APPENDIX 2

CRUISE PLAN R/V OCEANUS

Oregon State University
College of Earth, Ocean & Atmospheric Sciences

FILING DATE:	March 31, 2017
CRUISE NUMBER:	OC1705A
TITLE:	ETNP OMZ Methane
CONTRACT/GRANT NUMBER:	BIO OCE 1558916
PRINCIPAL INVESTIGATOR(S):	Frank Stewart, Jen Glass

PURPOSE: (Short, non-technical statement on how cruise relates to overall project)

The overarching goal of this project is to understand how marine microbes in low oxygen areas (oxygen minimum zones) contribute to the cyling of the greenhouse gas methane. This cruise will enable sampling of microbes and associated chemical (e.g., methane) in the world's largest oxygen minimum zone.

ITINERARY: (Include station positions and route waypoints.)

Please see attached.

WILL RADIOACTIVE METHODS BE USED? YES ☑ NO ☐

If so, list OSU radiation use authorization number:

WILL YOU BE BRINGING HAZARDOUS MATERIALS ABOARD? ☑ YES ☐ NO

If so, you are responsible for providing the Master with an Inventory of such materials & associated MSDS sheets.

SAMPLING PLAN:

Please see attached. We will sample in the Eastern Tropical North Pacific (ETNP) oxygen minimum zone (OMZ) at ~10 stations along two E-W transects starting from on-slope waters west of Manzanillo, Mexico and extending offshore (see proposed cruise track on page 3). At each station, general water column parameters will be assessed via

EQUIPMENT REQUIRED: (Should be included on Shared-Use Equipment request form)

Appendix 2

For additional space to list scientific personnel, see page 4.

SCIENTIFIC PERSONNEL TO BE ONBOARD: (Provide full legal name, gender & affiliation)	
Scientist in Charge:	Frank James Stewart (M, Georgia Tech)
Co-Chief Scientist(s):	none
Party Chief:	see page 4 for other personnel
Technicians:	none
Grad Students:	7 (please see page 4)
Undergraduate Students:	none
Observers:	Mexican collaborator (Claudia Remes)

OSU Marine Technician(s) Assigned to Cruise:	Brandon D'Andrea

USER SUPPLIED EQUIPMENT:	
Vans/Containers:	
Number:	0
Size:	
Estimated Weight:	
Location:	

OTHER BULKY HEAVY ITEMS:	
Location:	compressed gas cylinders (13)
Estimated Weight:	700 lbs

BILLING INFORMATION:	
Name:	Frank Stewart (C/O Nena Gray)
Address:	Engineered Biosystems Building, Room 2016
City, State, Zip	950 Atlantic Dr NW, Atlanta, GA 30332
Phone:	404-385-4107
Account Number (or number to reference):	3206ARP

DO YOU WANT CELLULAR/INMARSAT PHONE ACCESS: [✔] YES [ ] NO
Chief Scientist will be responsible for all charges – dedicated science phone.

Appendix 2
CRUISE PLAN R/V OCEANUS

Primary Project Discipline: (Select all that apply)

- ☐ Physical Oceanography
- ☐ Acoustics
- ☑ Chemical Oceanography
- ☑ Biological Oceanography
- ☐ Environmental Ecology
- ☐ Fish Investigation
- ☐ Climate/Meteorology
- ☐ Geology & Geophysics
- ☐ Mapping/Charting
- ☐ Ocean Engineering
- ☐ Training
- ☐ Transit/Non-science
- ☐ Pollution Assessment
- ☐ Other

Additional Cruise Info:

Please see attached pdf

SCIENTIFIC PERSONNEL TO BE ONBOARD:

	Full Legal Name	Gender	Affiliation	Function
1	Frank James Stewart	M	Georgia Tech	Chief Scientist
2	Cory Cruz Padilla	M	Georgia Tech	Graduate Student
3	Anthony Bertagnolli	M	Georgia Tech	Post Doc
4	Jennifer Blanchard Glass	F	Georgia Tech	Scientist
5	Abigail Marie Johnson	F	Georgia Tech	Graduate Student
6	Bo Thamdrup	M	U. Southern Denmark	Scientist
7	Clemens Schauberger	M	U. Southern Denmark	Graduate Student
8	erdís Guðlaug Rabölle Steinsdótt	F	U. Southern Denmark	Graduate Student
9	Michael Winslow Henson	M	Louisiana State	Graduate Student
10	Claudia Remes	F	UNAM-Sisal	Graduate Student
11	Philipp Friedrich Hach	M	ax Planck Institute of Marine Microbiolo	Graduate Student
12	Sean Andrew Crowe	M	U. British Columbia	Scientist
13	Emilio Guillermo Garcia Robledo	M	Aarhus U.	Post Doc
14	Brandon D ' Andrea	M	Oregon State	Marine Technician
15				Select from List
16				Select from List
17				Select from List
18				Select from List
19				Select from List
20				Select from List
21				Select from List
22				Select from List
23				Select from List
24				Select from List
25				Select from List
26				Select from List

