2014 Annual report for SCOR Working Group 144: Microbial Community Responses to Ocean Deoxygenation

Working group 144 was funded December 13-2013, and this report outlines working group 144 activity since.

Sunday, September 14, 2014

EXECUTIVE SUMMARY

SCOR Working group 144 *Microbial Community Responses to Ocean Deoxygenation,* is in its early days since the unofficial inaugural meeting at the ASLO ocean sciences meeting in late February, 2014. The inaugural meeting was well attended and was used to revisit the working group terms of reference and to plan the first official working group activity, a practical workshop held at Saanich Inlet/UBC in British Columbia, Canada. The practical workshop took place from July 13th, to 18th 2014, and was attended by 6 working group full members, 2 associate members (or their representative), and involved 12 additional student and technical participants. Notably, attendees included Ngappa Ramaiah from India, and Jung-Ho Hyun from South Korea, facilitating new interactions between Asian, European, and North American researchers. This practical workshop was enabled through ship time procured by working group members Steven Hallam and Sean Crowe. During the workshop, the working group ground truthed common standards for process rate and molecular microbial measurements and identified model ecosystems for future cross-scale comparative analyses. Completion of the workshop largely fulfilled the working group's terms of reference for year 1. A highlight of the workshop was the comparison of in situ sampling technologies to the conventional hydrocasts using sampling bottles that are recovered to the surface. This comparison addresses long-standing issues in research on low oxygen waters arising from artifacts produced through sample recovery. This in situ capacity was enabled through efforts by working group member Virginia Edgcomb to engage McLane Instruments in our working group activities, and was further enhanced through the involvement of Craig Taylor from Woods Hole Oceanographic Institute. Once all of the data products from the workshop have been produced, the working group will compile a report documenting the workshop outcomes. The working group has also constructed a website (http://omz.microbiology.ubc.ca/index.html) to enhance its visibility, the visibility of oxygen minimum zone research in general, as well as to facilitate information sharing between working group members and other researchers working on oxygen minimum zones. The report from the practical workshop will be accessible through the Working Group Website and will be submitted to SCOR along with the year 2 working group report. SCOR working group 143 participated in the practical workshop, forging links between the groups 143 and 144. Following the practical workshop, plans were laid for the working group meeting in year 2. This will take place in Warnemunde, and will be coordinated and hosted by Klaus Jurgens.

ACTIVITIES

Working group 144 conducted 3 principle activities during the reporting period: 1) Held an unofficial inaugural meeting at ASLO Ocean Sciences; 2) Conducted a practical workshop at Saanich Inlet; and 3) constructed a website. Details of these activities are described below.

I) Inaugural meeting held (02-26-2014).

Working group 144 held an inaugural unofficial meeting at the ASLO Ocean Sciences conference in Honolulu, February 26th. In total, 6 full members, and 4 associate members attended (Bess Ward, Steven Hallam, Sean Crowe, Klaus Jurgens, Virginia Edgcomb, Phyllis Lam, Veronique Garcon, Jung-Ho Hyun, Jody Wright, Raquel Vaquer-Sunyer, and Jon Kaye). The aims of this first meeting were to: 1) discuss the overall objectives of the working group; 2) discuss the annual agendas through the 4 years; and 3) plan the timing of the field workshop in Saanich Inlet for 2014. The outcome of the inaugural meeting was a July date for the year 1 workshop, plans for the year 2 meeting in Warnemunde Germany, with a possible field experience, and a revised plan for the year 3 meeting, which was changed at the request of SCOR from a working group meeting at a topical conference, to an educational workshop in Chile. Planning details outlined below.

1) Year 1 chairs Hallam and Crowe, in consultation with leadership coordinator Ward, have been planning the Year 1 workshop.
2) The workshop will take place at Saanich inlet and on the University of British Columbia campus
3) The Workshop will be divided into a practical field component, and a discussion forum.
4) Hallam and Crowe to secure 3-days of ship-time for the workshop
5) Working group member Edgcomb to secure novel in-situ sampling equipment that could be contributed by McLane Research Labs.
6) Crowe to acquire trace optical oxygen sensors for use in the workshop
7) Hallam to secure sequencing capacity
8) Experiments will be designed to ground truth incubation protocols to optimize the coupling of process rate and microbial community information. In particular, the experiments will test for various bottle effects and sampling artifacts related to O_2 contamination. These experiments will focus on carbon and nitrogen cycling in low oxygen waters.
9) A collaborative connection to be made with SCOR WG 143 to integrate N_2O and CH_4 measurements into the practical workshop
10) A cruise plan to be drafted
11) Expected 7 of our full members to attend the workshop, in addition to 3-5 associate members, including members from India and Korea.

