Project ID #: **4711-1101-06**
Project Title: **Toxicity of Deepwater Horizon Oil and Dispersants on Florida's Reef Biota**
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Institution: **College of Marine Science, University of South Florida**

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SCIENCE ACTIVITIES

1. SUMMARY

Coral reef communities are in decline worldwide, and the Florida reef tract is no exception. The Florida Keys National Marine Sanctuary Coral Reef Evaluation and Monitoring Project has reported an approximately 50% decline in stony coral cover between 1996 and 2006 (Callahan et al. 2007). Fortunately oil from the Deepwater Horizon blowout did not reach the Florida reef tract. However, a similar incident could do so in the future. If so, the inshore patch reefs will likely be most affected, both directly and secondarily because of their proximity to mangrove shorelines. Mangroves were the systems most impacted by the spill at Bahía Las Minas, Panama, with long-term consequences for reefs and other adjacent ecosystems (Keller and Jackson 1993).

Bleaching in corals and other organisms that host algal endosymbionts occurs when the host organism, the algal symbionts, or both (the holobiont), are stressed, causing either a loss of algal chlorophyll or the expulsion or digestion of the algae by the host. A variety of kinds of stress can induce bleaching, but photo-oxidative stress is the most common inducer of mass bleaching (Lesser and Farrell 2004; Lesser 2006; many others). Photo-oxidative stress can occur when there is prolonged exposure to too much sunlight, or when other physiological stress renders the holobiont unable to deal with the reactive oxygen species produced even under normal light capture (e.g., Weiss 2008). This can occur, for example, under elevated temperature or following exposure to toxic organic chemicals such as polycyclic aromatic hydrocarbons (PAHs).

Because organisms used to test lethal and sublethal effects on marine organisms are not appropriate models for understanding stress that can induce bleaching, the goal was to test and develop potential new "model organisms" to assess lethality and sublethal effects of crude oil and dispersants. The major outcomes of the research were a) to indeed identify a suitable model organism, Foraminifera of the genus *Amphistegina*, which occur in reef and carbonate shelf environments worldwide; b) to develop repeatable test protocols, despite an unanticipated complication; and c) that complication resulted in the discovery of the potential for chemically-induced dormancy in a major class of marine organisms. That discovery has wide ranging implications for understanding the environmental ranges and evolutionary histories of the most important group of organisms used in the petroleum industry as paleoenvironmental and biostratigraphic indicators.

2. RESULTS AND SCIENTIFIC HIGHLIGHTS

The objectives of the project were modified somewhat by two circumstances. The first was the withdrawal of the Torres group from the project, which removed the primary expertise in work with corals. Efforts to bring other collaborators into the project were unsuccessful, though recommendations were to utilize *Aiptasia* as a model organism, *Aiptasia* is common anemone often used as a model organism in physiological studies of Cnideria. Because of the delayed start of those experiments, that research is ongoing.

The second modification involved the chemicals tested in the study. A major area of the laboratory facilities in the College of Marine Science at the University of South Florida were selected for complete renovation, which resulted in the researchers of the Reef Indicators Laboratory being displaced to temporary laboratory facilities. Given extensive media reports of the health effects of exposure to the Macondo crude oil and Corexit dispersants, to limit the risk to researchers working in less than ideal facilities, the decision was made to begin tests using low toxicity ingredients of the dispersants, specifically propylene glycol.

The CMS Reef Indicators Laboratory primarily focuses on applications for the ubiquitous shelled marine protists, the Foraminifera, as bioindicators and paleoenvironmental indicators. For reef studies, the model organism most commonly used is the circumtropically most abundant foraminiferal genus that also hosts algal symbionts is the genus *Amphistegina*. This foraminifer has been shown to respond similarly to zooxanthellate reef-building coral species in its physicological dependence on algal symbionts for growth and calcification, and in its similar response to photo-oxidative stress that induces damage to or loss of the algal symbionts (i.e., bleaching). Thus, testing focused on the foraminifer *Amphistegina gibbosa*, which, with its Indo-Pacific sibling species *A. lessonii*, is being developed as a model organism for environmental research in subtropical/tropical coastal and coral reef environments.

