

# HARBOR BRANCH

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April 20, 2017

**To:** Harbor Branch Oceanographic Institute, Inc. Foundation Board

**From:** Anton Post, Ph.D., FAU HBOI Executive Director

**cc:** Daniel Flynn, Ph.D., FAU Vice President for Research  
Megan Davis, Ph.D., FAU HBOI Associate Executive Director for Academic  
Affairs and Economic Development  
Patrick Boles, FAU HBOI Associate Executive Director for Operations

**Re:** FAU Harbor Branch Highlights since January 2017 HBOIF Board Meeting

## 1. HBOI Foundation Research and Education Grant Updates – for Active Grants in FY2017

A. *Applying New Technologies to Transform Marine Natural Products Drug Discovery (PIs: Amy Wright, Esther Guzmán, Peter McCarthy, Shirley Pomponi) Grant Period of Performance 3/22/15-3/21/16; NCE End date: 3/22/17*

### **Specific Aim 1: Implement New High Content Screening Assays in Cancer Immunology**

*Milestone 1.1 Develop High Content Assay to identify Compounds that modulate cancer immune function*

- Two assays were developed: one that targets B7-H4, a costimulatory molecule whose expression enhances oncogenicity and inhibits apoptosis in pancreatic cancer cells and one that targets FoxP3, a receptor whose expression helps pancreatic cancer cells evade an immune response.

*Milestone 1.2 Assay a minimum of 250 materials from the HBOI Enriched Library and confirm activity*

- 961 peak library samples and 127 pure compounds were assayed in the FoxP3 assay. 58 fractions that exhibited more than 50% inhibition with less than 30% cytotoxicity were identified as potential hits. Activity for potential hits given to chemistry was confirmed by re-testing. No pure compounds fit our criteria for a hit.
- 910 peak library samples and 127 pure compounds were assayed in the B7-H4 assay. 101 fractions that exhibited more than 50% inhibition with less than 20% cytotoxicity were identified as potential hits. Activity for potential hits given to chemistry was confirmed by re-

testing. 19 pure compounds fit our criteria for a hit. Two pure compounds had their activity confirmed through a secondary assay.

*Milestone 1.3 Conduct initial chemical analysis of actives From Aim 1.2*

- 14 hits in the FoxP3 and 21 hits in the BH74 cancer immunology assays have been analyzed by HPLC and NMR identifying a number of chemically rich fractions for further investigation.
- Bioassay-guided fractionation of one of the BH74 hits led to a pure compound and the structure was identified. Confirmatory secondary assays are being conducted with other funding.

*Milestone 1.4 Prepare and submit a grant proposal to NIH to continue the biological screening and conduct full chemical investigation of active materials.*

- A grant proposal for submission to NIH is currently in preparation by Drs. Wright and Guzmán.

## **Specific Aim 2: Implement New Assays to Transform Antibiotic Drug Discovery**

*Milestone 2.1-2.3 Implement assays for bacterial biofilm disruption and quorum sensing inhibitors and screen materials in the assay.*

- An extensive series of experiments were conducted to create an assay for bacterial biofilm disruption using the High Content Imaging system. Although biofilms were being produced under lab conditions, and these could be visualized using an older established system, the new system proved to be unreliable due to the complexity of the required image analysis. Since much of the time allocated to this project was used in trying to establish this system without being able to generate useful results, our focus in the last quarter shifted to testing for antibacterial activity using conventional assays.
- Selected fractions prepared in Aim 3 were tested for antibiotic activity against the drug resistant pathogens MRSA and *Pseudomonas aeruginosa* and 33 actives were detected.
- The 33 fractions and the pure compound were sent to the Connell Laboratory at Rutgers for profiling against a panel of clinical MRSA and Enterococci strains, the ESKAPE pathogens and possibly some of the Select agents (*Bacillus anthracis*, *Yersinia pestis* etc). Data from this further testing will be used to support a joint grant proposal on discovery of natural products from marine sources. Testing is currently in progress.

*Milestone 2.4 Conduct initial chemical analysis of actives From Aim 2.3*

- One compound with activity against MRSA and *Pseudomonas aeruginosa* has been identified by bioassay-guided fractionation followed by NMR analysis from these hits. It has been sent to Rutgers.
- Dragmacidin G was assayed against MRSA and showed an MIC of 1  $\mu$ M with IC<sub>50</sub>s approximately 15 fold higher against mammalian cell lines suggesting a good selectivity index for MRSA. This work was published by Wright et al. in the journal *Marine Drugs* in January 2017 [Dragmacidin G, a Bioactive Bis-Indole Alkaloid from a Deep-Water Sponge of the Genus *Spongosorites Mar. Drugs* 2017, 15(1), 16; doi:10.3390/md15010016. <http://www.mdpi.com/1660-3397/15/1/16>]. The paper was published open access. The abstract has been viewed 1435 times and the full paper viewed 642 times since its publication on January 11, 2017. Dragmacidin G has also been sent to Rutgers.

