, applying models, observations, and experiments to develop innovative integrated ecosystem assessment and solutions for management of urbanization in the face of climate change. ***The ultimate goal is to identify, evaluate and predict ecosystem responses to the interactive effects of coastal urbanization and climate change, and improve management and resilience of ecosystem services in partnership with local stakeholders and communities.*** The Table below shows how RFA3 research will inform RFAs 1 and 2.

Links between CAChE subprojects/RFAs	**Subproject/RFA1: Detection Technologies for the Study of Environmental Stressors**	**Subproject/RFA2: Multi-Level Organismal Impacts and Responses**
Subproject/RFA3: Integrated Ecosystem Assessment	• Prioritize epigenetic and metabolic markers for further analyses based on population, community and ecosystem responses • Provide field validation of tracer methods for further development • Identify additional environmental metrics to better understand specific events	• Identify focal habitats and species for gene-environmental stressor interactions • Provide specific information on sex, size, or age-classes for further experimentation on organismal response • Provide spatio-temporal data for further research at the individual level

RFA 3: Innovating Integrated Ecosystem Assessment in an Urbanizing Coastal Environment

PROJECT DESCRIPTION

Along our coasts, dense urban areas transform the marine environment through resource exploitation, industrial and domestic pollution pathways and ocean sprawl resulting in a unique set of research challenges for the nascent field of urban marine ecology.[2,3] Further, while many urban contaminants remain unregulated and have largely unknown consequences on environmental health and human well-being, climate change is likely to exacerbate coastal urbanization pressures with detrimental effects for ecosystem structure and function[3,4]. Importantly, current urbanization impacts in combination with climate change will continue to disrupt the many ecosystem services - benefits people gain from the coastal environment - including food, coastal protection, recreation, water quality, and climate regulation[4–7] if current anthropogenic activities continue unabated. Importantly, current urbanization impacts, in combination with climate change, will continue to disrupt the many ecosystem services - benefits people gain from the coastal environment - including food, coastal protection, recreation, water quality, and climate regulation[5–8] if current anthropogenic activities continue unabated.

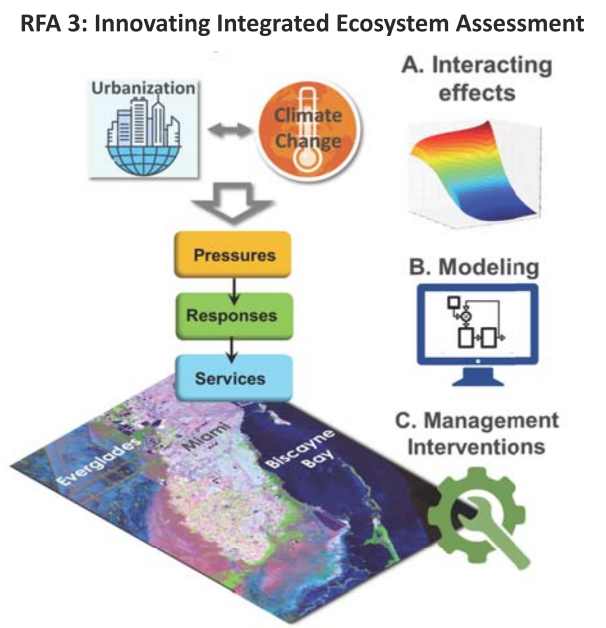

Figure 1. RFA 3 integrates across subproject themes to elicit drivers and interacting effects, model and forecast the influence of urbanization, and innovate ecosystem management interventions to reduce the impacts of urbanization to improve the provision of ecosystem services

The urbanizing landscape is a coupled human-natural system characterized by ecosystem pressures and states that feed back to the provisioning of ecosystem services.[9,10] Overarching pressures in our south Florida coastal urban environment are driven by coastal development, land use, and flood risk management that modulate hydrologic and nutrient regimes as well as freshwater delivery and contaminant loads against a backdrop of changing climate (Figure 1) How pressures cascade from genes to ecosystems and back to influence the provision of ecosystem services across coastal urban environments sets up a series of cutting-edge research questions that advances an emerging field - marine urban ecology.[3] In CRESTII, RFA3 will advance a quantitative understanding of *genes to ecosystems*-based understanding of coastal urbanization and climate change (*Subproject a*). We will model and predict how our coastal urban environment changes under scenarios of hydrologic management and climate change (*Subproject b*), and test management interventions to reduce the impacts of contaminant loads that disrupt ecosystems and their services (*Subproject c*).

