

**RESEARCH INSTITUTION: :”BASELINE AND OIL SPILL IMPACTED MARINE SPONGE MICROBIAL COMMUNITIES AND GENE EXPRESSION ANALYSIS WITH METAGENOMICS”**

**Jose V Lopez<sup>1</sup>**, Rebecca Vega Thurber, Peter McCarthy, Patricia Blackwelder, Marie Cuvelier  
1- Nova Southeastern University  
Oceanographic Center  
8000 North Ocean Drive  
Dania Beach, FL 33004

**SCIENCE ACTIVITIES**

1) General Summary

*Narrative (1 pages maximum): Please provide a brief overview of the project and goals supported during the conduct of this project. Be sure to highlight any ‘lessons learned’ that could be applied to the conduct of RFP-I and RFP-II projects (e.g., management, data support, logistics, etc.). Listing accomplishments against project activities, objectives and milestones in bulleted form is acceptable.*

The central aim of this proposal was to develop and characterize “sentinel” sponge species and their associated microbiota along with advanced molecular and genomic tools to assess the impact of oil contamination on Western Florida shelf reefs. Marine sponges and their associated microbes are excellent environmental sentinels. Using next generation transcriptomics and metagenomics we can simultaneously trace the direct impact of crude oil (and byproducts) and dispersants on both sponge physiology (a general marker of reef health) and microbial community dynamics (a general marker of regional seawater quality). Evaluating shifts in the composition and function of these organisms and their resident microbes will allow us to determine the overall effects of hydrocarbon loading in the water column that have resulted from the Deepwater Horizon oil spill.

Our primary approach was the application of modern “next generation” DNA sequencing and electron microscopic methods.

We also shifted our experimental strategy slightly from the original proposal, by focusing almost totally on an experimental approach. This was emphasized due to the availability of Dr Becky Thurber’s closed tank system at FIU.

Within the first quarter, we formed a working team comprised of the PI’s and postdoctoral research scientist Dr Marie Cuvelier, and NSU graduate student Emily Smith (see Table below).

Lessons learned – apply for more shiptime, and coordination with other BP PI’s for collections.

2) Results and scientific highlights

*Narrative (2 pages maximum): This should be a summary of significant results (positive and negative) and conclusions during the conduct of this project. Listing science results and highlights in bulleted form is acceptable. In each case, please explain the impact of the result.*

A major aim was to select and assess an appropriate candidate sponge species as our model taxa. This was not a trivial endeavor, as several taxa are available from the study area and a good match between the experimental applications and species is required. This process is ongoing as well as preliminary SEM ultrastructure microscopy. We were able to perform preliminary scanning electron microscopy of potential candidate species (*Agelas*, *Cinachyrella*, and *Ircinia*). A large number of filamentous bacteria appeared in the *Ircinia* sample.

We collected and archived marine sponges from Broward county reefs as potential candidates for metagenomic and experimental studies. Co-PI Dr Peter McCarthy from Harbor Branch Oceanographic Institute at Florida Atlantic University sent the two archived samples for NSU for analysis.

Co-PI Dr Pat Blackwelder performed scanning and transmission electron microscopy of potential candidate species (*Agelas*, *Cinachyrella*, and *Ircinia*). The EM studies have revealed unique structures within *Cinachyrella* that appear in both freshly-collected field samples, and in samples that have been maintained in aquaculture for up to 2-3 weeks. EM micrographs are attached.

- A Sanger clone library from the sponge *Cinachyrella* sp. showed many sequences most similar to *Chloroflexi* and *Actinobacteria*, with a few other sequences belonging to other bacterial groups.
- Graduate student Emily Smith prepared pilot RNA samples from control and treated *Cinachyrella alloclada*. Several of these samples have been sent to the Genomic Sciences Laboratory at North Carolina State University, (Raleigh NC 27606) for pilot Illumina platform RNA sequencing. After an unexpected delay of > 3 months due to purported machine problems at NC State, results have now been transferred to our lab totaling approximately 39 million high quality reads which have at least on reported alignment. The reactions have been analyzed by bioinformatics collaborator, David Willoughby of Ocean Ridge Biosciences. In the latter half of 2012, we submitted 16 more X2 and X3 replicates to Argonne National Laboratory for more RNA-sequencing. We are still awaiting the results and analysis.
- Three replicate oil dosing experiments (labeled as X1 – X3) were performed on the reef sponge *Cinachyrella alloclada* based on standard CROSERF protocols. Over 80 different sponge samples were exposed to 0.5 ppm oil, oil mixed with 10% Corexit, and Corexit 9500 only.
- Partial 16S rRNA gene sequences were amplified with universal PCR primers in order to globally amplify and characterize as much of the sponge microbial symbiont community as possible. Metagenomic amplicons were sent to the UKY core lab which used 454 Roche GS Titanium next generation DNA sequencing technology. The first X1 experiment generated a total of 153,000 raw sequence reads. This was further narrowed

to a final total of 97,267 high quality 16S rRNA reads. X2 and X3 experiments have produced similar DNA yields, for a total number of >388,000 16S rRNA sequences By the whole project.

