Two projects combined: (1) Microbial Activity in the Crustal Deep Biosphere, and (2) Collaborative research:

21/160

Slow Life Part 2

<table>
<thead>
<tr>
<th>PI</th>
<th>Beth Orcutt</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNOLS Staff</td>
<td></td>
</tr>
</tbody>
</table>
Lead Principal Investigator (PI)

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Co-Principal Investigators (CO-PIs)

<table>
<thead>
<tr>
<th>Full Name / Email</th>
<th>Organization</th>
<th>On voyage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stephanie Carr</td>
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<td>No</td>
</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Olivia D. Nigro</td>
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<td><a href="mailto:onigro@hpu.edu">onigro@hpu.edu</a></td>
<td></td>
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</tr>
<tr>
<td>Michael Rappe</td>
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<td>No</td>
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<td></td>
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Multi-SME Project

No

Short name

Slow Life Part 2

Website URL

-

Description of the Research Project

Two projects combined: (1) Microbial Activity in the Crustal Deep Biosphere, and (2) Collaborative research: Illuminating microbes and their viruses within the dark ocean crust through strain-level approaches

This project proposes to leverage existing subsurface infrastructure on the eastern flank of the Juan de Fuca Ridge with advances in single-cell based molecular and geochemical approaches to make fundamental new discoveries about the activity of life in the deep crustal biosphere. During a two-week research cruise, the research team will incubate crustal fluids in situ and in the laboratory with labeled substrates for tracking single-cell activity, coupled with radioisotope tracer activity and potentiostat measurements, with the objective of determining in situ and potential rates of activity and cellular physiology. The research will also identify which metabolisms active microorganisms utilize under in situ and laboratory conditions, the rates of these processes, and the microorganisms involved. The results are expected to provide explicit hypothesis testing of microbial activity and in situ microbial growth rates from the crustal deep biosphere to transform understanding of microbial activity in the crustal deep biosphere and generate critical information about the ability of life to survive under low energy conditions.

Summary of Field Work
This is a request for new shiptime to replace the 5.6 days lost to weather during AT42-11, to allow the project objectives for the funded work to be completed. We request 7 operational days with ROV Jason in single body mode to revisit the CORK observatories on the Juan de Fuca Ridge flank for conducting in situ experiments and collecting crustal fluid samples with third-party equipment (i.e. the Mobile Pumping System). Our objectives are to (1) conduct multi-day incubations to measure microbial activity with stable isotope tracers, (2) to collect filtered crustal fluid samples for assessing ecogenomics of subsurface crustal Bacteria, Archaea, and viral communities, (3) to collect large volume samples for viral induction experiments, and (4) to collect large volume samples for cultivation of novel thermophilic anaerobic microbial groups. Major tasks will be (1) the deployment and recovery of a free-flow chimney instrument and custom syringe samples on the top of the CORK at Hole U1362B for conducting an in situ experiment with stable isotopes, (2) collection and filtration of large volume fluid samples from multiple CORK horizons with the Mobile Pumping System connected to a Medium Volume Bag Sampler and Large Volume Bag Sampler from the University of Hawaii, (3) deployment and recovery of autonomous fluid filtration systems. The WHOI elevator will be needed to facilitate deployment and recovery of third-party equipment. Due to the need to conduct a multi-day in situ experiment as well as the time needed to turnaround the Mobile Pumping System samples, 7 days is the minimum amount of time needed to complete these tasks. We also request the use of a full-ocean depth Niskin rosettewith CTD sensors for collecting bottom seawater samples.

Biological oceanography
## Confirm Funding

<table>
<thead>
<tr>
<th>National Science Foundation</th>
<th>Ocean Science</th>
<th>Biological Oceanography (BO)</th>
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<tbody>
<tr>
<td>1737017</td>
<td>Funded</td>
<td></td>
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</table>
**Cruise Location**

General location  
IODP Holes U1362A/B on the eastern flank of the Juan de Fuca Ridge

Mobilization & Demobilization Ports

Preferred mobilization port  
Astoria (Oregon) - United States  

Preferred demobilization port  
Astoria (Oregon) - United States

Remarks  
Closest port in the USA to field site, and easy access from PDX airport. Lately, most cruises in this region have used Newport, OR, which is also fine.

Cruise Location Map

Remark  
The IODP Holes are within Canada’s Offshore Pacific Area of Interest, which will oversight from Canada’s Department of Fisheries and Oceans.

