Center for Oceans and Human Health (COHH): Addressing the Health of Florida Coastal Ecosystems and Communities.

Executive Summary

The effects of environmental change are evident in Florida coastal waters, where warming temperatures, sea level rise, changing precipitation patterns and so called "tropicalization" are creating documented ecological shifts. The Florida coastal region that is the focus of the proposed Center's research is the Indian River Lagoon (IRL) ecosystem, where ecological shifts have already begun to negatively affect the system, communities and human health through exposure to an increasing number of harmful algal bloom (HAB) events and suites of toxins. The IRL ecosystem is likely one of the most HAB impacted estuaries in the United States in terms of diversity of HAB species, number and extent of blooms, and resultant range of toxins that may ultimately affect the humans that live and work in the region. Understanding the connections between large scale environmental/climate change, the local environment and HABs, and resultant human health issues is the central mission of the proposed Center.

The specific aims of the overall project are:

1. Develop an Administrative Core that will manage and coordinate all Center investigators and activities, including guiding the Center's research progress and long term mission, assessing scientific progress and successes to achieving stated Center goals, coordinating external collaborations or opportunities that could enhance the Center's mission, providing regular communication and reporting to NIEHS/NSF program managers on Center research progress and activities, and organizing travel to COHH3 grantee meetings.

2. Develop three independent, but highly integrated Research Projects that will serve the Center's mission and science goals. The research projects are structured as a tiered approach to the problem, where Research Project 1 will provide better detection and identification of IRL HABs, elucidate the ecological drivers behind their occurrence, and ultimately use project results in an ecosystem level predictive model to better understand IRL HABs under the effects of environmental/climate change. Research Project 2 will investigate the environmental controls for the genetic expression of toxicity in HABs and the biosynthetic pathways for toxin production, potentially leading to the discovery of new or merging toxins in the system and new diagnostic tests. Research Project 3 will investigate the trophic transfer of HAB toxins in the IRL aquatic food web, while simultaneously assessing human exposure to HAB toxins via direct sampling of individuals through a collaboration with the Centers for Disease Control.

3. Develop a Facilities Core that will serve the three Research Projects by providing analytical data on the toxicity and suites of toxic compounds found in materials sampled by the research projects. This will be accomplished by conducting assays from project samples for toxicity against a panel of mammalian cell lines and conducting untargeted metabolomics of secondary metabolites (including toxins) in the same samples.

4. Develop a Community Engagement Core that will facilitate multidirectional communication between research investigators, state and local stakeholders and the broader community with the goal of improving environmental health literacy.

The success of the overall project will be determined by the research production of the Center (e.g. ecosystem model products, scientific presentations and peer-reviewed publications), new collaborations and funding leveraged as a result of Center activities (e.g. successful grant applications and new PI recruitment into the Center) and effective community engagement activities (e.g. well attended public symposia, positive and impactful press and media reports, demonstrated actions taken by water management or health professionals as a result of Center activities, and interactions with other COHH3 investigators or Centers).

Proposal Summary

Significance: The effects of environmental change are evident in Florida coastal waters, where warming temperatures, sea level rise, changing precipitation patterns and so called "tropicalization" are creating documented ecological shifts. The Florida coastal region that is the focus of the proposed Center's research is the Indian River Lagoon (IRL) ecosystem, where ecological shifts have already begun to negatively affect the system, communities and human health through exposure to an increasing number of harmful algal bloom (HAB) events and suites of toxins. In fact, the IRL ecosystem is likely one of the most HAB impacted estuaries in the United States in terms of diversity of HAB species, number and extent of blooms, and resultant range of toxins that may ultimately affect the humans that live and work in the region. The location and latitudinal extent of the IRL place its ecosystem within two biogeographical provinces (temperate and sub-tropical climate zones) providing a climate gradient within a connected environment that can be exploited as a model ecosystem to understand the connection between climate change, the local environment and HABs, and resultant human health impacts. Understanding these connections is the central mission of the proposed Center.

Project Premise: Through integrated research of the biological, physical, chemical, genomic, transcriptomic and toxicity characteristics of HAB events in the IRL ecosystem, it will be possible to develop ecosystem based models that can be used in understanding ecological shifts that are occurring as a result of environmental change to predict the response of HABs, with the ultimate goal of minimizing the health consequences to the people who live in the IRL watershed.

Project Products: The Center will produce integrated models of the IRL system that incorporate hydrographic, biogeochemical, and microbial diversity metrics to predict 1) short term dynamics of HAB signatures (e.g., species, toxicity, potential for toxicity through gene expression) as well as 2) long term effects associated with environmental/climate change. Results will be shared with those local agencies responsible for management strategies mitigating impacts on human health for people living in the surrounding area, both over the short and long term.

<u>*Background on the Indian River Lagoon (IRL) ecosystem*</u>: The IRL is a barrier island estuary comprised of three main waterbodies (Mosquito Lagoon, Banana River Lagoon and Indian River Lagoon), with the St. Lucie Estuary (SLE) linked to the southern reach of the IRL (Fig. 1). The IRL has a complex watershed within Florida including watershed connections to the St. Johns River and the Florida Everglades. Overall, the system spans 260 km or approximately 40% of Florida's east coast and ranges from ~ 1 to 8 km wide over its extent. The IRL is recognized as an "Estuary of National Significance" by the U.S. Congress, with an estimated annual economic value of ~ $8 billion (ECFRPC report, 2016). Its economic value results from industries such as tourism, commercial and recreational fishing, boating, water sports and aquaculture. The IRL ecosystem and its waters impact 45 cities and over 1.6 million residents.

Figure 1. Map of the Indian River Lagoon ecosystem and watershed, including the St. Lucie Estuary and Lake Okeechobee.

