Cruise Report for R/V *Atlantis* + ROV *Jason* Expedition AT42-11
Prepared June 2019

Slow Life in the Fast Lane: Microbial Activity in the Crustal Deep Biosphere

Expedition dates and Ports: 15 May 2019 to 28 May 2019, Newport, OR, to Newport, OR (mobilization: 11-14 May 2019, demobilization: 29 May 2019)

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ROV Expedition Leader:
Tito Collasius, Woods Hole Oceanographic Institution

Funding provided by:
Primary cruise support from NSF project OCE-1737017 to Orcutt
Complimentary science support funding: NSF OCE-1851582 and linked awards – Co-PIs Stephanie Carr, Olivia Nigro, and Mike Rappé*;* NSF OIA-1826734 and linked awards – Co-PIs Duane Moser, Beth Orcutt, Ramunas Stepanauskas, and Kai Ziervogel*;* NASA EXO18 grant 80NSSC19K0466 – PI Beth Orcutt

Cruise t-shirt Design by Josh Wood, Deep Carbon Observatory

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Summary

Site Name	Lat (N)	Lon (W)	Water depth (m)
Hole U1362A	47º 45.662	127º 45.674	2658
Hole U1362B	47º 45.499	127º 45.733	2658

The primary focus of research cruise AT42-11 aboard R/V *Atlantis* with ROV *Jason* was the study of the microbial biosphere in subsurface oceanic crust, with primary objectives including (1) seafloor and shipboard incubations of crustal fluids from IODPP Holes U1362A/B to identify rates of microbial activity and the active members of the microbial population (awards NSF OCE-1737017 and NASA 80NSSC19K0466 to Orcutt) and (2) collecting and filtering large volumes of crustal fluid for analysis of microbial ecogenomics and viruses as well as enrichment and culturing efforts (NSF OCE-1851582 and linked awards to Rappé, Nigro and Carr). Secondary objectives included downloading pressure data from the CORK observatories for collaborators; collection of water column samples for incubations to study microbial activity (NSF OIA-1826734 and linked awards); collection of crustal fluids, sediment push cores, and water column samples for studying thermophilic spore dispersal; collection of multi-year fluid samplers from the observatories; deployment of a flow meter on the observatories for a collaborator; and deployment of a top plug at Hole U1301A. These sites are located within Canadian waters.

In service of the primary objectives of the cruise, four dives of ROV Jason were completed during this cruise, totaling 79 hours of bottom time operational for science objectives. A total of 136 hours was lost from the program due to weather. ROV dives were supplemented by five deployments of the WHOI elevator to ferry equipment back and forth to the seafloor. One multi-day seafloor incubation of bottom seawater with spike solutions was completed, as was one 6.5-hr incubation of crustal fluid from Hole U1362B. Longer incubations times with the crustal fluids were not possible due to operational challenges with science equipment and loss of dive time due to weather. Three collections of fluid for shipboard spike incubations were completed (one of bottom water, two from Hole U1362B); collection of fluid from Hole U1362A for this purpose was not possible due to loss of dive time. In service of the ecogenomics, virus, and culturing efforts, a total of 352 L of raw crustal fluid was collected with the Mobile Pumping System from the CORKs, supplemented by 674L of fluid filtered *in situ* with the MPS and a record 21,300 total liters filtered *in situ* with the passive filtration units. This achieved primary objectives of getting samples from all horizons to assess spatial heterogeneity in the crustal aquifer, but unfortunately repeat sampling at Hole U1362A was not possible due to the lost time to achieve the objective of assessing temporal variability. All sampled CORKs were left sealed with valves closed, to enable continued pressure monitoring.

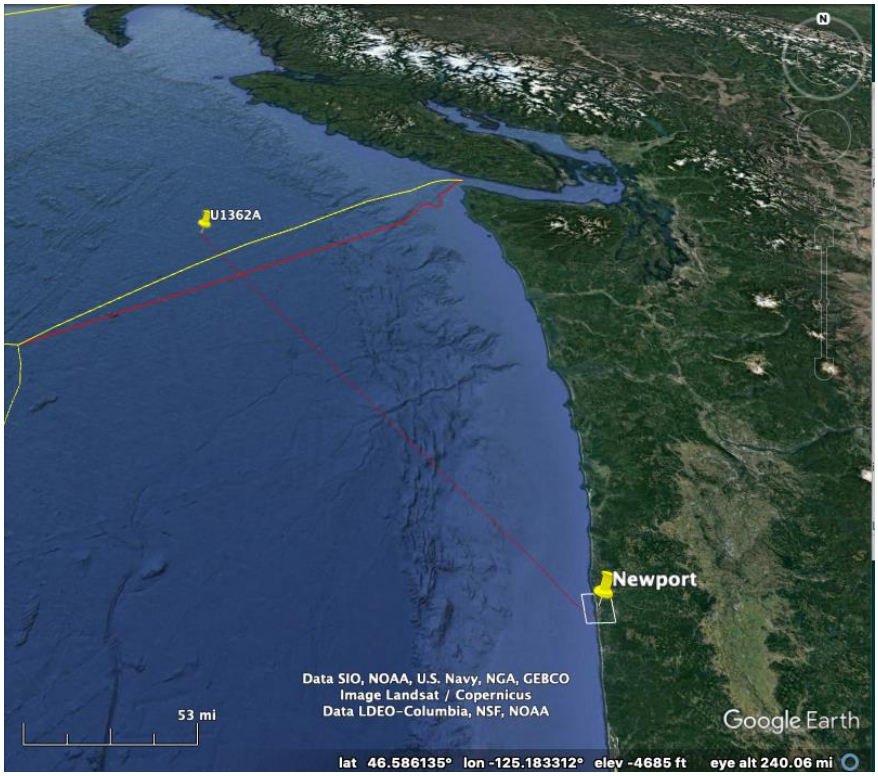
When primary objective operational conditions allowed, the following secondary objectives were accomplished: collection of five years of pressure data from Holes U1362A/B, collection of eight full water column profiles with the ship's CTD Niskin rosette, collection of three sediment push cores for the study of thermophilic spores, and collection of two multiyear OsmoSamplers from the same holes. Planned deployments of the flow meter at Hole U1362A and of the top plug at Hole U1301A were not possible due to loss of dive time.

This cruise report will be made publicly available through the Biological and Chemical Oceanography Database Management Office (BCO-DMO) project portal that has been established for the primary award (https://www.bco-dmo.org/project/700324, Project Coordinator: Beth Orcutt). The ship underway and CTD data will be made publicly available within the next 6 months through the Rolling Deck 2 Repository (R2R) database under cruise AT42-11: https://www.rvdata.us/search/vessel/Atlantis. Jason dive logs and frame grab imagery are immediately publicly available through the online "Jason Virtual Van" (http://4dgeo.whoi.edu/jason/). Video and still imagery described in the cruise report can be made available to interested parties upon request to the chief scientist. **All use of ROV *Jason* cruise imagery should include the following credit: Courtesy of RV *Atlantis* cruise AT42-11 ROV Jason dive ###, 2019, chief scientist Beth Orcutt, Bigelow Laboratory for Ocean Sciences, US National Science Foundation, © Woods Hole Oceanographic Institution; and an email should be sent to borcutt@bigelow.org and media@whoi.edu to document use.**

Following shore-based datasets generated by the primary awardees will be made publicly available through BCO-DMO. All nucleic acid sequence data generated from samples from this project will be made publicly available in a timely manner. We believe that these actions are consistent with the spirit of the Nagoya Protocol international agreement under the Convention on Biological Diversity (CBD) which entered into force in October 2014. The objective of the Nagoya Protocol is the fair and equitable sharing of the benefits arising from the utilization of genetic resources, including by appropriate access to genetic resources and by appropriate transfer of relevant technologies, taking into account all rights over those resources and to technologies, and by appropriate funding, thereby contributing to the conservation of biological diversity and the sustainable use of its components. Although Canada is a Party to the CBD, Canada is not currently a Party to the Nagoya Protocol; therefore, there is no comprehensive access and benefits sharing system in place to govern access to genetic resources or to facilitate the sharing of benefits arising from their use: https://www.canada.ca/en/environment-climate-change/corporate/international-affairs/partnerships-organizations/nagoya-protocol-access-genetic-resources.html. By publicly sharing "digital sequence information" (i.e. nucleic acid sequence data) gathered as part of this research cruise, we believe that we will be in compliance with the spirit of the CBD: https://www.cbd.int/doc/decisions/cop-14/cop-14-dec-20-en.pdf. All science party members should be aware that if Canada becomes a party to the Nagoya Protocol, there may be retroactive limits on the use of "genetic resources" collected during this research cruise when Access and Benefit Sharing arrangements are defined: https://www.abs-canada.org/about/faqs/.

Operationally, the ship and ROV *Jason* assets performed exceptionally well. One minor issue with ship operations (that the chief scientist is aware of) included a recurring problem with the dissolved oxygen sensor on the CTD Niskin Rosette. The SSSG were attendant to the issue and worked to resolve that the sensor data (i.e. voltage readings) were actually correct, but that an error with the calculation algorithms was causing the processed data to look spiky. The issue was not resolved with the vendor during the cruise, but manual processing of the data generated smoothed profiles. Another minor issue discovered during dive J2-1141 was competing navigation software being run from Top Lab during Jason navigation operations, which confounded the nav file. Luckily, since all our dives were at known fixed coordinates, this was not an issue for our dive program. The top lab program was shut down and the issue was

resolved. The ship's officers are commended for superbly maneuvering the ship during turbulent seas (with several days of steady winds above 25 kn), and the bosun and deck crew are commended for flawlessly executing elevator deployments and recoveries. ROV *Jason* operations were also very successful, and we were able to accomplish engineering dive needs as well as science operations. One bounce dive at the beginning of the dive program was quickly resolved (issue with o-ring in Reson). Most dives ended when the dive plan was completed, with the exception of dive J2-1142 ending early due to failure of the hydraulic pressure unit. The Jason group tracked minor issues with the winch levelwind during recoveries, but this did not impact science operations in any way. The LARS was expertly piloted during all deployment and recovery operations (including the exquisite first time deployment by Molly Curran). Minor ground faults were quickly resolved and did not affect the dive program. One of the Jason AFX failed on the last dive, but this was quickly resolved and did not affect the dive program as we were in layup mode during elevator recovery at the time. Notably, the new SeaLog program performed well and was easy to navigate compared to Virtual Van event logger, although there were minor glitches in capture of the Sulis camera frame grabs. The Jason data manager Tina Haskins is commended for tirelessly and enthusiastically managing data flows, in particular for making dive video clips and Sulis images immediately available after dives for the science party to make highlights. Pilots Chris Judge, Jimmy Varnum, and Korey Verhein are all commended for patiently and expertly handling delicate seafloor operations with new science equipment as well as patiently sitting through endless water pumping operations. Expedition Leader Tito is generously thanked for providing guidance to dive planning and offering assistance with science equipment modifications. It was a pleasure to work with the entire shipboard team.



Ship track (purple line) for AT42-11 as shown in Google Earth. Yellow line indicates EEZ demarcation between USA and Canada.

Personnel and Affiliations

(* indicates first oceanographic cruise; *n* = 10/21 science party members, 48%)

Science party

Beth N. Orcutt, Bigelow Laboratory for Ocean Sciences – *chief scientist*
Stephanie Carr, Hartwick College – *co-PI*
Olivia Nigro, Hawaii Pacific University – *co-PI*
Mike Rappé, University of Hawaii – *co-PI*

*Jorge Agobian, University of Miami RSMAS – *graduate student*
*Anne Booker, Bigelow Laboratory for Ocean Sciences – *postdoc*
Anirban Chakraborty, University of Calgary – *postdoc*
Jessica Choi, University of Pennsylvania – *postdoc*
Tim D'Angelo, Bigelow Laboratory for Ocean Sciences – *research associate*
*Kerry Dykens, University of New Hampshire – *graduate student*
Trevor Fournier, University of Alaska Fairbanks – *research technician*
Kelle Freel, University of Hawaii – *postdoc*
Andrian (Adi) Gajigan, University of Hawaii – *graduate student*
*Tylisha Gourdine, Hartwick College – *undergraduate student*
*Melody Lindsay, Bigelow Laboratory for Ocean Sciences – *postdoc*
*Duane Moser, Desert Resesrch Institute – *research scientist*
*Jacob Munson-McGee, Bigelow Laboratory for Ocean Sciences – *postdoc*
*Adam Price, University of California, Santa Cruz – *graduate student*
*Cherise Spotkaeff, University of Hawaii – *graduate student*
*Martin Van Den Berghe, University of Southern California – *graduate student*
Kai Ziervogel, University of New Hampshire – *research professor*

L to R: Beth Orcutt, Mike Rappé, Olivia Nigro, Anirban Chakraborty, Kelle Freel, Kerry Dykens, Jessica Choi, Jorge Agobian, Kai Ziervogel, Cherise Spotkaeff, Martin Van Den Berghe, Adi Gajigan, Trevor Fournier, Stephanie Carr, Jacob Munson-McGee, Ty Gourdine, Adam Price, Tim D'Angelo, Duane Moser, Melody Lindsay, Anne Booker

Jason group
Alberto (Tito) Collasius – Expedition Leader
James Varnum
Korey Verhein
Molly Curran
Drew Bewley
Manyu Belani
James Convery
Chris Judge
Tina Haskins
Andy Billings

Ship's crew
Master RV *Atlantis* – Al Lunt
Chief Mate – Peter Leonard
Second Mate – Shae
Third Mate – Molly Smith
COMET – Jim Painter
Bosun – Ronnie Whims
SSSG – Allison Heater, Emily Shimada
Able-Bodied Seaman – Jim McGill, Patrick Neumann
Ordinary Seaman – Clindor Cacho, Robert Arthur, Cecile Hall, Austin Castagno
Chief Engineer – JT Walsh
First Engineer – Phil Brennan
Second Engineer – Paul Ruh
Third Engineer – Bobby Dow
Oilers – Mike Spruill, Corey Lawton, Bruce Engert
Wiper/OS – Ryan Gregory
Cook: Mark Nossiter
Steward – Tanzi Edwards
Mess Attendant – Janusz Mlynarski

Day by day summary (all times local GMT-7 unless written otherwise):

Day	Activities
11 May	Start of mobilization in Newport, OR; loading of Jason equipment
12 May	Continued mobilization, continued loading of Jason equipment, WHOI containers moved off ship onto dock, science party pallet loading
13 May	Continued mobilization, gas tanks delivered
14 May	Final mobilization tasks. Safety briefing at 09:00, science party meeting at 18:30
15 May	Departure at 06:00 and underway under cloudy skies. Pilots departed 06:35. Transit at roughly 12 kn to site. Science party continued assembling equipment for loading on ROV Jason and elevator. Jason briefing and introduction to SeaLog software at 15:30. Pre-dive checks looked good.
16 May	Arrived on station 03:00. Minimal current and 10 kn winds. Waited for daylight for elevator launch. 06:12 start launch, but had to halt initial deployment due to loose equipment, but then elevator launched successfully 06:56. **Jason dive J2-1139** (launch 08:40) was aborted due to an issue with a sensor (on deck 09:46), but then **Jason dive J2-1140** launched successfully (10:10). The dive began with 6 hours of engineering operations, then moved immediately in to science operations at IODP Hole U1362B.
17 May	Dive J2-1140 continued at IODP Hole U1362B, with an elevator recovery in the morning (05:50 recovery) and Jason recovered in the evening (18:57). During 12-hr Jason turnaround period, **CTD casts 1 and 2** were completed (20:00 and 21:26 starts). Issues with oxygen sensor data, but collected a full water column cast above IODP Hole U1362B to begin incubation experiments
18 May	Started the day with elevator launch as scheduled 05:45 and continued with pre-dive check for 08:00 launch, but then launch delayed 12.5 hrs due to weather/swell. While waiting on weather (WOW), **CTD cast #3** conducted (13:12 launch) but no water samples collected due to continued issues with oxygen sensor, SSSG had Atlantis T-shirt sale, and science party had meeting. **Jason dive J2-1141** began at 20:30 with a brief recovery after first splashing into water to tighten science party equipment on the Jason basket.
19 May	Dive J2-1141 continued. Elevator released at 16:42, on surface at 18:10. Jason on deck by 18:18, then elevator recovered 19:06.
20 May	**Jason dive J2-1142** pre-dive operations began in the morning with an elevator launch at 06:53. Launch of Jason was slightly delayed due to science party equipment issues (UH filtering manifold missing a filter), but then got under way by 08:15. Although 24-hr dive was planned, operational plan changed part way through to accommodate earlier recovery due to deteriorating weather. Elevator released 13:54 and recovered 15:15. As revised plan got underway, failure of hydraulic pressure unit on Jason aborted dive; Jason on deck 18:40.
21 May	Continuing to WOW for Jason deployment. Sustained wind speeds increased from 5 to 14 m/s (10-27 kn), heave from 6-12 ft. Jason HPU issue resolved. **CTD Cast 4, 5, and 6** started 08:29, 14:12, and 18:37, respectively.
22 May	Continuing to WOW. Daily sustained wind speeds of 13 m/s (25 kn) with gusts up to 16 m/s (31 kn), heave from 6-12 ft. Weather decks secured so no CTDs possible.