a) Integrate novel sensing and sensing platforms across spatial scales and in near-real time with experimental and observational approaches to examine the interacting effects of coastal urbanization and climate change on ecosystem pressures, responses and services

Rationale. We will apply the integrated ecosystem services framework -Driver, Pressure, State, Impact, Response[11] developed for south Florida's coastal environment to evaluate the interaction of coastal urbanization and climate change. More specifically, we will examine relationships among: 1) variation in the quality, timing and location of contaminant loads and associated physico-chemical regimes 2) seascape structure and function, and 3) fish behavior and associated effects on community and ecosystem processes. We will also 4) disentangle the relative influence of these cumulative impacts with controlled experiments and 5) determine how extreme events modulate those responses. We build on the organismal-scale impacts of land-based contaminants and nutrients of RFA2 to quantify the cumulative direct and indirect effects on population dynamics, trophic linkages, ecological processes, and ecosystem services.

Subproject a.1. *Determine nutrients, dissolved organic matter (DOM), and contaminant inputs to Biscayne Bay from land-based sources.*

Urban landscapes contain unique sources of contaminants such as petroleum products (e.g., oil, gas), debris from ornamental vegetation, pet waste, and sewage from leaky septic and sewer systems.[12–14] The conversion of natural watershed habitat to impervious areas such as roads, rooftops, and parking lots and other infrastructure like stormwater systems designed to convey water and prevent flooding also affect the characteristics of a watershed, including hydrology, physiography, and biology, and can result in high infrastructure densities that also influence drainage (i.e., stream or pipe length per unit watershed area) in urban watersheds.[15,16] Taken together, the transport and release of contaminants from urban areas to coastal estuaries results in changes in water quality, clarity, nutrient balance, and ecosystem structure.[17,18] In addition to nutrients (nitrogen and phosphorus) and sediments, wastewater, septic systems and other sources often can contain fecal coliforms but also other unregulated chemicals such as Sucralose, pharmaceuticals, antibiotics.[19–21] Furthermore, a propensity of infrastructure failures and septic systems along the coastline of Biscayne Bay is thought to convey contaminants in diffuse groundwater flow[22] contributing to seasonal episodes of low oxygen, algae growth, and fish kills.

We will examine the influence of septic systems and wastewater infrastructure in delivering contaminants to surface and groundwater and their ultimate fate and transport to Biscayne Bay. We will sample groundwater and surface water along upstream to downstream gradients of regions containing septic systems, stormwater infrastructure, and dense urban land uses along canals for nutrients, coliforms, DOM and a wide variety of anthropogenic contaminants (e.g., plastics). Dissolved organic matter from groundwater and surface water will be analyzed using multi-dimensional spectrofluorometry[23] to identify organic signatures indicative of wastewater sources. Nutrients from wastewater sources versus fertilizer applications will be determined using nitrogen and oxygen isotopes of nitrates.[22] We will also develop a focused gray and green stormwater infrastructure (GSI) performance monitoring study to collect continuous, high temporal resolution data to quantitatively investigate the relative influence on contaminant quality and load and GSI system performance. The timing of freshwater inputs to the Bay from both canal and groundwater will also be compared with data collected from continuous monitoring sensors (dissolved oxygen, salinity, temperature, pH, fluorescence, chlorophyll) placed in the Bay.

Subproject a.2. *Response of seascape structure and function to urban coastal watershed drivers and associated physicochemical regimes.*

Multiple ecosystem services are associated with coastal urban environments including storm surge protection, coastline stabilization, nursery habitat for aquatic and avian organisms, sea level rise buffering, and contaminant storage.[24] All are undervalued by stakeholders, and uncertainty reigns the processes that govern them and their sustainability.[11,25,26] Biscayne Bay's many services are threatened by chronic contaminant loading. These pressures affect the structure, function and landscape configuration (expansion and loss of ecosystems from the coastline to the reef.[27] Consumer indicator species, due to their specific sensitivity to stressors and role in food webs and the provisioning of services, can help us detect changes in ecosystem services provision. For instance, Pink shrimp (*Farfantepenaeus duorarum*) act as an indicator of Everglades restoration success due to their relationship to salinity and configuration of seagrass habitats and their importance as a food resource to many upper trophic-levels that contribute to local and regional fisheries.[28,29] However, there are significant gaps in understanding of how various urban pressures impact coastal ecosystem states and services.

