Expedition Report for R/V Thompson + ROV Jason Expedition TN-421
TN-421: Slow Life Part 2: Microbial Activity in the Crustal Deep Biosphere

Expedition Dates and Ports: 31 July 2023 to 8 August 2023, Newport, OR, to Newport, OR
(mobilization: 29-30 July 2023, demobilization: 9 August 2023)

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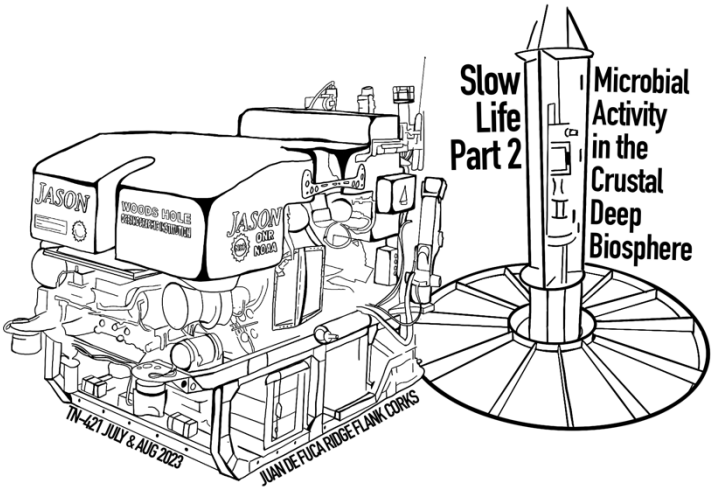

Funding for expedition:
Primary project: US NSF project OCE-1737017 to Orcutt
Complementary science support funding: US NSF OCE-1851582 and linked awards – Co-PIs Stephanie Carr, Olivia Nigro, and Mike Rappé

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Executive Summary

Table 1. Site locations

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 US National Science Foundation funded this Marine Scientific Research expedition of the Research Vessel (RV) *Thompson* with the remotely operated vehicle (ROV) *Jason II* to continue a multi-decade time-series study examining the unique characteristics of processes happening below the seafloor on the eastern flank of the Juan de Fuca Ridge (Table 1, Figure 1). This 9-day expedition departed on 31 July from Newport, OR, USA and returned back to Newport on 8 August. On this expedition, scientists used the ROV to collect sediment cores and sample pristine hydrothermal fluids via existing seafloor borehole observatories (CORKs). The borehole observatories (U1362A, U1362B) were drilled, cased, cored, and then instrumented with CORKs using the drillship *JOIDES Resolution* of the international scientific ocean drilling program called IODP. These instrumented sites allow continuous monitoring of pressure and temperature at depth, sampling of fluids and microbiological material, and measurement of fluid flow rate using autonomous instrumentation. The collected samples will be used to investigate the microbial diversity from this subseafloor realm, in order to better understand the functions that the microorganisms perform in the environment and how they connect to other habitats.

The target study area for this Marine Scientific Research is the subseafloor between known but poorly explored underwater mountains (seamounts) that exist within Canada's Offshore Pacific Area of Interest proposed for marine protection as the Tang.Gwan-hačx^{w}iqak-Tsigis (ThT) Marine Protected Area (MPA). The primary intention of the ThT is to protect the estimated 47 seamounts and 100s of seamount-like features within its borders. As a precaution for this expedition, an MPA Activity Plan was submitted to the Canadian Department of Fisheries and Oceans' (DFO) Marine Protected Area Management team. Ultimately, this document was not needed because the approval of the ThT as an MPA is still pending. Nevertheless, we treated our expedition as if we were working in an MPA and followed MPA guidelines. Additionally, we documented seafloor conditions and fauna during operations to share with DFO.

This area is also part of a long-term monitoring program of Ocean Networks Canada's (ONC) cabled array in the Pacific Ocean (i.e., the Cascadia Basin node). ONC scientists are leading an international program called SolidCarbon, which aims to leverage this study site for a potential demonstration of carbon capture and sequestration technologies. While no demonstration work occurred on this expedition, four Canadian scientists and students from ONC and the SolidCarbon team participated in the expedition to gain experience with the infrastructure and collect samples and data to inform baseline information for the project. In addition, two unofficial observers from First Nations sailed with the expedition, supported by ONC, to have

the opportunity for an immersive experience with scientific deep-sea research and a chance to connect with this unique environment and its inhabitants.

In service of the primary objectives of the cruise, five dives of ROV Jason were completed, totaling 55.5 hours of bottom time operations for science objectives. ROV dives were supplemented by a deployment of the WHOI elevator to ferry equipment back and forth to the seafloor and back. A total of 303 L of raw crustal fluid was collected with the Mobile Pumping System from the CORKs, supplemented by 372 L of fluid filtered *in situ* with the MPS. Passive filtration units filtered a total of 4866 L. This achieved the primary objectives of collected crustal fluid from all horizons for microbial growth rates experiments, enrichment cultures, and filtration for viruses and DNA.

The expedition also achieved many secondary goals. Pressure and temperature data were downloaded from CORKS U1362A and U1362B. The recovered datasets revealed that the data loggers installed in U1362A and U1362B recorded data from May 2019 to April 2022 and May 2019 to June 2020, respectively. The early terminations of data collection suggest that the batteries could no longer support the dataloggers. Additionally, we collected two full water column profiles with the ship's CTD Niskin rosette, collected 15 sediment push cores, and installed multiyear OsmoSamplers on CORKS U1362A and U1362B. The OsmoSamplers will collect crustal fluid samples from these sites to establish geochemical baselines, which will inform the ONC SolidCarbon project and partners.

Shipboard and ROV operations for the most part went smoothly, aided by exceptionally good weather on site, allowing all primary objectives to be accomplished. Electrical and mechanical issues with the ROV interrupted the first dive. Some time was also lost due to the need to re-do J-box cabling to have power and comms for the third-party ODI connector to allow data downloads. The final ROV dive was ended early due to issues with the winch; this precluded the chance to get replicate fluid sampling at one of the primary targets. The third-party Mobile Pumping System did not perform optimally on the first dive due to loose connections at the in-line oxygen optode sensor, but the issue was resolved for subsequent dives.

Processed and analyzed data from shore-based sediment and water analyses will be made available within two years (8/9/2025) on our respective BCO-DMO sites:

Orcutt: Project: Microbial activity in the crustal deep biosphere | BCO-DMO
Carr: https://www.bco-dmo.org/project/854575
Rappe: https://www.bco-dmo.org/project/854575
Nigro: https://www.bco-dmo.org/project/854575

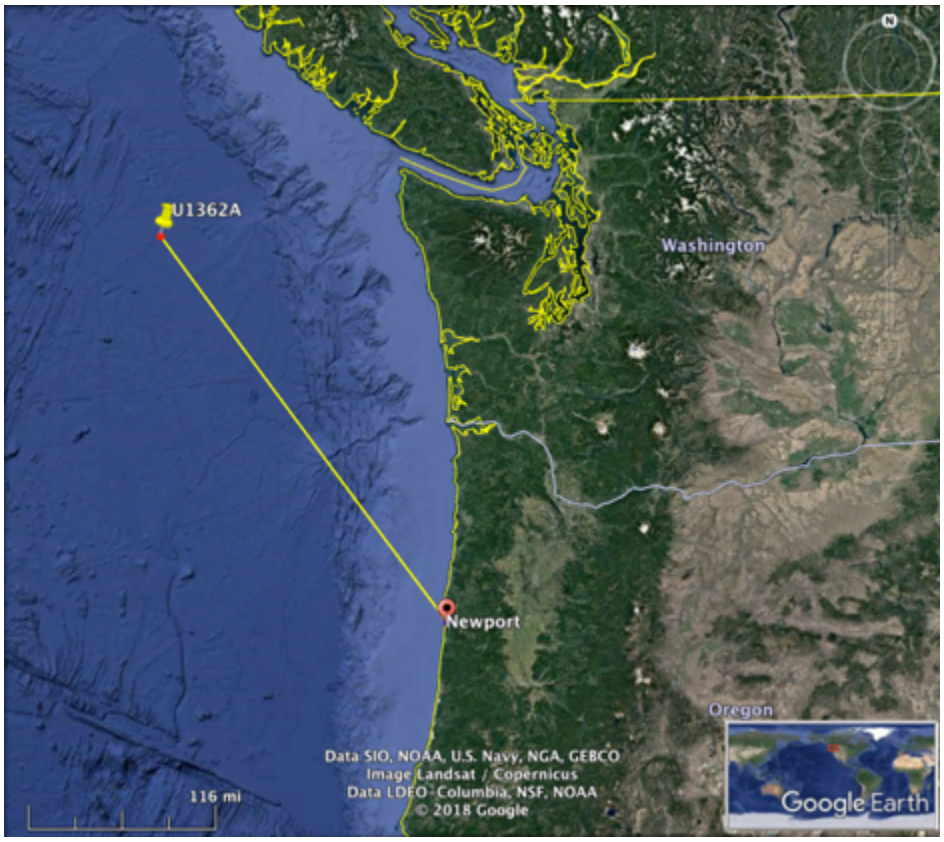


Figure 1 Cruise track for TN-421 to/from Newport, Oregon, as shown in Google Earth.