23 May	Continuing to WOW. Winds (10-12 m/s; 19-23 kn) and swell (<6 ft) down enough to allow CTDs. **CTD Cast 7 and 8** started at 08:53 and 14:06, respectively.
24 May	Continuing to WOW. Winds up (14-20 m/s; 27-39 kn) and swell up, weather decks secured.
25 May	Continuing to WOW. Sea state decrease after 6PM local. Deployed elevator at 20:05 local, and started **Jason dive J2-1143** at 21:10 local.
26 May	J2-1143 continued. Elevator recovered 04:53 local; 2nd elevator deployed 05:50 local and recovered at 11:53 local.
27 May	J2-1143 was off bottom at 06:30 and recovered beginning at 08:00, then the transit back to Newport, OR, port began.
28 May	Took on pilot offshore Newport at 06:30, alongside by 08:00, gangway down by 09:30 for demobilization to begin.
29 May	Second day of demob. All science personnel off the ship after last container picked up at 15:00.

Dive Summaries

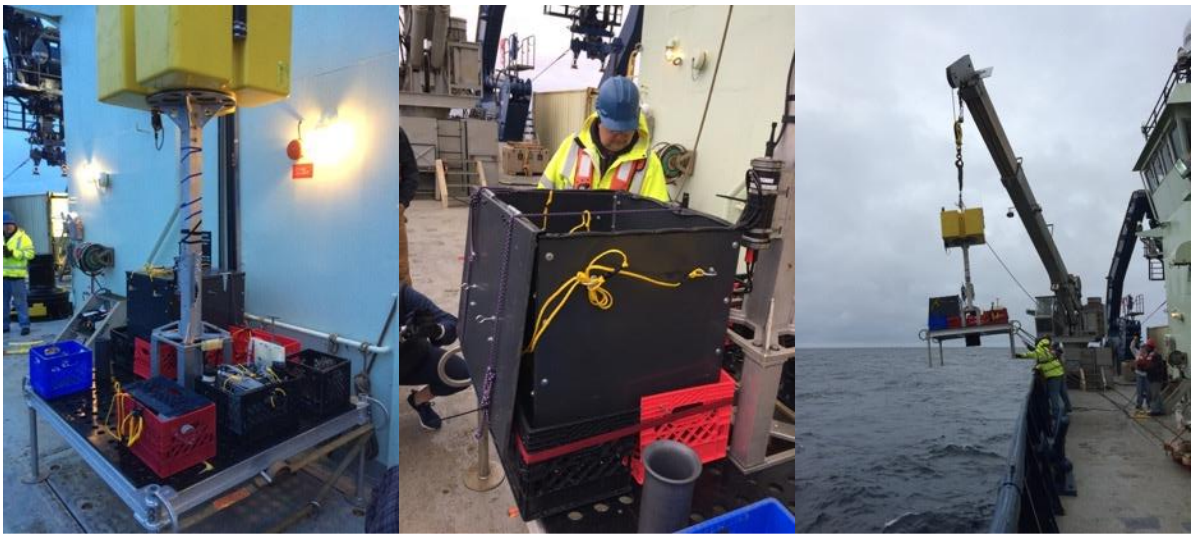
	Hours					
#	**Descent**	**Ascent**	**On Bottom**	**On deck**	**Not available**	**End of dive reason**
J2_1139	n.a.					
J2-1140	2:47	1:53	28:24	0	0	Time up
J2-1141	1:48	1:42	18:10	25:40	12:30 (WOW[a])	Time up
J2-1142	1:50	1:36	7:12	13:45	0	HPU failure
J2-1143	1:47	1:53	31:36	122:09	122:09 (WOW)	Time up
TOTALS			**85:22 (79+ Jason engineering dive time)**		**136:39**	

a: WOW, waiting on weather

Dive J2-1139 – aborted due to technical issues during descent. Issues resolved on deck and next dive immediately began. Descent stats for J2-1139 rolled in to J2-1140.

Dive J2-1140, Start May 16 2019 (all ROV times in GMT, local time GMT-7)

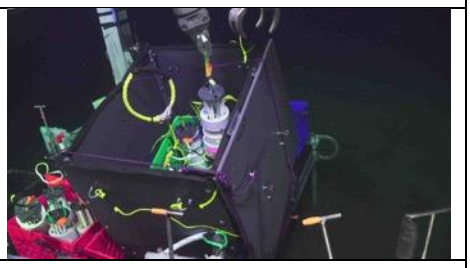
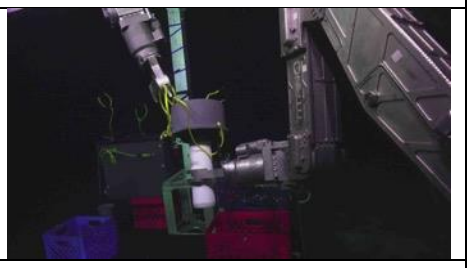
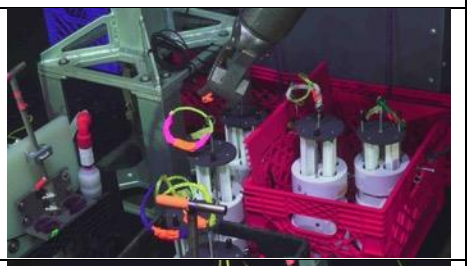
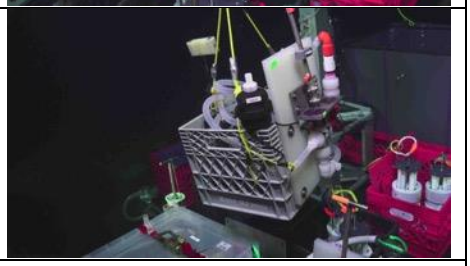
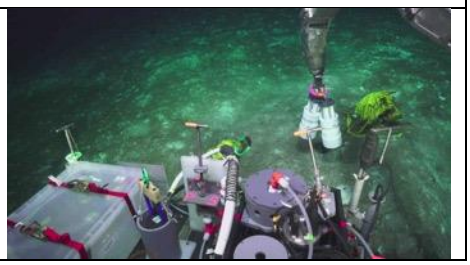
Overview: Very successful from a science operation standpoint. In the ~24 hours of science operations after the Jason engineering tests, we tested out new syringe sampling systems on the elevator and they worked very well, although our large box for incubating them needs some improvement for securing for deployment and seafloor operation/manipulation. Pressure downloads at the Hole U1362B CORK were successful, capturing 5 years of data, with no issues with the ODI connector or biofouling of the ODI data port. The UH Mobile Pumping System collected pristine fluids and in situ filters from the U1362B site (including 6 hours of in situ filtration, as we were ahead of schedule). Deployment of the UH virus in situ filtration unit went well. We recovered an OsmoSampler from the U1362B wellhead that had been sampling for 5 years. 4 out of 6 "squeezer" samplers from UAF worked to collect bottom water. We had a minor issue with the elevator not responding to acoustic release, but this was resolved.

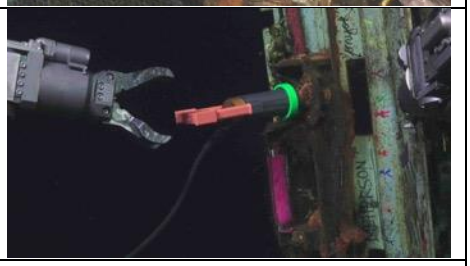
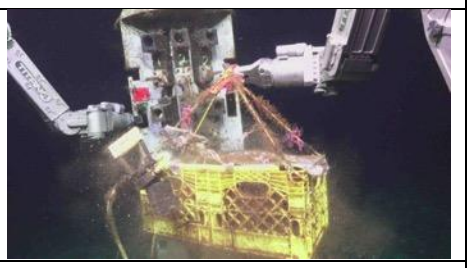
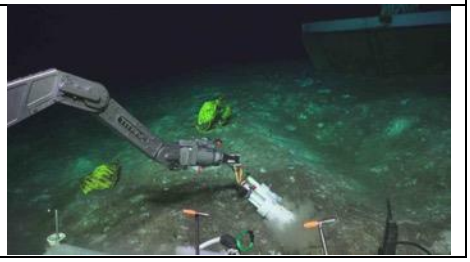


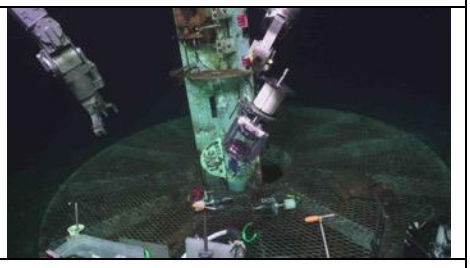
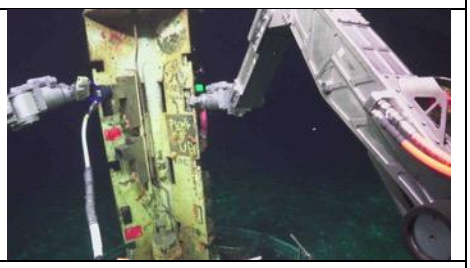
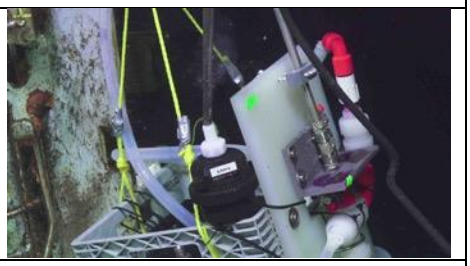
Elevator configuration before J2-1139/1140. Left: configuration the first time, with incubation box lid closed; Middle: reconfigured with lid open and tighter bungees. Right: Elevator being deployed.

Dive J2-1140 Jason basket pre-dive configuration.

Dive log J2-1140 (all times in GMT)

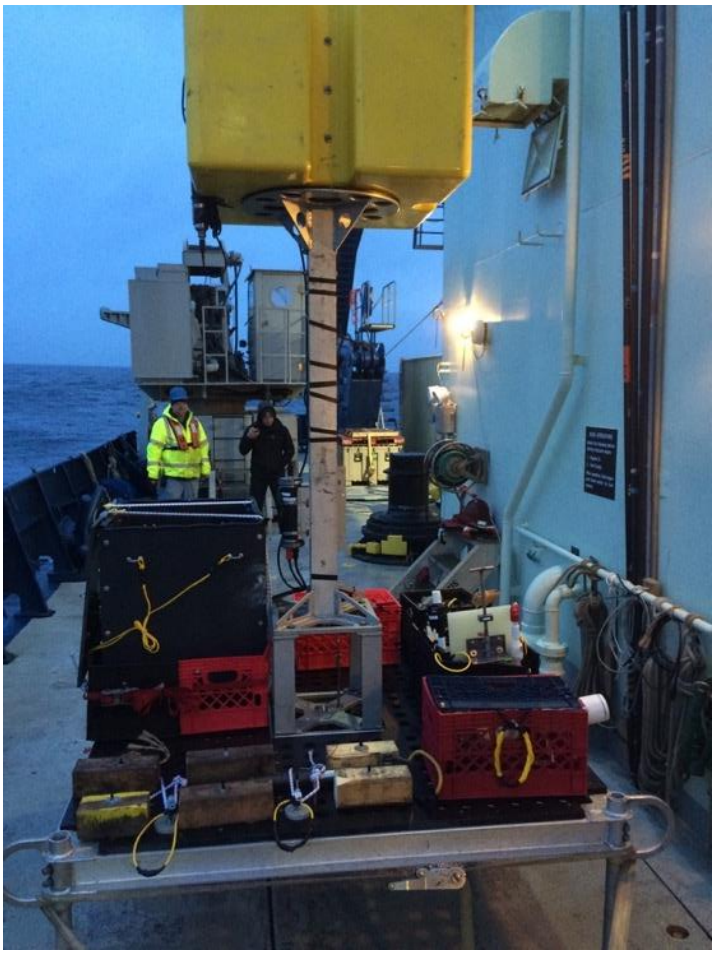
Time	Task	Representative Framegrab
17:10	Jason in water	
19:56	Jason on bottom, Begin Jason engineering dive	
02:30-	At elevator, fill all six syringe bundles inside Incubation Box by pulling on loops with **TWO COLORS** of tape; Unsecure syringe bundles by pulling on loops with **ONE COLOR** of tape, transfer each to milkcrate on elevator. Green syringe bundle did not fill all of the way.	
	Adjust bungees of incubation box and re-secure in milk crate braces	
03:40	Pick up CORK insert and place in Test Mount. Insert initially top heavy, but able to reorient in correct direction.	
	Pick up CORK Insert from Test Mount and re-stow on elevator	
04:00	Pick up two syringe bundles (Pink and Black) in starboard manip.	
04:10	Remove bungee from virus in situ filtration unit, pick up unit with port manip	
04:15	Transit 250 m to U1362B	
04:57	Hang virus in situ filtration unit on hook in MBIO bay	
05:00	Set Pink and Black syringe bundles in sediment by exploded float hard hats	

05:10	At pressure bay, turn valves CCW 180º for hydrostatic check	
05:14	In Osmo bay, T-probe measurement in outflow of OsmoSampler baskets (*max 8ºC*), close valve (CW 90 º) and disconnect Jannasch connector (turn CW 45º), put Jannasch connector in holster	
05:42	In Pressure Bay, turn valves back to formation	
05:45	Use toilet brush to clean ODI connector, connect ODI cable from Port Swingarm	
06:15	Turn on power to relay and begin to download data *(~2 hour*), Clear memory, reset clock, turn off relay	
08:21	disconnect ODI cable and return to Port Swingarm	
08:31	Pick up Yellow OsmoSampler	
08:36	Transit to elevator	
08:59	Stow Yellow OsmoSampler in empty milkcrate	
09:13	Try again to fill Green syringe bundle	
09:19	Secure Green and White syringe bundles in red milkcrate	
09:23	Pick up Blue and Yellow syringe bundles	
09:34	Transit back to U1362B	
09:58	Set down Blue and Yellow syringe bundles with Black and Pink ones	