The modified goals of the research with *A. gibbosa* were to determine lethal concentrations and effects ranges, as defined by the US Environmental Protection Agency, of two components of the Corexit dispersants, propylene glycol and 2-butoxyethanol. Preliminary experiments indicated that many specimens exposed to propylene glycol (v/v) at concentrations of 2% or higher appeared to be dead following 48-hour exposure, resulting in apparent LC50 of 3% and an initial effects range of 2-4%. When placed in filtered seawater, after 72-hours the actual LC50 was 6%. Using the methods developed, we found the apparent LC50 (more precisely, the acute effects or AC50) for *A. gibbosa* after a 48-hour exposure to propylene glycol is 3%; the initial acute effects range was 2-4%. After a 48-hour exposure to propylene glycol, followed by a 72-hour recovery period in filtered seawater, the LC50 was 6%, and the effects range was 2-8%. For 2-butoxyethanol, the AC50 following a 48-hour exposure was 0.2%, and the effects range 0.15-0.3%. After the 72-hour recovery period in filtered seawater, the LC50 was 1% and the effects range 0.32 -1.28%.

Another goal was the establishment of measures for chronic sub-lethal effects. Although differences in the increase in mean diameter over time (i.e., post-exposure growth) suggested some chronic effects, visual assessments indicated chronic effects in all concentrations of propylene glycol above control. This suggests that visually assessed health is a sensitive measure of chronic effects. It also means that the actual effects range of 48-hour exposure to propylene glycol on *A. gibbosa* was at least 0.5-12%, and that even the lowest exposures induced significant loss of symbiont color, i.e., bleaching.

To confirm the discovery of chemically-induced dormancy observed in the initial propylene glycol experiment, the observed dormancy effect in *A. gibbosa* was confirmed in repeated experiments, and during exposure to both propylene glycol and 2-butoxyethanol, despite differences in the relative toxicity of each chemical. This is the first experimentally observed occurrence of dormancy in a foraminifer in response to chemical exposure and, in the context of previous observations, suggests that dormancy may be a general response among the Foraminifera to acutely unfavorable conditions.

These results further demonstrate that *Amphistegina* spp. can be an effective bioindicator of water-borne pollutants on coral reefs. Exposure to chemicals causes measurable acute and lethal effects. In addition to acute effects and mortality, chemical exposure also produced sub-acute effects, causing foraminifers to express increasing visual signs of stress, with increasing concentration of exposure. While growth in exposed individuals did not prove to be linearly related to the concentration of exposure, making it seem unsuitable as a direct measure of the toxicity of exposure in the long term, the effects on foraminiferal health as determined by the increased visual stress markers may fill this role. Although further development of bioassay and bioindicator protocols will be required, the promise is there.

The refined methods reported in Ross (2012) are capable of differentiating between dormant and dead individuals, allow the study of both acute effects and lethal effects, and are shown to be effective for experiments exposing *A. gibbosa* to two different alcohols. These methods appear to be appropriate when using these protists in acute toxicology experiments, and strengthen the case for the use of *Amphistegina* as a low-cost, low-technology chemical bioassay.

Dormancy mechanisms, including diapause (e.g., suspension of growth and reduced metabolism induced by unfavorable conditions) and cryptobiosis (e.g., propagules or eggs that are essentially ametabolic), are widespread and diverse in the biosphere. Recognizing the diversity of mechanisms is integral to understanding these processes, ranging from distributions of organisms through time (e.g., biostratigraphy and paleoceanography), to potential responses to ongoing environmental changes, to triggers for release from quiescence (e.g., medical issues such as tumor growth and activation of protozoan parasites). Members of the protistan Class Foraminifera are ubiquitous in marine environments and are among the most widely utilized organisms in the Earth and Ocean sciences. The Foraminifera are essential to facets of environmental, evolutionary, sedimentological, paleoenvironmental, micropaleontological, biostratigraphic and paleoceanographic research, and continue to be widely used in economic geology.