The 5,700-km^2 IRL watershed includes parts of seven Florida counties, and its original extent has been expanded considerably by canals that drain inland areas, including a major canal linking Lake Okeechobee to the SLE. Six inlets connect the IRL with the Atlantic Ocean (Fig. 1). However, the Port Canaveral inlet yields little exchange because it has locks (not shown in Fig. 1). Along with relatively few inlets, the IRL has few large tributaries. Given its morphology and topography, the IRL ranges from micro-tidal in the north to being influenced by ~ 1 m tides in the south. Accordingly, circulation is driven primarily by wind in the north, which yields water residence times that vary from weeks to a year or more, while in the south, circulation is more strongly influenced by tides, leading to shorter residence times. Along with being large and complex, the IRL is also one of the most biologically diverse estuaries in North America (Swain 1995), in part because the system spans two biogeographical provinces (temperate and sub-tropical). The system is also a major spawning and nursery ground for numerous species of fish and shellfish, and is home to a resident population of dolphins and endangered Florida manatees.

Climate Change and the IRL Ecosystem: The location and extent of the IRL place its ecosystem within an ecological zone particularly susceptible to climate change, however, this feature also provides a climate gradient within a connected environment that can be exploited as a model system to better understand the effects of climate change. The IRL latitudinal ecotone between sub-tropical and temperate climates has been considered an important driver of its high floral and faunal diversity (Gilmore 1995, Schmalzer 1995), where compressed latitudinal gradients and complex geomorphology produce high habitat diversity and niche space that supports communities consisting of many different species adapted to tropical or temperate conditions. As a result, the composition of plankton, nekton, benthic fauna, and vegetation within and along the IRL all exhibit high species richness (including HAB phytoplankton species).

Climate change (e.g. rising temperatures) is drastically altering environmental gradients along the extent of the IRL and, consequently, the assemblage of species within this ecosystem. Sufficient change may ultimately lower diversity throughout the system if latitudinal gradients become less pronounced and habitats become more homogeneous across the region, i.e. where warmer conditions favor tropical species over those currently adapted to the temperate climate (“tropicalization”). For example, the range of mangroves along the Florida east coast has expanded northward through the IRL over the last 28 years as the frequency of extreme cold events has decreased (Cavanaugh et al. 2014). Tolerance to freezing sets the northern range limit for these plants, but also varies among species found within the IRL, suggesting that the relative abundance and range of species will change substantially in response to a warming climate (Cook-Patton et al. 2015).

Similar effects can be expected within the algal communities of the IRL, especially for the many phytoplankton species that are sensitive to temperature and its effect on water column stratification. Warmer conditions are likely to favor bloom forming cyanobacteria and dinoflagellates which are often toxic to humans and other wildlife (Paerl & Paul 2012, O’Neill et al. 2012). In fact, a recent shift towards intense blooms of picoplanktonic eukaryotes and cyanobacteria and away from traditional diatom dominated blooms has already been documented in the northern IRL, Banana River Lagoon, and Mosquito Lagoon (Badylak & Phlips 2004, Phlips et al. 2010, Gobler et al. 2013, Phlips et al. 2015). These changes may indicate that a state shift has already occurred within the IRL and extensive ecosystem restructuring is underway. Furthermore, the phenology of the IRL ecosystem is likely to be disrupted by long term climate change, causing mismatch among trophic levels and disruption of historically important ecosystem processes (Edwards & Richardson 2004). In addition to the ecological effects that climate change is causing, both the northern IRL and the SLE have been declared impaired due to excess nutrient inputs (Lapointe et al. 2015), and the resulting detrimental changes to water quality only increase the ecological stress on the system, which may be enhanced by the effects of climate change, as for example, by further fueling HABs that now occur to greater biomass and spatial extent.

Recent blooms in the northern portions of the IRL have been associated with record setting fish kills and high light attenuation that leads to wide spread seagrass mortality (Kenworthy & Fonseca 1996, Steward et al. 2005, Phlips et al. 2015). This negatively impacts the endangered manatee populations that depend on seagrass for a large portion of their diet (Lefebvre et al. 1999, Alves-Stanley et al. 2010) and can result in mortality of benthic fauna due to depleted oxygen levels (hypoxia). In the southern IRL, blooms of the toxic cyanobacterium *Microcystis aeruginosa* have become common and are associated with fresh water discharged from Lake Okeechobee (Phlips et al. 2012). Toxic algal blooms in the IRL can directly impact fish as well as manatees and dolphins leading to significant mortality events (Flewelling et al. 2005). Unfortunately, the extent to which these HAB toxins are affecting local human populations remains unknown.

Harmful algal blooms (HABs) and environmental change in the IRL ecosystem: As briefly described above, in recent years, the IRL ecosystem has experienced extreme and unprecedented HABs. In the northern IRL, the first unusual event occurred in 2011 when a "superbloom" of picocyanobacteria and a small Pedinophyte achieved an extraordinary biomass (chlorophyll-a concentrations >100 µg L^{-1}), spatial extent (nearly 81,000 ha of open water) and duration (up to eight months) (Phlips et al. 2015). This occurred after a prolonged drought between 2006 and 2010. In 2012, a "brown tide" comprised of *Aureoumbra lagunensis* affected large portions of the northern IRL. A second *Aureoumbra* bloom occurred in 2013, and since December 2015, large parts of the northern IRL have been experiencing another extreme *Aureoumbra* HAB (Fig. 2). These recent events have engendered substantial changes in the ecology of the IRL. The superbloom caused a loss of ~ 40% of the seagrasses mapped in 2009, a critical loss of habitat for numerous animals, and the on-going brown tide has caused numerous small fish kills and also one of the largest fish kills ever recorded in the IRL (http://www.cnn.com/2016/03/25/us/florida-fish-kill/?iid=ob_homepage_deskrecommended_pool&iref=obnetwork). While hypoxic waters due to bloom biomass are thought to be a primary cause of the fish kills, there is some concern that undescribed or unknown toxins may have also played a role, as many of the co-occurring picocyanobacteria and picoeukaryotes within these *Aureoumbra* HABs remain unidentified. It is also possible that *Aureoumbra* itself produces an unknown toxin (Gobler & Sunda 2012).

Figure 2. Images of northern IRL waters during the on-going Aureoumbra HAB (left panel) and waters in the St. Lucie Estuary (SLE) during the 2016 Microcystis HAB (right panel).