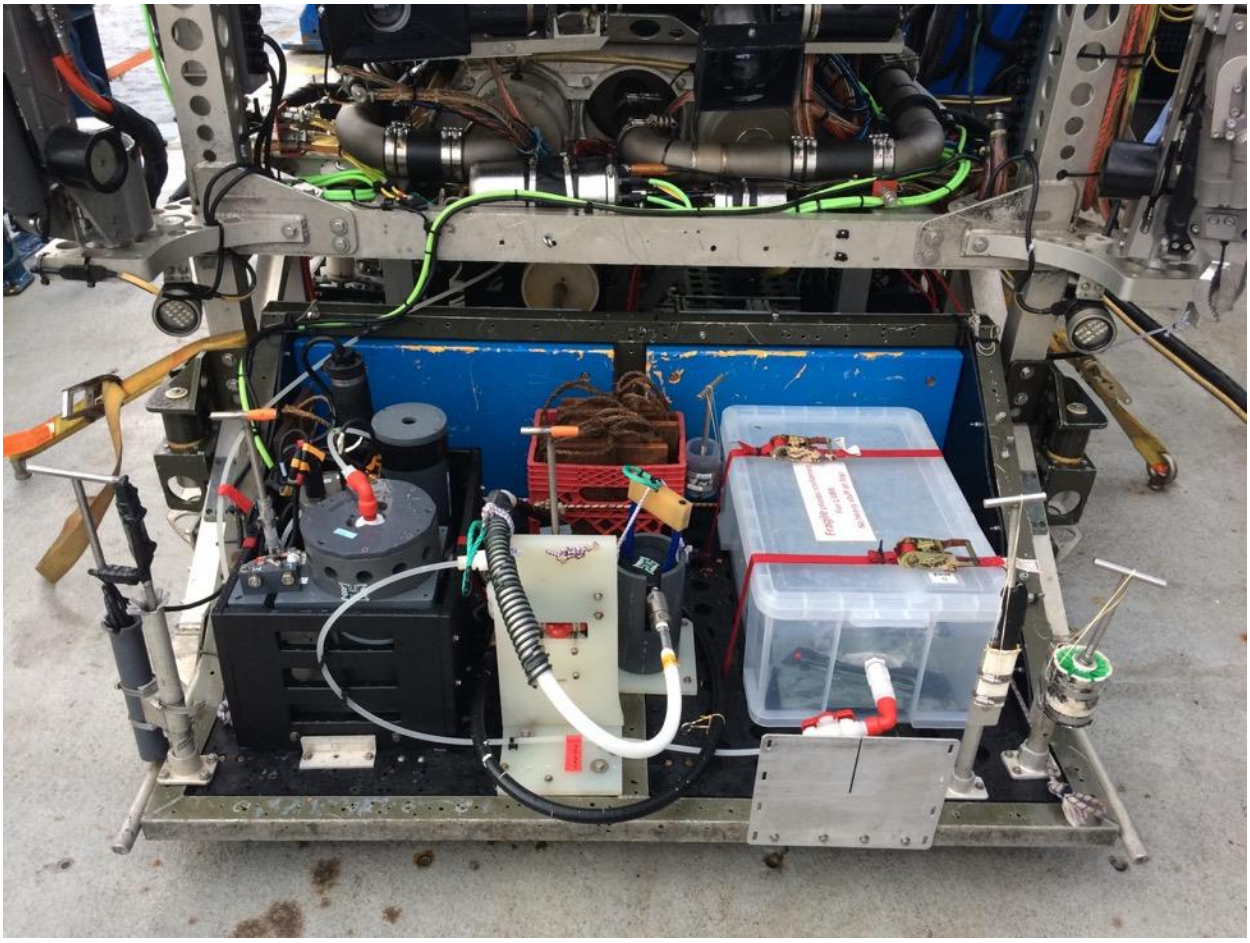
10:06	Tried communicating with elevator for release – not working	
10:09	From Starboard Swingarm, fire each squeezer and return to milkcrate; Stow Swingarm (*ONLY 4 WORKED*)	
10:37	Transit back to elevator	
11:27	**Release Elevator at 4AM local**	
12:38	Elevator on surface; Recover Elevator	
12:59	Transit to U1362B	
13:50	Use toilet brush to clean top Jannasch Coupler, Connect MPS Jannasch connector to same coupler, turn valve CCW 90º to open	
13:55	Turn on MPS to purge MPS line; End purge: oxygen 0.04µM, temp 18C	
14:37	Fill MVBS, LVBS, and in situ filtration (~0.5L/min * 6 hours = ~180 L); then turn off MPS.	
23:32	Power down MPS system and begin closing valves and detaching Jannasch connector	
23:39	Connect Virus In Situ Filtration Unit to same connector; Turn valve to open Virus In Situ Filtration Unit	
24:30	Jason off bottom by 5:30PM local; Recover Jason at 7PM local	

Dive J2-1141, Start May 18 2019 (all ROV times in GMT, local time GMT-7)

Overview: Another successful and efficient dive, although we had some challenging operations. We had a slight delay in launching Jason due to unsecure science equipment on the basket. We deployed an in situ virus filtration unit at IODP Hole U1362A, then moved to Hole U1362B for the majority of the dive. The virus filtration unit deployed on the prior dive was recovered, and fluid sampling/filtering with the MPS was again very successful (although we learned that we should collect these samples at the very end of the dive for better quality). We recovered the top plug from the top of the CORK without issue, and the new socket driver machined by the deck crew worked wonderfully for this task. Testing of our new incubation device equipment at the top of the CORK was not successful due to buoyancy and awkwardness issues in the high velocity flow, but it was an informative process and the piloting was extraordinary to handle challenging tasks. Despite this setback, we still managed to collect pristine crustal fluids with our new syringe bundle samplers and with squeezers. Minor issues during dive: Sullis framegrabber had some recording issues, starboard swing arm had trouble closing, lost toilet brush, ground faults during recovery.



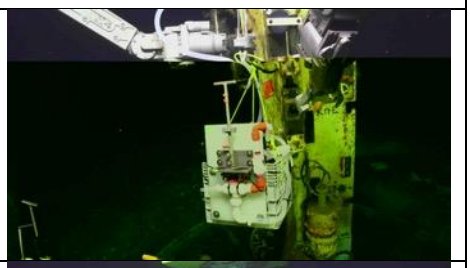
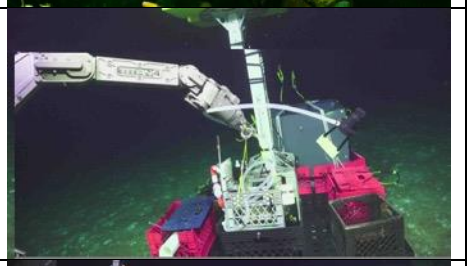
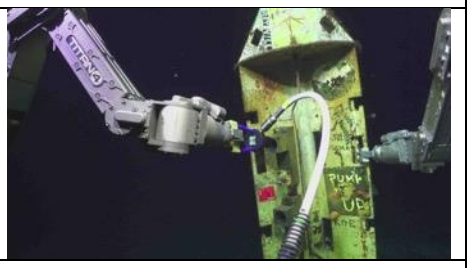
Elevator prior to launch for J2-1141.

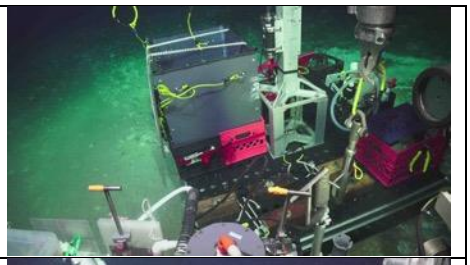
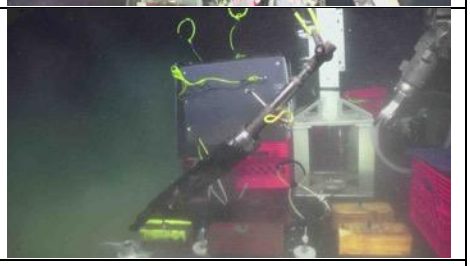
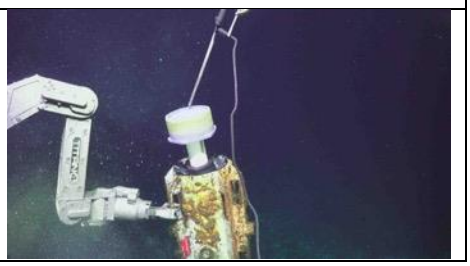


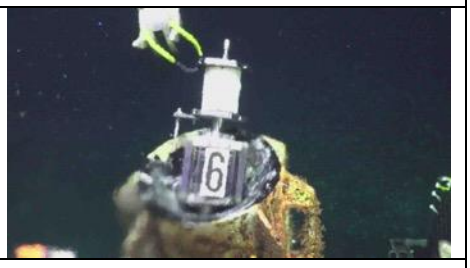
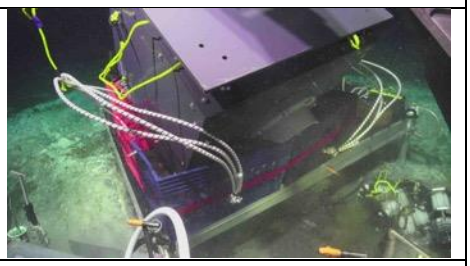
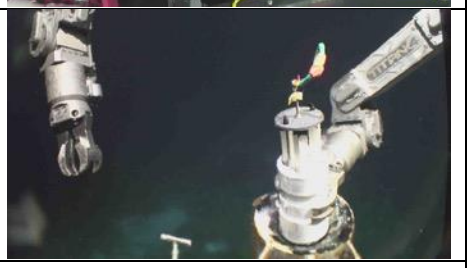
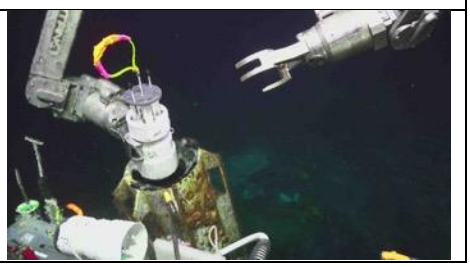
Jason basket configuration for J2-1141. Changes since prior dive: addition of knife (in front of LVBS) and new dog bolt socket tool (mounted to dive weight basket).

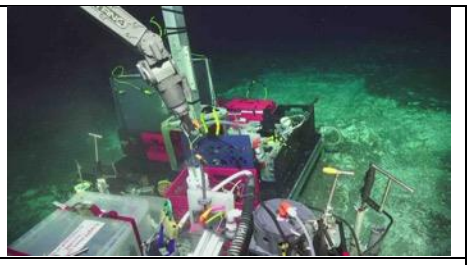
Dive log J2-1141 (all times in GMT)

Time	Task	Representative Framegrab
03:30	Begin Jason deployment	
	Return Jason to deck to secure LVBS	
03:54	Relaunch Jason, dive to elevator	
05:42	On bottom, transit to elevator	
05:55	Pick up virus in situ filtration unit from elevator	
05:58	Transit to U1362A	
06:23	Hang virus in situ filtration unit on wellhead, check start of flow meter (2257 liters)	
06:33	Use toilet brush to clean U1362A MBIO Bay lower port Jannasch Coupler; turn port CCW 90º to open; check for flow out of open port and allow to purge for 30 minutes (max temp ~8.2ºC)	

07:34	Difficulty to connect virus unit Jannasch connector to lower port, connected instead to top port	
08:09	Virus filtration unit valve turned to sample	
08:10	Transit to elevator	
08:38	Pick up elevator and move closer to U1362B wellhead	
09:09	At U1362B MBIO Bay, check flowmeter on virus in situ filtration unit (end 2515 liters); Turn valve to close port with virus in situ filtration unit connected (09:11); Disconnect Jannasch connector and stow in crate; remove unit milkcrate from CORK	
09:31	Stow and secure Virus In Situ Filtration Unit in milkcrate	
09:42	Pick up BLUE and YELLOW syringe bundles by hard hats	
09:48	Stow BLUE and YELLOW syringe bundles in milkcrate	
09:55	*Follow directions of Adi/Hawaii* - Use toilet brush to clean **top** Jannasch Coupler; Connect MPS Jannasch connector to same coupler; turn valve to open port	
10:04	*Follow directions of Adi/Hawaii* – something like, Turn on MPS to purge MPS line; Fill LVBS, MVBS, and in situ filters; then turn off MPS	
18:30	Detach MPS Jannasch coupler and stow in basket	

18:49	Pick up RS pulling tool and place on top of LVBS	
18:52	Pick up weight stack on top of CORK, dispose of away from CORK	
18:59	Pick up new dog bolt nut driver and loosen dogs at top of CORK (turn CW 3.75 times); Pick up toilet brush and clean top plug	
19:19	Pick up RS pulling tool, engage with top plug, Remove RS pulling tool + top plug (19:22)	
19:29	Stow and secure RS pulling tool + top plug in elevator	
19:36	Pick up CORK insert	
19:49	Attempt to install CORK insert into top plug, but it was not heavy enough to seat in the CORK throat against the flow coming out.	
19:54	T-probe in outflow of CORK insert; bring out starboard swingarm. Max temp 54.1ºC	

20:03	Remove CORK insert and measure T in open throat – max temp 54.9ºC.	
20:14	Fire squeezer samples in venting fluid. Squeezers 0, 2, 6, and 9 fired correctly; squeezers 1 and 4 did not. Then stow syringes and swingarm	
20:45	Trouble stowing starboard swingarm with squeezers	
20:52	Removed bungees from incubation box on elevator to enable access to syringe bundles, but box tipped when trying to remove bungees securing syringe bundles	
21:08	Box reseated and re-secured with bungees.	
21:26	Removed GREEN and WHITE syringe bundles from incubation box	
21:35	Fill GREEN and WHITE syringe bundles by holding over free flow at top of CORK	
22:01	Stow GREEN and WHITE syringe bundles in red milkcrate on elevator	
22:13	Remove PINK and BLACK syringe bundles from incubation box.	
22:26	Fill PINK and BLACK syringe bundles by holding over free flow at top of CORK. Note that PINK bundles expand and fill on its own, as if pressure from free flow pushed itself into check valve.	

22:46	Stowed PINK and BLACK syringe bundles in elevator in crate that held CORK insert. Moved bungee to secure incubation box. Stowed CORK insert on Jason basket, held down by Jason manipulator.	
23:11	Removed dive weights from elevator.	
23:45	Elevator released at 16:45 local	
23:52	Jason off bottom at 16:45 local	

Dive J2-1142, Start May 20 2019 (all ROV times in GMT, local time GMT-7)

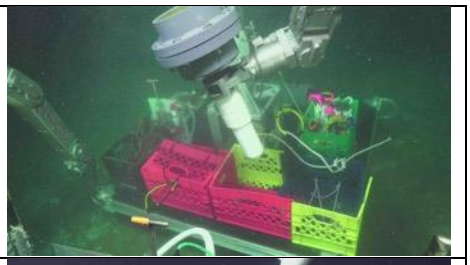
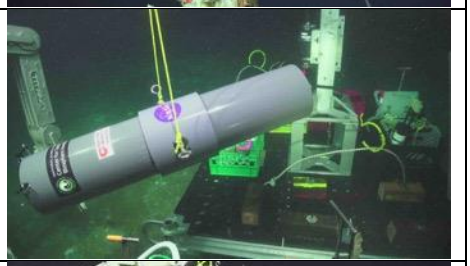
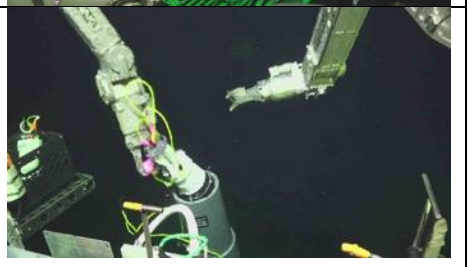
Overview: This short dive had limited sampling success due to operational issues with science equipment, shortening of dive program due to deteriorating weather, and then ultimate abortion of dive to hydraulic pressure unit failure. The dive began with operations at the top of the Hole U1362B CORK. Installation of the modified CORK insert was easy and went well. Installation of the modified incubation chimney also went well but was difficult due to poor weight control on the device versus high velocity flow; kudos to pilot Korey Verhein for accomplishing this task. Unfortunately, the modified syringe bundles with more weight added were still not heavy enough to sink in the chimney against the high velocity flow. One syringe bundle was triggered, but the rest were not attempted. The elevator was recovered earlier than planned to recover the syringe bundles to attempt further modification on them, in hopes of redeploying during the dive. An in situ filtration unit was successfully installed at U1362B, and then operations moved to Hole U1362A. Pressure valves were turned for hydrostatic checks, and the virus in situ filtration unit deployed on prior dive was disconnected. While setting up to pump fluids from U1362A deep, the hydraulic pressure unit on Jason failed, causing termination of the dive and inability to disconnect MPS from CORK. Thankfully, the MPS connector failed at the junction to the Jannasch connector on the CORK, without damaging the MPS instrument. With shortened dive program and equipment failure, we were not able to complete the dive tasks of MPS pumping and filtration at U1362A deep horizon, reconnection of the virus in situ filtration unit at U1362A deep horizon, collection of the U1362A OsmoSampler, download of pressure data from U1362A, and collection of water and push cores at Hole U1301A.