II) Saanich Inlet Workshop held (13-07-14 to 18-07-14)

The first official SCOR Working Group 144 activity was a practical workshop hosted by Hallam and Crowe at Saanich Inlet and at the University of British Columbia, Vancouver, BC. The workshop was a week long event that comprised informal meetings and round-table discussions in addition to 3-days of practical sampling activities and experiments on Saanich Inlet, a model coastal ocean analogue for marine oxygen minimum zones (OMZs). The overall objective of the field program was to test and compare sampling and incubation strategies for studies integrating molecular microbial observations with process rate measurements.

Participants:

Working Group Full Members: Steven Hallam, Sean Crowe, Virginia Edgcomb, Klaus Jurgens, Phyllis Lam, Nagappa Ramaiah

Associate Members: Jung-Ho Hyun, Jennifer Brum (on behalf of Mathew Sullivan)

Additional participants: Craig Taylor (WHOI), Maria Pachiadaki (WHOI), Hallam and Crowe lab students and staff (Monica Beltran-Torres, Celine Michiels, Stilianos Louca, Diane Fairley, Melanie Scofield, Andreas Mueller, Lora Pachomova, Kate Thompsen, and Kai Blumberg).

Planning:

The first day of the workshop (14-07-2014) comprised detailed planning and equipment set-up for the practical component the following days (Photo 1). Two in situ sampling devices from McLane labs were supplied by Edgcomb and Taylor including the commercially available PPS system (http://www.mclanelabs.com/master_page/product-type/samplers/phytoplankton-sampler) for in situ filtration, collection, and preservation of marine microbial biomass, and a pilot/beta version of an in situ incubation device (IPS), not yet commercially available. Experiments were designed to compare and cross-calibrate the in situ sampling devices with conventional bottle sampling protocols. A mooring was set-up to deploy the in situ incubation system over a 72 hour period. Additional experiments were planned to evaluate artifacts during conventional bottle-based sampling (eg O_2 contamination). A further set of experiments were designed to test bottle effects on microbial community composition and gene expression profiles during manipulations commonly imposed when making process rate measurements (bottle enclosure, labeled substrate addition). The IPS was configured to conduct 4 incubations with ^{15}N-labeled nitrogen compounds to determine rates and pathways of microbial nitrogen transformations. A 5th incubation was set up with ^{13}C-bicarbonate to determine rates of microbial dark carbon fixation. The IPS was configured to also collect biomass sampled directly from the incubation chamber and to preserve this biomass with RNA later for transcriptomic studies. Photo 1 below shows Taylor and Edgcomb configuring the IPS in Crowe's lab at UBC.

For comparison, parallel experiments were set up using conventional ex situ incubations in bottles. Planned ex situ process rate experiments included ^{15}N-label based measurements of microbial N transformations, ^{13}C-labed based dark carbon fixation, in

addition to measurements of bacterial production using ^{3}H-leucine and ^{3}H-thymidine. A number of the ex situ experiments were designed to evaluate bottle effects, the addition of labels on microbial community composition and transcription, as well as the effect of O_2 contamination as a result of bottle sampling. Rate measurements were set-up as a function of oxygen concentrations, and oxygen concentrations were to be monitored using sensitive (nM detection limits) oxygen optodes. ^{3}H-based measurements of bacterial production have been calibrated in oxygenated waters, but to our knowledge not in anaerobic OMZs. We therefore setup experiments to determine conversion factors for both thymidine and leucine based measures of bacterial production.

