GULF OF MEXICO RESEARCH INITIATIVE DATA MANAGEMENT PLAN TEMPLATE FOR ADDOMEx-2

SECTION 1: RESEARCH CONSORTIUM INFORMATION

1. Project Title

Aggregation and Degradation of Dispersants & Oil by Microbial Exopolymers-2

Consortium Summary

Research objectives and goals

The work proposed here will build on the previous findings of ADDOMEx. Specifically, research during ADDOMEx identified many of the bacteria and phytoplankton responsible for the copious production of transparent exopolymeric particles (TEP), exopolymeric substances (EPS), marine snow and marine oil snow (MOS) in the presence and absence of oil (as a water accommodated fraction; WAF), the dispersant Corexit, and Corexit-dispersed oil (as a chemically enhanced water accommodated fraction, CEWAF, and as a diluted form, herein called DCEWAF). The factors that influence or retard the subsurface scavenging of oil into MOS were determined and ongoing research is beginning to pinpoint the processes that promote decomposition of the oil post MOS formation. This improved understanding has expanded our ability to predict the behavior and footsteps of released oil, and the potential impacts of Corexit application, specifically with respect to Marine Oil Snow (MOS) processes (e.g., formation, fate). The following insights were gained during ADDOMEx but are not yet in current conceptual or numerical models: 1) reactive oxygen species (ROS; produced by sunlight or enzymes) mediate crosslinking of proteins in EPS to form aggregates. 2) details of the processes that control interactions between Corexit, oil and EPS in producing either sinkable MOS or dispersed gels promoting microbial degradation of oil compounds. 3) rapid oil oxidation and microbial degradation in water within a few days of exposure, especially at the surface of the ocean, and 4) rapid formation of microbial aggregates on oil droplets is enhanced in the presence of Corexit-dispersed oil.

The next step is thus to integrate ADDOMEx derived insights into a comprehensive conceptual model framework. Key experiments will generate measurements needed to improve numerical

modeling (in conjunction with FOMOSA and others) which will enhance prediction capabilities in order to guide the decision process of first responders.

The primary experimental goal of ADDOMEx-2 is to perform a series of "wrap-up" experiments intended to fill current knowledge gaps. All proposed experiments will be conducted and samples analyzed within year 1. These experiments will center around two main hypotheses:
(1) ***Particle formation and fragmentation is governed mainly by stickiness.***
(2) ***The fate of oil (chemically undispersed or Corexit dispersed) trapped within MOS is dictated by both chemical and microbial oxidation. Both processes lead to rapid oxidative alteration of the oil. This affects the sinking and dispersion of MOS and the associated oxidized oil.***
Sub-hypotheses will address further the mechanisms of the growth of nano- to micro- to macrogels and their role in dispersing oil, the factors that control MOS sedimentation, and the role of light versus microbially produced ROS in oxidation and crosslinking of MOS aggregates.

Furthermore, ADDOMEx-2 includes ***three critical synthesis-based activities***. The ***first*** is construction of a conceptual model detailing drivers and conditions leading to MOS formation and sedimentation events. This will strongly contribute to the legacy of the GOMRI program, which is the first time in history that such a targeted and focused study of marine snow, and marine oil snow specifically, has occurred. Towards meeting the goals of a GOMRI legacy, the proposed conceptual model will provide a *tool kit for responders addressing future oil spills with respect to MOS formation*. This conceptual model will translate the best available science into operative guidelines designed to meet the practical needs of different stakeholders. Scientists in ADDOMEx-2 will work within existing frameworks to reach stakeholders, acting primarily as a catalyst to bring various groups together to develop these products. Further, we will work with numerical modelers (FOMOSA team, Burd and Daly) to appropriately constrain these models but providing key parameters currently not available in the literature.

The *second synthesis-based activity* is an analysis of all microbial, chemical and physical data produced during ADDOMEx and ADDOMEx-2 to determine linkages between taxa across all three domains of life and between taxa and their environment by performing a network analysis. Appropriate data from other GoMRI funded research will be incorporated as well, producing results that span many experimental conditions and result in identification of relationships between taxa crucial to MOS formation and oil degradation, regardless of experimental design.

The *third synthesis-based activity* proposed for ADDOMEx-2 is a an international workshop in year 2 with invited papers, presentations and stakeholder meetings which will lead to several types of publication materials designed to appeal to the variety of stakeholders – academic peer reviewed manuscripts, including a special issue of a relevant journal dedicated to MOSSFA, and publications targeted at a general audience to be produced and distributed with the help of Sea Grant and others, and a website devoted to the conference and publications.