In the southern IRL and SLE, recent extreme HAB issues have revolved around large blooms of *Microcystis aeruginosa* and other co-occurring, toxic cyanobacteria, which begin in Lake Okeechobee and are transported into the SLE during freshwater releases designed to prevent local flooding around the Lake (Fig. 2). Once in the SLE, blooms persist in the estuary's low salinity waters and are fueled by nutrients from the local SLE watershed (Lapointe et al. 2015). In addition to creating dense surface mats that clog waterways, give off noxious/toxic aerosols and potentially cause hypoxia/anoxia events, *Microcystis* blooms produce microcystins, potent hepatotoxins that have been linked to dermal, respiratory, and hepatic effects among both animal and human populations (e.g. Dawson 1998, Carmichael 2001). Furthermore, *Microcystis* and other cyanobacteria have been reported to produce the neurotoxins, saxitoxin and β-Nmethylamino-L-alanine (BMAA). The toxin BMAA, which is potentially linked to amyotrophic lateral sclerosis and symptoms resembling Alzheimer's (Cox et al. 2003, 2005, Holtcamp 2012) may be a significant emerging threat in south Florida coastal waters (Brand et al. 2010). This suite of toxins creates serious concerns for human health, and has led to local warnings regarding exposure, closure of beaches and waterways, and state of emergency declarations within the IRL region. For example, as a result of the 2016 SLE *Microcystis* bloom, on June 29, 2016, Martin County issued a formal declaration of emergency based on negative impacts to the economy, public health and ecosystem viability, and submitted a request for a federal disaster declaration. A critical concern for local water managers was that the acute and/or chronic exposure risks of *Microcystis* and other cyanobacteria toxins to aquatic life and humans in and around the region have yet to be documented or scientifically evaluated. Thus, an improved understanding of the ecology and persistence of cyanobacteria blooms and the distribution, release, and bio-accumulation of their toxins represent the key challenges for local scientists, public health officials and water managers (and the proposed Center's research). Over the past decade, reoccurring cyanobacterial HABs have impacted the southern IRL/SLE, with 2016 being one of the worst blooms on record, and since the growth of *Microcystis* blooms are greatly enhanced by warm temperatures (25 - 30 °C) (Lehman et al. 2008, Davis et al. 2009, Liu et al. 2011) there is obvious concern that further warming of waters induced by climate change may only increase the intensity and duration of blooms that now plague the SLE.

Historically, beyond the recent large scale, extreme HAB events described above, the IRL is also subject to many other recurrent HABs. For example, over the past few decades, *Pyrodinium bahamense*, a motile dinoflagellate that produces saxitoxin (a potent neurotoxin), has bloomed regularly during the summer in the northern IRL (Phlips et al. 2004, 2015). As a result of these recurrent *Pyrodinium* HABs, pufferfish harvesting is banned in the system due to the large number of human cases of saxitoxin pufferfish poisoning (Deeds et al. 2008). These HABs have also resulted in the only regular monitoring of toxins in the IRL, where the Florida Department of Environmental Protection tests shellfish for saxitoxin on an approximately monthly basis in the summer from a number of locations, and restricts shellfish harvesting when required.

Karenia Brevis, a bloom forming dinoflagellate that produces brevetoxin, and is a persistent and widespread problem on Florida's west coast for both wildlife and humans (e.g. Brand & Compton 2007) also blooms in the IRL, with a widespread HAB reported as recently as 2007, and with persistent brevetoxin detected in the IRL system long after the large bloom dissipated (Hitchcock et al. 2012). The HAB forming diatom *Pseudo-nitzschia pseudodelicatissima* has also been reported to bloom in the IRL (Phlips et al. 2004). This diatom is associated with amnesiac shellfish poisoning through the production of domoic acid. The toxic dinoflagellate species *Gyrodinium pulchellum* produced several HABs with associated fish and invertebrate kills in the IRL during the 1990s, and was found to produce both the neurotoxin hemolysin, and hemolytic toxin hemagglutinin (Steidinger et al. 1998). There is also concern that toxic dinoflagellates from the genus *Cochlodinium,* which already occur in the IRL (Badylak & Phlips 2004) and whose HABs have been globally expanding in the last decade (Kudela & Gobler 2012) may begin to produce regular HABs in the IRL system.

Filamentous cyanobacteria of *Lyngbya spp.* commonly bloom throughout the IRL as mats and epiphytes on seagrasses (Capper & Paul 2008). This cyanobacteria can produce a suite of toxins including lyngbyatoxins, microcolins and malyngolides (Paerl et al. 2008, Arthur et al. 2009). Toxic epiphytic dinoflagellates also occur in the IRL, and include toxic species of *Prorocentrum* and the ciguatoxin producing *Gambierdiscus toxicus* (Badylak & Phlips 2004). *Gambierdiscus toxicus*, which favors warm tropical waters, and is responsible for ciguatera fish poisoning in humans, may soon find the IRL more tolerant to its growth as the IRL becomes more tropical in environment due to climate change. Unfortunately, these potential "benthic" HAB species are not routinely sampled in the IRL.

More recently, during the summer of 2016, significant blooms of *Scrippsiella trochoidea, Chattonela subsalsa* and *Alexandrium monilatum* occurred in the central and southern IRL (PI Sullivan, unpublished field observations). While the *Scrippsiella* bloom was considered a nuisance bloom (but could have potentially caused hypoxia due to its biomass), the latter two species produce saxitoxins, brevetoxins and other ichthyotoxins. In fact, *Alexandrium monilatum* has been reported to create sporadic HABs and fish kills in the IRL since the 1950s (Howell 1953, Norris 1983).

In summary, there are nearly 40 different types of HAB species reported in the IRL (Badylak & Phlips 2004, Phlips et al. 2015) and while toxicity has been demonstrated in the system for a number of these species, climate change and related ecological effects may cause even more of these species to produce widespread HABs and/or genetically express toxicity (if they do not currently). In addition, climate change may allow other toxic/bloom species that currently do not occur in the system to become resident in the IRL and further increase the number of HAB events, similar to the very recent occurrence of *Aureoumbra* in the IRL and its ecologically devastating blooms in the system.