We will evaluate multi-scale seascape structure (e.g., gray and natural infrastructure, habitat amount and spatial configuration), and associated primary and secondary structure and productivity, organic and inorganic carbon and nutrient status, sediment-water column flux of and accumulation in plants and sediments, of various types (e.g., seawalls, revetments, mangroves, marsh and near-shore aquatic, seagrasses, tidal flat, benthic submerged, coral-associated ecosystems).[30] We will also evaluate patterns across ecological systems exposed to different levels of runoff (freshwater delivery and contaminants) using field measurements, field sensors and chambers, across spatial scales with a combination of broad- and fine-scale mapping and over time using historical datasets.[31] Effects on food web structure and energy flow pathways will be examined using stable isotope analysis and source-contribution models (e.g., Bayesian mixing models), comparing dietary diversity and energy sources across stressor gradients and associated changes in seascape configuration.[32–34] We will apply a matrix-based method for estimating direct and indirect impacts and interaction strength among ecosystem pressures, states and services.[35]

Subproject a.3. *Effects of habitat degradation on fish behavior and food web structure.*
Of particular interest is the impact of predictable and episodic shifts in important abiotic factors from tidal forcing (e.g., turbidity, salinity, tidal mixing, water quality and velocity) and water management activities in structuring these processes. Prey perceive information about immediate threats using cues through a series of sensory modalities such as vision and pressure fluctuations[1-5] that either emanate from a predator's swimming movement or from anti-predatory behaviors of conspecifics.[6] However, the characteristics of the environment can dramatically affect how accurately aquatic organisms identify and evaluate threats,[1,7] or gain predator information from risk-aware conspecifics. Water turbidity and habitat characteristics can reduce prey's ability to perceive the presence of a predator,[8,9] while fluctuations in hydrodynamic conditions can contribute to the loss of perception of predation cues and ultimately the overall risk of predation.[10-12] Human "noise" disturbances (e.g., shipping, boating activities, coastal construction and dredging, seismic studies for oil and gas exploration, renewable energy, and military exercises) also have effects which may be substantial given the dependency of fish on underwater sound throughout their life history across an array of marine seascapes, including estuaries.[9,10,11,4,5,6,7]

Shifts in ecosystem dynamics across seascape gradients influencing habitat characteristics will be examined through: 1) variation in the temporal and spectral[13] characteristics of specific features of underwater soundscapes indicative of soniferous animal behaviors, densities, and distributions and 2) imaging acoustics[20,21] coupled with machine learning techniques for adaptively quantifying predator and prey responses.[22] These methodologies are robust across varied estuarine conditions and are not light-limited offering unique opportunities to non-invasively examine community dynamics during day and night periods. The aim of this project is to explore how natural (i.e., tidal) and episodic (disturbance) physicochemical variation and noise disturbances influence primary consumer (i.e., forage fish) distribution, behavior and interactions with their predators.

Among contaminants, the physical suspension and deposition of sediments can increase the pelagic larval duration of fishes,[36] changing the dispersal and connectivity within meta-populations. We will investigate the impacts of urban sedimentation on changing larval dispersal through a range of approaches, including biophysical modeling[37] on a range of marine organisms (e.g., the bicolor damselfish, *Stegastes partitus*, as a model reef fish). Pollution such as increased sedimentation can impact ecological processes, such as grazing by herbivorous fishes. For instance, herbivory of seaweeds is a critical process on reefs as it facilitates coral settlement and growth, thus increasing reef resilience.[38] However, accumulating sediment can reduce grazing pressure and facilitate seaweed growth as parrotfishes and other herbivores avoid sedimented areas.[39,40] We hypothesize that sediment plumes originating from activities in Biscayne Bay (e.g., from dredging, boating, port and cruise ship activities) will influence food web dynamics in adjacent ecosystems.

Using NOAA monitoring data, we will examine the spatial extent and drivers of sedimented algal cover on the Florida reef tract, and we will use models of benthic dynamics[41,42] to quantify the effects of sediment on fish grazing and reef function.