Personnel and Affiliations

Table 2. *Shipboard scientific staff for TN-421 and listing of shore-based collaborators. Green shading, OCE-1737017 group; purple shading, complimentary OCE-1851582 group; blue shading, Canadian First Nation observers. Ocean Networks Canada, ONC .*

#	Last Name	First Name	PI Group	Role	Email
Shipboard					
1	Orcutt	Beth	Orcutt	Chief Sci.	borcutt@bigelow.org
2	D'Angelo	Tim	Orcutt	Res. Assoc	tdangelo@bigelow.org
3	Budner	Michael	Orcutt	Undergrad.	budner.m@northeastern.edu
4	Melissa	Herring	Orcutt	Grad. Stu.	herring.m@northeastern.edu
13	Rappé^	Mike	Rappé	Co-PI	rappe@hawaii.edu
14	Freel	Kelle	Rappé	Postdoc	kfreel@hawaii.edu
15	Spotkaeff	Cherise	Rappé	Technician	cspotkae@my.hpu.edu
16	Mochimaru	Hanako (Hana)	Rappé	Res Sci	hanakom@hawaii.edu
15	Lin	Tina	Self	Res Sci	tinalinht@ntu.edu.tw
17	Ramfelt	Oscar	Rappé	Grad. Stu	oramfelt@hawaii.edu
18	Nigro^	Olivia	Nigro	Co-PI	onigro@hpu.edu
19	Hunckler	Julia	Nigro	Grad. Stu.	juliahunckler@gmail.com
20	Carr	Stephanie	Carr	Chief Sci	carrs@hartwick.edu
21	Brown	Sierra	Carr	Undergrad.	Browns3@hartwick.edu
14	Daisernia	Andrew	Carr	Undergrad.	Daiserniaa@hartwick.edu
16	Chakraborty	Anirban	Chakraborty	Ass. Prof	anirbanc@isu.edu
17	Ellis	Bronwyn (Rhys)	Chakraborty	Undergrad.	rhysellis@isu.edu
18	Sevgen	Serhat	Tutolo	Grad. Stu	serhat.sevgen@ucalgary.ca
19	Robutka	Hannah	Coogan	Grad. Stue	hannahrobutka@uvic.ca
20	Lauer	Rachel	Lauer	Ass. Pro	Rachel.lauer@ucalgary.ca
21	Putra	Haidar	Lauer	Undergrad.	mhaidar.putra@ucalgary.ca
22	Joseph	Jessica	ONC	observer	Kassidygeorge@oceannetworks.ca
23	George	Kassidy	ONC	observer	jessjoseph@live.ca
Shore-based collaborators					
	Lindsay	Melody	self		mlindsay@bigelow.org
	Becker	Keir	self		kbecker@earth.miami.edu
	Fisher	Andy	self		afisher@ucsc.edu
	Wheat	Geoff	self		wheat@mbari.org
	Tutolo	Ben	Tutolo		benjamin.tutolo@ucalgary.ca
	Coogan	Laurence	Coogan		lacoogan@uvic.ca
	Du Preez	Cherisse	self		Cherisse.DuPreez@dfo-mpo.gc.ca
	Mahmoudi	Nagissa	Self		Nagissa.mahmoudi@mcgill.ca

Figure 2. Shipboard scientific staff for TN-421. Back row: Michael Budner, Serhat Sevgen, Julia Hunckler, Oscar Ramfelt, Tim D'Angelo; 3rd Row: Kassidy George, Haidar Putra, Hannah Robutka, Tina Lin, Rachel Lauer, Andrew Daisernia, Kelle Freel, Olivia Nigro, Jessica Joseph; 2nd Row: Beth Orcutt, Anirban Chakraborty, Sierra Brown, Stephanie Carr, Hanako Mochimaru; 1st row: Melissa Herring, Rhys Ellis, Cherise Spotkaeff, Michael Rappé.

Table 3. Additional TN-421 personnel: ROV Jason team (A), Shipboard Science Support personnel (SSSG, B) and R/V Thompson Ship Crew (C)

A.

Jason group
Alberto (Tito) Collasius
Korey Verhein
Mario Fernandez
Hugh Popenoe
Scotty McCue
Fred Denton
Matthew Schwartzman
Adam Ensminger
Tom Trudel
Ronnie Whims

B.

Shipboard Science Support Personnel
Stephen Jalickee
Elizabeth Ricci

C.

Ship's Crew	
Pamela Blusk	Able-Bodied Seaman
Bernadette Castner	Able-Bodied Seaman/ 3rd Mate
Todd Crump	Chief mate
Raven Frisell	Deck Cadet
Eric Haroldson	Captain
Joanna Hoyt	Able-Bodied Seaman
Mark Johnson	Chief Engineer
Brian Jones	Second Cook
Joesph Kaser	Oiler
Ava Kelley	Able-Bodied Seaman
Maria LaPointe	Able-Bodied Seaman
Cassi Lehfeldt	Oiler
Andrew Minshall	Third Engineer
Victor Ottoboni	2nd Mate
Alexander Padgett	Oiler
Terence Singerline	Mess
David Stanford	Able-Bodied Seaman
Daniel Stewart	Engineer Cadet
Benjamin Tague	First Engineer
Aaron Walker	Able-Bodied Seaman
Tyler Wickesberg	Second Engineer
Freda Zacharias (Liz)	Steward

Expedition Day by Day Summary

Summary by Stephanie Carr

Table 4: Day by Day Summary. All times local GMT-7.

29 July	Start of mobilization in Newport, OR; science party pallet loading
30 July	Continuation of mobilization. Science Party begins assembling equipment on ROV Jason II.
31 July	Final mobilization tasks. Safety briefing at 10:30. Departure at 13:00 and underway under cloudy skies. Transit at roughly 12 km to site. Fire drill at 14:16. ROV briefing and introduction to SeaLog software at 15:30. Science meeting at 19:00. Science Party continued assembling equipment for loading on ROV Jason.
1 Aug	Arrive on station at 10:00. Electrical issues with the ROV Jason winch prevented an ROV deployment. A CTD was cast at 12:15 to collect bottom water for ONC group, Nagissa Mahmoundi, Chakroborty and Lin. After repairs and a successful weight test with the winch, we had our first Jason dive, J2-1507. Jason was lunched at 11:10 pm.
2 Aug	J2-1507 dive continued. We arrived at U1362B and started pressure download procedures. An observed oil leakage caused us to abort dive J2-1507 at 03:30. A manipulator was quickly replaced and the ROV was launched again at 08:00 J2-1508). J2-1508 followed the remaining J2-1507 plan, starting with an attempt to download pressure data at U1362B. We were unable to receive data from the CORK and rescheduled the effort. The MPS pumped crustal fluid from the MBIO line with the LVBS, but a problem with the McLane manifold prevented the collection of an *in situ* filter and MVBS bags. An attempt to deploy a passive *in situ* filter was made, but passive filtering was not observed and the filter unit was recovered. The ROV was recovered at 19:40. Crustal fluids from the LVBS bag were subsampled that evening in the ship's laboratories.
3 Aug	The day began with an J2-1509 an 08:00 elevator launch. The elevator included Osmo samplers for the Ocean Network Canada group and 3 *in situ* filter units for Nigro. The ROV was launched to U1362A at 8:30. The 27-hour dive was very successful. Starting at the elevator near U1362A, the ROV picked up a passive *in situ* unit, carried it to U1362B, and successfully deployed the unit in the MBIO Bay of U1362B. A second attempt to download pressure data at U1362B was successful and push cores were collected in that area for Chakroborty and Moochimaru. The ROV transited to U1362A for another successful pressure download.
4 Aug	J2-1509 continued at U1362A. Crustal fluid from the shallow and deep horizon were filtered *in situ* using the McLane manifold The LVBS and MVBS bags were filled at the U1362A MBIO Bay, deep horizon. An *in situ* passive filter unit was deployed in the deep horizon of U1362A. An attempt to deploy an *in situ* passive filter at U1362A shallow was unsuccessful as the rotating purge-to-

	sample T-handle detached from the front of the unit. The unit was recovered with the ROV for repairs at 15:25. Crustal fluids and sediment cores were subsampled that evening in the ship's laboratories.
5 Aug	The day began with a 04:24 launch of the ROV at the elevator near U1362A. Osmo Samplers were recovered from the elevator and were hung in the U1362A Osmo bay (deep horizon). The repaired *in situ* passive filter was hung in the MBIO shallow port. The second Osmo Sampler was retrieved from the elevator and carried to U1262B where it was hung in the Osmo Bay. From there the ROV transited to U1301 to collect push core samples and to fire the Niskin bottle for Chakroborty. The ROV transited back to U1362A to pump water into the LVBS and 5 MVBS bags from the shallow horizon. The final MVBS bag was filled with fluid from the U1236B bioline in the MBIO bay. The ROV was recovered at 00:18 Local time.
6 Aug	After the ROV recovery, crustal fluids and sediment cores were subsampled during the early morning in the ship's laboratories. A CTD was launched at 02:00 and recovered at 06:00 for Lin and Sevgen/Tutolo. The ROV J2-1511 dive was launched at 12:14. During the transit to the bottom, a concerning sound was heard from the winch. It was not possible to troubleshoot the problem while the ROV was in the water. Collection of the *in situ* filters became our most immediate priority. All three units were collected and stowed on the elevator. An attempt to remotely recover the elevator around noon was unsuccessful. Instead, Jason manually released the wights from the elevator. The ROV and elevator were recovered at approximately 19:30 and 20:00 pm, respectfully. The passive filters were preserved in the ship's laboratory spaces. Trouble shooting of the winch continued and it was decided to end science dives for this expedition.
7 Aug	The ship begins transit back to Newport. Science party is at 13:00 followed by a showing of "Blue Planet: The Deep" in the lounge.
8 Aug	Arrive at dock at 07:00. Demobilization day.
9 Aug	2nd demob day. Science party moves off the ship.

Dive Summaries

Summary by Stephanie Carr

Table 5. Dive Summary: All times GMT.

Dive #	Launch time	Start Ops on bottom	End Ops	On Deck	Total Ops time (hrs)	End of dive reason
J2-1507	2023/08/02 06:10	2023/08/02 08:09	2023/08/02 10:15	2023/08/02 11:43	2:06	Manipulator failure
J2-1508	2023/08/02 15:17	2023/08/02 17:05	2023/08/03 00:48	2023/08/03 02:39	7:43	Completed goals
J2-1509	2023/08/03 15:35	2023/08/03 17:21	2023/08/04 20:24	2023/08/04 22:25	27:03	Completed goals
J2-1510	2023/08/05 11:24	2023/08/05 13:13	2023/08/06 05:15	2023/08/06 07:18	16:02	Completed goals
J2-1511	2023/08/06 19:14	2023/08/06 22:36	2023/08/07 01:11	2023/08/07 03:00	2:35	Winch Troubles

ROV Jason Dive J2-1507 and J2-1508

J2-1507 Dive Summary

Our first objectives focused on collecting pressure data and crustal fluids from the CORK observatory at Hole U1362B, which is a single-horizon observatory. Originally planned to be a 20-hour dive, Jason was recovered after 4 hours due to an oil leak in the hydrologic of one of the manipulators. The manipulator was replaced and we continued our objectives on the next dive.