### **Specific Aim 3: Enable discovery of novel chemotypes from the HBOI Organism Repository through expansion of the enriched fraction library**

*Milestone 3.1 Ferment 30-50 microbes from the HBOI collection and conduct fractionation to add materials to the Enriched Fraction Library*

- For the microbiology side of this project the focus was on the fungi that are held in the Harbor Branch Marine Microorganism Culture Collection and a novel method was tested for the induction of natural product production in which a fungus is grown on a solid medium in the presence of a competing bacterium. Using this method 333 samples were generated that will be added to the Peak Library.

*Milestone 3.2 Extract and conduct fractionation on 25-50 unusual, chemically rich organisms and add materials to the Enriched Fraction Library*

- Fractionation was completed on 37 macro-organisms with 760 fractions added to the enriched fraction library.

*Milestone 3.3 Submit fractions from 3.1 and 3.2 to HBOI assays and partner laboratories for broad based bioassays*

- Selected samples (those richest in chemistry) have been tested as follows: 1) for modulation of the cancer related immune modulatory proteins BH74 and FoxP3; 2) against the bacterial pathogens MRSA and *Pseudomonas*; 3) in a newly developed antitumor assay that uses 3D-tumor spheroids as models of intact tumors. Active fractions have been identified in all assays.
- Agreements were put in place for shipping of samples to two new partners: the Connell/Russo laboratory at Rutgers (broad antibacterial screening including ESKAPE pathogens and Select Agents) and the O'Connor laboratory at Washington State University (Toxoplasmosis and *Cryptosporidium*).

- Samples have been shipped to the Connell/Russo laboratory

## **Specific Aim 4: Develop innovative approaches to produce natural products and understand factors driving biosynthesis**

### *Milestone 4.1 Optimize conditions for gel microdroplet encapsulation of at least 10 species of sponges*

- Production of gel microdroplets has been optimized. The design for the microfluidic chips has been simplified, and new microfluidic chips for production of the microdroplets have been made. Encapsulation of cells from model sponge species is in progress.

### *Milestone 4.2 Create sponge cell mixtures from dissociated cells from at least 10 different sponge species pairings*

- Metabolite profiles of 18 sponge species were evaluated, and based on these data, mixtures of cells from 7 species in 11 different species pairings were prepared. As part of Specific Aim 3, Dr. Wright analyzed species mixtures by LC-MS. The samples showed differences in chemistry that we are continuing to explore. An unexpected outcome resulted during evaluation of results of high content imaging of sponge cell metabolism: results of an independent test for protein expression suggest that sponge cells may arrest cell cycling in response to DNA damage. This is a finding that is important to both establishment of a sponge cell line as well as to a broader understanding of mechanisms of DNA damage and repair.

### *Milestone 4.3 Develop at least one gel microdroplet bioassay to identify novel bioactive species*

- This aim has not yet been completed, however, a PhD student is developing a GMD antimicrobial assay as part of her thesis research.

### *Milestone 4.4 Submit grant proposal to the NIH, NSF, EU, or Dutch NWO for continued support of the research*

- This research has been leveraged with grant support from NOAA (CIOERT-Vulnerable Sponge Ecosystems). Based on our recent exciting results related to cell cycle arrest and DNA repair, Drs. Pomponi and Guzmán will be preparing a proposal to submit to NIH and/or NSF.

## **B. Love Your Lagoon: Seagrass Nursery for Indian River Lagoon Restoration (PI: Dennis Hanisak, Co-PI: Paul Wills) Grant Period of Performance 4/20/15-6/30/16; NCE End Date 6/30/17**

Since the last update the initial planting of *Halodule* (plugs of seagrass transported from the field in pots) has been completed in all six of the seagrass nursery tanks in November, after the end of growing season. The experimental tanks continue to be maintained, **which includes** keeping epiphytes in check and fertilizing with slow-release fertilizer (Osmocote) as a source of nutrients.

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Over the winter, there was some observed thinning in seagrass biomass in the tanks, similar to what occurs seasonally in the IRL. Over the spring, it is expected to see expansion of seagrass in the tanks, as that is the time of year seagrass growth is maximal in the IRL.