Subproject a.4. *Relative influence of land-based contaminants and mechanisms driving responses across the seascape.*
Coupled with the climate change, mechanistic drivers of community to ecosystem responses and their relative influence are difficult to determine,[4] limiting the ability to manage for the provisioning of services. Experimental approaches to disentangle mechanisms and evaluate the direction, magnitude, and rate of responses to independent drivers and their additive, antagonistic or synergistic effects are key knowledge needs to management interventions aimed at sustaining the provisioning of coastal services.[3] Experimental approaches offer controlled conditions to advance our understanding of antagonistic and synergistic effects of climate change and hydrologic management, leading to high-impact discoveries[43,44] that guide restoration recommendations of the National Research Council's committee on Everglades Restoration progress it.[45] Further, these experimental approaches enable testing how legacy contaminants interact with climate change and hydrologic management to modulate the release of inorganic nutrients or other contaminants and carbon dioxide as a result of changes in temperature and episodic freshwater discharges from urban canals and stormwater infrastructure.[46]

We will apply experimental approaches including small-scale bench-top benthic sediment-water column core incubations,[46] mesoscale outdoor tank experiments,[47] and *in-situ* field-based benthic microcosm,[48,49] and mesocosm[43] chamber studies generate response functions for key ecosystem attributes. We will assess mechanistic drivers of nutrient and carbon uptake/release from sediments and into water column, ecosystem structure, metabolism, nutrient flux and uptake) to enhance our understanding of drivers of long-term cumulative effects of contaminant release, hydrologic management and climate change, and inform modeling of changes in ecosystem structure and function.

Subproject a.5. *Contaminant quality, loading and influence on population structure and function with climate change and extreme events.*

Along with other climate change effects, there is an urgent need to assess how urban coastal ecosystems respond to stressors associated with extreme climate events (ECEs), interacting with other urban press stressors (e.g., eutrophication and pollution).[1,2] Recent findings also suggest that urbanization can increase autochthonous production and bioavailability of organic matter and nutrients, particularly during warm and/or wet months when urban runoff is increased[14,16,50] with significant long-term implications for fish populations, fisheries, and fishing communities.[5–8] ECEs such as hurricanes, droughts, floods, and temperature anomalies, are likely to become a stronger driver of coastal and marine ecosystem dynamics.[3,4] In low-lying coastal watersheds, diurnal and seasonal extreme tides, that have been increased by sea-level rise and coastal storms, can interact with runoff to influence the timing, quality, and bioavailability of organic matter and nutrients while increasing the exposure of aquatic ecosystems and biota to other types of novel contaminants from urban watersheds. Despite the high socioeconomic value of coastal fisheries, the degree to which this ecosystem service is impacted by the interaction of ECEs and altered urbanized watersheds remain unknown. To address this critical knowledge gap, we propose an integrative approach coupling biogeochemistry, hydrology, ecology, fisheries, and climate to describe how ECEs affect contaminant quality and loads and assess coastal nektonic communities and related recreational and commercial fisheries within the urban ecosystem context of Biscayne Bay. Specifically, with our integrative approach, we ask: 1) How do ECEs and freshwater management interact to influence contaminant quality and loading and subsequent extreme ecological responses of nektonic communities? 2) How does recreational fishing catch structures respond to ECEs and associated ecosystem disturbances modulated by contaminant quality and loading (e.g., seagrass die-off, algae blooms)?

We will complement existing sampling and sensor deployment with high resolution auto sampling and event sampling to differentiate canal, stormwater, and near-shore water quality and characterize changes in contaminant loads in relation to variation in the frequency, extent and location of urban flood events. Event sampling (with high rainfall, predicted high tides and storm events) will be augmented by: 1) citizen science sampling campaigns in partnership with local government and non-profit organizations and 2) customized, low-cost sensing platforms (~$100, aerial and surface) designed and built by our students, and 3) building on previous work on the optimal deployment of inexpensive water vehicles.[51] We will also assess nektonic communities' response complemented by historic time-series records that help elucidate direction and rate of change and isolates signal from noise to detect and inform modelling of regime-changes.

b) Apply ecosystem pressure, response and services relationships to model and assess how regional hydrologic management, watershed land use, and climate change influence contaminant loads to disrupt ecosystem services