J2-1508 Dive Summary

This dive continued the objectives of J2-1508. The dive began with an unsuccessful download of pressure data, due to dead batteries in the CORK. Next, the Mobile Pumping System (MPS) was used to sampling pristine crustal fluids from the subseafloor. The umbilical in the MBIO bay was purged and the LVBS bag was filled. A leak at the MPS optode prevented *in situ* filtering for metagenomics and the filling of the MVBS bags. The *in situ* virus passive filtration unit was deployed, but returned to the ship when warm fluid was not observed to flow through the unit.

Table 6. ROV Jason Vehicle Configuration for J2-1507 and J2-1508 deployment.

Location	Instruments, Sampling Tools
Front porch	Mobile Pumping System (MPS) – 3rd party Large Volume Bag Sampler (LVBS) – 3rd party Toilet Brush – 3rd party Knife High-temperature probe
Starboard Swing Arm	6-gallon milkcrate with 1x Nigro passive filtration unit – 3rd party
Port Swing Arm	6-gallon milkcrate with ODI connector cable – 3rd party
Aft	Medium Volume Bag Sampler (MVBS) + McLane pump – 3rd party
Niskins	2x 5L

Table 7. Third Party equipment in-water weights for J2-1507 and J2-1508 deployment.

Item	Deployment	Recovery
MVBS + McLane Pump	34	34
MPS	33	33
LVBS	6	6
Nigro virus passive filtration unit	16	16
ODI connector	3	3

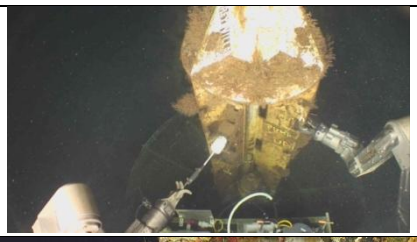
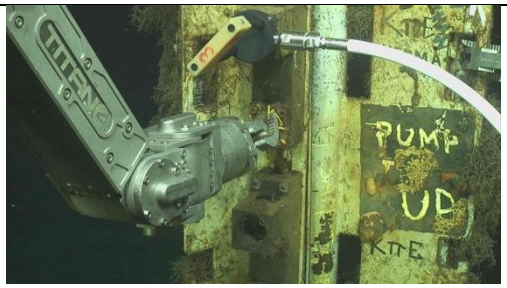
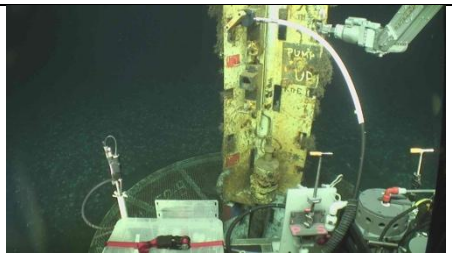
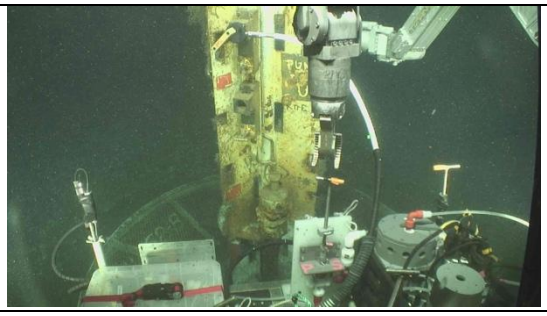
Figure 3. Front porch and swing arm of ROV Jason for dive J2-1507 and J2-1508.

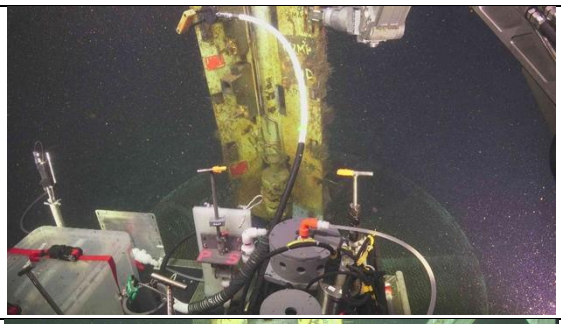
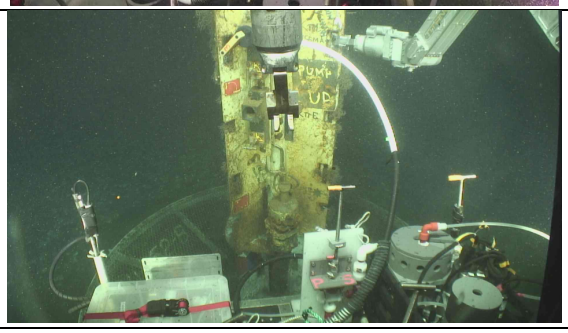
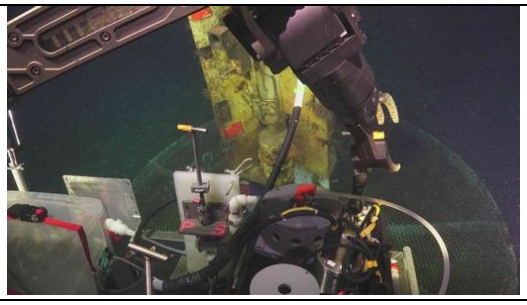
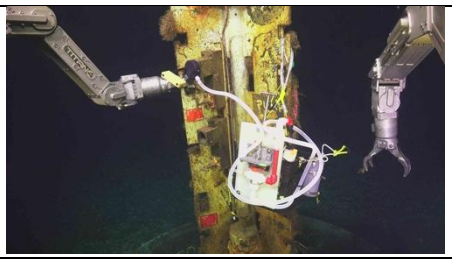
Table 8. Dive log J2-1507 (all times in GMT).

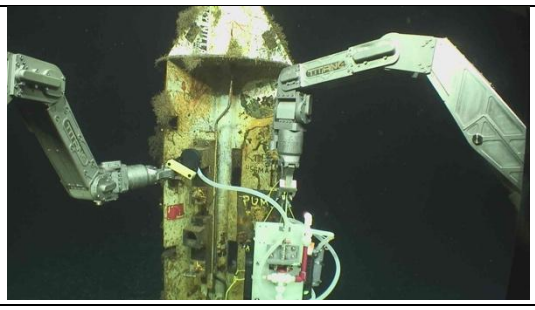
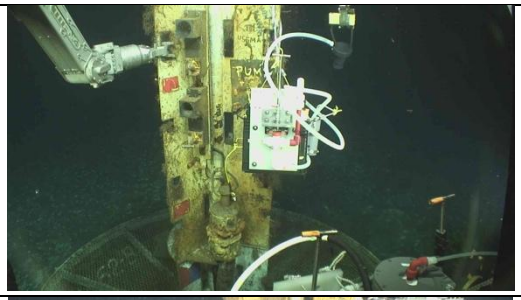
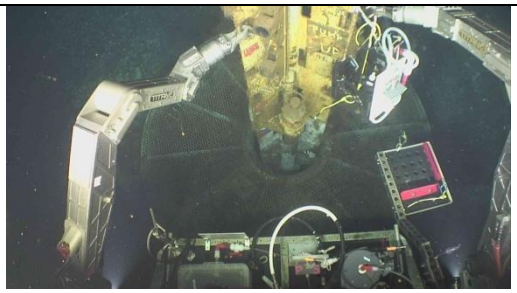
Time	Task	Representative Frame grab
Aug 1st 06:10	Deploy Jason to U1362B, track to bottom	
8:09	Arrive at bottom	
8:19	Arrive at CORK U1362B	
8:22	360˚ video of CORK U1362B	
8:35	Close up of Pressure Bay	
8:29	Close up of Chemistry Bay	
8:33	Close up of MBIO Bay	
8:36	Octopus in pressure bay	
8:42	Turning pressure values 180˚ CCW	
9:17	Turning pressure values 180˚ CW, values back to formation.	
9:57	Loss of oil observed	
10:11	Beginning recovery	
12:00	Jason on deck	

Table 9. Dive log J2-1508 (all times in GMT)

Time	Task	Representative Framegrab
Aug 2 15:17	Jason in the water	
17:05	Arrive at U1362B	
17:16	Connecting ODI for data download.	
17:26	Pressure data is not responding to shipboard software, unplugging and retrying	
17:58	Pressure download needs trouble shooting. Effort aborted.	
18:05	Repositioning to MBIO bay	

18:10	Cleaning MBIO bay with toilet brush	
18:14	Connect MPS Jannasch connector	
18:22	Turn top MBIO bay value	
18:25	Powering on MPS	
18:30	Flush pump	
18:35	Shimming water observed from MPS	
19:26	Turning LVBS handle from P to S	

19:28	LVBS pumping	
20:19	Turning handle from S to P	
20:20	Powering McClane	
20:21	Turning other handle 108 degrees	
22:55	Set filtration unit to purge	
23:16	MVBS not working well, abandoning effort	
23:17	Powering down McClane	
23:18	Turning off power to MBS	
23:38	Hang viral filtration unit.	
23:53	Filtration unit connected and shimmering fluids observed	

23:59	Turning t-handle CW 180˚	
Aug 3 0:01	Shimmering water observed	
0:07	T-handle back to purge, no sign of shimmering fluids.	
00:25	Disconnect viral filtration unit	
00:29	Stow viral filtration unit	
00:45	Closing the CORK value	
00:48	starting Jason Recovery	

ROV Jason Dive J2-1509

J2-1509 Dive Summary

J2-1509 operations began with an elevator launch with 5 pieces of 3rd party equipment. The ROV was launched shortly after to the Hole U1362B CORK. With the ROV configured to supply power to the CORK, pressure data was successfully downloaded. Then *in situ* passive filter unit #3 was deployed in the MBIO bay. A batch of sediment push cores and the Niskin samples were

taken near the U1362B site. Next the ROV transited to Hole U1362A. There pressure was successfully downloaded. Then, several hours were spent filtering water *in situ* with the MPS at the MBIO shallow and deep horizons. LVBS and MVBS bags were filled with the MPS. *In situ* passive filtration unit #2 was deployed in the MBIO deep horizon. A broken T-handle was observed on *in situ* passive filter unit #1, which was targeted for the shallow horizon. The unit was returned to the surface for repairs.