Innovation:

The Center will have a number of innovative approaches to research and scientific products:

1) Developing and refining advanced remote sensing techniques for the detection, monitoring and targeted sampling of HABs in a large and complex ecosystem. This is critical area of research for a number of funding agencies (e.g. NASA, NOAA)
2) Adapting and refining a fully coupled mechanistic ecosystem-hydrodynamic model developed by Center collaborators at the St. Johns River Water Management District (SJRWMD) to predict future growth and transport of HAB events under the effects of environmental/climate change.
3) Screening for new or emerging HAB toxins using cell based cytotoxicity assays and LC-MS based secondary metabolomics analysis.
4) The integration of toxicity and metabolomics analysis with metagenomic and metatranscriptomic data to understand the functional (expressed) features of the genomic landscape of the IRL.
5) Screening to identify potential new genes responsible for toxin production and biosynthesis, with the long term goal of the development of gene expression-based assays for the fast detection of toxins in coastal environments.
6) Initiating novel bio-monitoring studies designed to provide opportunities for extracting tissue samples from live aquatic animals for toxin analyses during non-bloom and bloom periods (most studies of uptake for HAB toxins have principally relied on access to dead animals during bloom periods). This novel approach will be preemptive, and permit collection of critical ambient baseline levels outside of bloom events that are needed to interpret the direct impacts of HABs on ecosystem health.
7) Studies on animal models that will quantify trophic position (via stable isotopes) and ecosystem-level interactions for multiple large-bodied indicator species previously unexplored in the IRL system. These data will reveal mechanisms of toxin transfer.
8) Merging individual-based methods (i.e., biotelemetry) with environmental data such as HAB spatial-temporal distributions. Despite acoustic telemetry being recommended as a valuable tool to answering linkages between spatial ecology and the presence of HAB toxins for several years, no studies have yet examined this relationship.
9) The proposed research will fill a significant gap in knowledge and analytical capability of detecting HAB toxins (and in particular, cyanobacteria toxins) in human populations. While the health effects associated with HABs have been relatively well established, a limited number of epidemiologic studies have assessed the impact of HABs on human health. Additionally, the characterization of potential symptoms related to toxin exposure among the local population will be a novel contribution and fill an important data gap.

Approach:

Understanding the connection between environmental change, the local ecosystem and HABs, and resulting human health impacts is the central mission of the proposed Center. The IRL is an economically and ecologically important estuary that appears at, or very close to, a tipping point with respect to the effects of climate change and the increasing number and severity of HAB events. As the scope of the HAB problems are increasing, the public is demanding information and action from water quality managers and policy makers from surrounding counties and districts on the underlying causes, and the current and future risks to health from these blooms (http://www.mynews13.com/content/news/cfnews13/news/article.html/content/news/articles/cfn/2016/3/29/brevard_county_fish_.html). Thus, how the potential toxicity and spread of these HABs will effect, both now and in the future, the over 1.6 million residents who live and work around the IRL is a primary Center goal. This is a critical time for IRL research, as no other federal projects or state or local agencies are conducting the required integrated approach to understanding the scope of the problem. The proposed Center will provide that mechanism, and has been both highly encouraged and recognized by scientists and managers from local agencies as a major need for the region (see Letters of Support). To achieve these advances in our understanding of the environmental and human health impacts of HABs and environmental/climate change in the region, we must conduct fundamental research into the environmental distributions and drivers of the HABs and their toxicology, along with concurrent investigations of health impacts (Codd et al. 1999). To that end, we have assembled a tightly integrated Center, with independent projects that work at different tiers of the problem to serve the Center's overall mission. A schematic of the relationships between the different Center Cores and Research Projects, their basic functions, and how communication and data will be exchanged is presented in Figure 3.