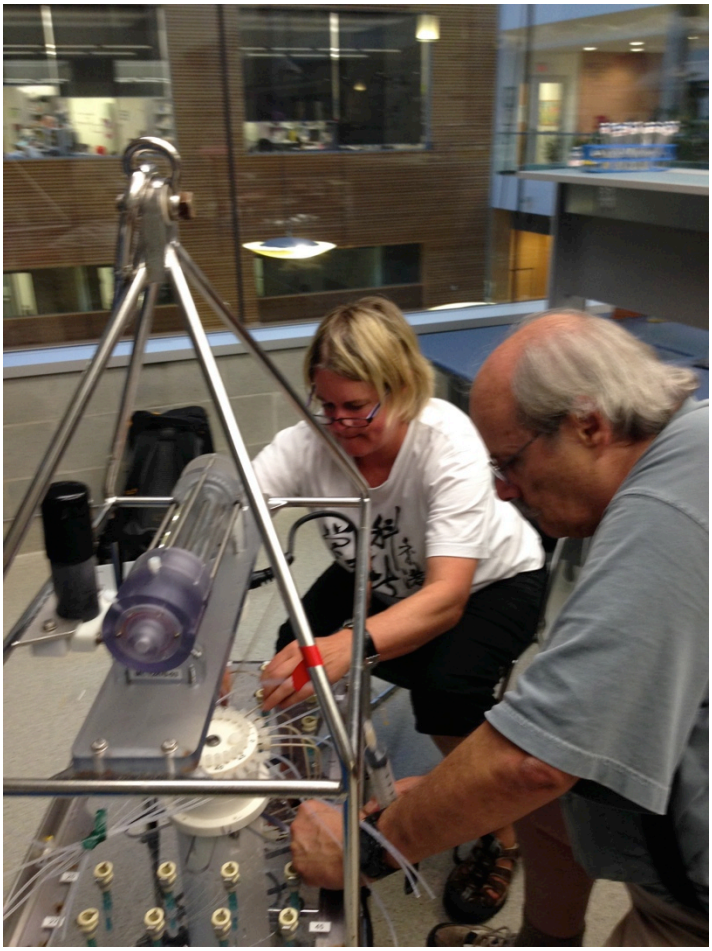


Photo 1. Taylor and Edgcomb configure McLane Labs IPS in Crowe's lab at UBC.

Comparisons of molecular microbial studies conducted on in situ recovered material vs. bottle recovered, surface filtered, material were also planned. Experiments to evaluate different filter combinations were planned since the PPS cannot accommodate the Sterivex filters typically used for ex situ filtering. Filters used on the PPS (and IPS) are 0.4 µm poresize, whereas the sterivex filters are 0.2 µm. Samples were to be collected and preserved for protein, RNA and DNA based profiling of microbial community composition and expression.

Execution:

Planned experiments were commenced the following day (15-07-2014) and conducted over a period of 4 days. Hallam and Crowe secured ship time on the RV Strickland based out of the University of Victoria to support the working group activities with 3 days of sampling. The working group deployed the moored IPS system in Saanich inlet on the first day of the practical component (photo 2 below). The mooring was configured for a 3 day deployment that would enable conduction of the 5 consecutive in situ incubations. The IPS was deployed at 165m depth, just below the upper boundary of Saanich Inlets anoxic, sulphidic deep waters, which was confirmed with CTD and chemical profiling prior to deployment. Following deployment of the mooring, bottle sampling for ship-board filtration and collection of biomass commenced. 3 depths, 150m, 165m, and 185m were sampled. These depths were targeted to capture a gradient in redox conditions. Following sample collection with bottles, the in situ PPS work commenced (photo 3)—in situ samples of microbial biomass were collected from 3 depths.

Photo 2. Edgcomb explaining IPS system to workshop participants

Sampling the 3 depths with the PPS took roughly 4 hours. Over this 4 hour period, bottle samples were filtered directly aboard for RNA and protein based molecular microbial analyses. Finally, bottle sampling to recover water for the ex situ incubations was collected from 5 depths; 135m, 150m, 165m, 185m, and 200m. Water samples were also collected from all depths sampled for a standard suite of nutrient analyses. Following

recovery of water for bottle based incubation studies, working group members returned to UBC to initiate label based process rate measurements and filter remaining water samples for DNA based molecular analyses. All attending working group members participated in the ship based activities, aided by Hallam and Crowe's students and technicians, as well as Craig Taylor, Maria Pachiadaki, and Jennifer Brum (on behalf of Mathew Sulivan).