Cruise Plan – OC1705A
ETNP OMZ Methane
Version date: March 31, 2017

A) Summary schedule

Mobilization:	Mazatlan, May 2-3
Depart Mazatlan:	May 4, Thursday by 0900
Transit to Station 5T:	May 4-5
Arrival at 5T (start of sampling):	May 5 afternoon
Sampling along transects:	May 5-18
Depart sampling area:	May 18 morning
Transit to San Diego:	May 18-22
Dock in San Diego:	May 22, Monday by 1100
Demobilization:	May 22-23

B) Summary of operations

We will sample in the Eastern Tropical North Pacific (ETNP) oxygen minimum zone (OMZ) at ~10 stations along two E-W transects starting from on-slope waters west of Manzanillo, Mexico and extending offshore (see proposed cruise track on page 3). At each station, general water column parameters will be assessed via CTD equipped with sensors for fluorometry, PAR, and dissolved oxygen. Seawater collections for microbial biomass and chemical measurements will be done via rosette casts to discrete depths spanning the oxycline and suboxic zones. More extensive sampling (2-3 days per station) will be done at ~3 process stations (see Table below). At these sites, additional seawater samples will be collected for shipboard stable isotope and microcosm experiments to measure chemical (methane, nitrogen, sulfur, oxygen) transformation rates and community compositional and transcriptional responses to seawater amendments. These seawater collections will also be used as inocula for incubations to enrich for target microorganisms. We anticipate ~6-12 CTD/rosette casts per process station and 1-3 casts per non-process (survey) station, with most casts to depths of less than 800 m. At ~5 stations (likely including the 3 process stations), depending on time and operational capabilities, we will also collect sediment samples using either the MC400 or gravity corer.

C) Participants

Name	Role	Lab	Institution
Frank James Stewart	Chief scientist	Stewart	Georgia Tech
Cory Cruz Padilla	Grad student	Stewart	Georgia Tech
Anthony Bertagnolli	Post-doc	Stewart	Georgia Tech
Jennifer Blanchard Glass	Co-PI	Glass	Georgia Tech
Abigail Marie Johnson	Grad student	Glass	Georgia Tech
Bo Thamdrup	Co-PI	Thamdrup	U. Southern Denmark
Clemens Schauberger	Grad student	Thamdrup	U. Southern Denmark
Herdís Guðlaug Rabölle Steinsdóttir	Grad student	Thamdrup	U. Southern Denmark
Michael Winslow Henson	Grad student	Thrash	Louisiana State

Claudia Remes	Grad student	Prieto-Davo	UNAM-Sisal
Philipp Friedrich Hach	Grad student	Kuypers	Max Planck Institute
Sean Andrew Crowe	Co-PI	Crowe	U. British Columbia
Emilio Guillermo Garcia Robledo	Post-doc	Revsbech	Aarhus U.
Brandon D'Andrea	Marine tech	NA	Oregon State

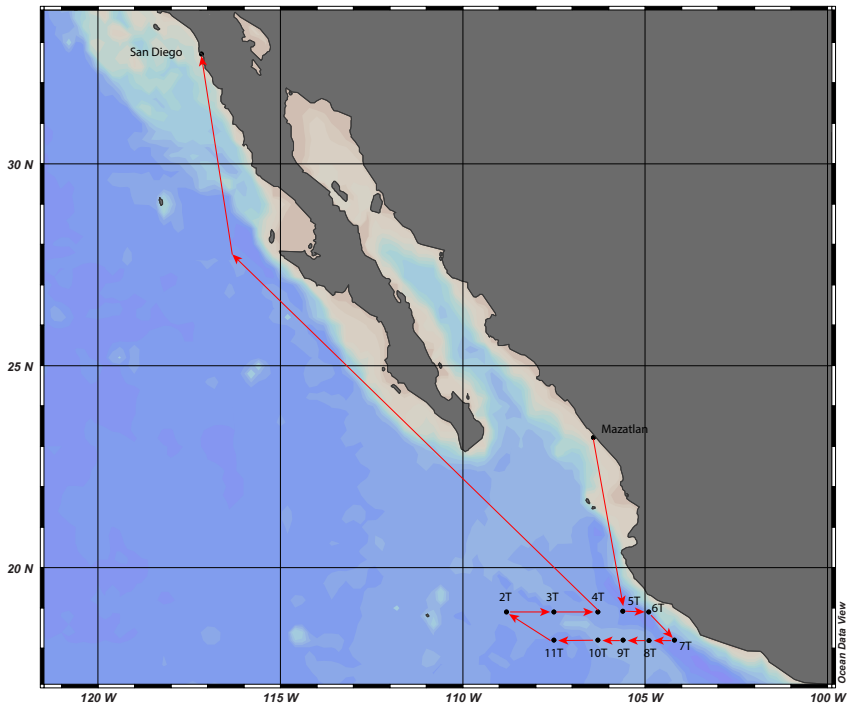
D) Participant special considerations (dietary constraints, etc)