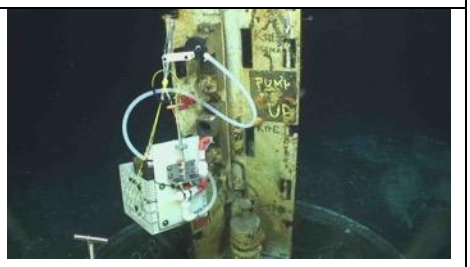
Elevator configuration for J2-1142, including modified CORK insert (left) and incubation chimney (right).

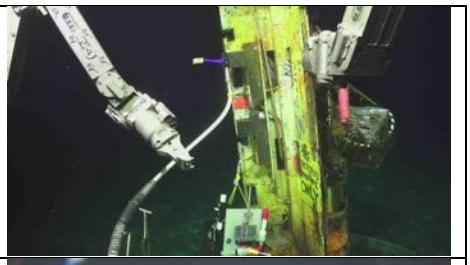
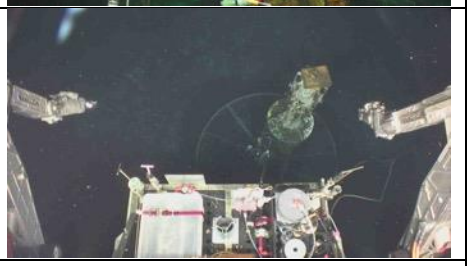
No changes to Jason basket from prior dive.

Dive log J2-1142 (all times in GMT)

Time	Task	Representative Framegrab
	Deploy elevator to U1362B, track to bottom	
15:16	Deploy Jason to elevator	
17:09	Jason on bottom	
17:19	Pick up elevator and transit to U1362B	

17:50	Pick up CORK insert	
17:56	Install CORK insert into throat of CORK. Slid in easily, but not fully sealed (bushing adapter resting on CORK throat bevel). T probe inside CORK insert (max temp 61.3ºC)	
18:07	Pick up CORK chimney with lanyard. Not weighted correctly to hang vertically. Luckily bungee for securing syringe bundles at top was attached, and this was used as a lift point.	
18:20	Install CORK chimney onto CORK insert. Task difficult due to light weight of chimney and high velocity flow out of CORK. Excellent piloting slow eased the chimney into place. T-probe outflow may temp 62.1ºC.	
	Burp of black stuff out of CORK	
18:49	Pick up WHITE and PINK syringe bundles from milkcrate on elevator, transfer to Port SwingArm on top of ODI cable	
19:04	Lower PINK syringe bundle into top of incubation chimney while holding with starboard manip, pull back on plunger with port manip. Realize that syringe bundles are way too light to sink into chimney against the high velocity flow coming out. Decide to end this operation and return WHITE and PINK syringe bundles to elevator, and to return elevator to surface early for modifying syringe bundles.	
19:46	While deliberating, noticed a slug of black particle rich fluid burped out of CORK outflow	

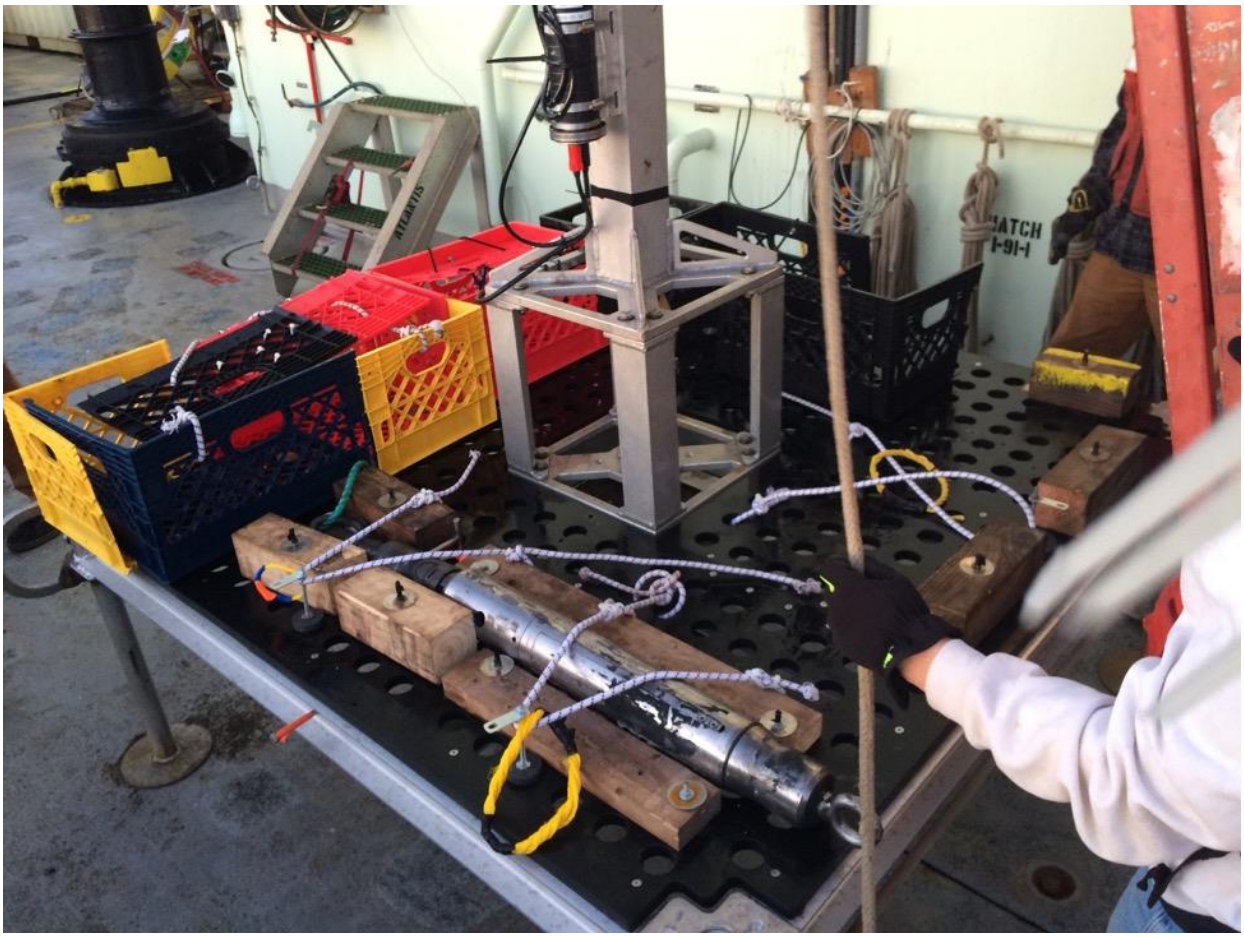
19:49	Head to elevator to stow WHITE syringe bundles in red milkcrate	
20:04	Went over to U1362B CORK to pick up PINK and BLACK syringes laying on seafloor for recovery, then decided to leave them in place a bit longer	
20:09	Secure syringe bundles in elevator and pick up Adi's in situ filtration unit.	
20:16	Adi's in situ filtration unit hung on U1362B CORK MBIO Bay. Cleaned top port with toilet brush, install Jannasch connector, remove plug from unit, turn connector port valve to open, confirm flow coming out of purge line, T probe purge 9.5ºC max, turn valve from purge to sample on unit, verify start of flow meter at 2255 and that it is moving. Clocking 2L/min.	
20:53	Move to elevator to recover, no dive weight removed as there was minimal payload for this unplanned recovery	
20:58	Release elevator. On surface at 15:00 local. Transit Jason to U1362A when ready.	
22:38	Arrive at U1362A	
22:47	In U1362A pressure bay, turn lower two valves to hydrostatic (top valve for seafloor already open to hydrostatic)	
22:55	At U1362A Osmo Bay, T probe in outflow of Black OsmoSampler (max temp 8ºC). Due to deteriorating weather, stopped further operations with OsmoSampler to finish pressure work for quicker departure.	
23:17	At U1362A Pressure Bay, turn lower 2 valves back to formation. *Note: issue with sealog so this event was not recorded.*	
23:28	At U1362A MBIO bay, T probe on virus in situ filtration unit. Max temp 6.2ºC. Turn valve to purge on virus filtration unit, check value on flow meter (2471), disconnect Jannasch connector and stow in unit.	

23:32	At U1362A MBIO Bay, connect MPS Jannasch connector to top port coupler; turn valve to open port. Turn on MPS to purge MPS line. Optode reading 3.5 µM oxygen, T 11.5ºC.	
23:45	**About this time, lost hydraulic pressure unit on vehicle (not recorded in SeaLog), no manipulator control. Cannot disconnect Jannasch connector from port, MPS connector line ripped at junction to Jannasch connector while Jason coming off bottom**	
00:21	Jason off bottom	
01:56	Jason on deck	

Dive J2-1143, Start May 25 2019 (all ROV times in GMT, local time GMT-7)

Overview: This was an incredibly successful final dive of the program. Operations began at IODP Hole U1362B CORK, with collection of squeezer samples from the modified CORK chimney and recovery of a passive in situ filtration unit for bacteria (which filtered nearly 16,500 liters!). The seafloor crustal fluid incubation experiment was finally successfully started within the modified CORK chimney after modifying the syringe bundles to have more weight and less surface area. All syringe bundles were recovered after 6.5 hours of incubation at high T and P, followed by removal of the CORK chimney and re-installation of the top plug into U1362B. Operations at IODP Hole U1362A included recovery of the broken Jannasch connector, removal of a passive in situ viral filtration unit, collection of push cores, download of pressure data, recovery of an OsmoSampler, and a record 15.5 hours of in situ filtration and bag filling with the Mobile Pumping System.

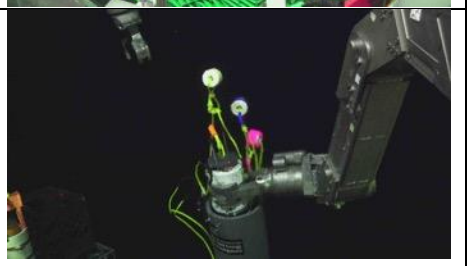
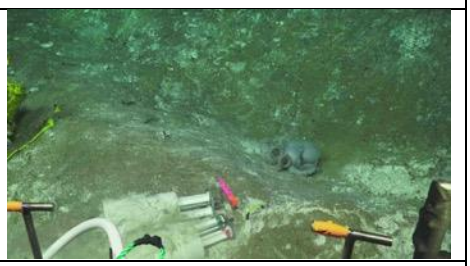
Layout of first elevator deployed before J2-1143, showing cradles for recovering equipment and syringe bundles for deployment.

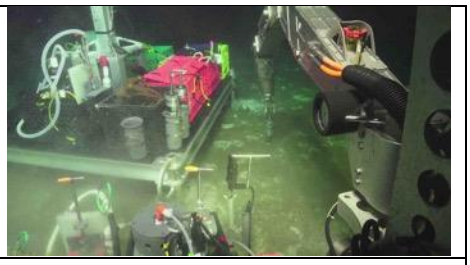
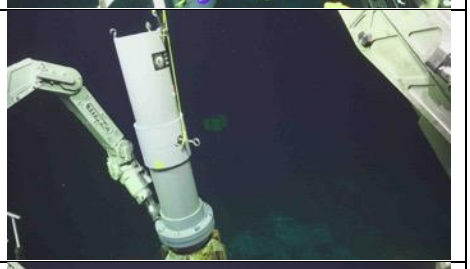
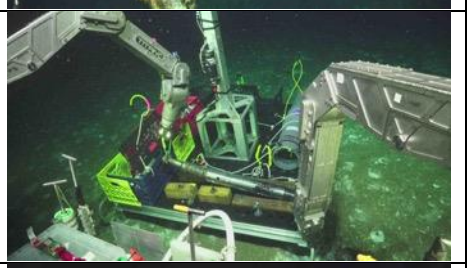


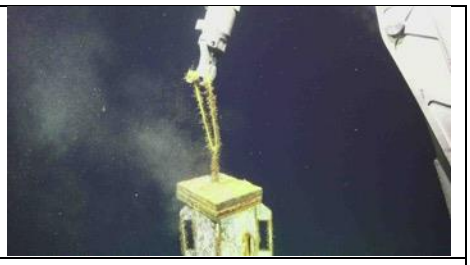
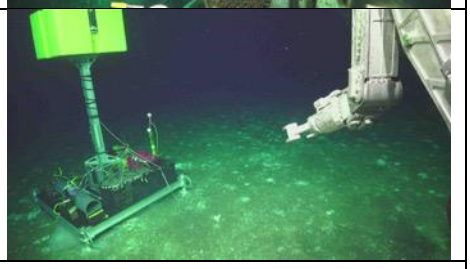
Layout of second elevator of J2-1143, showing GS running tool + top plug and cradle for recovering CORK chimney.

Dive log J2-1143 (all times in GMT)

Time	Task	Representative Framegrab
05/26 03:05	Deploy elevator to U1362B, track to bottom	
04:10	Deploy Jason to elevator	
06:00	Pick up elevator and move to U1362B	
06:24	Fire squeezers in top of U1362B CORK chimney. Squeezers 2, 9, 3, 4 triggered correctly; 0, 6 did not trigger correctly. Squeezers stowed in elevator 1.	

07:13	At U1362B MBIO Bay, Adi's filtration unit had clocked 16,481 liters before we turned filtration unit valve to purge. Closed valve on CORK, disconnected Jannasch connector, removed unit from CORK and stowed on elevator.	
07:36	Remove syringe bundles from elevator	
07:57	Begin inserting syringe bundles in CORK chimney. Finally heavy enough to sink into chinney. Trigger and lower PINK syringe first, then BLUE. Then trigger WHITE and remove to swingarm before triggering and lowering YELLOW. PINK and BLUE bundles caught on ledge inside chimney, had to be jostled to sink. Took about 30 minutes to complete.	
08:40	Pick up BLACK and PINK syringe bundles that have been on the seafloor. Secure PINK, BLACK and WHITE syringe bundles in elevator	
08:53	Pick up elevator and move to U1362A	
09:42	At U1362A MBIO Bay, close valve on CORK where Jannasch connector was ripped off dive before, and disconnect broken connector	
09:49	Remove Olivia's viral filtration unit from wellhead, stow in elevator	

09:59	Collect 3x push cores near elevator, ~20m from CORK	
10:26	Elevator released. On surface 04:53 local	
12:50	2nd elevator launched 05:50 local.	
14:16	Pick up elevator and move to U1362B	
14:30	Begin removing syringe bundles from CORK chimney, YELLOW then BLUE then PINK. Stow in elevator.	
14:57	Remove CORK chimney from insert, stow in elevator	
15:08	Remove CORK insert from CORK top, stow in elevator	
15:15	Pick up GS running tool + top plug from elevator. Cotter pin fell out of T-handle.	
15:29	Insert Top plug into U1362B CORK top, doesn't seal all of the way. Push it down with manip. Tighten dog bolts by turning 3+ turns CCW. Remove T-handle and disengage running tool by pulling collar back. Still shimmering water around top plug, so will put a dive weight on it later. Stow running tool in elevator.	