One of the biggest challenges in using a closed system for this nursery is that the water temperature in the summer gets quite high (exceeding 35 °C at times). Our initial budget did not allow for any type of temperature control to alleviate that problem, therefore, last summer the impact of high temperatures were mitigated by shading the tanks. This kept the water temperature within the temperature range that *Halodule* can tolerate, yet provided sufficient light for survival. This solution may have been suboptimal for growth. Looking ahead to this summer, this month funds were rebudgeted to address this issue. By June, there will be an on-line system that will provide adequate temperature control to eliminate high temperatures as a stressor in the nursery. This should improve the ability to cultivate seagrass year-round in the HBOI seagrass nursery.

Lastly, the PI has been communicating with Florida Fish and Wildlife Commission and St. Johns River Water Management District about providing support to continue the nursery effort and/or conduct a pilot-scale demonstration for transplanting seagrass from our nursery into the IRL in the coming year. By June, it will be known if funds will be available for FY 2018 from these sponsors.

## *C. Love Your Lagoon: Indian River Lagoon Graduate Research Fellowships (PI: Peter McCarthy) Grant Period of Performance 5/16/16- 5/5/17*

Through the 2016 Love Your Lagoon Gala event, the Harbor Branch Foundation raised \$111,350 in support of the Graduate Research Fellowships. Combined with a small carry forward (\$3,775.55) from the 2014 Fellowships, these funds supported Fellowships up to a total of \$115,125.55.

A Request for Proposals was issued on March 4<sup>th</sup>, 2016 and proposals were received from 14 FAU students who are performing research relevant to the Indian River Lagoon while working with a member of the FAU-HBOI faculty. Requests totaled \$131,578.97.

Following an internal review, all fourteen students received funding for their projects: \$62,830 (54.6%) was committed to student research assistantship salary; \$3,597.00 (3.1%) was for tuition; \$6,625.50 (5.8%) was for travel related to experimental work and presentation of research at scientific conferences; and \$42,073.05 (36.5%) was for the purchase of necessary small equipment and supplies related to the graduate students' research projects.

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The funds are available to the students until May 5<sup>th</sup>, 2017 after which the students will provide a report of their progress. This will be compiled into a full report on the use of the funds. To date, \$101,604.64 (88.25%) of the award has been expended. The students presented their work at both the Indian River Lagoon Symposium and at FAU's 8<sup>th</sup> Annual Graduate and Professional Research. Many of the students attended the 2017 Love Your Lagoon Gala event and we were very pleased to be told that proceeds from this event will again be used to support the Indian River Lagoon Graduate Research Fellowships; the review of the 2017 LYL Gala proceeds applications is currently in progress.

## 2. FAU HBOI Research and Education Selected Highlights

- a. Mission Ocean Discovery, Ocean Discovery Visitor Center: To date there have been 1,852 visitors, tours have been fully book, Marine Science Fridays have been full, and gift shop is doing well. The plan is to keep the ODVC open through the summer and fall. There will be a Link summer intern (G. Barbarite and D. Hanisak co-mentors) and the intern will be involved in advancing a LOBO and Marine Ecosystem Health exhibit.
- b. President Kelly and Carolyn Boltin-Kelly announced at the FAU Gala that a portion of the proceeds will support the new MS Marine Science and Oceanography degree and Marine and Coastal Affairs undergraduate fellowships.
- c. MS Marine Science and Oceanography degree program update: The proposal/budget moved through a series of committees over the past few months with the goal to launch the degree fall 2017. The last committee: University Faculty Senate passed the degree. The last steps will be approval by Board of Trustees on May 16 and BOT. This is a joint degree with College of Science and HBOI.
- d. On the HBOI website under research personnel there is a table that can be downloaded or referenced that shows all the faculty, project managers, postdocs, and affiliated faculty and their areas of expertise.
- e. HBOI, USDA-Aquaculture Research Service (ARS), NOAA Aquaculture and prominent researchers and industry members (28 in all) attended the March 23-24 Marine Fish Aquaculture Scoping Workshop. A white paper report is being developed and will be a key reference document for advancing marine fish aquaculture in the US.
- f. Development – Spring Annual Appeal letter will be sent out the end of April.
- g. Large proposal submissions: NIH/NSF Center for Ocean and Human Health (\$5M); EDA Tech Runway HBOI (\$2M), and NOAA Sea Grant (\$1.5M) – Aquaculture Commercialization.
- h. Bonefish & Tarpon Trust held its annual board meeting at HBOI on 8 Feb 2017. HBOI and FIT researchers provided progress reports for their \$1M research project sponsored by BTT.