Table 10. ROV Jason Vehicle Configuration for J2-1509 Deployment

Location	**Instruments, Sampling Tools**	**In-water weight (lb)**
Basket	Mobile Pumping System (MPS) – 3rd party Large Volume Bag Sampler (LVBS) – 3rd party Toilet Brush – 3rd party; Knife; High-temperature probe	33 6 n.a.
Starboard Swing Arm	4-gallon milkcrate with 6x pushcores	
Port Swing Arm	6-gallon milkcrate with ODI connector cable – 3rd party	3
Aft	Medium Volume Bag Sampler (MVBS) + McLane pump – 3rd party	34
Niskins	2x 5L	n.a.

Table 11. Third Party equipment in-water weights for J2-1509 deployment.

Item	**Deployment**	**Recovery**
“long” green OsmoSampler	35	0
“short” blue OsmoSampler	20	0
Passive filtration unit	16	16
Passive filtration unit	16	16
Passive filtration unit	16	16

Figure 4. Third party equipment on Jason for dive J2-1509: The starboard swing arm (left), front porch (middle) and aft (right).

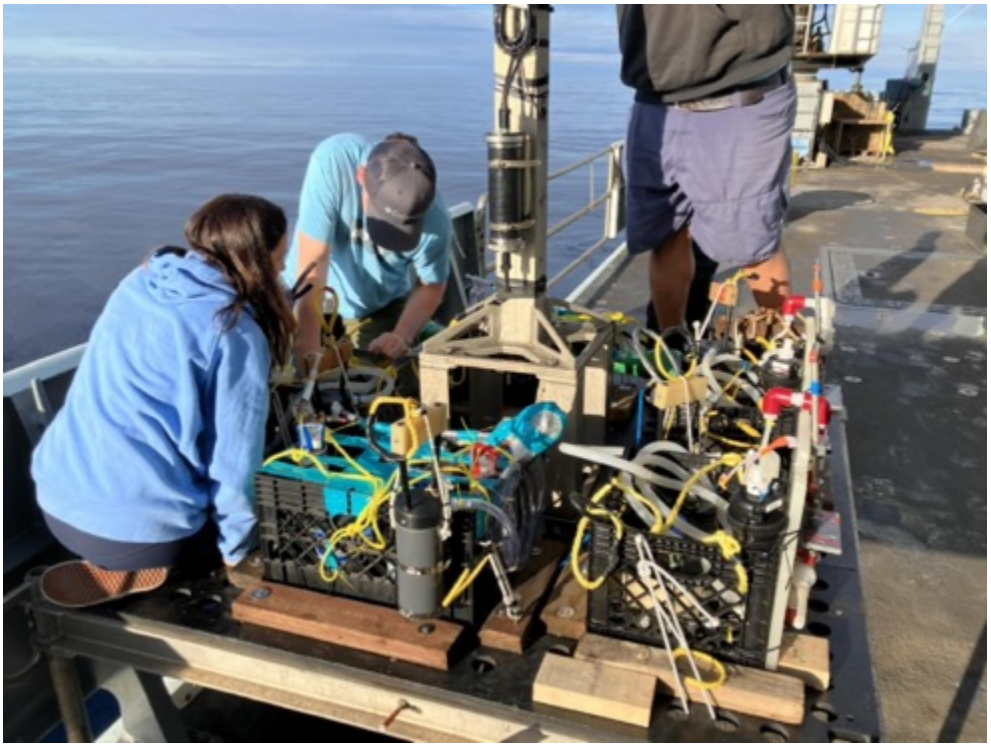
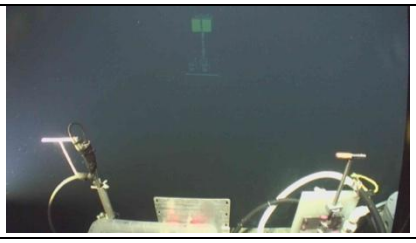
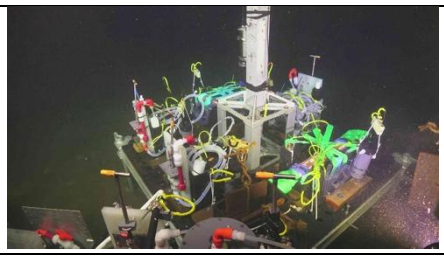
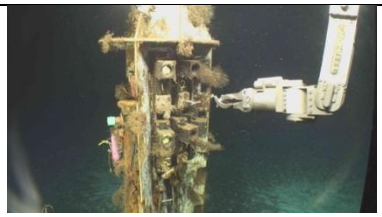
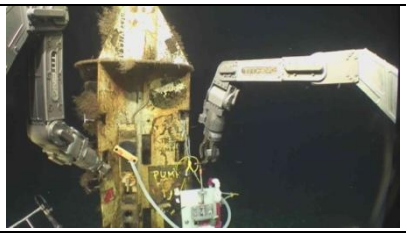
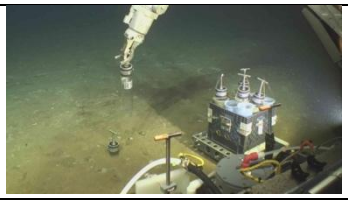


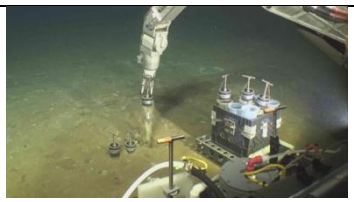
Figure 5. Elevator configuration for dive J2-1509.

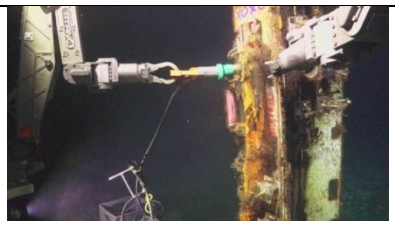
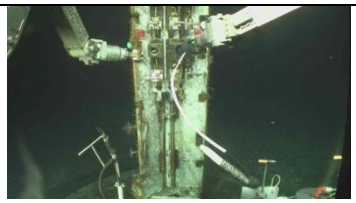
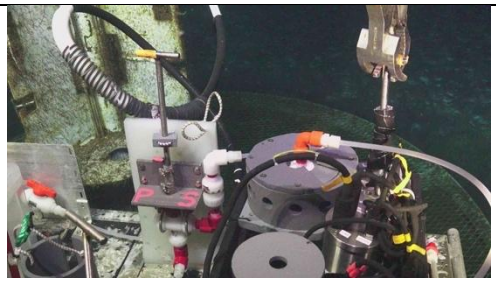
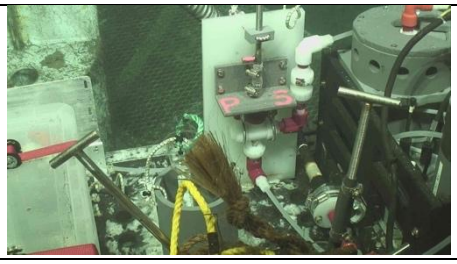
Table 12. Dive log J2-1509 (all times in GMT)

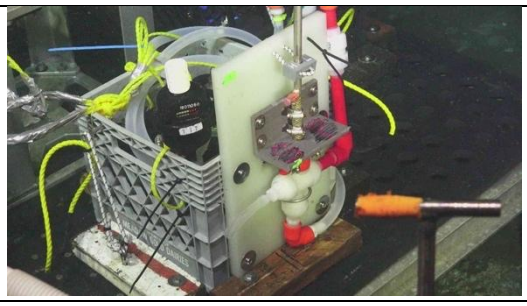
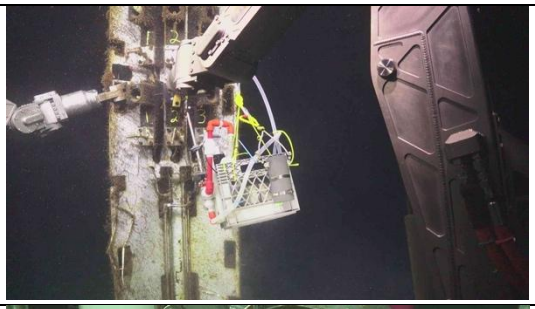
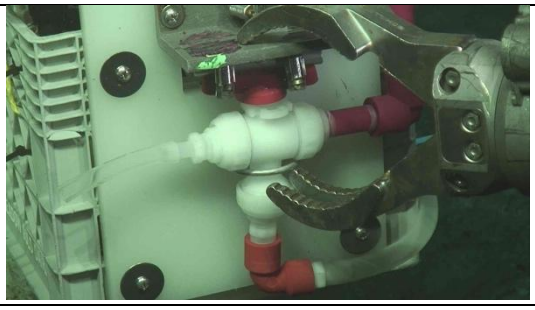
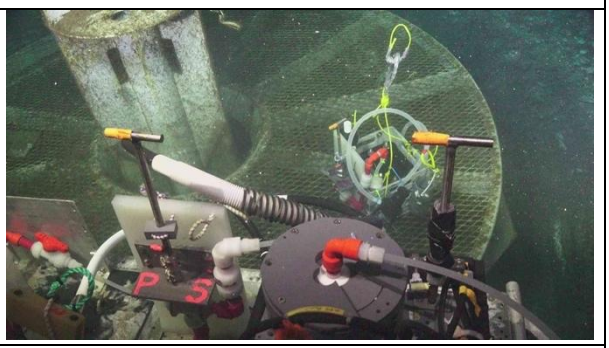
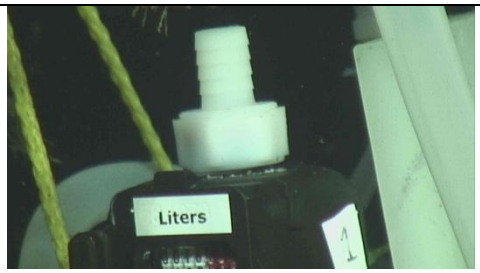
Time	Task	Representative Framegrab
Aug 3rd 17:33	Jason off Deck	
17:20	Elevator in sight	
17:21	Jason on bottom	