16:06	Weight stack put on top of top plug, shimmering stops	
16:16	Pick up elevator and transit to U1362A	
16:57	At U1362A OsmoBay, close valve on Black Osmosampler, disconnect Jannasch connector, remove OsmoSampler, stow in elevator	
17:20	2nd elevator released with pull, requires a little nudge out of the sediment	
17:40	While sitting off bottom waiting for elevator recovery, there was a failure of the AFX in the Jason control van and loss of power to the vehicle. The system was back up and running within 15 minutes and the dive continued.	
18:45	2nd elevator on deck, begin transit to U1362A	
19:53	At U1362A, clean ODI port with toilet brush, then start pressure download at 20:05. Pressure download complete at 21:26	
21:44	At U1362A Osmo Bay, connected MPS to lower middle port 2 to sample shallow horizon. Cleaned with toilet brush first. Filled 3x MVBS bags 22:38 and one in situ filter at 0.5L/min for 4 hours.	

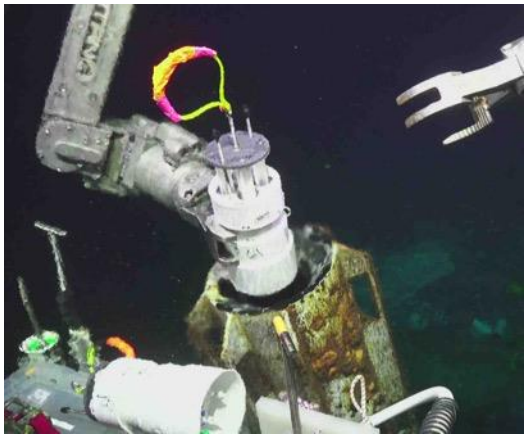
May 27 03:09	At U1362A MBIO Bay, connected MPS to upper port bio line to sample deep horizon. Cleaned with toilet brush first. Filled LVBS bag at 03:41. Began in situ filtration at 0.5L/min at 04:06 and paused at 11:33.	
11:34	Began filling MVBS bags. Difficult to see filling as lights on aft cam stopped working. At 12:42, switched back to in situ filter until end of dive	
13:15	MPS shut down sequence begins, valve on CORK closed	
13:29	Jason off bottom	

Seafloor and shipboard incubation experiments summary

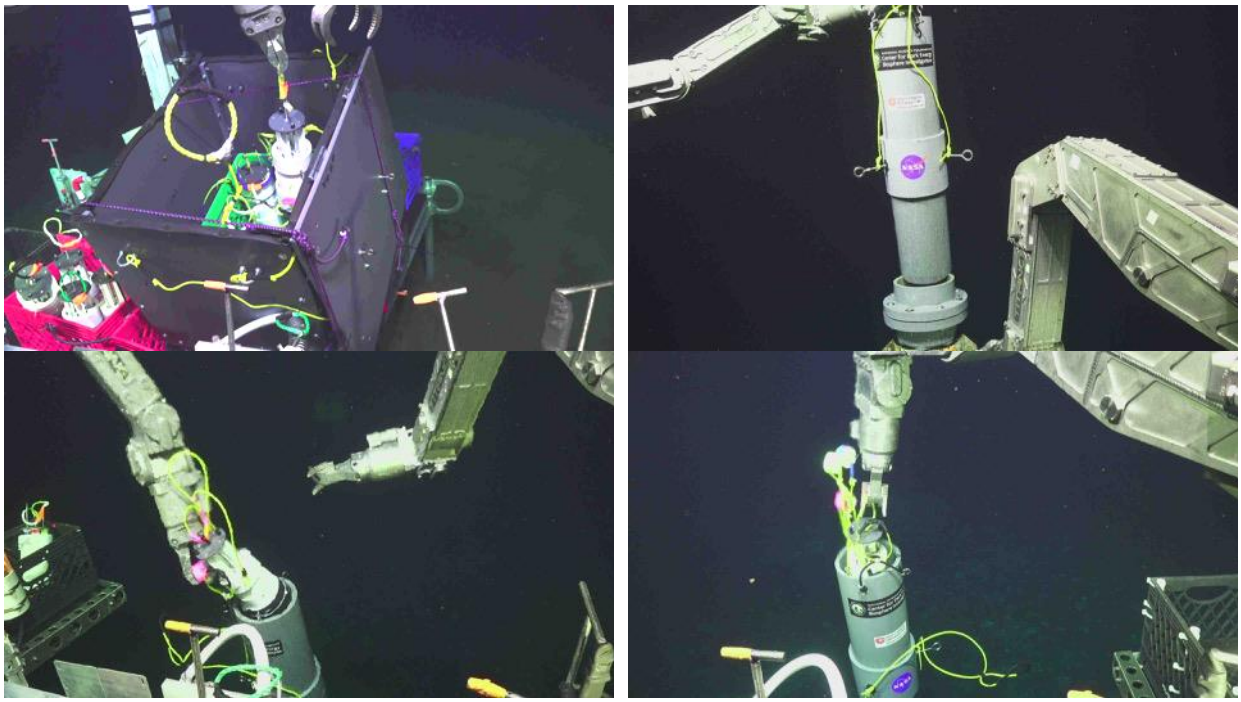
Summary by Orcutt group (Booker, Lindsay, & Orcutt) and Martin Van Den Berghe (MVDB)

The primary objective for this cruise was to conduct seafloor and shipboard incubations of crustal fluids with spike solutions (with ^{2}H, ^{15}N, and ^{13}C-labeled substrates plus other stimulants or inhibitors of microbial processes) to determine rates of microbial activity and growth and identify active members of the crustal fluid microbial communities. The methods for accomplishing this objective included (1) deployment of custom syringe samplers prefilled with spike solutions, for incubating on the seafloor within the anoxic 62ºC+ crustal fluid, and (2) collection of pristine crustal fluids with squeezer samplers (provided by collaborator Geoff Wheat) for shipboard experimentation replicating the seafloor incubations. A secondary collaborative objective with MVDB was to use this same incubation approach (with different stable isotope tracers) to examine silica dissolution kinetics from crustal materials associated with microbial activity.

For the seafloor incubation systems, these consisted of bundles of 4x 100ml syringes preloaded with spike solutions, which were then filled on the seafloor to ~80ml incubation volumes. For the Orcutt group syringes, spike solutions with stable isotopes diluted 1:10 when filled. Each Orcutt group syringe bundle consisted of a background treatment (10% D_2O and 5 µM $^{15}NH_4Cl$ final concentrations), a 13C-bicarb treatment (0.04 mM final concentration + D_2O and $^{15}NH_4Cl$ as above), a 13C-acetate treatment (0.04 mM final concentration + D_2O and $^{15}NH_4Cl$ as above), and a nitrate stimulation (0.1 mM final concentration + D_2O and $^{15}NH_4Cl$ as above). Syringe bundles for MVDB were prefilled for a 1:1 dilution with a 29-Si spike solution for studying mineral dissolution kinetics.



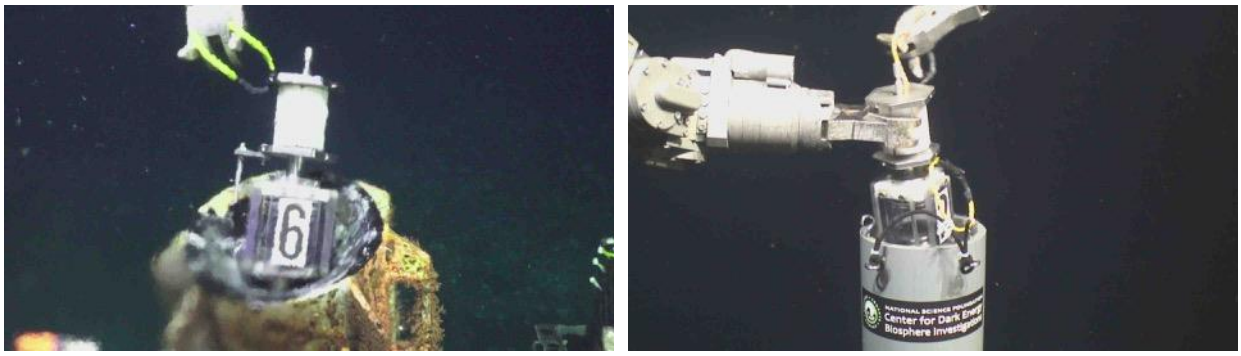
This cruise was the first time that this incubation system was deployed, so unsurprisingly it took a few tries to work out the kinks in the deployment approach. Pleasingly, the operation of the syringe bundle actuation concept (i.e. pulling back on the syringe plungers with the ROV manipulators) worked on the very first attempt. Initial plans to simultaneously deploy and trigger 6x syringe bundles at once inside a large PVC box fitted to the top of Hole U1362B were abandoned after attempts on dives J2-1140 and J2-1141 revealed that the box was too light and unwieldy to manipulate. In replacement, a 8” diameter PVC pipe chimney was designed for insertion into the top of the CORK. This concept worked well, although it could have benefitted from better placement of a lift bridle. The syringe bundles had to be weighted down to enable sinking inside the chimney against the high velocity flow existing the CORK, but this was finally successful on the last dive, enabling a ~6.5-hour seafloor incubation period within the high-temperature CORK fluids. While this is far shorter than the planned deployment length of 4 days, we are optimistic that we will still see a signal.



Example photographs of the in situ incubation experiment system. The original PVC box concept was abandoned as the box was unwieldy, although the method for deploying the syringe bundles bungeed within a milkcrate worked, as did the actuating of the syringe filling (top left). An alternative method was attempted on dive J2-1142, wherein a cylindrical chimney was mounted on an insert put into the open CORK top (top right). While the chimney concept worked, the syringe bundles were originally too light and had too much surface area to sink in the chimney against the high velocity flow (bottom left). Adding weight to the syringe bundles allowed for a successful in situ incubation on dive J2-1143 (bottom right).

Shipboard, the Orcutt group syringe bundles were dismantled at room temperature in a glove bag (for crustal fluids) or the cold room (for bottom seawater control samples) and dispersed for shore based single cell sorting (for nanoSIMS and activity-based single cell genomics), potential sulfate reduction rate measurement (by collaborator Tina Treude), and geochemical analysis. The MVDB syringe bundles were also dismantled shipboard within a glove bag and immediately processed for shore-based analyses.

For parallel shipboard incubations with pristine crustal fluids (or bottom seawater), custom "squeezer" syringes (from collaborator Geoff Wheat, prepped by participant Trevor Fournier) were triggered within freely venting hydrothermal fluids from Hole U1362B (or on the CORK platform, for bottom water) and recovered with ROV Jason. Shipboard, the squeezer contents for Orcutt lab experiments were combined in a 1L bottle inside an Argon-filled glove bag and then redistributed for incubation experiments. For the MVDB experiments, fluids were mixed with ^{29}Si spike solution and olivine pieces for shipboard silica dissolution experiments.



Example photographs of squeezer syringes being deployed with the Hole U1362B CORK open top plug (left) and incubation chimney (right).

Key times for syringe bundles with spike solutions incubated on the seafloor.

Bundle color	**User**	**Filled date/time (GMT) + dive**	**Move to BSW time (GMT) + dive**	**Off bottom date/time (GMT) + dive**
U1362B bottom seawater (start J2-1140)				
White	Orcutt	20190517 02:30	n.a.	20190517 11:27
Yellow	Orcutt	20190517 02:30	n.a.	20190518 23:45
Blue	Orcutt	20190517 02:30	n.a.	20190518 23:45
Black	Orcutt	20190517 02:30	n.a.	20190526 10:26
Green	MVDB	20190517 02:30	n.a.	20190517 11:27
Pink	MVDB	20190517 02:30	n.a.	20190526 10:26
U1362B CORK top (start J2-1141; note: fired in 62ºC crustal fluid and then immediately incubated in BSW)				
White	Orcutt	20190519 21:35	20190519 21:35	20190519 23:45
Black	Orcutt	20190519 22:26	20190519 22:26	20190519 23:45
Green	MVDB	20190519 21:35	20190519 21:35	20190519 23:45
Pink	MVDB	20190519 22:26	20190519 22:26	20190519 23:45
U1362B CORK chimney open top (start J2-1142; note: fired as above)				
Pink	MVDB	20190520 19:04	20190520 19:04	20190520 20:58
U1362A CORK chimney incubation (start J2-1143)				
Pink	MVDB	20190526 07:57	20190526 14:30	20190526 17:20
Blue	Orcutt	20190526 07:57	20190526 14:30	20190526 17:20
Yellow	Orcutt	20190526 07:57	20190526 14:30	20190526 17:20
White	Orcutt	20190526 07:57	20190526 07:57	20190526 10:26

Squeezer samplers collected

Squeezer	Contents	Filled date/time (GMT)	Off bottom date/time	User
J2-1140				
0, 2, 3	U1362B bottom SW	20190517 10:22	20190517 24:30	Orcutt group
1	U1362B bottom SW	20190517 10:22	20190517 24:30	MVDB
8, 7	Did not fire	n.a.	n.a.	n.a.
J2-1141				
0, 2, 6	U1362B CORK top	20190519 20:43	20190519 23:52	Orcutt group
9	U1362B CORK top	20190519 20:43	20190519 23:52	MVDB
1, 4	Did not fire	n.a.	n.a.	n.a.
J2-1143				
2, 3, 9	U1362B CORK top	20190526 06:24		Orcutt group
4	U1362B CORK top	20190526 06:24		Ziervogel
0, 6	Did not fire	n.a.	n.a.	n.a.