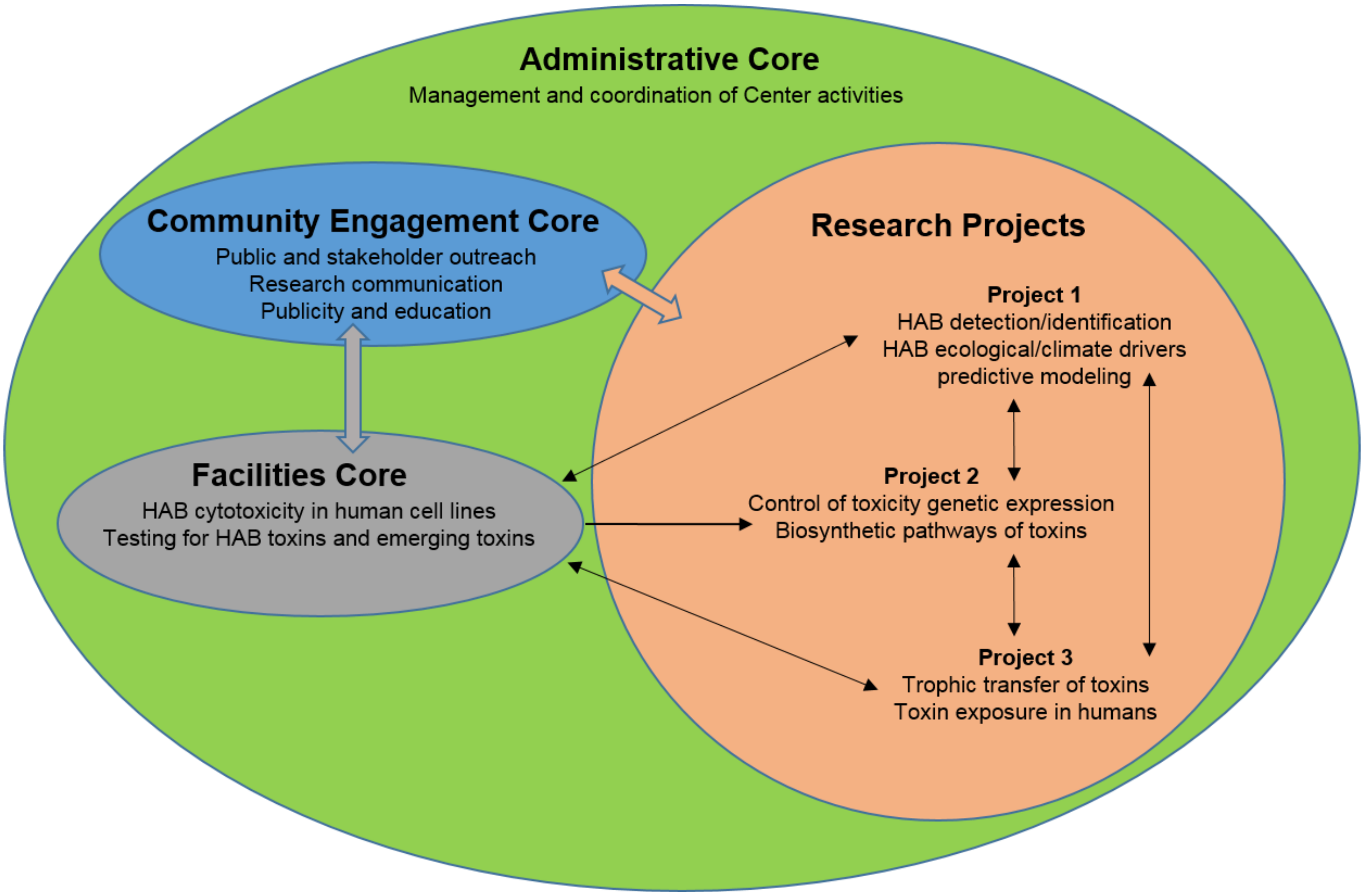

Figure 3. Schematic of the Center, its basic functions and communication/research interactions.

The Administrative Core (AC) will provide overall management and coordination of Center research and activities, including an active role in the community engagement core. In addition to management, the AC will assess both the scientific and community outreach successes of the Center and provide guidance to explore new collaborations or research directions that will enhance the Center's stated mission and goals. The AC Director and Co-Director, Dr. Sullivan and Dr. Wright, respectively, have the complimentary scientific experience required to effectively manage the scope of the Center's proposed research projects, where Dr. Sullivan has extensive experience (over 30 years) in HAB and oceanographic research problems and Dr. Wright has extensive experience (over 35 years) in biochemistry and biomedical research. The success of the AC will be determined by the research production of the Center (e.g. ecosystem level predictive models and other scientific products, scientific presentations and peer-reviewed publications), new collaborations and funding leveraged as a result of Center activities (e.g. successful grant applications and new PI recruitment into the Center) and effective community engagement activities (e.g. well attended public symposia, positive and impactful press and media reports, demonstrated actions taken by water management or health professionals as a result of Center activities, and interactions with other COHH3 investigators or Centers). The details of the specific aims and research strategy of this core are found in separate documents of this proposal package.

The Community Engagement Core (CEC) will facilitate multidirectional communication between research investigators, state and local stakeholders and the broader community with a goal of improving environmental health literacy. The health concerns identified by the facilities core and research projects will be addressed through various media outlets including a dedicated web presence, informational pamphlets, and direct communication via public lecture series and interactive displays at the FAU-HBOI Ocean Discovery Visitors Center. The specific aims of the CEC are:

1. Directly interact with Center research investigators to facilitate the interpretation of their findings. The CEC will work to fill a knowledge gap about the health risks associated with HAB events and environmental toxins. Information regarding significant health hazards will be identified and translated in a manner that is meaningful and relevant to a variety of audiences.
2. The CEC will utilize targeted methods to ensure that pertinent information reaches the general public. Communication efforts will be critical in providing a deeper understanding of environmental health, while engaging the community in prevention and safety practices.
3. To build and deepen partnerships with community organizations, stakeholders and agencies in order to improve and advance environmental health. The CEC will disseminate the findings to policy makers and public health officials and health care practitioners so that they can make informed decisions that will impact the community well beyond the scope of this project.

The CEC Director, Dr. Barbarite, is the Mission: Ocean Discovery Coordinator at FAU-HBOI. She has extensive experience in public education and outreach and oversees the FAU-HBOI Ocean Discovery Visitors Center (http://www.fau.edu/hboi/community/odc.php). To carry out the proposed objectives, she will work closely with a graduate student assistant funded by the Center and the experienced Communications team employed by FAU-HBOI (http://fau.edu/hboi/newsroom/newsroom.php). Center Director Sullivan and Co-Director Wright will work directly with Dr. Barbarite, to develop and continually update the outreach and community engagement plan of the CEC.

<u>The Facilities Core</u> (*Cytotoxicity and Metabolomics Core*) (FC) will provide data to the Research Projects on the toxicity and suites of secondary metabolites (including known and new toxins) found in project samples and materials. The specific research aims of the FC are:

1. Profile extracts from HABs in a panel of cell lines to define toxicity and provide data back to all three Research Projects.
2. Conduct untargeted metabolomics of secondary metabolites to identify toxins and other metabolites that correlate with HAB dynamics in samples collected in Projects 1 and 3 and provide data back to all three Research Projects for inclusion in systems based analysis.
3. Identify novel toxins in the system using database dereplication, bioassay-guided fractionation and spectroscopic identification.
4. Communicate data on toxicity and the secondary metabolome to all Projects and collaborate closely with the project investigators to correlate the data produced by the Core with data produced in the Research Projects.

Using cell based assays, the FC will identify HABs that show significant toxicity. The FC will also conduct liquid chromatography–mass spectrometry (LC-MS) based secondary metabolomics analysis with the goal of defining whether known toxins are present and consistent with the cytotoxicity observed. If a HAB is toxic and known toxins are not observed, samples will be further analyzed for the presence of previously unrecognized or emerging toxins in the system. The FC will also provide a MS-based secondary metabolomics profile of the system that can be incorporated into the larger modeling and meta-genomic and meta-transcriptomic signatures of the systems. These data may identify compounds associated with various stages of HABs (initiation, persistence, termination) and associated toxicity that can be used as predictive markers.

The activities of the FC will be conducted jointly by the Cell Biology Laboratory of Dr. Esther Guzmán (Core lead) and the Natural Products laboratory of Dr. Amy Wright. Dr. Guzmán has led the HBOI cancer drug discovery program for the past eight years and has extensive experience in testing for cytotoxicity. Dr. Wright is the Director of the State of Florida Center of Excellence in Biomedical and Marine Biotechnology and has over 35 years' experience in the identification of known and novel secondary metabolites from many different types of organisms both macro organisms and microbial. She has conducted two studies on natural products of bloom forming cyanobacteria in the IRL.