- Still confirming

E) Proposed waypoints and cruise track

- Station 5T (*) will be our first waypoint after Mazatlan. 5T is ~265 nm from port, or ~30 hrs at 9 knots (24 hrs at 11 knots). Thus, estimated arrival at 5T is afternoon of May 5th.
- Station 4T (**) will likely be our last waypoint before leaving for San Diego. 4T is ~1010 nm from San Diego, or ~4.7 days at 9 knots (3.8 days at 11 knots).
- Thus, for a late morning arrival in San Diego on May 22, we should leave the sampling area on the morning of May 18.
- ***The total time in the sampling area is ~13 days (including transit between transect stations, estimated at ~48 hrs).***
- Please note, however, that the exact cruise track (i.e., order of stations visited) may be revised depending on water column conditions at the time of sampling in May.

Station*	Lat N**	Long W**	Station type	Operations
Mazatlan	**23 13.200**	**106 25.200**	Departure port (May 2-4)	Mobilization
5T*	18 55.230	105 12.590	Survey station (<1 day)	CTDs
6T	**18 54.001**	**104 54.040**	**Process station (2-3 days)**	CTDs, MC400
7T	18 12.023	104 12.160	Survey station (<1 day)	CTDs, MC400
8T	18 11.885	104 53.804	Survey station (<1 day)	CTDs
9T	18 11.998	105 12.006	Survey station (<1 day)	CTDs
10T	**18 12.047**	**106 17.797**	**Process station (2-3 days)**	CTDs, MC400 (if depth not limiting)
11T	18 12.077	107 29.955	Survey station (<1 day)	CTDs
2T	**18 54.072**	**108 48.015**	**Process station (2-3 days)**	CTDs, MC400 (if depth not limiting)
3T	18 54.201	107 29.922	Survey station (<1 day)	CTDs
4T**	18 53.873	106 17.977	Survey station (<1 day)	CTDs, MC400 (if depth not limiting)
San Diego	32 42.900	117 09.750	Return port (May 22)	Demobilization


San Diego
Mazatlan
30 N
25 N
20 N
120 W
115 W
110 W
105 W
100 W
2T
3T
4T
5T
6T
7T
8T
9T
10T
11T
Ocean Data View

Radioisotope use plan Oceanus cruise OC1705A

a. The proposed work will investigate the rates, pathways and the genetic information behind the rates and pathways of microbial community metabolism in the oxygen minimum zone of the Eastern Tropical North Pacific. The work will take place between May 4th and May 23rd of 2017, and the probability of repeat work at a later date is uncertain. The cruise will take place in the Eastern Tropical North Pacific due East of Manzanillo between ETNP OMZ stns. 6 and 11 near 18° 54.0′ N, 104° 54.0′ W. The cruise will mobilize out of Mazatlan and demobilize in San Diego. Equipment and radioisotopes will be loaded in San Diego prior to the cruise.

b. Sean Crowe, Assistant Professor at the University of British Columbia will take administrative responsibility for the radio isotope work. Sean has more than 10 years of experience working with radioisotopes both on land and at the Sea. Additional scientific personnel working with radioisotopes include Bo Thamdrup, Professor at the University of Southern Denmark (trained user of radioisotopes with more than 25 years of experience on land and at sea) and Emilio Garcia-Robledo, Postdoc at Aarhus University (trained user of radioisotopes with more than 5 years of experience).

c. A list of all radioisotopes to be involved, chemical and physical forms of each, total of each in possession on the cruise, total of each to be "in use" at any time.

Radioisotope	Physical form	Chemical form	Specific activity	Total activity
^{35}S	dissolved ion	SO_4^{2-}	9-37 GBq/mmol S	185 MBq
^{14}C	dissolved ion	HCO_3-	1.85 GBq/mmol C	185 MBq
^{3}H	gas in N_2	CH_4	0.74 GBq/mmol CH_4	74 MBq

d. A description of proposed activities in sufficient detail to permit determination of types and magnitudes of radiation hazards involved.