Key times (GMT) for MVDB syringe bundles processed for experiments

Location/Bundle/Time	Bottom Water/Green/T0	Bottom Water/Pink/9+day	U1362B/Green/T0	U1362B/Pink/2nd T0	U1362B/Pink/6 hour
Syringes prepped	20190516 04:00	20190516 04:00	20190518 09:00	20190520 02:30	20190525 22:30
Deploy dive	J2-1140	J2-1140	J2-1141	J2-1142	J2-1143
Deployed in ocean	20190516 15:00	20190516 15:00	20190518 13:00	20190520 14:00	20190526 03:00
Actuated	20190517 02:30	20190517 02:30	20190519 21:35	20190520 19:04	20190526 08:00
Retrieval dive	J2-1140	J2-1143	J2-1141	J2-1142	J2-1143
Off Bottom	20190517 11:27	20190526 10:26	20190519 23:45	20190520 20:58	20190526 14:00
Retrieved on deck	20190517 13:00	20190526 12:00	20190520 02:30	20190520 22:00	20190526 19:00

Key times (GMT) for MVDB squeezer incubations (see table above for squeezer collection info)

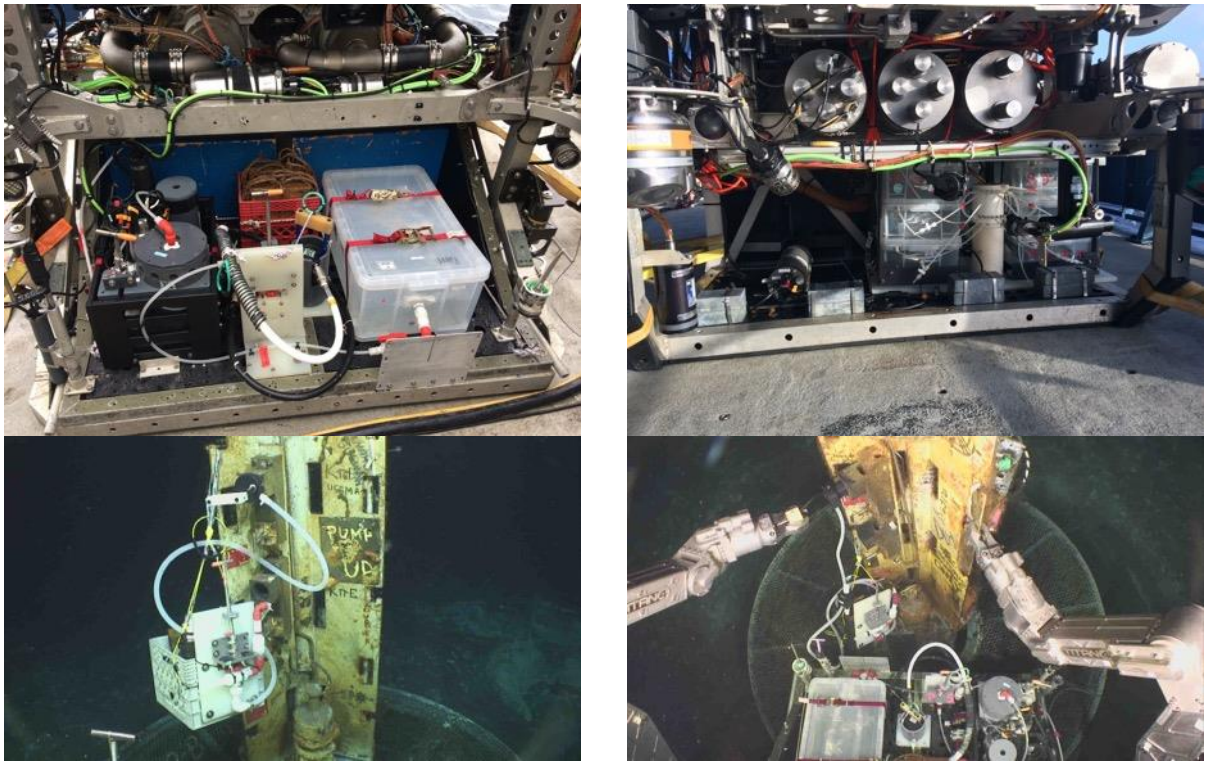
Location	Bottom seawater	U1362B crustal fluid
Dive	J2-1140	J2-1141
Start incubation (T0)	20190518 03:30	20190520 02:00
Time point T1	20190519 09:30	20190521 08:30
Time point T2	20190520 15:30	20190522 17:00
Time point T3	20190520 22:30	20190523 21:30
Time point T4	20190523 03:00	20190525 03:00
Time point T5	20190524 09:30	20190526 10:00
Time point T6	20190525 17:30	20190527 15:00
End	20190525 20:20	20190527 16:00

Summary of CORK fluid sampling via Mobile Pumping System and *in situ* filtration

Summary by Mike Rappé

Collaborators Carr, Nigro, and Rappé led efforts in sampling fluid delivery lines from CORK wellheads U1362A and U1362B. Whole fluids were collected for ship- or lab-based processing using the Mobile Pumping System (MPS) coupled to the Medium Volume Bag Sampler (MVBS, mounted in the rear Jason basket) and Large Volume Bag Sampler (LVBS, mounted in the forward Jason basket), while microbial and viral biomass was collected *in situ* using a combination of filtering opportunities including the McLane manifold that services the MVBS, the In Situ Viral Filtration sampler (led by Co-PI Nigro), and In Situ Microbial Filtration sampler (led by participant Andrian Gajigan). The In Situ Filtration samplers were deployed and recovered via elevators. Raw fluids served three primary interests: (i) inoculum for a variety of enrichment and microbial isolation experiments, (ii) filtration for microbial nucleic acids, and (iii) tangential flow filtration of viral-sized particles to create a viral concentrate that will be used for challenging enrichments and isolates to isolate viruses. In addition to these primary uses, crustal fluids were also distributed to a variety of collaborators.

Example photos of the Mobile Pumping System (MPS) mounted on the front of Jason (top left photo, left side) along with the Large Volume Bag Sampler (LVBS, top left photo, right side), and the Medium Volume Bag Sampler (MVBS) and McLane manifold unit mounted in the back of Jason (top right photo). Photos on the bottom row are examples of the In Situ Filtration Units connected to the CORK fluid horizons with Jannasch connectors.



When sampling was possible (i.e. on dives J2-1140, J2-1141, and J2-1143), these systems worked especially well, collecting several hundreds of liters of fluids from each CORK horizon, and passing thousands of liters of fluids through filters *in situ* (with volume based on the flow rates estimated from pump speeds for filters connected to the McLane unit, and based on visual inspection of flow meters installed on the passive units). On the MPS, flow rates were maintained at ~0.5 L min^{-1} when pumping from CORK PTFE fluid delivery lines, achieving temperatures of ~20°C and O_2 of << 1 μM during active sampling. Notably, deployment of the In Situ Microbial Filtration sampler achieved a record volume of fluid filtered *in situ* (i.e. 16,500+ Liters).

While there were great successes in recovering fluids and filters from each of the target CORK horizons (i.e. U1362A shallow, U1362A deep and U1362B deep) on three dives during the cruise for examining spatial heterogeneity in the crustal fluid deep biosphere, a primary objective to collect replicate samples from each horizon to examine temporal variability was not possible due to early termination of a dive due to mechanical issues (i.e., dive J2-1142) and the loss of additional dives from weather.

Additional samples collected from the MVBS, where possible:

Clumped isotopes: From MVBS bag #9 recovered from U1362B bioline on dive J2-1140, a 50ml bubble of Argon gas was injected by Orcutt into the foil-lined bag in the cold room to allow gas equilibration. After 24 hours, a 20ml bubble was extracted from the bag and transferred to an evacuated glass serum vial. A similar process was repeated for MVBS bag #4 from U1362A bioline (deep) on dive J2-1143, with the exception that the entire 50ml bubble (this time of nitrogen) was extracted and split into 2x evacuated glass bottles. After removal of the gas bubble, whole water samples were collected by additional investigators. The gas samples are being sent by Nigro to collaborators at UCLA for clumped isotope analysis of methane, along with whole water samples collected from U1362A bioline.

Microbial eukaryotes: From the bags described above, 3x 250ml whole water samples were collected by Orcutt into amber jars, fixed with 6.75ml of cold formaldehyde, and stored cold for shore based microscopic analysis of microbial eukaryotes by collaborator Sarah Hu. A similar set of samples was collected from CTD cast at4211005 for bottom water comparison.

Poised potential experiments: From the bags described above, Orcutt collected 5x 1L samples into muffled glass bottles for shore based poised potential experiments to examine microbial metal cycling processes. 3x 38ml glass bottles containing sterilized polished basalt chips were also filled to examine biofilm formation from crustal fluids. Samples were stored under nitrogen headspace at room temperature.

Crustal fluids sampled with the MPS and in situ filtration devices on cruise AT42-11.

Dive	**J2-1140**[1]	**J2-1141**	**J2-1143**	
CORK horizon	U1362B_bio	U1362B_bio	U1362A_bio_deep	U1362A_SS_shallow
In situ McLane 0.2μm filter	134 L	210 L	210 L	120 L
LVBS	60 L	49 L	47 L	--
MVBS	60 L	66 L	60 L	10 L
In situ viral filter[2]	2,300 L	2,500 L[3]	--	--
In situ microbial filter	--	16,000 L	--	--

[1]Dive numbers refer to deployment.
[2]Also includes a 0.2 m pore-sized prefilter for microbial analysis
[3]Deployed at U1362A_deep

Distribution of subsamples from MVBS and LVBS fluid samplers. Deep ocean bottom seawater was also collected via CTD casts to serve as control samples for crustal fluid analyses.

Dive	**J2-1140**	**J2-1141**	**J2-1143**	
CORK Horizon	U1362B_bio	U1362B_bio	U1362A_bio_deep	U1362A_SS_shallow
Raw fluids - culturing	2.8 L	2.8 L	10 L	10 L
Cryopreserved fluids, DMSO	3 x1mL	1 x1mL	3 x1mL	3 x1mL
Cryopreserved fluids, glycerol	56 x1mL	58 x1mL	50 x1mL	50 x1mL
Viral & cell counts	0.2 L	0.2 L	0.2 L	0.2 L
Microbial nucleic acids	114 L	114 L	47 L	10 L
Viral nucleic acids	31.6 L	40 L	55 L	--
Chemistry (Wheat)	0.1 L	0.1 L	0.1 L	0.1 L
CH_4/CO measurements	0.05 L	0.05 L	0.05 L	0.05 L
Spore experiments (Hubert/Chakraborty)	9.5L	3 L	5 L	--
Clumped isotope analysis, gas bubbles/raw water (Nigro/Orcutt)	--	2x/--	2x/10 L	--
Poised potential experiments (Orcutt)	--	5 L	5L	--
Viral flow cytometry (J. Martinez-Martinez)	0.03 mL	--	0.03 mL	0.03 mL
Si dissolution (Van Den Berghe)	--	1 L	--	--
Eukaryotic microscopy (Hu)	--	0.75 L	0.75L	--

Crustal fluid gas chemistry summary

Section by Stephanie Carr

Dissolved methane and carbon monoxide concentrations were measured in crustal fluid and bottom seawater samples. For crustal fluid samples, 20ml glass vials were filled without headspace from the MVBS bags in a glove bag. Samples were poisoned with 100µL of 10M NaOH, then a 2ml headspace of nitrogen was displaced into the vials. Samples equilibrated at room temperature for 24 hours, then were warmed at 58ºC for at least one hour before measurement. For bottom seawater, water was collected from CTD Cast 5 (at4211005) on deck

and processed as above. Concentrations were measured using an SRI 310C Gas Chromatograph equipped with FID and methanizer (to convert CO to CH_4). 1ml headspace samples were injected and separated on a 3" mol sieve 5A column with column temperature held at 40ºC for 2 mins, ramped to 45ºC at 2.5ºC min^{-1} and held at 45ºC for 5 min, then ramped to 60ºC at 5ºC min^{-1}. Gas concentrations were calibrated versus standard curves made from Restek gas standards #34505 and 34478 using standard $PV = nRT$ equation formulations, and complete conversion of CO to CH4 was verified by comparing the two standards to each other.

Collection	Site/Horizon	Vial number	µM CH_4	µM CO
J2-1140	U1362B Bio (deep)	J2-1140-0017	27.8	BDL[a]
		J2-1140-0018	30.8	BDL
		J2-1140-0020	37.4	BDL
J2-1143	U1362A Bio (deep)	J2-1143-0896	7.7	BDL
		J2-1143-0894	10.1	BDL
		J2-1143-0893	9.6	BDL
J2-1143	U1362A SS (shallow)	J2-1143-0548	3.5	BDL
		J2-1143-0545	1.9	BDL
		J2-1143-0543	5.1	BDL
CTD AT11005	Bottom water	AT4211005-0299	0.18	BDL
		AT11005-0297	0.16*	BDL

a: BDL, below detection limit, determined as three times the signal-to-noise of blank injections at 0.16 µM ± 0.035 µM (*n*=4 blank measurements).* this value is at the detection limit.

Crustal fluid microbial culturing experiments summary

Section by Stephanie Carr, Jessica Choi, and Kelle Freel

Raw crustal fluid was immediately subsampled from MVBS bags within a glove bag and anoxically transferred into serum vials with stoppers filled with the various anaerobic media, which were incubated on board at various temperatures. At the end of the cruise, enrichment replicates were divided and shipped overnight at room temperature to Hartwick College (Carr and Gourdine), the University of Pennsylvania (Choi and Ileana Pérez-Rodríguez), and the University of Hawaii (Freel and Rappé) for shore-based evaluation.

Media name	**Target**	**Inoculation source**			**Incubation Temp ˚C**
		CORK	Horizon	Dive	
Modified DSMZ 1210a (with iron citrate) "Thermosulfurimonas Medium"	Autotrophic iron reducers	U1362B U1362B U1362A U1362A	Bio line Bio line Deep Shallow	1140 1141 1143 1143	75, 65, 55 75, 65, 55 75 75
Modified DSMZ 141 "Methanogenium Medium (H_2/CO_2)"	Methanogens	U1362B U1362B U1362A U1362A	Bio line Bio line Deep Shallow	1140 1141 1143 1143	75, 65, 55 75, 65, 55 75 75
Modifeid DSMZ 1278 "Archaeoglobus Sulfaticallidus Medium"	Autotrophic sulfate reducers	U1362B U1362B U1362A U1362A	Bio line Bio line Deep Shallow	1140 1141 1143 1143	75, 65, 55 75, 65, 55 75 75
Modified SME Medium	Autotrophic nitrate reducers	U1362B U1362B U1362A U1362A	Bio line Bio line Deep Shallow	1140 1141 1143 1143	75, 65, 55 75, 65, 55 75 75
	Autotrophic sulfur reducers	U1362B U1362B U1362A U1362A	Bio line Bio line Deep Shallow	1140 1141 1143 1143	75, 65, 55 75, 65, 55 75 75
Autotrophic carboxydotrophic media	Autotrophic carboxydotrophs	U1362B U1362B U1362A U1362A	Bio line Bio line Deep Shallow	1140 1141 1143 1143	75, 65, 55 75, 65, 55 75 75
Dissimilatory carboxydotrophic media with yeast extract	Heterotrophic carboxydotrophs	U1362B U1362B U1362A U1362A	Bio line Bio line Deep Shallow	1140 1141 1143 1143	75, 65, 55 75, 65, 55 65 65
Dissimilatory carboxydotrophic media with pyruvate	Heterotrophic carboxydotrophs	U1362A U1362A	Deep Shallow	1143 1143	65
Dissimilatory carboxydotrophic media with autoclaved *Thalassospira*	Heterotrophic carboxydotrophs	U1362A U1362A	Deep Shallow	1143 1143	65
Modified DSMZ 1278 with yeast extract	Heterotrophic sulfate reducers	U1362A U1362A	Deep Shallow	1143 1143	65
Modified DSMZ 1278 with pyruvate extract	Heterotrophic sulfate reducers	U1362A U1362A	Deep Shallow	1143 1143	65
Modified DSMZ 1278 with autoclaved *Thalassospira*	Heterotrophic sulfate reducers	U1362A U1362A	Deep Shallow	1143 1143	65
Modified DSMZ 1278 with carbon monoxide	Heterotrophic sulfate reducers	U1362A U1362A	Deep Shallow	1143 1143	65

CTD sample collection and casts summary

Section by Duane Moser, Kai Ziervogel, Jacob Munson McGee

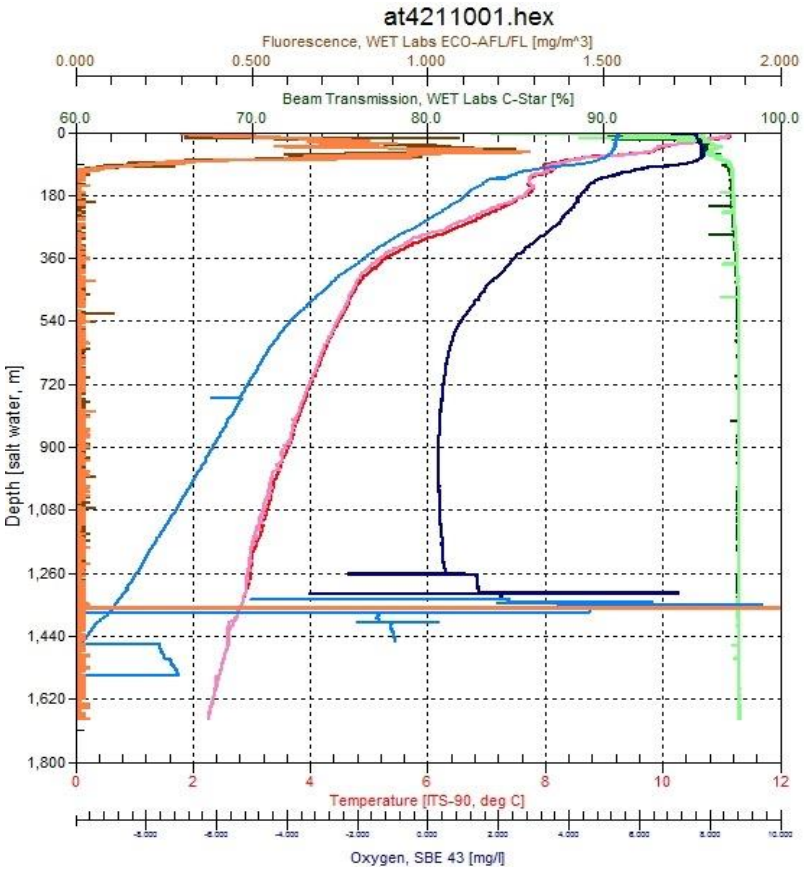
WHOI's 24x 10L bottle CTD Niskin rosette sampler was used during periods of opportunity to collect water column samples for various groups. The primary user group was "microG2P" consisting of Kai Ziervogel (lead), Kerry Dykens, Duane Moser, and Jacob Munson-McGee. Additional users included the "CaNiRa" team, Anirban Chakraborty, and minimal sampling by the Orcutt group. Team scientists participated in the operation of the CTD system (e.g. cocking of sampling bottles, operation of taglines during deployment, stowage after deployment) after appropriate training from Atlantis' SSSG.