Rationale. Due to climate change, and intensifying anthropogenic influence, boundary regions at the ocean- interface are experiencing systematic trends in environmental stresses and changes in the frequency and magnitude of rapid-onset shocks, such as hurricanes and tropical storms. This environment is inherently nonstationary,[52] and communities at this interface are highly vulnerable to flooding from storm surge and likely intensifying rainfall due to low land elevation, dense populations, and high property values. The multi-directional flow of water (i.e., terrestrial runoff moving seaward and tides, sea level rise, hurricanes moving landward) creates an exchange between the various, generally unbounded coastal regions and thus, parts of the coast large spatial gradients. Water quality, that condition that determines the human-derived beneficial or detrimental consequences of the water condition, is one of the most influential aspects of these transfers. For instance, eutrophication of coastal waters can lead to harmful algal blooms (HABs), cause human and ecological health concerns, reduce our shoreline aesthetics, and greatly diminish the "tourist draw" and economic value of urban coasts.

Subproject b.1. *Coupled coastal hydrodynamic-surface water-groundwater modeling to simulate changes in freshwater delivery and contaminant loads with climate change, regional watershed management and local wastewater infrastructure.*

In many metropolitan areas such as Biscayne Bay, there is evidence that the frequency of coastal flooding is increasing due to rising sea levels,[53] Several factors contribute as compounding effects to coastal flooding, including: (a) astronomical tides, storm surge, and wave effects; (b) extreme inland rainfall and flood discharge from upstream contributing areas; and in some cases, (c) rising groundwater levels attributable to sea level rise. These integrated effects of flooding, known as Compound Flooding have emerged as a fundamental challenge for coastal ecosystems,[54] Florida has also recently experienced multiple sewer overflows and the issue is expected to worsen due to its growing population, urban development, climate change and aging infrastructure. Sewer overflows introduce pathogenic microorganisms posing human health concerns, and bring excessive nutrients that contribute to algal blooms and fish kills. Understanding and simulating the fate and transport of sewer overflows is critical for evaluating the impacts on ecosystem processes and services. We hypothesize that the nonstationary nature of these compounding effects has implications for physical, biological, and chemical processes in the coastal environment which are not well understood.

Our objective is to develop and apply a system of coupled models to address interactions of driving factors and assemble and analyze existing data to understand and predict compound flooding in coastal fringe areas. The proposed system of coupled models will include (a) coastal hydrodynamic models to simulate both surge, waves, and run-up;[55] (b) surface water hydrology model for simulating the rainfall-runoff processes;[56] (c) groundwater model, MODFLOW[57]; and (d) watershed models of hydrology and water quality[58]and the Environmental Fluid Dynamics Code (EFDC)[59]; (e) wetland models to simulate changes in both physical and biological processes in natural areas such as mangroves;[60,61] and (f) GSI models to predict the dynamic as well as long-term performance of GSI systems under varying future scenarios.[62] This dynamical modeling will be preceded by the assembly and analysis of a comprehensive database on compound flooding for calibration, using machine learning[63] and multivariate statistical analysis.[64] The database will leverage cutting-edge LiDAR technologies, remote sensing, hydrologic, hydrodynamic, as well as existing water quality datasets. Outputs from this modeling will be used to drive food web and ecosystem modeling.

Subproject b.2. *Modeling of freshwater delivery and contaminant load impacts on aquatic primary producers, food web structure, function and services.*

Dynamics of primary producers. Seagrass growth and survival depends upon the interaction of multiple drivers (e.g., temperature, salinity, submarine irradiance, inorganic carbon, nitrogen, phosphorus and community processes (epiphytes and herbivory,[65]). Environmental monitoring of inflows, water quality, and seagrass attributes offers the foundation to connect these multiple drivers to potential fluctuations in seagrass seascapes.[17,66,67] We propose an integrated ecosystem modeling assessment that links seagrass habitat stressors and uses multiple datasets collected by multiple bay partners to simulate modeling of seagrass production to assess the effects of environmental drivers and explore potential management, climate and restoration scenarios.[68–72] The seagrass simulation framework includes: 1) a spatial database for the bay shoreline, bathymetry, and seagrass distribution and abundance; 2) a physical model including a box model for transport and dynamic predictions of volume, depth, and submarine light; and 3) an ecosystem modeling component. The work will allow us to couple dynamic modeling with the functionality of GIS and spatial analysis,[72,73] examine spatial patterns in drivers and seagrass habitat over a range of scales, and forecast potential changes in seagrass coverage with changes in regional water management and coastal development,[72] and develop into a user tool that helps translate modeling results into useful information for stakeholders