<table>
<thead>
<tr>
<th>Science station number</th>
<th>Station Type</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Sea depth</th>
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</thead>
<tbody>
<tr>
<td>1 - Sampling Station</td>
<td>Sampling Station</td>
<td>47° 00.00’ N</td>
<td>127° 00.00’ W</td>
<td>~2612m</td>
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</table>
## Cruise Location Analysis

### Exclusive Economic Zones

<table>
<thead>
<tr>
<th>Country</th>
<th>EEZ Name</th>
<th>Stations</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States</td>
<td>United States Exclusive Economic Zone</td>
<td>Sampling Station</td>
</tr>
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</table>

### Marine Protected Areas

<table>
<thead>
<tr>
<th>MPA</th>
<th>MPA Regions</th>
<th>Stations</th>
</tr>
</thead>
</table>
**Cruise Dates**

Preferred science start date
8/1/2022

Earliest acceptable science start date
6/1/2021

Latest acceptable science start date
9/30/2022

Date(s) Or Date Range To Avoid

Remarks
we would prefer to sail in 2021, but recognize the schedule is probably full. Summer is ideal time for this region. We were bumped into early May in 2019 and lost >50% of operational time to weather.

Science Days
7

Load Days
1

Unload Days
1

Transit Days
2

Total days
11
Ship Requirement

Preferred Ship
- RV Atlantis
- RV Roger Revelle
- RV Sikuliaq
- RV Thomas G. Thompson
- RV Kilo Moana
- RV Sally Ride

Please give details for your choice above
A global or ocean class vessel capable of supporting the ROV Jason or similar ROV

We need a regional/ocean/global class vessel capable of using ROV Jason and enough deck space for using the WHOI elevator. Jason is the preferred NDSF asset due to existing interfacing design of the Mobile Pumping System, the payload allowable for the MVBS and LVBS, and time needed on bottom for filtering large volumes of fluid. A similar ROV platform could be considered if it can interface with the Mobile Pumping System and support payloads needed. HOV Alvin is not preferred due to unknown interfacing with third-party systems, payload and time-on-bottom limitations, and physical discomfort issues for the PI, but we are willing to consider this if Jason is not available.

Does your project require a multi-ship operation?
No

Total Science Party Size
13

Number of different science teams
4

Number of Operator Provided Technicians needed
1

Total
14

Remarks
Four PIs, each bringing at least one student, postdoc or technician to assist with sample collection, plus two additional staff to handle the third-party equipment turnaround, and two scientific collaborators from Canada
Pooled and Portable Equipment

Remotely Operated Vehicle
Jason/Medea

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth of usage</td>
<td>1000+m</td>
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Please discuss your requirements for the above equipment.

Our field program requires the use of ROV Jason or similar capacity ROV that can interface with the third party Mobile Pumping System and associated peripherals.

We need ROV Jason to operate the third party Mobile Pumping System, Medium Volume Bag Sampler, and Large Volume Bag Sampler that were successfully used on AT42-11.
Ship Fitted Equipment

Dynamic Positioning (DP)

Fiber Optic Cable (0.681)

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Please describe how the project will use the 0.681 Power Optic Cable.</td>
<td>..</td>
</tr>
<tr>
<td>Please indicate the maximum depth.</td>
<td>..</td>
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Explain the rationale for the choices and state the flexibility to potentially use other equipment.

global or ocean class vessel capable of supporting the ROV Jason or similar ROV

Other ship fitted equipment that may affect scheduling

We need at least 20 feet of bench space for preparing experiments, plus access to an environmental cold room, sink, fume hoods, refrigerators, freezer, ultralow temperature freezer, and the liquid nitrogen system. We will need to use hazardous chemicals including formaldehyde, glutaraldehyde, and compressed gases.

We note that the new Universal Fluid Obtainer (UFO) designed by NDSF will not allow the collection of the large volume of fluids (10s to 100s of liters) needed to complete our project objectives, although it could be helpful to have as a backup in case of failure of the third-party Mobile Pumping System.

use of a full-ocean depth Niskin rosette with CTD sensors for collecting bottom seawater samples.
User Supplied Equipment

Science Supplied Equipment

- Over the side handling system requirements

Please list the number of science provided vans.

Does your equipment require an ITAR or EAR license? No
**Marine Environmental Impact Assessment**

Are there any known seasonal environmental constraints for working in the area? (E.g. Breeding season)
No

Are any items of deployed equipment planned to remain unrecovered? (E.g. Anchors).
No

Is any of the material not planned to be recovered hazardous?
No

Are any substances to be purposefully introduced into the water column to support the science?
No

Will any marine organisms be intentionally sampled/captured/killed (including invertebrates and flora)?
No

Do you plan to recover and import any biological organisms or samples?
No

Will you bring any hazardous materials for the cruise?
No

Will your project be using radioisotopes?
No

Will your project be conducting natural abundance isotope work?
No

Will your project require a Marine Mammal Protection Act Incidental Harassment Authorization?
No
Costings

Ships

<table>
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<th>Rate</th>
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<tr>
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<td>Ship Cost</td>
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<td>Unload Cost</td>
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<td><strong>Total</strong></td>
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Other Cost

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