The Research Projects are composed of three independent, but highly integrated studies. A summary of the primary aims of each of the projects and their approach and relationship to the other projects is given below. Detailed descriptions of the projects are found in their research strategy documents of the proposal package.

Research Project 1: *Environmental drivers of HAB ecology in the IRL*.

Research Project 1 has two overall goals: 1) Define the scope of the HAB health threat in the IRL using advanced remote sensing techniques and real-time and autonomous environmental measurements combined with an intensive and targeted sampling program; and 2) Integrate project results into an existing IRL predictive ecosystem level model for use with HAB forecasting using mechanistic and statistical probabilistic approaches based on both historical environmental records and data collected through this project.

Background: Several Florida state agency based HAB monitoring efforts are currently on-going within the IRL, but are significantly constrained by budgetary limitations. The Florida Fish and Wildlife Conservation Commission (FWC) and Florida Department of Environmental Protection (FDEP) normally conduct water sampling only in response to large bloom events that have already developed, and exhibit high chlorophyll biomass or discolored water in the system. Samples are taken, the dominate HAB species are identified (but not always, e.g. Phlips et al. 2015) and assays for common toxins such as microcystin and saxitoxin may be conducted (emerging or unknown toxins are not addressed). This information is provided to the Florida Department of Health which may issue advisories based on toxicity levels. However, even in these cases, the true extent of the health threat is ambiguous. The number, location, and frequency of sample collection varies greatly depending on the number of bloom events that are actually detected. Because of this variation in effort, the collected data is difficult to use for assessing long term trends or ecological patterns of the distribution and abundance of HAB species and their toxins. More troubling, the health risks from HABs that are not evident from visual detection or high chlorophyll measurements, as for example, benthic/epiphytic blooms of toxic algae, or HABs that are not biomass or chlorophyll dominants (e.g. “cryptic blooms”, McManus et al. 2007) can be entirely missed by the agencies. Routine monitoring of phytoplankton (all species, toxic or not) at defined locations is conducted only by the St. Johns River Water Management District (SJRWMD) in the northern part of the IRL on a (usually) monthly basis (the SJRWMD is a collaborator on this project). This regular monitoring of dominant phytoplankton species allows for better insight into algal population dynamics and will be used as historical and on-going supplementary data, but sampling frequency is low relative to the possible growth dynamics of important HAB species, and only covers part of the ecosystem.

Continuous monitoring of parameters such as chlorophyll, temperature, salinity, and nutrients is conducted by several networks of in situ sensors in the IRL. In the northern half of the IRL, the SJRWMD maintains 5 sensor platforms spread out over approximately 125 km in the Mosquito Lagoon, Northern IRL, Banana River Lagoon, and Central IRL (http://www.sjrwmd.com/watershedfacts/). Further south, Center collaborator, Dr. Hanisak and FAU-HBOI, maintain a network of 10 Land Ocean Biogeochemical Observatory (LOBO) moored sensor platforms (http://fau.loboviz.com/) in the southern IRL and SLE covering a distance of approximately 50 km. The Ocean Research and Conservation Association (ORCA) in coordination with FDEP has also deployed 25 small autonomous sensor platforms throughout the extent of the IRL to monitor chlorophyll, nutrients, and other environmental parameters (http://www.teamorca.org/orca/orca-water-monitoring.cfm). In aggregate, these platforms provide high temporal resolution observations, but low spatial resolution relative to the overall size of the IRL, and very limited biological information beyond bulk chlorophyll concentrations. However, these publically available data are valuable as both historical and on-going monitoring of basic IRL environmental parameters that can supplement the ecological and modeling analyses of the proposed project.

This project will assess HAB dynamics in the IRL and associated environmental drivers in a rigorous manner for the first time. The scope of environmental characterization will be unprecedented for such a region, where synoptic remote sensing approaches will be used to adaptively guide collection of discrete samples for a broad array of lab analyses including metagenomic microbial community composition in the water column and benthic interfaces, HAB species assemblages, biogeochemical state, and toxin profiles. Predictive models will be developed that will quantitatively link these measured environmental variables to HAB initiation, growth, transport, and senescence. Mechanistic and probabilistic modeling approaches will provide complimentary predictive capabilities in space and time, enabling broader scope for predictability and increased confidence for overlapping space-time realms.

The specific aims of Research Project 1 are:

1. Apply ocean color remote sensing from UAVs and Landsat and Sentinel satellite imagers to spatially map phytoplankton distributions on small scales (< m to 60 m) throughout the IRL.
2. Directly characterize environmental hydrographic, biogeochemical, phytoplankton composition, physiology, and optical parameters in-water with state-of-the-art, real-time instrumentation.
3. Assess phytoplankton community structure and health with custom holographic imaging microscopy, flow cytometry, and conventional light microscopy.
4. Assess nutrients, nutrient N^{15} isotopes, and organically bound carbon, nitrogen, and phosphorous to understand the effects of nutrient composition and source on HAB growth dynamics.
5. Assess microbial community composition associated with the sediment/water interface, water column, and macrophytes by applying metagenomic shotgun and targeted 16S/18S rRNA sequencing techniques, while supplying similar water/benthic samples to Research Project 2 for further omics analysis.
6. Apply microbial diversity metrics and community-based indicators (CBIs) to assess shifts in microbial composition before, during and after blooms and links to HAB toxicity.
7. Test, validate, and refine a fully coupled mechanistic ecosystem-hydrodynamic model developed by collaborators at the SJRWMD that will be adapted to predict growth and transport of HAB events.
8. Develop and test a probabilistic multi-variate ecosystem niche model based on historical records and our extensive data sets to predict and understand environmental drivers of HABs.

The primary impact of this project will be a far deeper understanding of how environmental/climate change and associated environmental drivers control HAB dynamics and toxicity. The predictive model products for HAB initiation, growth, and dispersion within the IRL will be used by local agencies including the St. Johns River Water Management District (SJRWMD), South Florida Water Management District (SFWMD), Martin County, and IRL National Estuary Program (NEP) (see Letters of Support) to more effectively manage resulting human health threats.