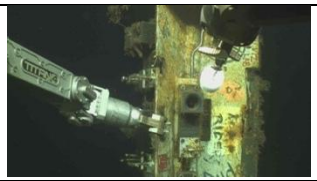
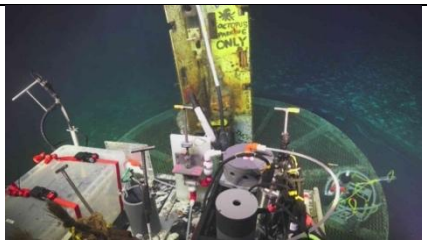
17:25	Dropped weighs and moved elevator to U1362B	
17:54	Picked up viral passive filter unit 3	
18:06	Hang viral in situ filtration unit 3 in MBIO bay	
18:15	Open top MBIO valve	
18:47	Connect virus in situ filtration unit Jannasch connector to U1362B MBIO Bay, top port	
18:38	In situ filter purged for 10 mins.	
18:48	Filtered handled turned from purged to filter. Shimmering flow observed.	
19:07	Flow meter observed. Flow rate is very slow. Meter starts at 16,488.	
19:24	Connection made to ODI port in pressure bay.	
19:25	ODI Relay Opening	
19:29	Power on- communication made	
19:32	Downloading	
20:15	Pressure Download Complete	
20:16	Powering down ODI & Holstered.	

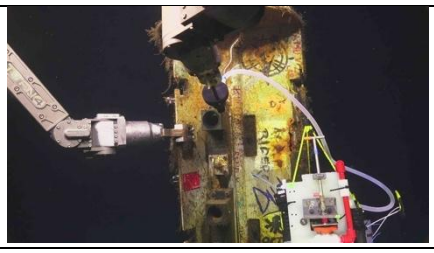
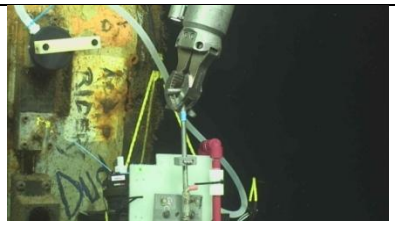
20:24	Check flowmeter of viral in situ filter unit. Still at 0016488 L	
20:30	Moving viral in situ filter unit to purge	
20:34	Shark eggs found	
20:41	Moved to Osmo Bay, opened value 2. Slow flow visualized.	
21:03	Measured temperature, after 5 mins, reading is 7.5 ˚C	
21:15	Measured temperature in MBIO Bay, 9.1˚C	
21:23	Turning in situ filter valve to sample	
21:43	Closed valve at Chem Bay 2	
21:52	Fire 1 L Niskan	
21:54	Landing for sediment landing near U1362B	
21:56	Pushed in PC4 (first recorded as PC1, in error)	
21:57	Pushed in PC5	

21:59	Pushed in PC6, retrieved and stored in forward starboard hole.	
22:06	Pushed in PC1	
22:07	Pushed in PC2	
22:07	Pushed in PC3	
22:08	Retrieved PC3	
22:09	Retrieved PC2	
22:10	Retrieved PC1	
22:12	Moving elevator to U1362A	
22:58	360 video of U1362A	
23:06	Turn bottom of three pressure values ccw180˚to open position	
23:11	Top of three values appears to be turned to seafloor already.	
23:08	Turning middle valve to seafloor, 180˚ ccw	
23:42	Turning bottom of three valves cw to formation	
23:43	Turning middle of three valves cw to formation	
8/4 00:05	Cleaning ODI port with brush	

00:13	Connecting ODI connector	
00:20	Start pressure download	
01:21	End pressure download	
01:21	Disconnected ODI connector.	
1:36	U1362A MBIO Bay, cleaning lower port 2 with toilet brush for MPS Jannash connector.	
01:41	U1362A MBIO Bay, Open lower port #2	
02:14	U1362A MBIO Bay, Connecting MPS to lower port #2	
02:20	Supplying power to the optode	
02:29	MSP power on.	
02:30	Shimmering fluids from purge valve	
02:33	Begin flushing MPS	
03:30	Provide power to McLane	
03:31	Turning 180˚ degrees on T handle to direct water to McLane	
03:54	Start filtering in situ filters. 3.0 uM optode oxygen reading.	
08:40	Turning McLane valve from M to L	
08:45	MPS power down	
08:48	Disconnect MPS Jannasch connector	
08:56	Jason returns to elevator	

08:59	Jason Picks up in situ passive filter unit #1	
09:17	Hang in situ passive filter in MBIO bay	
09:29	Connect in situ filtration unit with Jannasch connector to shallow line via port 2.	
09:43	When T-handle is turned, it appears that the read top of the stop cock has popped off the plumbing of the filter's handle. Problem cannot be solved at the seafloor. Decide to bring unit back.	
10:00	Disconnect virus in situ filtration unit	
10:04	Viral filtration unit left on CORK platform.	
10:05	Transit to U1362B to retrieve other in situ filter unit (#3) that we expect is not working given a lack of flow from the U1362B CORK	
10:55	Check the filtration unit at U1362B. It appears that a few hundred liters have been pumped. Decide to leave unit #3 there and return to U1362A.	

11:38	Arrived back to U1362A	
11:46	Closed value 2 in Chem Bay	
11:58	Cleaned top valve with brush.	
12:03	Connect Jannasch connector to port top port in MBIO bay.	
12:06	MSP optode and pump powered on	
12:13	Purging MPS	
12:45	Turning MPS T-handle 180˚to sample	
12:49	Start LVBS filling	
13:14	End filling LVBS	
13:21	Mclane filter started two mins ago	
18:07	End filtering in situ filter.	
18:06	Start filling MPS bags.	
19:36	Start powering down McLane	
19:36	Turning MPS handle 180 cw to purge.	
19:37	Powering down optode	
19:38	Disconnecting Jannasch connector	
19:42	Transiting to elevator to pick up passive filter unit #2.	
19:47	Picking up the in situ passive filter unit.	
19:54	Back to CORK 1362A	
19:56	Attaching passive in situ filter unit #2 to Deep horizon	

19:59	Connect virus in situ filtration unit Jannasch connector to MBIO Bay, deep horizon, top port.	
20:01	Shimmering water observed from purge line.	
20:04	Turn filter unit T-handle 180 degrees ccw	
20:16	Picking up the broken in situ passive filter unit (#1) from CORK platform to stow on ROV	
20:20	Secured filtration unit #1 to front porch.	
20:24	Off Bottom	

ROV Jason Dive J2-1510

J2-1510 Dive Summary

J2-1510 was launched at 04:00, local time to the Hole U1362A CORK. First an OsmoSampler was retrieved from the seafloor and deployed at U1362A, Osmo bay, deep horizon. The second OsmoSampler was carried to and deployed U1362B, Osmo Bay. The ROV then transited to and collected push cores in a transect at U1301A. A Niskin was also fired near U1301A. The ROV transited back to U1362A to collect crustal fluid. Using the MPS MBIO deep horizon fluid was collected into the LVBS and 5 MVBS bags. The final in situ passive filter was deployed at the U1362A shallow horizon. Finally, the ROV transited back to U1362B to fill one MBVS bag with fluid from U1362B MBIO bay .

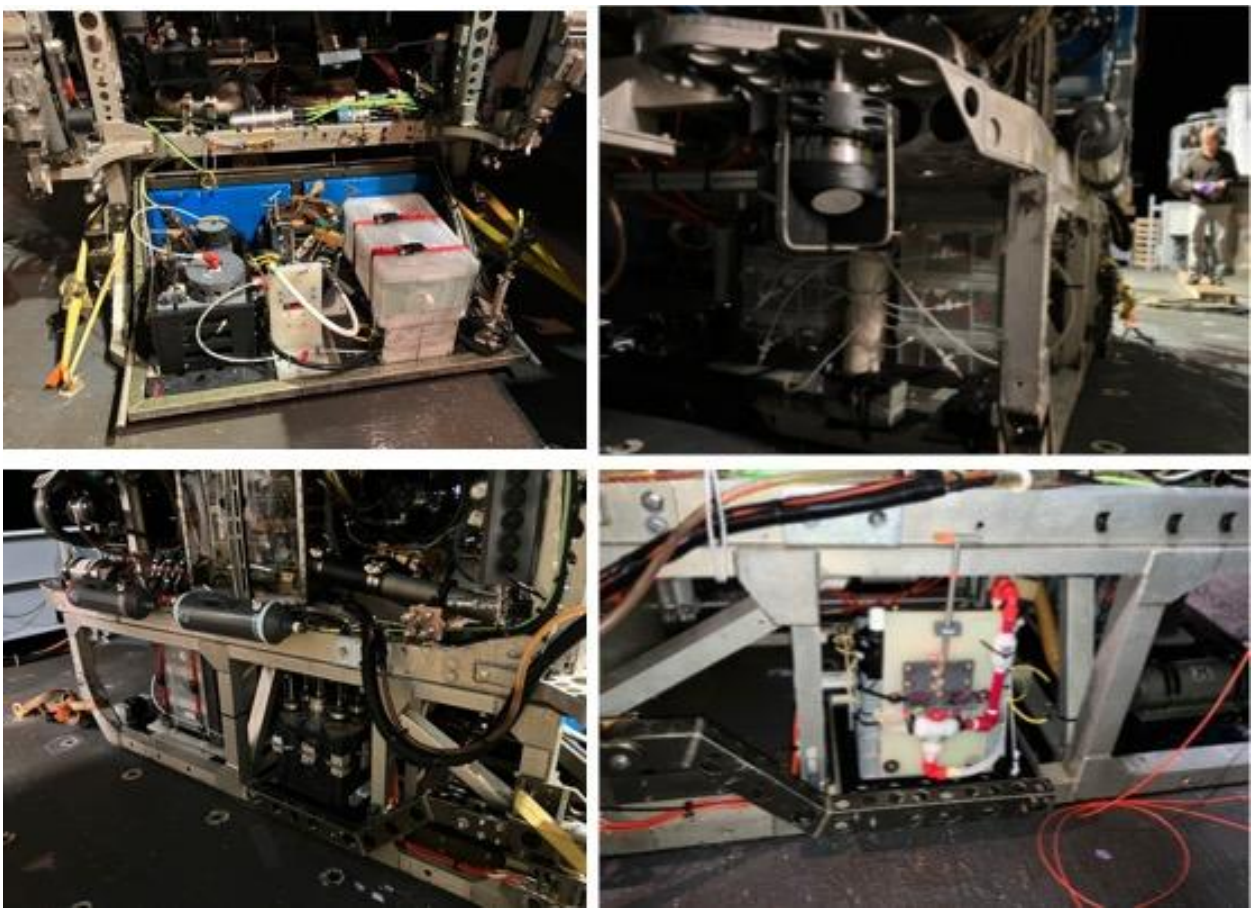
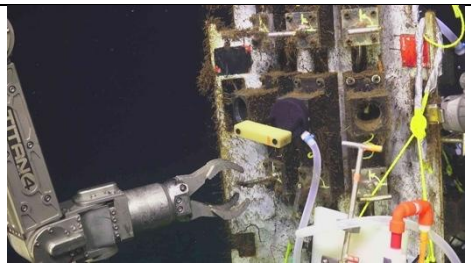