Sea state was a concern for most casts. A kink was observed after Cast 6 near the wheel block and the cable needed to be cut and re-wired prior to Cast 7 by SSSG. Casts 1 – 5 had sporadic but serious issues with data display, primarily affecting oxygen readings but also affecting conductivity and other probes as well. These were eventually traced back, in part, to several unneeded corrections that were turned on in the set-up file. SSSG removed these instructions from the set-up file, but there were still issues with wavering oxygen values below about 1,500 m and with the upcast oxygen measurements running higher than downcast values below 1,500 m.

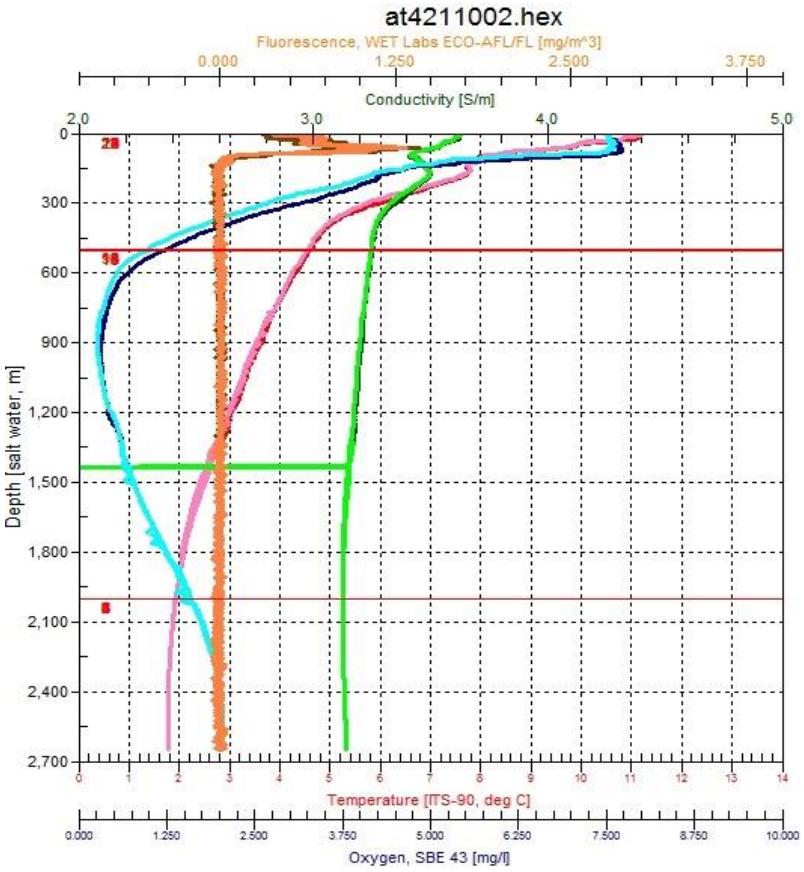
The system consists of a custom-built SBE 9plus Seabird instrument package operating with SBE11plus Firmware Version ">= 5.0". Sensors on the CTD included:
Conductivity (Calibrated: 2018-08-16),
Temperature (Calibrated: 2018-08-16),
Pressure (Digiquartz with TC; Calibrated: 2014-04-08 and verified 2019-02),
Fluorometer (WET Labs ECO-AFL/FL; Calibrated: 2016-11-22),
Transmissometer (WET Labs C-Star, Calibrated: 2016-12-06 and 2018-03-25)
Turbidity (WET Labs, ECO-NTU, Calibrated 2016-11-22)
PAR (SBE QSP200L4S PAR Sensor, Calibrated 2017-08-01)
OSBE 43 Oxygen Probe (Calibrated: 2018-09-06)

CTD logs (*all times local, GMT-7*)

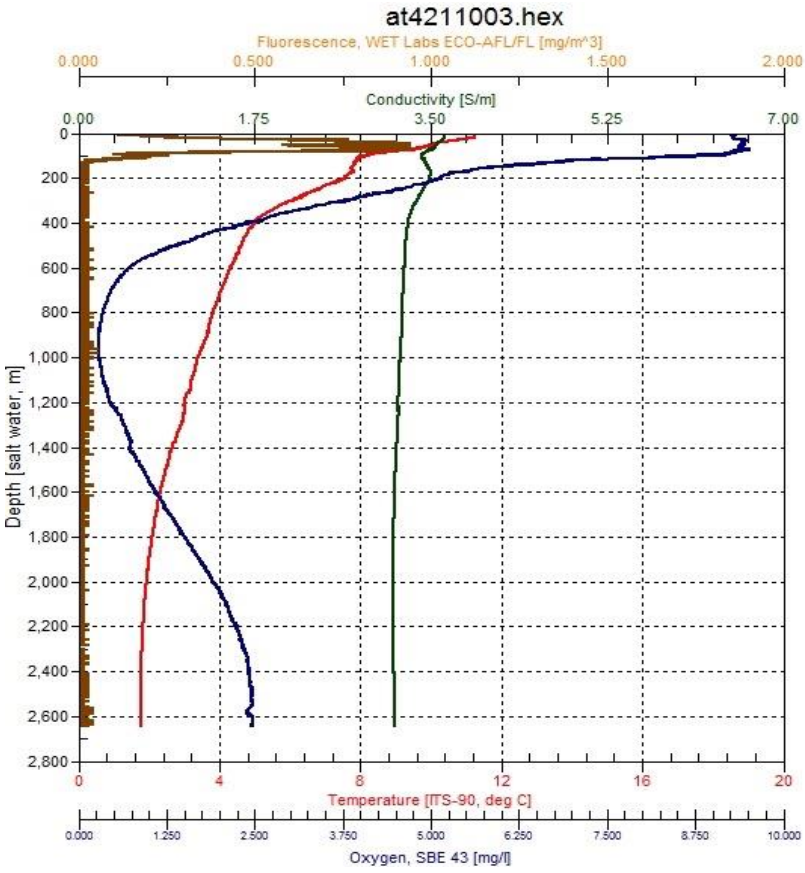
Cast	Date	Launch time	Recovery time	Latitude	Longitude
at421101	20190517	20:04	n.a.	47.7585777	-127.762139
Comments: problems with sensors, not used. Cast at421102 started immediately after without recovery					
Depth (m)	**Bottles**	**Experiments**			
n.a.	n.a.	n.a.			



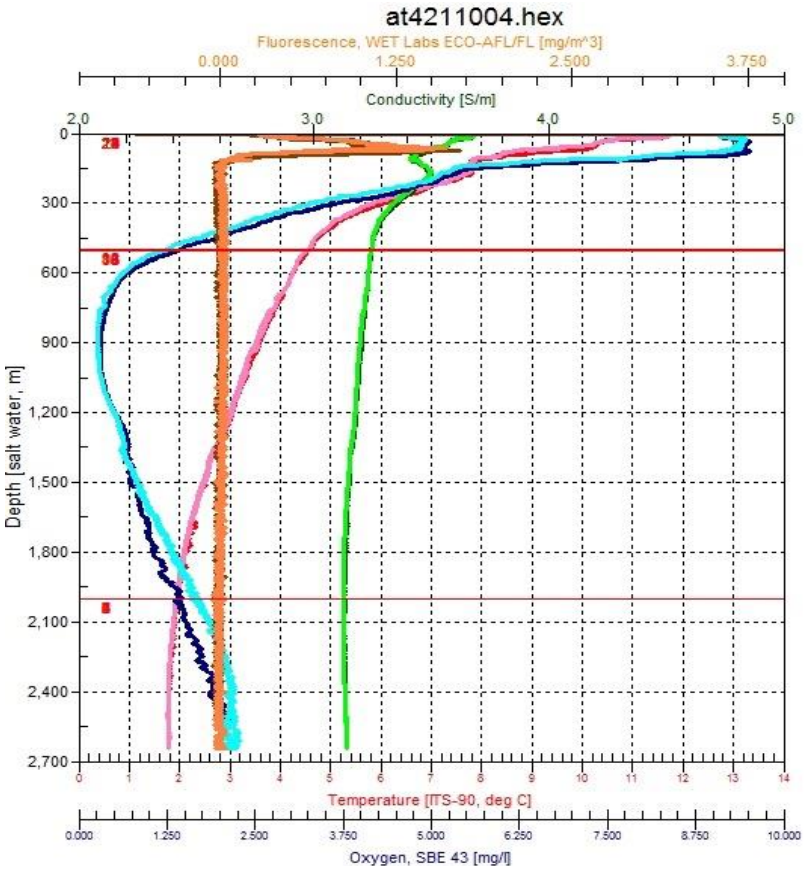
Cast	**Date**	**Launch time**	**Recovery time**	**Latitude**	**Longitude**
at421102	20190517	21:12	23:25	47.7585777	-127.762139
Comments: Problems with oxygen sensor data					
Depth (m)	**Bottles**	**Experiments**			
2000	1-8	MicroG2P group - 30 incubations: 6x 2L incubations per amendment in 4˚C cold room sampled at 24 and 72 hours for Nutrients, SCG, biomass, hydrolytic enzyme activities			
500	9-16	MicroG2P group - 30 incubations: 6x 2L incubations per amendment in 4˚C cold room sampled at 24 and 72 hours for Nutrients, SCG, biomass, hydrolytic enzyme activities			
5	17-24	MicroG2P group - 30 incubations: 6x 2L incubations per amendment in 11.2˚C incubator sampled at 6 and 24 hours for Nutrients, SCG, biomass, hydrolytic enzyme activities			



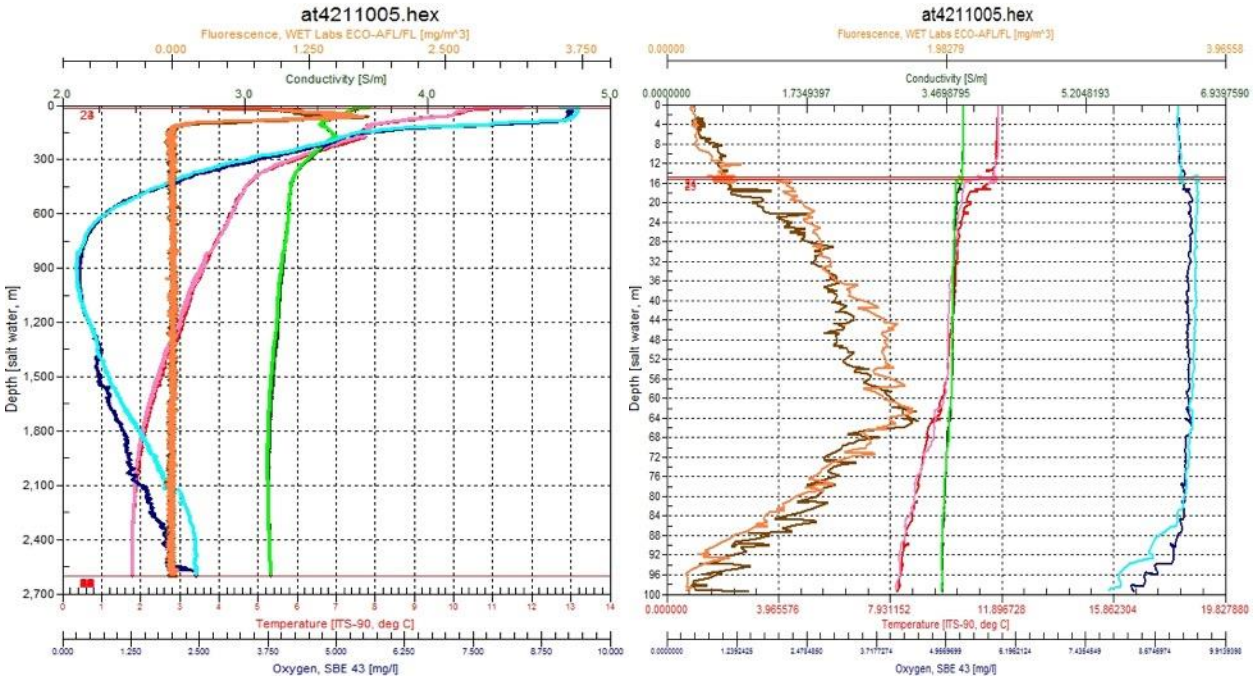
Cast	**Date**	**Launch time**	**Recovery time**	**Latitude**	**Longitude**
at421103	20190518	13:15	15:27	47.764639	-127.758492
Comments: Oxygen sensor issues, upcast data not usable					
Depth (m)	**Bottles**	**Experiments**			
2650	1-12	Chakraborty collected water for incubation experiments at home lab			
0	13-18	Water discarded			



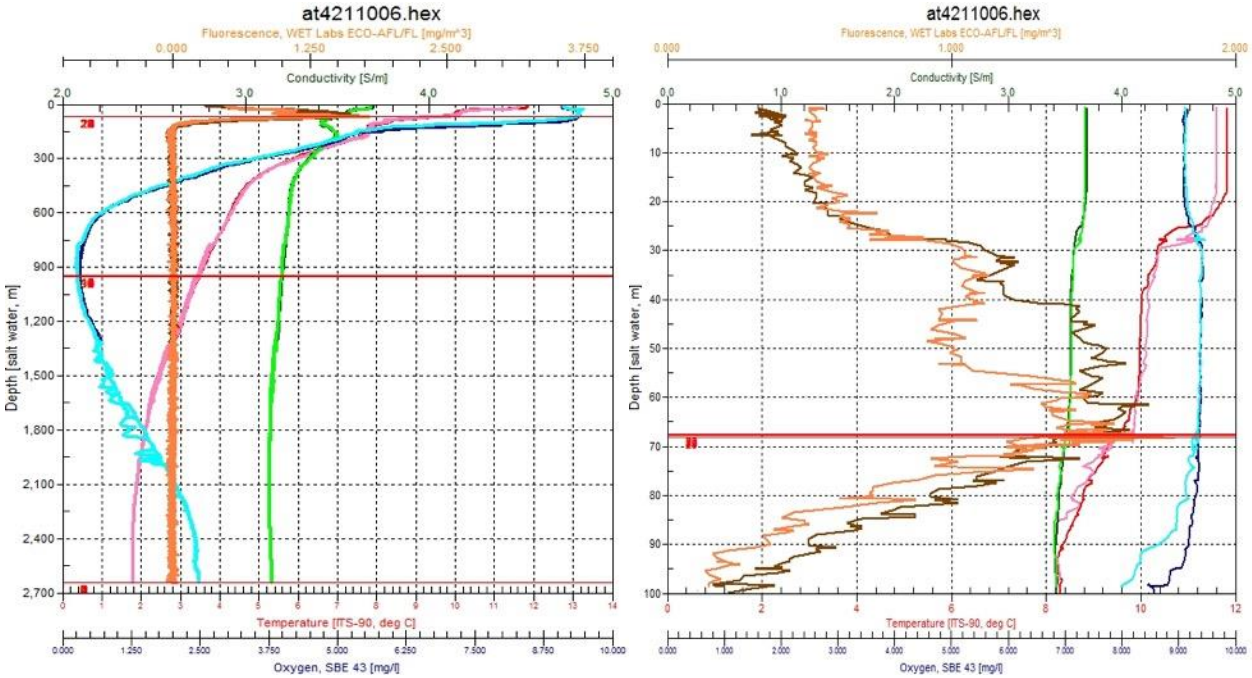
Cast	**Date**	**Launch time**	**Recovery time**	**Latitude**	**Longitude**
at421104	20190521	8:45	10:50	47.758302	-127.762269
Comments: Sensors replaced before cast					
Depth (m)	**Bottles**	**Experiments**			
2000	1-7	MicroG2P group - 15 incubations: 3x 2L incubations per amendment in 4˚C cold room sampled at 72 hours for Nutrients, SCG and biomass			
500	8-16	MicroG2P group - 30 incubations: 6x 2L incubations per amendment in 4˚C cold room sampled at 24 and 72 hours for Nutrients, SCG and biomass			
0	17-24	Water discarded			