While observations of diapause-like responses in benthic foraminifers have at least a 40-year history, the observations of Ross (2012) appear to be the first report of a dormancy- or diapause-like response to chemical exposure. The physiological characteristics of dormant foraminifers and the range of taxa that can become dormant are virtually unknown. Thus, this discovery opens an entirely new arena for research with a wide range of potential implications.

3) Cruises & field expeditions

Ship or Platform Name	Class	Chief Scientist	Objectives	Dates
R/V Mola Mola	Dayboat	Hallock Muller	Collections of specimens for experiments	3/6/2011
R/V Mola Mola	Dayboat	Hallock Muller	Collections of specimens for experiments	6/13/2011
R/V Mola Mola	Dayboat	Hallock Muller	Collections of specimens for experiments	12/17/2011
R/V Mola Mola	Dayboat	Mendez-Ferrer	Collections of specimens for experiments; collection of fluorometry data	3/26/2012
R/V Mola Mola	Dayboat	Hallock Muller	Collections of specimens for experiments; collection of fluorometry data	05/29-30/2012
R/V Mola Mola	Dayboat	Hallock Muller	Collections of specimens for experiments; collection of fluorometry data	6/3/2012
R/V Mola Mola	Dayboat	Mendez-Ferrer	Collections of specimens for experiments; collection of fluorometry data	07/11-12/2012

4) Peer-reviewed publications, if planned

See section on student and post-doctoral publications

5) Presentations and posters, if planned

See section on student and post-doctoral publications

6) Other products or deliverables

None

7) Data

See Ross (2012).

PARTICIPANTS AND COLLABORATORS

8) Project Participants

First Name	Last Name	Role in Project	Institution	Email
Pamela	Hallock Muller	Principal Investigator	University of South Florida	pmuller@usf.edu
Joseph	Torres	Co-PI	University of South Florida	jjtorres@usf.edu

MENTORING AND TRAINING

9) STUDENTS AND POST-DOCTORAL PARTICIPANTS

First Name	Last Name	Post-doc / PhD / MS / BS	Thesis or research topic	Institution	Supervisor	Expected Completion Year
Natasha	Mendez-Ferrer	PhD student	Assessing photo-oxidative stress in corals and foraminifera	USF - CMS	P. Hallock Muller	2015
Adrienne	George	PhD student	Coral diseases & responses of *Aiptasia* to dispersant chemicals	USF - CMS	P. Hallock Muller	2014
Benjamin	Ross	PhD student	Environmentally-induced dormancy in foraminifera	USF - CMS	P. Hallock Muller	2015
Benjamin	Ross	MS student	Responses to Chemical Exposure by Foraminifera	USF - CMS	P. Hallock Muller	2012
Heidi	Toomey	MS student	Chlorophyll Fluorescence and Stress in *Archaias angulatus*	USF - CMS	P. Hallock Muller	2013
Brittany	Lyp	BS - Environmental Science	Undergraduate intern	USF-SP	P. Hallock Muller	2012
Kasi	Techton	BS - Environmental Science	Undergraduate intern	USF-SP	P. Hallock Muller	2014

10) STUDENT AND POST-DOCTORAL PUBLICATIONS, IF PLANNED

- Published, peer-reviewed bibliography – None to date
- Theses or Dissertations Competed

Ross, B. 2012. Responses to Chemical Exposure by Foraminifera: Distinguishing Dormancy From Mortality. M.Sc. Thesis, University of South Florida. Not yet available online.

- Manuscripts submitted or in preparation

Ross, B., Hallock, P., Torress, J.J., Van Vleet, E.S. Response to Chemical Exposure: Discovery of a Diapause-Like Mechanism. Manuscript in preparation for submission to *Nature* in early 2013 (backup will be *Journal of Foraminiferal Research*)

Ross, B. Hallock, P. A Model Organism for Toxicity Testing for Reef Environments: Protocols for Toxicity Tests with *Amphistegina* spp. Manuscript in preparation for submission to Ecotoxicology.