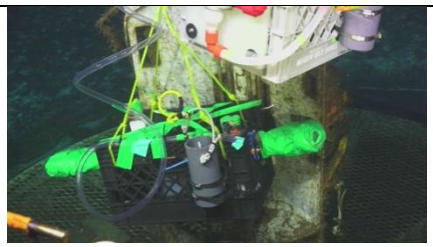
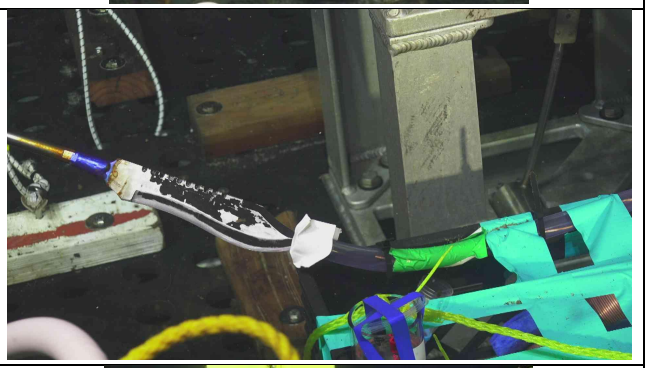
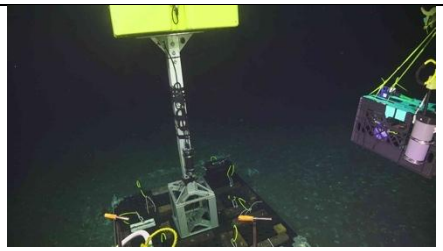
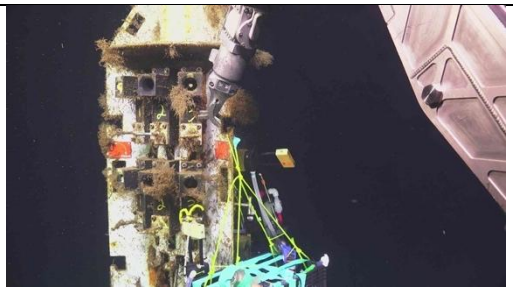
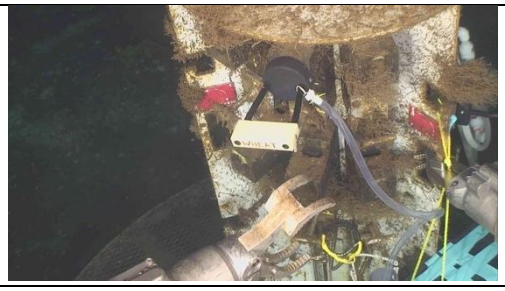
Figure 6. Third party equipment configurations on the ROV Jason: front porch (top left), aft (top right), starboard swingarm (bottom left), port swingarm (bottom right).

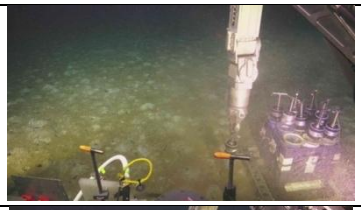
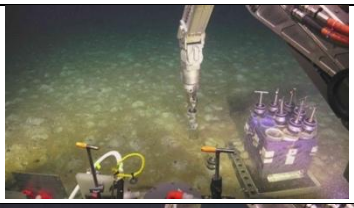
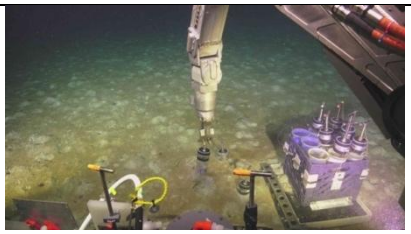
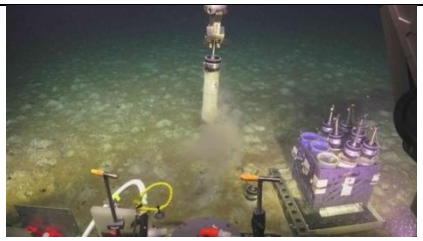
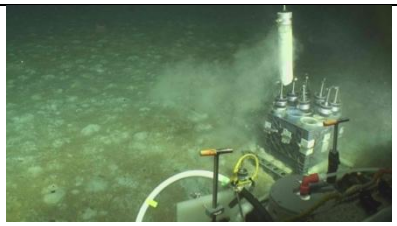
Table 13. ROV Jason Vehicle Configuration for J2-1510 Deployment

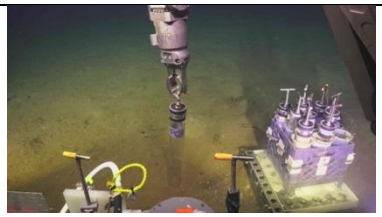
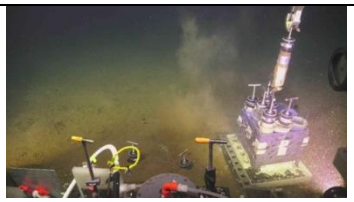
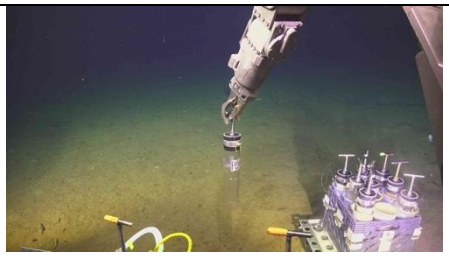
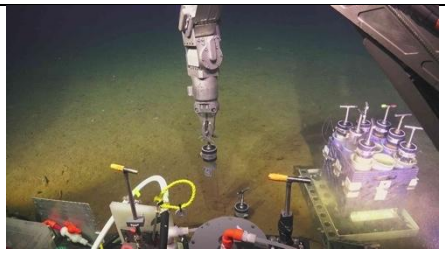
Location	**Instruments, Sampling Tools**	**In-water weight (lb)**
Basket	Mobile Pumping System (MPS) – 3rd party Large Volume Bag Sampler (LVBS) – 3rd party Toilet Brush – 3rd party; Knife; High-temperature probe	33 6 n.a.
Starboard Swing Arm	4-gallon milkcrate with 6x pushcores	9
Port Swing Arm	Passive filtration unit– 3rd party	16
Aft	Medium Volume Bag Sampler (MVBS) + McLane pump – 3rd party	34
Niskins	2x 5L	n.a.

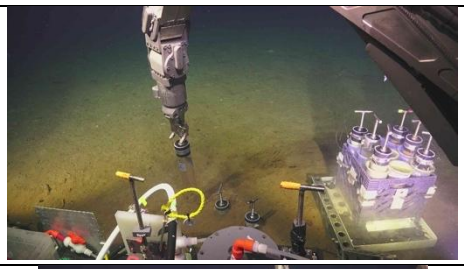
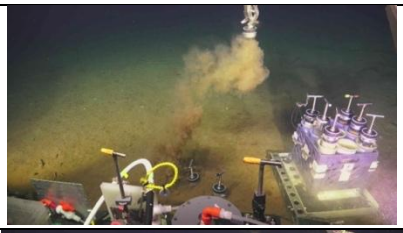
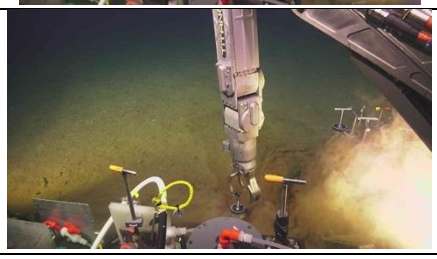
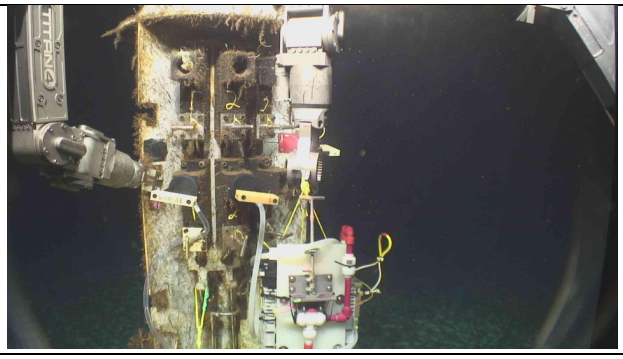
Table 14. Dive log J2-1510 (all times in GMT)