The highly interdisciplinary Investigator team for this project includes Drs. Twardowski (Project Lead), Sullivan, and Moore (UNH), established experts in ocean color remote sensing techniques, HAB detection, and HAB dynamics, Dr. Lapointe, a seminal expert in HAB ecology with over 20 years' experience studying HABs in the IRL and related nutrient dynamics, Drs. McCarthy, Dickens, and O'Corry-Crowe, with expertise in metagenomics and bioinformatics, and Drs. Jiang and Jacoby (SJRWMD), providing expertise in modeling coupled ecosystem-hydrodynamics in the IRL. The primary outcome of this project will be a far deeper understanding of how environmental/climate change and associated environmental drivers control HAB dynamics and toxicity. Predictive models for HAB initiation, growth, and dispersion within the IRL will be used by local agencies (i.e. SJRWMD, Martin County Commission, South Florida Water Management District, IRL National Estuary Program) to more effectively manage resulting human health threats. Results will have the potential to dramatically improve assessments of related human health threats for the region and directly support the underling research of the other two research projects.

Research Project 2: *Refining the functional dynamics of toxic algal blooms in coastal waters.*

Research Project 2 has the overall goals of 1) identifying genes that are associated with the toxin production in HABs that may impact human health and 2) understanding the dynamics of this functional gene expression in relation to ecological dynamics of HAB events. This will be achieved through describing the microbial metatranscriptomic landscape of the IRL, identifying the potentially functional biosynthetic components of the metagenomic landscape, and integrating these data to identify transcriptionally active components of the biosynthetic pathways. Samples for this research project will be collected in conjunction with the field efforts of Research Project 1, and also require specific results from Research Project 1 to interpret the underlying ecological and environmental drivers behind the omics project findings.

The specific research aims of Project 2 are:

1. Describe the metatranscriptomic landscape of the Indian River Lagoon.
    1.1. Develop a molecular workflow for the combined extraction of RNA and DNA from water in a form suitable for high-throughput sequencing.
    1.2. Develop a bioinformatics workflow to assemble messenger RNA transcriptome of prokaryotic and eukaryotic microbes in the IRL and measure their abundance.
    1.3. Develop a bioinformatics workflow to annotate assembled transcriptomic units.

2. Identify functional features of the metagenomic landscape of the IRL.
    2.1. Compare the annotated transcriptomic units with OTU data from Research Project 1 to identify FTUs.
    2.2. Perform differential expression analysis of the FTUs to find FTUs associated with HABs and integrate with other environmental data available from Research Project 1.

3. Identify the HAB toxin biosynthetic components of the multi-omic landscape
    3.1. Use biosynthetic screening to identify prokaryotic gene clusters that may produce toxins.
    3.2. Investigate the largely elusive (to date) biosynthetic pathways for toxins produced by eukaryotic dinoflagellates and diatoms.
    3.3. Identify transcriptomic activity of predicted gene clusters in the metatranscriptomic landscape.
    3.4. Identify differential expression of the identified functional members of the gene clusters that are associated with HAB events.

In order to identify the components of the biological molecular environment and changes associated with HABs in the IRL, it will be necessary to understand the functional, i.e. transcribed, aspects of the genomic environment. This will allow an examination of the differential expression of these components in relation to HAB events, and identification of functioning biosynthetic pathways of the microorganisms associated with toxin production in the HABs. Information about the metagenomic landscape of the IRL from the research results of Research Project 1 and toxicity and metabolomics analysis from the Facilities Core, will be combined with the metatranscriptomic information, to go from the identification of operational taxonomic units (OTUs), a standard metric in metagenomics, to the identification of functional taxonomic units (FTUs), allowing an examination of the relationship between the metagenomic environment and the metatranscriptomic environment as a whole. Samples will also be screened to identify the presence of biosynthesis genes and pathways for HAB toxins with the aim of identifying the functional components responsible for the toxin production of the HABs. In the long term, the integration of metagenomic data, metatranscriptomic data and the screening of toxicity and toxin genes may allow identification of new genes responsible for toxin production.

The outcome of this research project will describe the most abundant microbial gene transcripts, their potential function, which taxonomic units are responsible for the production of these transcripts, and the presence of potential toxin-producing transcripts in these samples. This should lead to a better understanding of the functional microbial landscape of the IRL, and how this landscape changes in relation to HAB ecological dynamics. Ultimately, this could lead to the development of novel gene expression-based assays for the fast detection of toxin risks in coastal environments.

The investigative research team of this project includes Drs. Dickens (Project Lead), O'Corry-Crowe and Wang. Dr. Dickens has extensive experience of bioinformatics and computational biology (15 years), he has worked on large international genome projects, developed methods for analysis data from non-standard and new genomes, and the application of genome and transcriptome analysis to many aspects of human health and disease, from type-2 diabetes and heart disease, cancer biology and parasitology. Until recently he lead the core bioinformatics and an experimental sequencing team at the Wellcome Trust Centre for Molecular Parasitology, developing novel analysis pipelines and bespoke sequencing methods for non-standard parasite samples and non-model organisms. Dr. O'Corry-Crowe has applied molecular techniques to study population viability and individual fitness of organisms in the ILR since 2007. Crowe has strong expertise in molecular biology and the preparation and sequencing of nucleic acid material from water, marine organisms and also from difficult samples (such as ancient DNA, aDNA). He led a molecular ecology research team at NOAA for 15 years, and now leads the Population Biology and Behavioral Ecology (PBBE) program and established the aDNA lab at HBOI-FAU. His latest projects incorporate multiple -omics fields to study host-pathogen interactions in aquatic environments. Dr. Wang's research group focuses on the biosynthesis and biocatalysis of marine natural products, especially those with potential applications in medicine and agriculture. His research includes cloning gene clusters from culture-dependent or -independent sources, determination of biosynthetic pathways, metabolic engineering of pathways, and functional and structural elucidation of key catalysts in pathways of interest. He is especially interested in the exploration of new methods to identify novel natural products from cryptic gene clusters in microbial genomes and from uncultivable microorganisms in marine habitats.