- Dr Marie Cuvelier has completed the analysis of XI- X3 data, applying state of the art 16S rRNA analyses, such as QIIME. For example, she has performed comparisons of taxonomic identifications using UCLUST and CDhit options. Rarefaction analysis showed interesting differences between seawater and sponge microbial communities. For example, among all seawater samples, the seawater control had the highest number of predicted OTU’s (operational taxonomic units), while among sponge samples, the oil-dispersant sample at the 24 hr timepoint showed the highest microbial diversity. The analysis also show fairly wide changes in cyanobacteria and actinobacteria diversity in control samples. Only minor trends suggest the greatest increases in bacterial diversity occur in oil/dispersant treated samples. For the most part there are no major shifts in the bacterial community, which refutes the working hypothesis of this project. Consequently, most physiological changes likely stem from within the sponge host

We expect that a minimum of two manuscripts will result from this research.

We have also tested new primers for optimal and most comprehensive amplification of 16S rRNA gene fragments. Through a collaboration with Earth Microbiome Project (EMP), new universal primers have now been utilized.

Table of sponge subsamples taken from each treatment and the intended application of each sample in Experiment X1.

	<b>Oil (WAF) (0.2 gm/L)</b>	<b>Chemically enhanced (CE) –WAF</b>	<b>Dispersant only (Corexit 9500A)</b>	<b>Untreated seawater control</b>
<b>RNA</b>	Yes	Yes	Yes	Yes
<b>DNA</b>	Yes	Yes	Yes	Yes
<b>Electron microscopy</b>	Yes	Yes	Yes	None
<b>FISH</b>	Yes	Yes	Yes	None
<b>Water samples for bacterial filtration/genomics</b>	Yes	Yes	Yes	Yes

### 3) Cruises & field expeditions

N/A

<b>Ship or Platform Name</b>	<b>Class (if applicable)</b>	<b>Chief Scientist</b>	<b>Objectives</b>	<b>Dates</b>

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- 4) Peer-reviewed publications, if planned (Note: a special section will focus on student and post-doctoral publications)
  - a. Published, peer-reviewed bibliography (Copies of the papers are requested)
  - b. Manuscripts submitted or in preparation (Please note target journal, and anticipated date of publication or submission)

Manuscripts In progress as described above.

- 5) Presentations and posters, if planned (Please provide copies of each) (Note: a special section will focus on student presentations)

Title	Presenter	Authors	Meeting or Audience	Abstract published (Y/N)	Date
Life at the bottom: Marine Sponges as Microbial Incubators and Bioindicators,	Jose Lopez	R Thurber, P Blackwelder	“Climate-Sustainability Lecture Seminar Series”, Nova Southeastern University	N	Feb 10, 2012
Synergistic Effects of Crude Oil and Corexit Dispersant on a Sponge Holobiont System	Jose Lopez	Marie Cuvelier, Peter Larsen, Patrica Blackwelder, Emily Smith, Pete McCarthy, Jack A. Gilbert, Yonggan Wu, David Willoughby, Rebecca Thurber	Annual Meeting of the Society of Integrative and Comparative Biology, San Francisco, CA	Y	January 2, 2013
Synergistic Effects of Crude Oil and Corexit Dispersant on a Sponge Holobiont System	Jose Lopez	Marie Cuvelier, Peter Larsen, Patrica Blackwelder, Emily Smith, Pete McCarthy, Jack A. Gilbert, Yonggan Wu, David Willoughby, Rebecca Thurber	Gulf Of Mexico: Oil Spill & Ecosystem Science Conference, New Orleans LA	Y	January 19, 2013

- 6) Other products or deliverables

*Please list (for example: maps, models, tools) and indicate where they can be located/obtained.*

- Novel sponge model
- 16S rRNA gene and RNA-seq EST sequences. These will be deposited into various databases such as GenBank, Dryad, MG-RAST
- Electron micrographs of sponge models and microbial symbionts
- Master’s thesis (NSU) – Emily Smith

7) Data

*Reporting on data is done separately through communications with Harte Research Institute; however, please provide a spreadsheet indicating the metadata and ancillary information on the location and status of the archived samples. Also, indicate if there are any issues with respect to data archiving schedule and plan.*

**PARTICIPANTS AND COLLABORATORS**

8) Project participants

*Please list the participants of your project, their role(s)\* and contact information. This includes some personal information that we will hold closely and for limited purposes. We ask for demographic data – relating to gender, race, and citizenship – so that we can: gauge whether the GoMRI program is fairly reaching and benefiting everyone regardless of demographic category; ensure that those in under-represented groups have the same knowledge of and access to programs, meetings, vacancies, and other research and educational opportunities as everyone else; and we can monitor involvement of international investigators. We will use the demographic data for statistical purposes only. Submission of demographic data is voluntary, but basic data such as name, contact information, and role in the project is required. No personal information will be released. **Note: Student/educational information will be collected elsewhere in this report.***