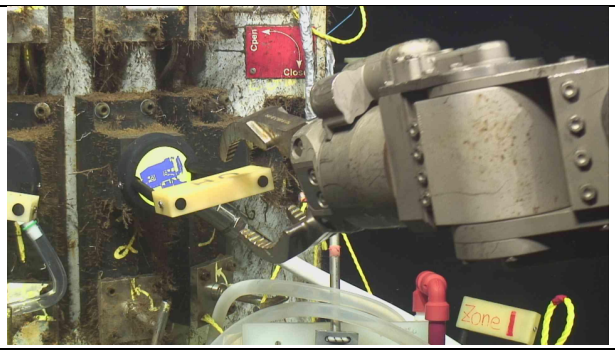
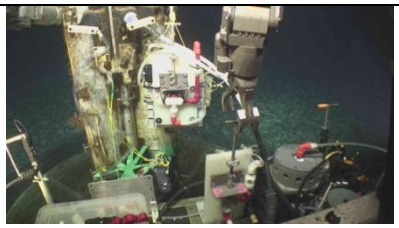
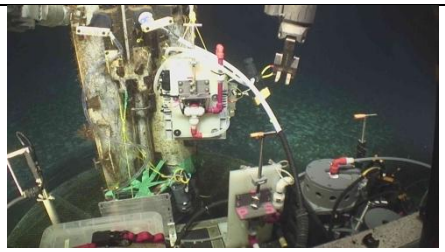
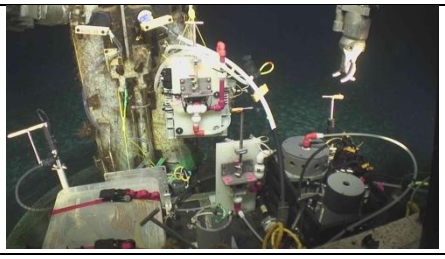
Time	Task	Representative Framegrab
Aug 5th 11:03	Jason off Deck	
11:02	LVBS box is moving, returned to deck	
13:13	Arrived at bottom close to U1362A	
13:24	Checking flow meter on passive filter unit	
14:02	Successfully attached deployed 3rd passive filter unit on Osmo bay lower valve 2 shallow.	
14:07	Confirmed flow through purge line of filter unit 3	
14:21	Successful switch to sample mode. Confirmed via simmer of water	
14:53	At elevator. Green Osmo sampler has tape on end of it that should have been removed. Using night to stab hole through tape.	
14:57	Moving the green Osmo sampler to U1362A	
15:03	Arrived at U1362A Osmo bay	
15:13	Hooking up the milk crate	
15:19	Cleaning port with toilet brush	
15:24	Open lower port 2 in Osmo bay	
15:34	U1362A Osmo Bay Lower 1 Value now open, connecting Jannisch connector	

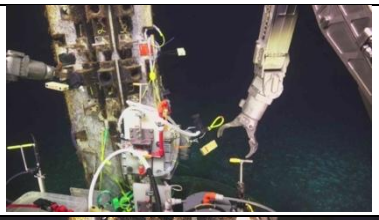
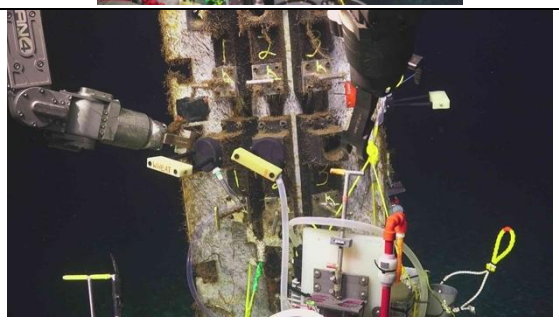
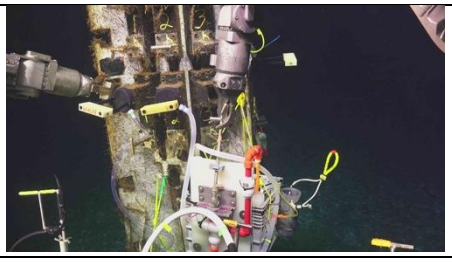
15:34	Shimmering confirmed	
15:46	At elevator. Cutting tape off second OsmoSampler.	
15:50	Carried the blue OsmoSampler to U1362B	
16:14	Arrived at U1362B	
16:17	Hung OsmoSampler on CORK	
16:20	Connecting Jannisch connector to value top 2 on Osmo Bay. (Previous cleaned during a previous dive).	
16:29	Second attempt to connect Jannisch connector	
16:30	Shimming confirmed out OsmoSampler (video taken)	

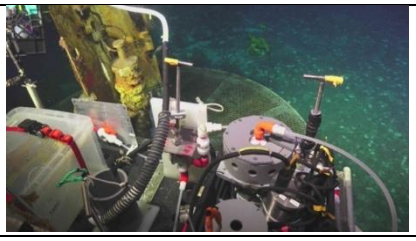
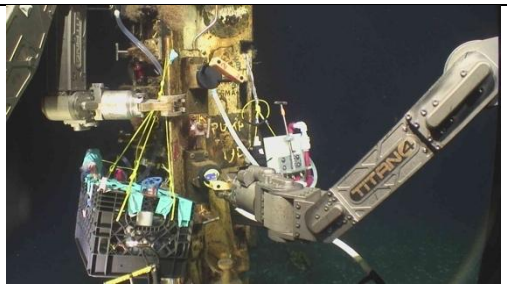
16:33	U1362B Passive filter unit #1 at 18,05X L	
	Transit to 1301A	
17:20	Approaching U1301A. Crustal Fluid flow around the base of 1301A.	
17:40	Insert U1301A Transect A pushcore #1	
17:44	Insert U1301A Transect A pushcore #2	
17:47	Insert U1301A Transect A pushcore #3	
17:49	Pullout and Stow U1301A Transect A pushcore #3	
17:51	Pullout and Stow U1301A Transect A pushcore #2	

17:52	Pullout and Stow U1301A Transect A pushcore #1	
17:56	Transecting from point A to point B.	
18:11	Landing at point B	
18:17	Insert U1301A Transect B pushcore #7	
18:26	Insert U1301A Transect B pushcore #8	
18:34	Insert and stow U1301A Transect B pushcore #9	
18:34	Stow U1301A Transect B pushcore #8	
18:36	stow U1301A Transect B pushcore #7	
18:38	Transiting to transect Point C	
18:43	Arriving at point C	
18:43	Insert U1301A Transect C pushcore #4	
18:50	Insert U1301A Transect C pushcore #5	

18:51	Insert and stow U1301C Transect B pushcore #6	
18:51	Stow U1301A Transect B pushcore #6	
18:53	Stow U1301A Transect B pushcore #5	
18:53	Stow U1301A Transect B pushcore #4	
18:56	Heading back to CORK to do Niskin Bottles	
19:33	Flow is observed around bottom of CORK. Temp 2.65 C	
19:47	1 L Niskin fired	
19:49	Pulling rope for other Niskins	
19:52	Transiting to 1362A	
21:10	Arrived at U1362A	
21:15	Disconnect passive filter from shallow horizon	
21:16:57	Turn passive filter handle 180˚ to purge	

21:19	Hans connector deployed into lower port 2 of Osmo Bay	
21:25	Optode Powering	
21:28	MPS power	
21:29	We have shimmering water.	
22:19	Still purging. Oxygen levels are still 2.4 and temperature 12.6. This is odd, given that the lines were already purged the day before. There are concerns of seawater infusion. Values still falling but decide to sample LVBS anyway to move us along. Will monitor samples for MVBS bag and will decide which will be best for culturing.	
22:38	T Handle to S. Temp and oxygen values continue to fall.	
23:20	LVBS bag appears full, slowing down pump. Readings from the MPS: Oxygen 1.5% temp 12.3˚C	
23:22	MVBS has been turned 180 cm	
23:22	Powering on MClane	
23:24	Far right t-handle after turning 180 ccw	

23:27	1000 rpm filtering 0.7 L/min into middle bag. Oxygen 2.7µM, temp 7.6	
8/6 00:59	Finished pumping 5 bags of MVBS	
01:00	Right hand T-handle on porch turned 180˚C	
01:00	Powered down MPS	
01:01	Powered down optode	
01:02	Handle turned ccw 90˚ and Jannasch connector removed from lower valve #2	
1:05	Passive in sit filter unit connected to lower value #2. Shimmer fluid observed.	
1:07	T-handle on passive in situ filtration unit turned to sampling position	
1:17	Ronnie took a picture of the flow meter of the device	
01:18	Transit to U1326B	
01:55	Arrived at U1326B	
02:28	U1362B MBIO Bay top valve turned cw 90˚ to off position.	
02:37	U1362B MBIO Bay lower valve turned ccw 90˚to open position. Shimmering fluid confirmed.	
02:40	Cleaning lower valve with toilet brush.	