Research Project 3: *Establishing shared routes of exposure to HAB toxins in the IRL: the intersection of ecosystem and human health.*

Research Project 3 has the overall goals of 1) understanding HAB toxin trophic transfer in various components of the IRL food web during bloom and non-bloom seasons, as well as refining the trophic roles and distribution patterns of large-bodied "indicator" species through analyses of diet and movement patterns and 2) simultaneously assessing human exposure to HAB toxins via direct sampling of individuals through a collaboration with the Centers for Disease Control (CDC).

The specific research aims of Research Project 3 are:

1. Determine HAB toxin concentrations in IRL marine life from various taxonomic groups through novel bio-monitoring surveys.

2. Evaluate trophic transfer mechanisms of HAB toxins from primary producers to consumers through analyses of diet and stable isotopic signatures.

3. Reveal spatial response and exposure rates of mobile consumers to HAB blooms via analyses of historical biotelemetry data and new acoustic tagging initiatives.

4. Assess potential impacts of HAB toxin exposure on human populations through time-series analyses of hospital data during bloom and non-bloom periods.

5. Conduct direct evaluation of concentrations of HAB toxins among tissues from local residents during and post bloom events in south Florida.

This research will fulfill a major scientific knowledge gap in understanding IRL ecosystem toxicology, bioaccumulation and trophic transfer through integration of wildlife and human-based empirical studies. To best identify potential uptake pathways to humans, the project will use both air-breathing and fully submerged animal models from various trophic guilds: piscivores (dolphins, sharks), invertivores (rays, fishes), and herbivores (green sea turtles). The project will simultaneously assess human exposure to environmental toxins via direct sampling of individuals. These individuals will also be queried for their physical interactions with the IRL, including consumption behavior of fisheries resources (finfish, shellfish, etc.). The scope of the current center proposal will enable the team to integrate quantitative temporal and spatial data on the HABs, including duration, intensity and precise spatial distribution into the human health assessment. These data will be combined with results obtained from marine indicator species that interact with the same resources, with an overall goal of evaluating HAB toxin exposure pathways and trophic transfer mechanisms in the IRL system.

This research project has assembled a team of scientists with significant complementary expertise: Dr. Ajemian (Project Lead) is a fish ecologist that studies trophic interactions and movement ecology, Dr. Page-Karjian is a wildlife veterinarian specializing in health and ecology of marine vertebrates (particularly sea turtles), Dr. Fire (FIT) is a biological oceanographer with specific training in marine mammal toxicology and food web dynamics, and Mr. Schaefer, MPH is an epidemiologist with strong experience and training in environmental and human health . His research has included both human and wildlife population health studies in and along the IRL. Much of this research team is currently collaborating on other associated projects that will leverage the results developed herein.

Center Summary: The proposed, highly integrated Center will fill a critical need for understanding the significant health threats associated with HABs in the IRL ecosystem, and how these threats will be effected by environmental/climate change. The major products of the Center will include an in-depth characterization of HAB ecology in the IRL system (e.g. distributions, physiology, environmental drivers, taxonomy with metagenomics, toxicity), the refinement of an ecosystem level model to predict the effects of environmental/climate change on the occurrence, distributions and transport of HABs in the IRL ecosystem, a better understanding of the functional microbial landscape of the IRL, and how this landscape changes in relation to HAB ecological dynamics, as well as a better understanding of functional genetic components responsible for the toxin regulation and biosynthesis, a new understanding of HAB toxin trophic transfer in important components of the IRL aquatic food web (i.e. indicator species) and lastly, epidemiological studies on the direct exposure of regional human populations to HAB toxins. The Center will actively seek to educate and engage the public on Center results and work with numerous state and local stakeholders to fill a major scientific need required to protect the health and safety of the region's population.

Outlook for Center Development: The research of the Center will be responsive and flexible to new collaborations and research directions that will enhance the Center's stated mission of understanding how environmental/climate change will impact human health. For example, in addition to understanding the effects of climate change on IRL HAB problems, rising sea levels will change both the circulation patterns and the hydrology of the IRL ecosystem. The IRL has known issues with mercury and other metal contamination in sediments and wildlife (and likely humans) (e.g. Trocine & Trefry 1996, Strom & Graves 2001, Durden et al. 2007). Changing hydrology and circulation patterns could move/transport these contaminates to new areas of the ecosystem and pose increased risks to human populations. In addition, increasing precipitation and freshwater runoff with rising water temperatures could increase the occurrence/abundance of known aquatic pathogens in the IRL that favor warm waters with lower salinity (e.g. species of *Vibrio* or other pathogenic bacteria, Schaefer et al. 2009, Barbarite 2016). The current structure of the proposed Center would be responsive to researching these problems through the adaptation of different protocols and methods, but with a similar tiered research approach. For the *Vibrio* issue specifically, results from planned metagenomics assays should detect and map their occurrences in the IRL, however, understanding associated pathology or variations related to environmental changes would require additional research and collaborators. It is important to note that some of the Center's investigators have already conducted (Dr. Barbarite -*Vibrio* in the IRL), or are actively conducting research (Schaefer – mercury exposure in local pregnant women) into these issues. In addition to these potential future research directions, a number of Center investigators either have pending proposals or are actively funded to conduct research on HABs in the IRL system through other federal agencies and foundations (e.g. NOAA, EPA, NASA, Everglades and HBOI Foundations). These existing projects will be leveraged to help Center research. Future projects that can enhance or further leverage Center research will continually be developed by Center investigators. New PIs/investigators that can fill knowledge gaps or enhance Center research goals will be recruited as needed.

FAU-HBOI has recently been designated to become one of FAU's four "Research Pillars" (https://www.fau.edu/research/institutes-pillars/index.php): 1) ocean and environmental science (FAU-HBOI); 2) sensing and smart systems; 3) neuroscience; and 4) healthy aging. Under this research structure there are additional resources and incentives to collaborate and develop projects with other colleges and researchers throughout the large FAU system (6 campuses with over 180 degree programs and hundreds of faculty). The proposed Center fits into the new FAU pillar concept of expanding collaboration and leveraging expertise across campuses to increase the research enterprise of FAU. In addition to the development of new internal lines of research and leveraged funding to expand Center research through the pillar, external collaborations will be developed under the guidance of the Center's internal and external steering committees.