^{35}S Use

- ~2 MBq of ^{35}S-SO_4^{2-} is injected into 250 mL stoppered serum bottles using a μ1 syringe (x ~14 at once)
- the 250 mL serum bottle is incubated for a period of 36 hours
- the serum bottles are opened and 10mls of seawater is removed and frozen in 15 mL Falcon tubes
- 20% Zn-acetate, and a HS- carrier, are added to the serum bottles to precipitate reduced sulphur
- the serum bottle contents are vacuum filtered through glass fibre filtes
- glass fibre filters are rinsed with 3^5S free seawater and frozen in Falcon tubes
- residual ^{35}S-SO_4^{-2} in the filtrate waste is precipitated from the seawater with BaCl2 and the resulting BaSO4 filtered out of the seawater and stored for disposal in home lab

^{14}C Use

- Water samples will be transferred to 50 mL serum bottles and closed by rubber stoppers (30 per station)
- 42 µL of the ^{14}C-HCO_3 stock will be injected to each bottle (42 µCi/sample, 1.25 mCi per Station)
- Samples will be incubated during 8 hours in a gradient of light
- Samples will be filtered to retain all the particles using polycarbonate filters of 0.2 µm pore size (expected ^{14}C amount in the filters <0.1 µCi). Filters will be rinsed with 10 mL of a 1 mM HCl solution to remove the residual ^{14}C-HCO_3 and preserved for later analysis.
- The filtered water will be acidified by adding 200 µL of concentrated HCl in order to remove the t residual ^{14}C-HCO_3 as CO_2 gas. This process will be done in the fume hood and the resulting release of ^{14}C-CO_2 will be lower than the limits established by the OSU's Policy On Radioisotopes. 5 mL of the acidified solution will be preserved in 15 mL falcon tubes and the rest will be discarded as acidified sea water waste as the expected content will be lower than 0.05 µCi per sample

^{3}H Use

- In the fume hood, 25 kBq of ^{3}H-CH_4 (~10 μL) is injected into seawater samples in glass vials closed with butyl rubber septa (Exetainers and serum bottles) using a microliter syringe. Each experiment involves up to 100 vials.
- The vials are incubated for up to 72 hours at ~12 °C. Incubations are terminated by the injection of 100 μL $ZnCl_2$ solution (50% w/v).
- After fixation, the headspace of each vial is flushed with pressurized air (supplied by an aquarium air pump) for 30 minutes using injection needles (25 gauge) for in- and outlet, in order to remove the remaining ^{3}H-CH_4. This is done in the fume hood.
- The vials are stored cold until shipment and further analysis in the home lab.

e. All radioisotope use will be restricted to and confined within the Radvan. No other OCEANUS facilities will be used in connection with the radioisotope work.

f. A short discussion of pertinent safety devices and procedures: 1. lab coats, gloves, shoe covers, other apparel, 2. portable GM survey meters to be provided by users, 3. proposed survey frequency and techniques, 4. personnel dose reduction equipment to be provided by users (i.e. shields, forceps, long pipets, vial opening devices, etc.), 5. fume hoods for gaseous isotopes, and 6. storage - freezers & refrigerators (located on van drawing).

g. A waste disposal plan. Note that the OSU license does not permit discharge of radioactive wastes to the ocean; all waste must be packaged and brought ashore for disposal. Disposal of radioactive waste by OSU involves a separate fee; fee schedule is available on request.

SYDDANSK UNIVERSITET
UNIVERSITY OF SOUTHERN DENMARK

To whom it may concern

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February 22, 2017

Professor Bo Thamdrup is a registered, trained and experienced user of radioactive isotopes in ecological experiments. His isotope work at the Institute of Biology has comprised experiments with ^{3}H and ^{14}C.

All work with radioactive isotopes at the Department of Biology, University of Southern Denmark, is carried out under a specific permission from the Danish Health Authorities.

All scientific and technical staff and students must demonstrate adequate knowledge about Danish laws and procedures concerning work with radioactive isotopes before they are given the permission to use these in experiments.

Yours sincerely

Poul Bjerregaard
Professor

In charge of work with radioactive isotopes at the Department of Biology and responsible towards the Danish Health Authorities.

AARHUS UNIVERSITY

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To whom it may concern

Aarhus March 3, 2017

This letter certifies that Dr. Emilio Garcia-Robledo has the necessary training for handling and use of ^{14}C. His training has been obtained under my supervision and follows the rules given by the Danish Agency for Health from which I am certified as a radiation coordinator.

Yours sincerely,

Bente Aa Lomstein

Bente Aagaard Lomstein
Radiation coordinator

THE UNIVERSITY OF BRITISH COLUMBIA

Risk Management Services

Sean Crowe

has successfully completed the Canadian Nuclear Safety Commission requirements in

Radionuclide Safety and Methodology

Including Receiving Class 7 Dangerous Goods

E. Sedgwick

Ted Sedgwick
RMS Radiation Safety Advisor

Rosemary Redfield
Chair, Advisory Committee on
Radioisotopes and Radiation Hazards

Course Date: 06/28/2013

Certificate No: RA-2013-129195

a place of mind