The expected outcomes correspond to *GRI Theme 2* (70%) Chemical evolution and biological degradation of the petroleum/dispersant system and *GRI Theme 3* (30%) Environmental effects of the petroleum/dispersant system on the water column in coastal waters and organisms, in order to address the science of ecosystem recovery. **This project is investigating the detailed steps of water-column processes that lead to sinkable MOS formation and oxidative loss terms for**

oil through controlled laboratory experiments which were developed during the course of ADDOMEx.

2. Lead Principal Investigator and Contact Information

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4. Data Manager Roles and Responsibility.

Kathy Schwehr has a 50% effort for data management. She will make decisions regarding data management in collaboration with Antonietta Quigg, Peter Santschi, and consortium researchers as needed. Kathy Schwehr will be in charge of day to day management. Consortium researchers helping with data submission from each co-PI group are Jessica Hillhouse (Quigg); Andrew Irwin (Finkel); Shawn Doyle (Sylvan); Clarence Le (Chin); Kai Ziervogel and Uta Passow for their group; Maya Morales (Knap/Wade); Hongmei Chen and Derek Waggoner (Hatcher).

5. Data Submission to GRIIDC

The quality assurance and control (QA/QC) will begin with each researcher. After dissemination to the ADDOMEx consortia, further review and discussion will evolve. Ultimately, final QA/QC will rest with the Program Manager, Antonietta Quigg. Individual PIs are responsible for submitting their own data to GRIIDC. Alternatively, Kathy Schwehr will submit data when PIs ask for assistance.

6. Data Management Training

Addomex 2 will be in compliance with GOMRI directives for training and will maintain updates.

7. Communicating Data Submission Requirements

GRIIDC dataset submission guidances have been emailed to the consortia along with the GRIIDC website address for these. Any new requirements will be emailed to the consortia and announced during all-hands phone conferences and meetings.

8. Data Storage and Backup

a) Data will be stored on individual's computers or data storage systems, then backed up on the TAMUS data repository on Google Drive. From there it will QC'ed by Kathy Schwehr, Peter Santschi (Deputy Addomex Program Director), the consortia, and Antonietta Quigg (Addomex Program Director). Finally, the data will be submitted to GRIIDC.

b) The data will be backed up daily by each PI, sent to the TAMUS Google Drive, backed up daily by Google.

c) The data will be backed up daily, and weekly by the DM to an external hard drive.

9. Ethics and Compliance

a.) There are no known ethical or privacy issues for the protection of privacy, confidentiality, or security that may apply to the data from this project.

b.) There are no known IRB or HIPPA issues that might preclude sharing data.

c.) There is no applicable case for protection of the identity of participants in this grant research.

d. Although no issues are anticipated, if a situation concerning data sharing, ethics, or compliance develops, the GRIIDC team will be consulted regarding appropriate means to comply. Antonietta Quigg (Addomex 2 Program Director) and Peter Santschi (Deputy Addomex2 Program Director) will manage any concerns.

SECTION 2: METHODS INFORMATION

1. Research Cruises

Not applicable at this time.

2. Non-Cruise Field Work

Not applicable at this time.

3. Environmental Sample Lab Analysis

Not applicable at this time.

4. Microcosms/Mesocosms

a) Microcosms

R6.x807.000:0003	EPS mechanisms in aggregation & dispersion of oil: Fragmentation of Skeletonema grethae-775 snow – Aggregate characteristics	Uta Passow
R6.x807.000:0004	EPS mechanisms in aggregation & dispersion of oil: Fragmentation of Skeletonema grethae-775 snow – Disaggregation dynamics	Kai Ziervogel

Microcosm: Produce marine snow/marine oil snow with Skelotenoma grethae-775 and varying combinations of water accommodated fraction (WAF) of oil, oil, or oil and Corexit.

a) Roller table growth of marine snow aggregation, measurements of size, Transparent Exopolymers (TEP), Particulate Organic Carbon (POC)
b) Measurements of size distribution changes with turbulence

R6.x807.000:0009	Microbial Community Gene Expression, Mixed Isolate Bottle Experiment, Fall 2018, qPCR measurements	Shawn Doyle, Jason Sylvan
R6.x807.000:0031	Microbial Community Gene Expression, Mixed Isolate Bottle Experiment, Fall 2018, micrographs	Shawn Doyle, Jason Sylvan

Microcosm: Bottle experiment exposing mixed isolates of microbes to oil slick and truly dissolved oil.

a) qPCR for gene expression
b) microscopy images for aggregate growth monitoring

R6.x807.000:0013	Concentration-dependent responses of *Thalassiosira pseudonana* and *Pseudo-nitzschia sp*. to polycyclic aromatic hydrocarbon exposure	Laura Bretherton, Andrew Irwin

Microcosm: Grow *T. pseudonana* and *Psuedo-nitzchia sp.* at a range of PAH concentrations.