*\* We understand one person may fulfill more than one role; please list all applicable roles using the following standardized titles: Principal Investigator, Co-Principal Investigator, Scientific Participant, Technician, Lab Assistant, Administrative Support.*

First Name	Last Name	Role in Project	Institution	Email	Gender	Race	Citizenship
Jose	Lopez	PI	Nova Southeastern University	<a href="mailto:joslo@nova.edu">joslo@nova.edu</a>	M	Pacific Islander	USA
Rebecca	Vega-Thurber	Co-PI	Florida Intl University	<a href="mailto:rvegathurber@gmail.com">rvegathurber@gmail.com</a>	F	Caucasian	USA
Peter	McCarthy	Co-PI	FAU at Harbor Branch Oceanographic Inst.	<a href="mailto:PMCCART5@hboi.fau.edu">PMCCART5@hboi.fau.edu</a>	M	Caucasian	USA
Patricia	Blackwelder	Co-PI	Nova Southeastern University	<a href="mailto:pblackwelder@rsmas.miami.edu">pblackwelder@rsmas.miami.edu</a>	F	Caucasian	USA
Piero	Gardeneli	consultant	Florida Intl	<a href="mailto:gardinal@fiu.edu">gardinal@fiu.edu</a>	M		USA

			University					
Gretchen	Bielmyer	consultant	Valdosta State University	Gkbielmyer	F	Caucasian	USA	
Smith	Emily	Graduate research assistant	Nova Southeastern University	Es874@nova.edu	F	Caucasian	USA	
Willoughby	David	Bioinformatics Consultant	Ocean Ridge Biosciences	davidw@oceanridgebio.com	M	Caucasian	USA	
Wu	Yonggan	Bioinformatics Consultatant	Ocean Ridge Biosciences	yongganw@oceanridgebio.com	M	Caucasian	USA	
Gilbert	Jack	Bioinformatics Consultatant	Argonne National Laboratory	<a href="mailto:gilbertjack@anl.gov">gilbertjack@anl.gov</a>	M	Caucasian	USA	

## MENTORING AND TRAINING

### 9) Student and post-doctoral participants

*Please list the student participants of your project, their educational role, and other information. This includes some personal information that we will hold closely and for limited purposes. We ask for demographic data – relating to gender, race, and citizenship – so that we can: gauge whether the GoMRI program is fairly reaching and benefiting everyone regardless of demographic category; ensure that those in under-represented groups have the same knowledge of and access to programs, meetings, vacancies, and other research and educational opportunities as everyone else; and we can monitor involvement of international investigators and students. We will use the demographic data for statistical purposes only. Submission of demographic data is voluntary, but basic data such as name, contact information, and research area is required. No personal information will be released.*

First Name	Last Name	Post-doc / PhD / MS / BS	Thesis or research topic	Institution	Supervisor	Expected Completion year	Gender	Race	Citizenship
Marie	Cuvelier	Post-doc	Metagenomic analysis	FIU	R Thurber	Dec 2012	F	C	
Emily	Smith	MS	Gene expression of dosed sponge samples	Nova	J Lopez	Dec 2012	F	C	

### 10) Student and post-doctoral publications, if planned

- a. Published, peer-reviewed bibliography (Copies of the papers are requested)
- b. Manuscripts submitted or in preparation (Please note target journal, and anticipated date of submission or publication)

Smith, E. Willoughby, D., Blackwelder, P., McCarthy, P.J., Gilbert, J., Cuvelier, M., Vega-Thurber, R., Lopez, J. Transcriptome analysis of marine sponge *Cinachyrella sp.*, possible new model organism for oil and dispersant ecotoxicology. BMC Genomics. In Preparation – target submission date May 2013

- 11) Student and post-doctoral presentations and posters, if planned (Please provide copies of each)

Title	Presenter	Authors	Meeting or Audience	Abstract published (Y/N)	Date

- 12) Images

*Please attach high-resolution image and provide details including a description of the image, location, credit, date, etc. Of note: Image may be used in GoMRI promotions, make sure you have rights to use the image. Note: GoMRI will establish a Flickr site to share these images through the GoMRI website and with media and the public.*

Electron micrographs and their descriptions are attached. All SEM photo copyrights are owned by Dr Patricia Blackwelder (Nova Southeastern University).

- 13) Continuing Research

*If you are continuing this research under another grant, please include granting authority and title of award and a very brief synopsis (2-3 sentences).*

We have obtained additional funding through an internal Nova Southeastern University Presidential Faculty Research and Development Grant. The project is a continuation of the FIO project entitled “Developing diagnostics for oil exposure in marine sponges” but will primarily be used to support the last stages of graduate student Emily Smith’s MS thesis. This project will focus on the bioinformatics analysis of the first set of Illumina RNA-seq data.