11) STUDENT AND POST-DOCTORAL PRESENTATIONS AND POSTERS

Title	Presenter	Authors	Meeting or Audience	Abstract published	Date
Acute and Sub-acute Effects of Exposure to Dispersant Chemicals on the Larger Foraminifer *Amphistegina gibbosa*	Ross, Benjamin (poster)	Ross, B., Hallock, P.	Florida Association of Benthologists Annual Meeting	No	Oct-11
Assessing acute and long term effects of chemical exposure in Foraminifera: Challenges and new directions	Ross, Benjamin (best poster award)	Ross, B., Hallock, P.	76th Annual Mtg., Florida Academy of Sciences	No	Mar-12
Developing protocols for testing toxic chemicals on the symbiotic foraminifer, *Amphistegina gibbosa* d'Orbigny	Ross, Benjamin (oral)	Ross, B.	JAMSTEC Field Workshop on Living Foraminifera, Japan	No	Jul-12
Responses to chemical exposure by Foraminifera: Distinguishing dormancy from mortality	Ross, Benjamin (oral)	Ross, B., Hallock, P.	Geological Society of America Annual Meeting	Yes	Nov-12
Photo-oxidative stress and bleaching in the foraminifer *Amphistegina gibbosa* d'Orbigny: A PAM fluorometry approach	Mendez-Ferrer, Natasha (oral)	Mendez-Ferrer, N., Hallock, P.	Autónoma de México, Puerto Morelos, México.	No	Jan-12
Photo-oxidative stress and bleaching in the foraminifer *Amphistegina gibbosa* d'Orbigny	Mendez-Ferrer, Natasha (poster)	Mendez-Ferrer, N., Hallock, P.	76th Annual Mtg., Florida Academy of Sciences	No	Mar-12
Quantifying photic stress in algal symbionts of larger benthic foraminifers.	Mendez-Ferrer, Natasha (poster)	Mendez-Ferrer, N., Hallock, P.	Geological Society of America Annual Meeting	Yes	Nov-12

12) IMAGES

None of particular interest.

13) CONTINUING RESEARCH

The equipment purchased for the project are providing resources for the graduate student participants to continue their dissertation research while funded by other sources such as fellowships and teaching assistantships. Thus, the funding has already supported completion of one Master's Thesis, and will ultimately contribute to a second thesis and three doctoral dissertations. Natasha Méndez-Ferrer was awarded a small research grant from the Cushman Foundation for Foraminiferal Research to continue field observations of photo-oxidative stress in foraminifers, for comparison with laboratory assessment of test specimens. Ben Ross has applied for an EPA fellowship to continue his research on developing *Amphistegina* as a model organism for testing stressors of coral reefs. P. Hallock Muller has submitted an NSF proposal for the team to continue work on Ross's exciting discovery of chemically-induced dormancy in benthic foraminifera.

REFERENCES

Callahan, M., J. Wheaton, et al. (2007). 2006 Executive Summary EPA Steering Committee Meeting July 2007, Florida Fish and Wildlife Conservation Commission Fish and Wildlife Research Institute.

Lesser, M. P. (2006). Oxidative stress in marine environments: Biochemistry and physiological ecology. Annual Review of Physiology. 68: 253-278.

Lesser, M. P. and J. H. Farrell (2004). Exposure to solar radiation increases damage to both host tissues and algal symbionts of corals during thermal stress. Coral Reefs 23(3): 367-377.

Ross, B. 2012. Responses to Chemical Exposure by Foraminifera: Distinguishing Dormancy From Mortality. M.Sc. Thesis, University of South Florida. Not yet available online.

Weis, V. M. (2008). Cellular mechanisms of Cnidarian bleaching: stress causes the collapse of symbiosis. Journal of Experimental Biology 211(19): 3059-3066.