a) PAHs
b) Physiological phytoplankton responses

R6.x807.000:0014	Physiological responses of marine phytoplankton exposed to oil and Corexit in culture experiments, Jun 2018	Laura Bretherton, Andrew Irwin

R6.x807.000:0034	Physiological responses of marine phytoplankton exposed to oil and Corexit in culture experiments, Jun 2018, 18S RNAseq	Laura Bretherton, Andrew Irwin

Microcosm: Grow *Micromonas pusilla*, *Tetraselmis astigmatica*, *Ochromonas sp.* and other groups of phytoplankton in different concentrations of oil measured as estimated oil equivalents.

a) RNA
b) Physiological phytoplankton responses

R6.x807.000:0022	Microcosm: Generation of EPS by phytoplankton and bacteria with and without polysaccharide inhibitator at various concentrations of WAF; enzyme, protein, polysaccharide assays; particle size	Manoj Kamalanathan, Antonietta Quigg
R6.x807.000:0027	Microcosm: Generation of EPS by phytoplankton and bacteria with and without polysaccharide inhibitator at various concentrations of WAF; SEM micrographs	Manoj Kamalanathan, Antonietta Quigg
R6.x807.000:0028	Microcosm: Generation of EPS by phytoplankton and bacteria with and without polysaccharide inhibitator at various concentrations of WAF; CLSM micrographs	Manoj Kamalanathan, Antonietta Quigg

Microcosm: Generation of EPS by phytoplankton and bacteria with and without polysaccharide inhibitor at various concentrations of WAF

a) No polysaccharide inhibitor (dichlorobenzonitrile (DCB): protein and polysaccharides in WAF and control
b) With polysaccharide inhibitor (dichlorobenzonitrile (DCB): protein and polysaccharides in WAF and control

R6.x807.000:0023	Microcosm: Flask experiment of Phytoplankton-Bacteria interaction in a natural community using control and WAF treatments; non-radioactive experiment; assays for protein, carbohydrates, enzymes.	Manoj Kamalanathan, Antonietta Quigg
R6.x807.000:0024	Microcosm: Flask experiment of Phytoplankton-Bacteria interaction in a natural community using control and WAF treatments; radiolabeling experiment; activities of bacteria, phytoplankton, oil	Kathy Schwehr, Peter Santschi
R6.x807.000:0029	Microcosm: Flask experiment of Phytoplankton-Bacteria interaction in a natural community using control and WAF treatments; non-radioactive experiment, flowcytobot	Manoj Kamalanathan, Antonietta Quigg
R6.x807.000:0030	Microcosm: Flask experiment of Phytoplankton-Bacteria interaction in a natural community using control and WAF treatments; non-radioactive experiment, 16s sequencing	Manoj Kamalanathan, Antonietta Quigg

Microcosm: Flask experiment of Phytoplankton-Bacteria interaction in a natural community using control and WAF treatments; radiolabeling and non-radioactive experiments.

a) Physiological responses, protein, polysaccharides, enzyme assays
b) Flowcytobot

c) 16s sequencing data
d) Radioisotope activity of labeled bacteria, labeled oil component, labeled phytoplankton

b) Mesocosm

Description: MICROX, a mesocosm to quantify microbial oxidation and degradation of oil. The aim of M6 is to determine the detailed chemistry involved in the degradation of the WAF and DCEWAF, and the particles using large volumes. The degradation rates in preceding experiments were so fast that there was not enough material for major extractions. In this case we will sacrifice tanks at T0, T0.5, T1, T3, T6, T8 and T12 (T=time in days).

R6.x807.000:0005	Estimated Oil Equivalents, EOE, for MICROX, a mesocosm to quantify microbial oxidation and degradation of oil. Analytical measurments.	Terry Wade, Tony Knap
R6.x807.000:0006	Hydrocarbon biodegradation indicator measurements for MICROX, a mesocosm to quantify microbial oxidation and degradation of oil. Analytical measurments.	Terry Wade, Tony Knap
R6.x807.000:0007	Time series of nutrients in MICROX, a mesocosm to quantify microbial oxidation and degradation of oil. June 20 2018. Analytical measurments.	Terry Wade, Tony Knap

The analysis above will be completed at different times, so it is separated into 3 datasets for increased availability to those who will need to share it.

a) EOE
b) Biomarkers, PAHs
c) Nutrients

R6.x807.000:0008	Microbial Community Composition and Structure, in MICROX, a mesocosm for studying the microbial oxidation and degradation of oil, June 2018, 16S/18S	Shawn Doyle, Jason Sylvan

a) 16S/18S and a set of transcriptomes from water column
b) 16S/18S and a set of transcriptomes from aggregates.