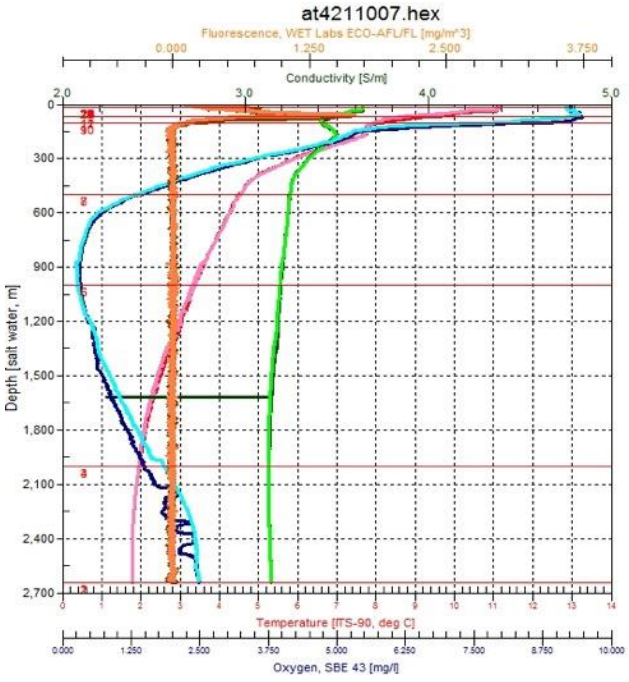
Cast	**Date**	**Launch time**	**Recovery time**	**Latitude**	**Longitude**
at421105	20190521	14:24	17:35	47.760917	-127.761144
Comments: Changed cables and sensor settings before cast					
Depth (m)	**Bottles**	**Experiments**			
2601	1-22	Rappé			
10	23-24	Rappé			



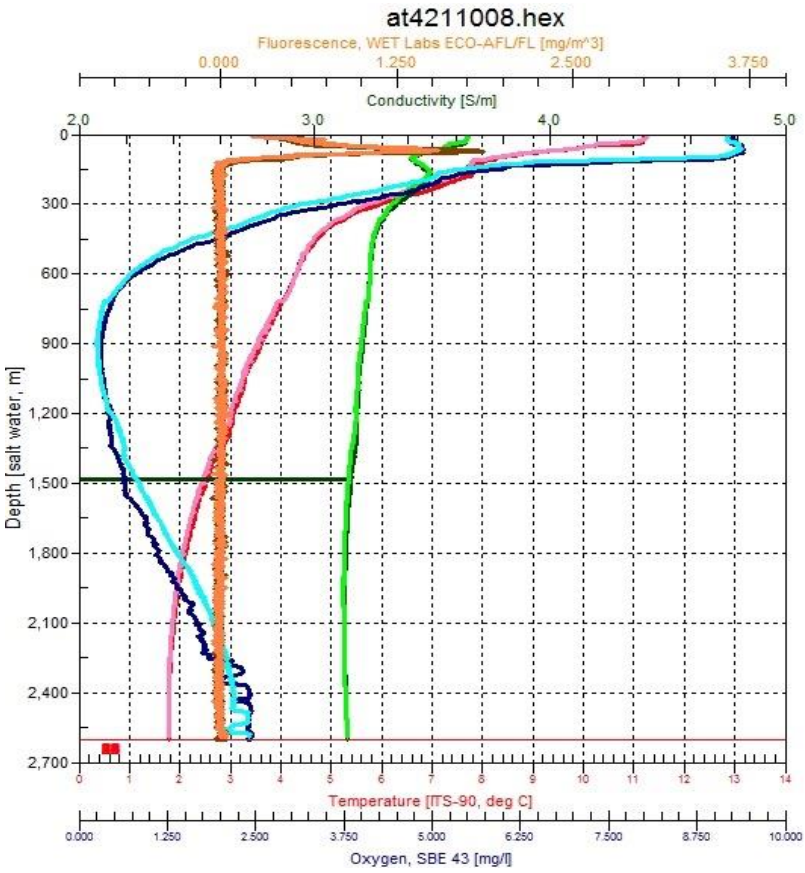
Cast	**Date**	**Launch time**	**Recovery time**	**Latitude**	**Longitude**
at421106	20190521	18:40	21:10	47.760950	-127.761452
Comments:					
Depth (m)	**Bottles**	**Experiments**			
2645	1-8	MicroG2P group – Bottom water collected for onboard enzyme assays and cell abundance; Chakraborty collected water for incubation experiments at home lab; D'Angelo collected water from bottom depth for Orcutt group for rock chip incubations and sample collection for Sarah Hu			
950	9-16	MicroG2P group – oxygen minimum zone water collected for onboard enzyme assays and cell abundance			
65	17-24	MicroG2P group – Deep chlorophyll maximum water collected for onboard enzyme assays and cell abundance on whole water and size fractionated samples (0.8 um)			



Cast	**Date**	**Launch time**	**Recovery time**	**Latitude**	**Longitude**
at421107	20190523	09:00	11:05	47.759791	-127.760428
Comments: Cable reterminated before cast					
Depth (m)	**Bottles**	**Experiments**			
2650	1-2	MicroG2P group - Water filtered onto 0.2-um filter for microbial community analysis			
2000	3-4	MicroG2P group - Water filtered onto 0.2-um filter for microbial community analysis			
100	5-6	MicroG2P group - Water filtered onto 0.2-um filter for microbial community analysis			
500	7-8	MicroG2P group - Water filtered onto 0.2-um filter for microbial community analysis			
105	9-10	MicroG2P group - Water filtered onto 0.2-um filter for microbial community analysis; Water collected for onboard enzyme assays and cell abundance			
65	11-12	MicroG2P group - Water filtered onto 0.2-um filter for microbial community analysis			
10	13-24	MicroG2P group - Water filtered onto 0.2-um filter for microbial community analysis; 30x incubations: 6x 2L incubations per amendment in 11.2˚C incubator sampled at 0, 6 and 24 hours for Nutrients, SCG, and biomass			



Cast	**Date**	**Launch time**	**Recovery time**	**Latitude**	**Longitude**
at421108	20190523	14:10	15:11	47.758904	-127.761096
Comments:					
Depth (m)	**Bottles**	**Experiments**			
2645	1-24	Rappé			



Summary of sampling for thermophilic spores

Summary by Anirban Chakraborty

As a companion project to the primary funded projects, collaborators from the University of Calgary joined the cruise to examine the abundance and biogeography of thermophilic spore-forming anaerobic bacteria that remain dormant in permanently cold sediment. These spore-forming thermophiles belong to the so-called rare biosphere and are not detected in nucleic-acid-based diversity assays of sediment or bottom water. Additionally, these dormant endospores remain unaffected by selective pressure on the seabed, which makes them ideal model organisms for studying passive dispersal. Subseafloor petroleum reservoirs and mid-ocean ridge spreading centers are warm environments that host anaerobic microbial communities that could potentially supply these organisms to the seafloor via fluid flow. As the CORKs at the cruise study site allow access to high-temperature anoxic crustal fluids, as well as their dispersal into nearby sediment when the crustal fluids leaked from the boreholes (for example, see Wheat et al. 2010 *G*^{3}), samples of crustal fluid, sediment and bottom water were collected opportunistically to evaluate the hypothesis that that thermospores are inhabitants of deep hydrothermal fluids and are distributed to nearby seawater and sediments via when these fluids are ejected into the ocean.

Raw crustal fluid samples were collected from CORK boreholes U1362B (deep bioline; total volume = 12.5 L from dives J2-1140 and J2-1141) and U1362A (deep bioline; MVBS bag #4; total volume = 5 L, Jason Dive J2-1143; May 26, 2019) using the MVBS filled by the MPS on Jason. MVBS bags were stored in the cold room upon recovery to allow gas equilibration for 12-24 hours with a bubble of nitrogen or argon gas (for clumped isotope analysis), then fluid samples were withdrawn into 1-L sterile polycarbonate bottles and subsequently stored at 4°C. These fluid samples will be utilized to conduct incubation experiments to resuscitate viable endospores at various temperatures.

Seawater from the bottom of the water column (water depth=2645 m, total volume = 9 L) near to borehole U1362B was collected from Cast at421103 and at421106. Seawater was sampled from 10-L Niskin bottles directly into sterile 1-L polycarbonate bottles and stored at 4°C for incubation experiments.

Three sediment push cores were collected 20 m away from CORK U1362B (20-cm deep; Jason Dive J2-1143; May 26, 2019). Due to high water content within the sediment, sectioning cores at depth intervals was not possible. Instead the sediment was collected in bulk within sterile WhirlPak bags and stored at 4°C for incubation experiments. In addition to bulk sediment, triplicate aliquots of 1.5-ml sediment were frozen at -20°C for DNA-based community assessment.

CORK hardware servicing summary

Summary by Jorge Agobian and Beth Orcutt

The CORK top plug at Hole U1362B was removed on dive J2-1141 to enable the *in situ* incubation experiments for the Orcutt group. The weight stack was lifted off the CORK top, and the toilet brush was then used to clear off the black rust that had formed under stagnant conditions. A newly machined socket driver was used to loosen the dog bolts on the outside of

the CORK (note: thanks to Jason and Atlantis deck crew for quick work to make a new tool, since the science party accidentally did not bring the needed tool), then the RS pulling tool was inserted into the top plug to remove it. This process worked without a hitch. Fluids immediately began venting from the CORK top, as expected. The top plug + RS pulling tool were recovered via elevator.

The U1362B top plug was cleaned shipboard and then re-deployed with the GS running tool on the 2nd elevator on dive J2-1143. After inserting the tog plug, disconnecting the running tool, and retightening the dog bolts, fluids were still weeping around the top plug. The former weight stack was retrieved from the CORK ROV platform and redeployed on top of the top plug; this stopped the weeping of fluids out of the CORK top.

Due to operational constraints during the cruise that prevented additional dives, we were unable to deploy the top plug U1301A as planned as a secondary objective of the cruise (note: this hole had been left open after the AT26-18 cruise due to operational constraints on that cruise). We were also unable to pull the top plug from Hole U1362A as planned for secondary objectives to deploy a flow meter on this CORK.

CORK pressure data download summary

Summary by Adam Price

The CORKs at Holes U1362A/B installed during IODP Expedition 327 in 2010 included pressure loggers monitoring formation pressure at various horizons within the CORKs, as well as seafloor pressure (see Fisher et al. 2012 *Scientific Drilling*). The last download of data from these loggers occurred in 2014 during cruise AT26-18. During AT42-11, pressure data was downloaded from both CORK pressure loggers after performing a hydrostatic check (note that the hydrostatic check at U1362A was completed on dive J2-1142). See the dive logs above for specific details on when valves were turned and ODI loggers connected.

CORK	Dive	Date/Time [a]	Data Start [b]	Data End [b]	Offset (s)	Filename
U1362B	1140	2019/05/18 06:13	2014/08/14 17:28	2019/05/17 04:56	-90396.824	19p1362b_1.raw
U1362A	1143	2019/05/26 20:02	2014/08/12 20:38	2019/05/26 20:01	+3925.00	19p1326a_1.raw

[a] Time when download started. [b] as reported in converted data file

CORK OsmoSampler recovery summary

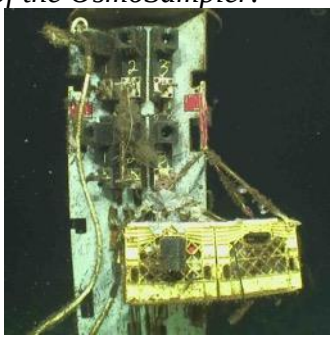
Summary by Trevor Fournier

Two osmo sampler systems were deployed during R/V Atlantis/Alvin Expedition AT26-18 in 2014. A dual milk crate bundle designated as "Yellow OsmoSampler" was deployed on Alvin dive 4758 on wellhead U1362B on 20140820. Another dual milk crate bundle designated as "Black OsmoSampler" was deployed on Alvin dive 4759 on wellhead U1362A on 20140821. Each OsmoSampler had three OsmoSampler types (see Wheat et al. 2011 DOI: 10.2204/iodp.proc.327.109.2011): Standard (2x 300m Teflon coils in series + 1 OsmoPump), gas (2x 300m Copper coils in series + 1 OsmoPump), and an MBIO (2x 300m Teflon coils for sample + 1x 300m Teflon coil with fixative (ethanol) + 2 OsmoPumps).

Yellow OsmoSampler was recovered on dive J2-1140 on 20190518; and Black OsmoSampler was recovered on dive J2-1143 on 20190526. Before recovery, hydrothermal fluid outflow from the OsmoSamplers lines were both confirmed visually and with the temperature sensor (max temp ~8ºC). The MBIO coils from both OsmoSamplers were disposed of by the chief scientists due to concerns about possible mercuric chloride content as well as certainty that the systems did not pump as intended (based on prior tests at other sites).

For the Yellow OsmoSampler, the Standard OsmoPump was a 5-membrane type (8" height x 3.75" diameter). The salt reservoir was ¾ full of salt and the DI reservoir was 123 psu, suggesting overpumping in the ~5 year deployment. The Standard coils 1 (closest to intake) and 2 (closest to pump) was connected in the correct orientation, and they were sectioned in 1.2m length intervals at sea. The Yellow copper coils were also attached as above to a 4-membrane pump (7" height x 4" diameter) that had salt in the salt reservoir and 8 psu in the DI reservoir, suggesting minimal over-pumping. The coils were clamp sealed shut for transport to shore. A small octopus was accidentally recovered in the Yellow OsmoSampler basket, and it sluggishly moved when prodded. The octopus was transferred to a plastic bag and frozen by the chief scientist at -80ºC for shore-based analysis by collaborators.

Photos of the Yellow OsmoSampler recovered on Dive J2-1140 from Hole U1362B. Left: view of OsmoSampler still connected to the Hole U1362B CORK OsmoSampler bay deep horizon. Middle: View of the Standard and Gas OsmoSamplers on one side of the OsmoSampler. Right: View of the MBIO OsmoSampler and the small octopus (under the yellow rope) on the other side of the OsmoSampler.



For the Black OsmoSampler, the Standard OsmoPump was a 4-membrane type. The salt reservoir was 2/3 full of salt and the DI reservoir was 4 psu, suggesting minimal overpumping in the ~5 year deployment. The Standard coils 1 (closest to intake) and 2 (closest to pump) was connected in the correct orientation, and they were sectioned in 1.2m length intervals at sea. The Yellow copper coils were also attached as above. The coils were clamp sealed shut for transport to shore.

Photos of the Black OsmoSampler recovered on Dive J2-1143 from Hole U1362A. Left: view of OsmoSampler still connected to the Hole U1362A CORK OsmoSampler bay deep horizon. Right: View of the Standard, Gas, and MBIO OsmoSamplers.