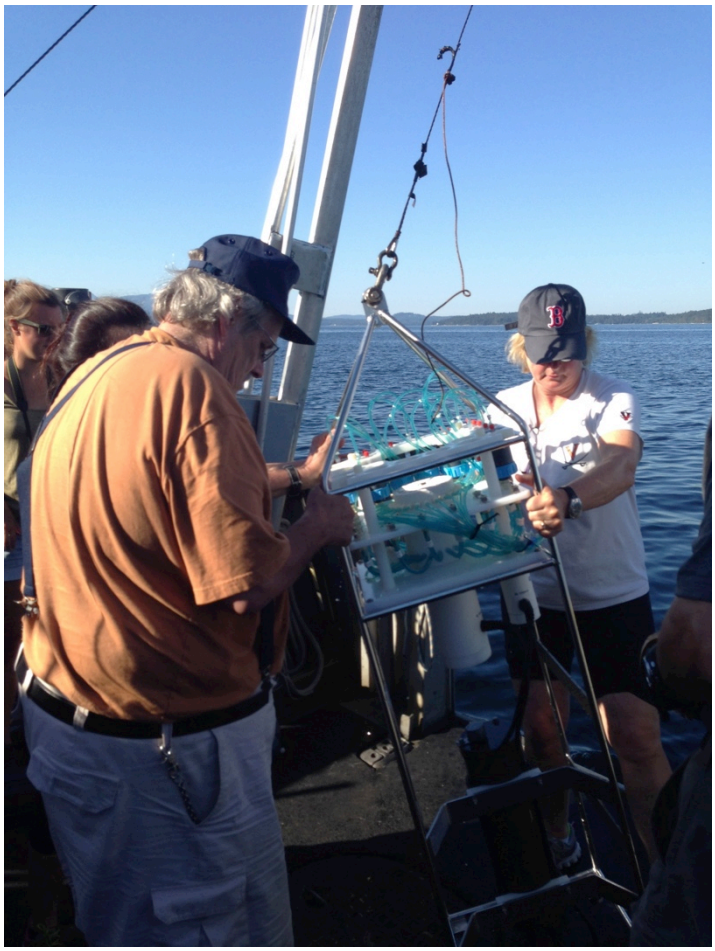


Photo 2. Edgcomb and Taylor deploying PPS system on RV Strickland

At UBC Lam supervised and conducted ^{15}N-based measurements of microbial N-transformations aided by Crowe's Ph.D. student Michiels. Hyun supervised and conducted ^{3}H-thymidine, and ^{3}H-luecine based measurements of bacterial production rates and calibration of carbon conversion factors. Crowe's students Michiels and Thompson aided in Bacterial Production measurements. Jurgens and Crowe conducted measurements of dark carbon fixation. Crowe measured oxygen concentrations using trace optodes in a subset of the bacterial production, and dark carbon fixation bottles. Lam and Michiels also measured O_2 in a subset of the N-transformation rate measurements. Hallam and Brum supervised and conducted filtration aided by Beltran-Torres and Hallam lab technicians and students.

On the second practical day (16-07-2014) some working group members were at sea to collect samples from additional depths to fill out the profile (10m, 75m, and 135m). Other working group members finished lab based incubations and filtration.

Resolution:

The final day of scheduled working group activities (17-07-2014) comprised largely of a round table discussion with all working group members. Thompson acted as round table rapporteur. Round table discussion topics included:

- Producing a data matrix for the practical aspects of the workshop
- An activity summary
- Workshop report and task allocation
- Planning for future working group activities and the next working group meeting