R6.x807.000:0010	Molecular level characterization of oil and aggregate oxidation products: MICROX, a mesocosm for studying microbial oxidation and degradation of oil, Exp. June 2018 FTICR-MS data	Pat Hatcher, Derek Waggoner
R6.x807.000:0011	Molecular level characterization of oil and aggregate oxidation products: MICROX, a mesocosm for studying microbial oxidation and degradation of oil. June 2018 GCxGC-MS data	Pat Hatcher, Derek Waggoner

Full characterization of the particles and the water column chemistry. This mesocosm is primarily dedicated to large volume sampling for the Hatcher team to characterize oxidation

products. The datasets are from different analytical instruments in different time frames, so it is more effective to collect these data as they becomes available.

a) FTICR-MS
b) GC x GC-MS

R6.x807.000:0016	Rates of 14C Hydrocarbon oxidation by Microbial Communities, Mesocosom Exp, Fall 2018, oxidation rate measurements	Amanda Achberger, Jason Sylvan
R6.x807.000:0017	Characterization of cell concentrations and microaggregate abundance and size, Mesocosom exp, Fall 2018, abundance counts	Amanda Achberger, Jason Sylvan
R6.x807.000:0018	Fluorescent In Situ Hybridization of Microaggregates, Mesocosom Exp, Fall 2018, micrographs	Amanda Achberger, Jason Sylvan
R6.x807.000:0019	Microaggregate Microbial Community Structure, Mesocosom experiment, Fall 2018, SSU rRNA sequences	Amanda Achberger, Jason Sylvan
R6.x807.000:0020	Microaggregate Microbial Community Metagenome, Mesocosom exp, Fall 2018, sequence data	Amanda Achberger, Jason Sylvan

These are significantly different types of data that will be acquired over varied time periods and so they are structured to be in different datasets. These are side lab experiments performed a samples from MICROX mesocosm. Each dataset name is representative of the measurements that will be included in it.

5. Pure Lab-Based Studies

a) Yes.

R6.x807.000:0001	Adhesive force measurements between EPS	Wei-Chun Chin

Adhesive force measurements between EPS Force measurement data between various types of EPS under different environmental conditions. Force measurements between various types of EPS aggregates under different environmental conditions. AFM or laser tweezer will be used to assess the attractions between various EPS particles (aggregates).

a) Measurements of adhesive force
b) Microscopic images

R6.x807.000:0002	Aggregation process for nanogels, microgels and larger aggregates	Wei-Chun Chin

Aggregation process for nanogels, microgels and larger aggregates Images and image analysis for the formation pathways of marine oil snow, growing from nano-gels to micro- to macroaggregates. Images and image analysis for the formation pathways of marine oil snow, growing from nano-gels to micro- to macroaggregates will be collected.

a) Images and image analysis.

R6.x807.000:0015	Change in molecular weight of aggregates in artificial seawater formed during irradiation and during irradiation with Reactive Oxygen Species, size fractions of organic carbon	Peter Santschi, Luni Sun
R6.x807.000:0032	Change in molecular weight of aggregates in artificial seawater formed during irradiation and during irradiation with ROS, protein and carbohydrate concentrations	Peter Santschi, Luni Sun
R6.x807.000:0033	Change in molecular weight of aggregates in artificial seawater formed during irradiation and during irradiation with ROS, molecular weight by SDS-page	Peter Santschi, Luni Sun

Change in molecular weight of aggregates in artificial seawater formed during irradiation and during irradiation with ROS,

a) size fractions of organic carbon
b) protein and carbohydrate concentrations
c) molecular weight by SDS-page

6. Modeling

Not applicable.

7. Mapping

Not applicable.

8. Remote Sensing and Aerial Imagery

Not applicable.

9. Images

Images for data will be taken of microscope research, i.e., primarily of aggregates by Confocal Laser Scanning (CLSM), Scanning Electron (SEM), Atomic Force (AFM).

Others may be taken depending on research results.

10. Video

Some may be taken depending on research results. No large files (>1 GB) are anticipated at this time.

11. Social Surveys and Interviews

Not applicable.

12. Economics

Not applicable.

13. Other Methods and Data Types

Not applicable.

SECTION 3: DATASET INFORMATION FORMS

The following pages contain the current DIFs with assigned UDI.

Unique Dataset Identifier (UDI)	Dataset Title	Primary Point of Contact (As provided in Dataset Information Form)
R6.x807.000:0001	Adhesive force measurements between EPS	Wei-Chun Chin
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R6.x807.000:0014	Physiological responses of marine phytoplankton exposed to oil and Corexit in culture experiments, Jun 2018	Laura Bretherton, Andrew Irwin
R6.x807.000:0015	Irradiation of proteins causes aggregate formation through cross-linking	Peter Santschi, Luni Sun
R6.x807.000:0016	Rates of 14C Hydrocarbon oxidation by Microbial Communities, Mesocosom Exp, Fall 2018, oxidation rate measurements	Amanda Achberger, Jason Sylvan
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