Food Web Dynamics. Examining the ecosystem-scale effects of the multifaceted impacts of pollutants on population dynamics, behavior, and physiology of marine species represents a challenging research agenda. However, food web models provide an exciting tool to draw together many of the organismal-scale insights generated in RFA2 and RFA3. Thus, we propose to assess trophic linkages and build food web models across bay and adjacent habitat- mangroves, seagrass beds, coral reefs,[74] Food webs will be parameterized using trophic linkage analyses as well as published data sources, and will be coupled across habitat types through known interactions (e.g., fish inter-habitat movements,[75,76]). These food web models will provide a framework to investigate the impacts of pollutants on metrics such as energy flow and trophic efficiency. For example, sediment can change fish predator-prey interactions,[36] diesel and anionic surfactants can change fish metabolic performance,[77] and pharmaceuticals can alter animal functions through physiological and behavioral effects.[78] These coupled food web models will provide an opportunity to examine how urban impacts in one habitat cascade across tropical marine seascapes. Food web models can also be used to examine the potential for the effects of extraction, including fishing and collection for other purposes (e.g. recreational fishing bait;[79–81]). More specifically, we will use a combination of historical reconstruction and fishery-independent data to parameterize food webs models to estimate potential pink shrimp catch, and the sustainability of this fishery, in Biscayne Bay under different climate change scenarios and considering alterations to food web components (e.g., predators and competitors). These food web model simulations based on Biscayne Bay's pink shrimp population dynamics will allow us to expand our knowledge on how ecosystem functioning is influenced by pressures operating independently or interactively at different spatiotemporal scales (e.g., fishing, nutrient and sediment loads, freshwater management); especially, within the context of urban coastal landscapes.

c) Develop and test ecosystem management interventions to reduce coastal urbanization impacts and enhance provision of ecosystem services

Rationale. Fundamental alteration of the watershed through conversion of natural to gray infrastructure results in excess nutrients, sediment and other pollutants discharged into nearby water bodies. These challenges are exacerbated in coastal areas such as Biscayne bay because of the integrated effects of urbanization, climate change, and hydrogeological conditions (e.g., high water tables and low elevations), negatively affecting coastal water quality and ecosystems. Further, the provision of novel habitat (hard substrata) along sedimentary shores can have a number of ecological consequences including altering local and regional biodiversity by modifying natural patterns of dispersal of species, or by facilitating the establishment and spread of exotic species.[2] Reversing this trend is an emerging area of research that cuts across disciplines of engineering, ecology, and hydrology, and requires integrated field experimentation, water quality monitoring, geospatial analyses and modeling. Building from subprojects a and b, complemented with field experiments, deployment of high-resolution sensing networks, and geospatial analyses of fine-scale infrastructure and land use, we will evaluate how different forms of ecosystem management interventions modulate the transformation and retention of contaminants and carbon sequestration; prevent seagrass loss, algal blooms, and unsuitable abiotic regimes for faunal species. We will leverage the characterization of seascape structure and function to identify potential management interventions.[82]

Subproject c.1: *Testing green infrastructure for ecosystem recovery.*
Significant acreage of coastal ecosystem area, including mangrove, coastal marsh, and seagrass had been lost across the Biscayne Bay by the 1970s.[31] In the last 2 decades, increasing water quality degradation has resulted in up to 90% loss of seagrass in some areas.[66] For seagrass ecosystems, their capacity to reduce sediment resuspension, sequester nutrients, oxygenate sediments and the overlying water column are processes that contribute to the overall resilience of ecosystem services.[83,84] With a resurgence of public and government interest in recovering the health and ecosystem services of Biscayne Bay, specific recommendations endorsed by Miami-Dade County government[85] include experimental and pilot studies to recover lost habitat and their services. Investments in large-scale federal programs and local-scale projects to create and restore ecosystems to recover and enhance coastal ecosystem services[86,87] have advanced well beyond scientific understanding of their performance, yet many uncertainties persist, including genetic and biological responses of individual organisms (RFA 2;[88]). These include hydrologic restoration of coastal wetlands as part of the Comprehensive Everglades Restoration Program (CERP), an interdisciplinary and interagency team charged with re-creating fresher, estuarine conditions along the western shoreline of Biscayne Bay, including mangrove forests and sawgrass marshes known to sequester nutrients, trace metals, and contaminants.[83,84]