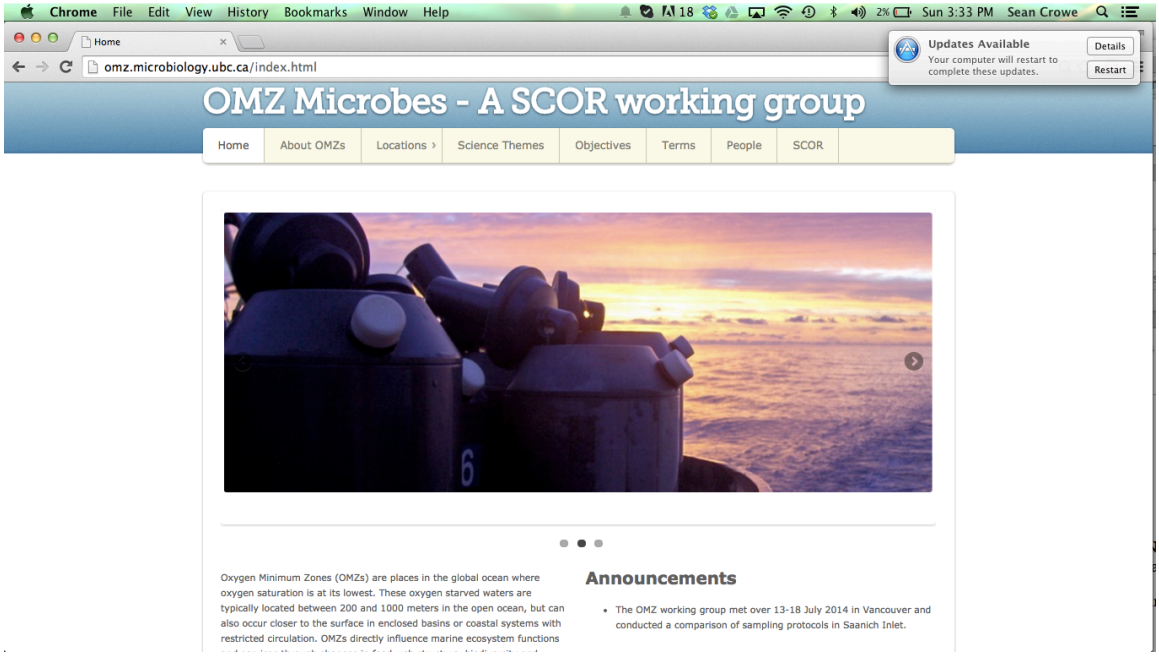
On resolution the working group decided to produce a report (over and above this annual Working Group Report) that documents the findings of the workshop and contains all of the data generated. This report would be used to inform discussions at the second working group meeting and will form the basis for the white paper to be produced at this second meeting. Plans were made to produce all data products resulting from the practical workshop well in advance of the second meeting so that they could be both included in the workshop report and used effectively at the second working group meeting. The second working group meeting was planned for the summer of 2015, to be held in Warnemunde Germany. This meeting will be hosted by Jurgens, who will lead working group activities in the second year.

Other activities conducted the final day included additional sampling of Saanich Inlet, mooring recovery, and synergistic interactions with SCOR working group 143. Philippe Tortell from WG 143 participated in the round table discussion, explaining the terms of reference for WG 143. Ways in which the two groups could interact were discussed, and it was decided that representatives from WG 143 would be invited to the WG 144 meeting in 2015. Samples of trace gases were also collected to directly link the Saanich Inlet workshop to WG 143's objectives to standardize and calibrate trace gas measurements. Mooring recovery was unfortunately unsuccessful due to a combination of adverse weather conditions and equipment failure. An additional day of ship time was acquired for an attempt at recovering the mooring and the sunken IPS system. Ngappah and Crowe along with technicians from the Hallam lab spent an additional day trawling unsuccessfully for the lost most mooring. Rescue operations are still underway, and Edgcomb and Taylor have enlisted the help or a recovery engineer from WHOI.

Overall, the practical workshop facilitated collection of critical data to inform discussion at the working group meeting in year 2, and the ultimately a white paper identifying standards and opportunities in the study or microbial communities in expanding oxygen minimum zones.

III) Website construction

Crowe and Hallam have supervised with input from working group members the construction of a working group 144 website put together by Hallam's technician Kellogg. The website can be viewed on the world wide web at http://omz.microbiology.ubc.ca/index.html. The website is under constructions, but some sample screen shots are included below.



STATUS OF FULFILLING TERMS OF REFERENCE

SCOR WG 144 set the following term of reference for its first year of activities:

1. In year 1 of the working group we will convene a practical workshop in Saanich Inlet, a seasonally anoxic fjord off the coast of Vancouver Island British Columbia, Canada, to ground truth common standards for process rate and molecular measurements and identify model ecosystems for future cross-scale comparative analyses.

With the completion of the practical workshop just 7 months following the inaugural meeting of working group 144, the working group is well on track for fulfilling terms of reference for year 1. Remaining tasks include some data processing and analysis, data compilation, and the preparation of the workshop report.

PLANS FOR THE COMING YEAR IN RELATION TO THE TERMS OF REFERENCE AND CAPACITY BUILDING

Capacity building activities scheduled for this year center around the involvement of Working Group Members from India and Korea in the Saanich inlet Workshop. Interaction with members from Namibia and Chile will take place largely through the website construction with oxygen minimum zones from these regions featuring prominently. All working group members are contributing information to the website. We also plan to have all working group members participate in the meeting planned for Warnemunde in 2015.

ANY SPECIAL REQUESTS FOR EXTRA FUNDING

None currently identified, however, the costs of the practical workshop exceeded our current budget.