Thus, we will establish a series of multi-scale physical and biological management experiments and interventions to test management techniques for ecosystem recovery. Using experimental flumes,[89] we will test the water quality and contaminant regulation capacity of hardened shorelines (seawall) as compared to restored, created and hybridized vegetated shorelines.[90–93] Using sediment management techniques including beneficial use of dredged or other material[94] we will conduct pilot experiments to increase submerged land elevation to reduce water column light attenuation and test techniques for restoring seagrass habitat. We will also conduct pilot tests to examine the potential for restoring habitats that support filter feeding organisms to sequester nutrients and reduce nutrient concentrations in the water column in both field and outdoor laboratory mesocosm experiments.[47]

BROADER IMPACTS

RFA3 will support the overall broader impacts of CREST-CAChE II. Specifically, students will co-develop research projects with RFA3 faculty and be guided by a near-peer mentoring team of postdocs and senior graduate and undergraduate students. They will learn innovative ecosystem assessment methodologies and solutions for management of urbanization in the face of climate change. They will also learn methods for engaging the community and decision-makers in comparing potential future scenarios. In addition, RFA3 research will inform the micro-credentialing workshops offered to graduate and undergraduate students. Through our partnerships network, students will gain the skills to communicate and translate their data into actionable information for decisionmakers and stakeholders.

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Undergraduate and Graduate Mentoring Plan

The graduate and undergraduate mentoring plan will build upon the successes of the mentoring activities in CREST-CAChE I. There are three primary areas of mentoring of both undergraduate and graduate students that will be enhanced in CREST-CAChE II: a) immersive convergence research, b) journal club, and 3) near-peer mentoring.

The summative evaluation found that graduate and undergraduate student participation with the CREST-CAChE community was instrumental in their socialization into the scientific field. In particular, access to expertise outside of the students' primary discipline significantly strengthened their research. Much of this socialization occurred through the informal social networks within the CREST-CAChE community. To strengthen this interdisciplinary component, CREST-CAChE II will promote **immersive convergence research** in which students co-develop their individual research projects with faculty in each of our three Research Focus Areas. The formalization of the interdisciplinarity of students' research will capitalize on the social networks within the community and enhance the socialization process.

In the summative evaluation, graduate students described the positive role of participation in the CREST community in terms of fostering a sense of belonging and mitigating isolation. Graduate students perceived CREST as the kind of nurturing environment that had been found in the literature to support cultural identity and mitigate a sense of isolation. Therefore, the second mentoring strategy will be the continuation of the biweekly **student journal club**, which is led by PIs Crowl and Teutonico and open to all students. No other faculty are permitted. This semi-structured space provides opportunities for students to share their research and receive the kind of meaningful recognition that has been found to contribute to the development of STEM identities among underrepresented students. The student journal clubs will provide professional development workshops on career skills, including science communication, data visualization, research presentations, resume preparation, fellowship applications and STEM career pathways. The journal clubs are a forum to socialize students in their role as scientist and to integrate them into the overall, cross-institutional program.

To ensure student engagement in all of the opportunities offered by CREST, we will use a **near-peer mentoring** approach. Research teams of faculty, graduate students and senior undergraduates will mentor their junior peers. Mentoring will focus on interdisciplinary research, courses, credentialing and career pathways, as well as the challenges students face outside the classroom. Mentors will be trained using the model developed by FIU's STEM Transformation Institute and Provost's ADVANCE Institutional Transformation program that prepares mentors to navigate both the cognitive and non-cognitive domains, as many of our students face challenges as first generation, diverse and/or economically limited students. The summative evaluation demonstrated the positive influence of near-pear mentoring. This opportunity to introduce younger students to scientific research had a positive influence on their investment in the field and to their commitment to their own research. While the intended influence of near-peer mentoring is from mentor to mentee, the preliminary findings of the summative evaluation indicate that the socializing influence may be reciprocal in that the mentors reported receiving positive benefits as well. Therefore, resources invested in these models may be doubly effective.