02:49	MPS Jannasch connector successful connected to MBIO Bay lower valve.	
02:49	Power on the optode	
02:51	Power up MPS. Oxygen 1.5 uM temp 11.5	
02:54	Shimmering water from purge port.	
03:09	Power McLane, Oxygen 0.6 µM, 15.9˚C.	
03:55	T handle was turned from LVBS purge to McLane manifold.	
03:23	MVBS bag filling	
03:26	Power down McLane	
0327	Turn MVBS handle 180˚ CW to MPS purge.	
03:28	Power down McLane and Optode.	
03:30	Closing U1362B MBIO bay bottom valve closed.	
03:33	Opening U1362B Upper MBIO bay 90˚CW to open.	
03:37	Disconnect MPS Jannasch connector.	
	Time looking for animals	
07:17	Jason on Deck	

ROV Jason Dive J2-1511

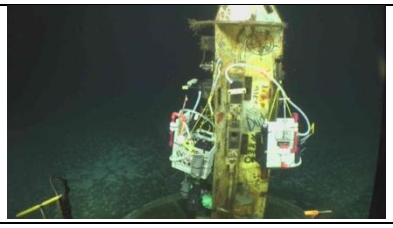
J2-1511 Dive Summary

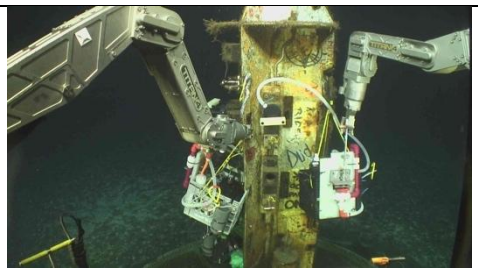
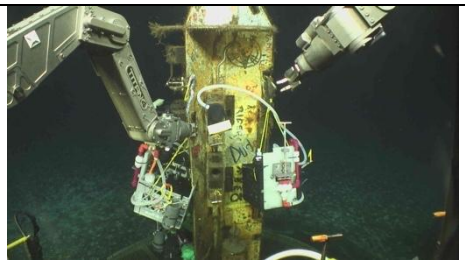
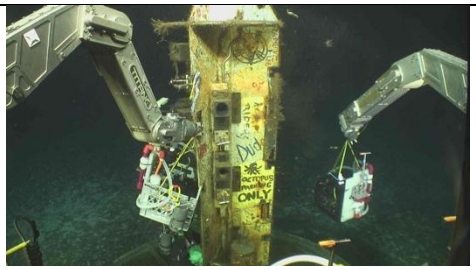
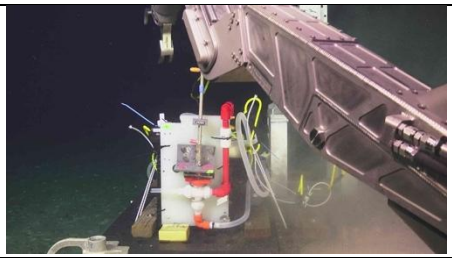
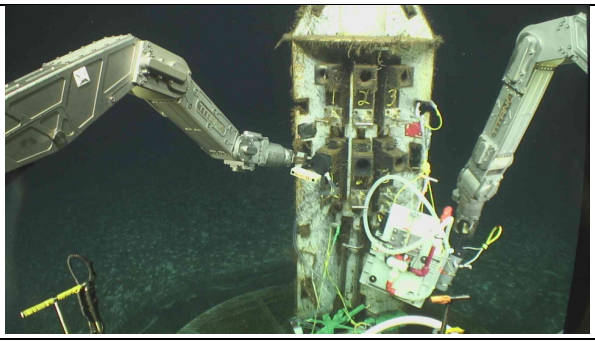
J2-1511 was the final dive of TN-421. During the launch it became apparent that there was a problem with the ROV winch. After some testing, the decision was made to prioritize retrieving the *in situ* passive filter units. The ROV arrived at Hole U1362A first removed both passive *in situ* viral filtration units and stowed them on the elevator. The ROV then carried the elevator to U1362 to retrieve the final *in situ* passive filter unit. The ROV manually released the elevator weights. Both the elevator and ROV were recovered without a problem.

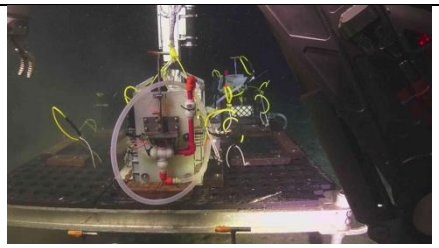
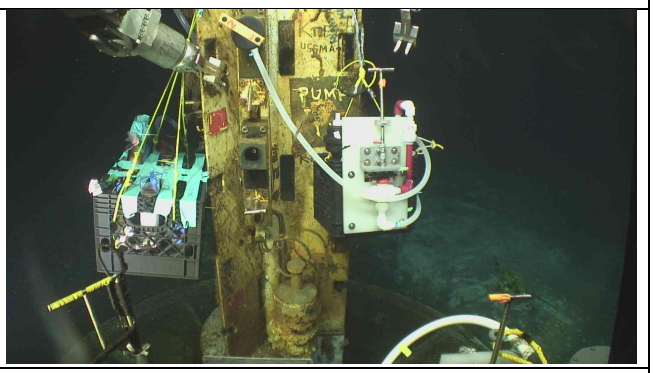
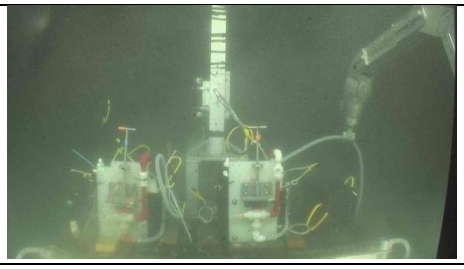
Table 15. ROV Jason Vehicle Configuration for J2-1511 Deployment

Location	Instruments, Sampling Tools	In-water weight (lb)
Basket	Mobile Pumping System (MPS) – 3rd party Large Volume Bag Sampler (LVBS) – 3rd party Toilet Brush – 3rd party; Knife; High-temperature probe	33 6 n.a.
Starboard Swing Arm		0
Port Swing Arm		0
Aft		0
Niskins	2x 5L	n.a.

Table 16. Dive log J2-1511 (all times in GMT)

Time	Task	Representative Framegrab
Aug 6th 3:46		
19:12	Jason off deck	
19:14	Jason in the water	
20:59	An unusual sound detected in the winch. Engineering team trouble shoots.	
22:17	Problem not resolved. Given that we are 300 m to the bottom, Jason group plans to retrieve the passive filter experiments.	
22:36	On bottom	
22:40	Arrived at U1362A	

22:50	MBIO Bay, Deep, in situ filter #2, T-handle turned cw 180˚ to purge, shimming water observed in purge.	
22:51	MBIO Bay, top deep valve turned cw 90˚ to closed.	
22:53	Filter Jannasch connector disconnected.	
22:55	Not easy to stow Jannasch connector, unit moved from CORK and dropped.	
23:11	Carrying the passive filter unit to the elevator.	
23:19	Passive filter Unit #2 secured on the elevator.	
23:23	Arrived back at U1362A to collect second viral unit from Osmo Bay, lower #2 (Shallow).	
23:25	Handle on passive unit #3 turned to purge position.	
23:26	Handle on Cork U1362A Osmo Bay #2, lower, closed.	

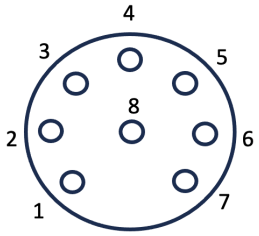
23:26	Disconnected Jannasch connector for passive filter unit #1.	
23:29	Transiting to elevator.	
23:31	Arrived at elevator	
23:36	Stowed passive filter unit #1	
23:40	Begin to transit to U1362B with elevator.	
15:46	Finished moving elevator.	
Aug 7th 00:24	U1362B MBIO bay. Change T-handle of passive filter unit to purge.	
00:26	Disconnect virus in situ filtration unit Jannasch connector (top valve)	
00:28	Pick up viral filtration unit, return to elevator.	
00:37	Stow passive in situ filter device on the elevator.	
00:40	Preparing to release the elevator to surface	
00:46	Sent burn release, but didn't get a response. Waiting 10-15 mins.	
00:50	Send release command many times without response.	
01:10	Removed dive weights. Elevator released from seafloor.	
01:11	Off Bottom.	

CORK downloads and hydrogeology summary

Summary by Rachel Lauer and Haidar Putra

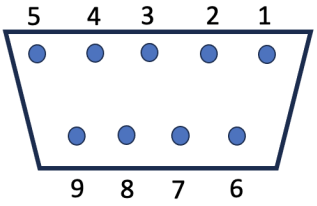
A secondary objective of cruise TN-421 was to download pressure and temperature data from the CORK observatories installed at holes U1362A/B and share the downloaded data with collaborators. Prior to each download, a hydrostatic check was performed where both seafloor and formation valves are opened, then closed followed by a 30-minute delay. The first download was attempted following a hydrostatic check on dive J2-1507. After several attempts to wake up the logger, it was determined that a download would require supplying pressure to the logger, as it was not able to transmit under its own power. As a result, the download was rescheduled until after the Jason crew was able to drain the Junction box and rewire the communications to support providing power to the loggers. Both CORKs were installed during IODP Expedition 327 in 2010, so it was anticipated that the loggers in both U1362A and 1362B would require external power for a successful download. A description of the pin configuration used to successfully supply power and communications from the surface to the seafloor is included below (Figure 7) .

Seacon Female AWM-8X-FS



Pin	Wire	Relay	J-Box
1	White/Up +	5	555
2	Black/Up -	4	554
3	Green/unused	X	X
4	Red/12V +	16	622
5	Orange/Ground	15	621
6	Blue/Down +	3	552
7	Brown/Down -	2	551
8	Shield/Gnd	1	550

DB9 Female



Focal ODI RS422 Adaptor

Ethernet	DB9
(2) Orange	(2) Rx +
(3) Black	(5) GND
(4) Red	(1) Rx -
(5) Green	(3) Tx +
(6) Yellow	(4) Tx -

Ethernet Port

Figure 7. Wiring configuration used to supply power from Jason van to seafloor for downloads.

After reconfiguring the junction box to supply power, data from both CORKs were successfully downloaded during dive J2-1509. Following each download, the logger memory was cleared, and loggers were reconfigured to sample at a 10-minute interval. Inspection of the converted data indicated that both loggers had stopped logging since the last download in 2019, as indicated in Table 17. Additional information regarding the downloads, and when valves were turned is included in the dive logs above.

Table 17. Timing, duration, and size of downloaded data from 1362A/B

CORK	Dive	Date	Data Start (UTC)	Data End (UTC)	Blocks downloaded	filename
1362A	J2-1509	03-Aug-23	26-May-19 8:30 PM	22-Apr-22 2:00 PM	63490	23p1362a_1.raw
1362B	J2-1510	03-Aug-23	18-May-19 8:19 AM	28-Jun-20 11:46 AM	33792	23p1362b_1.raw

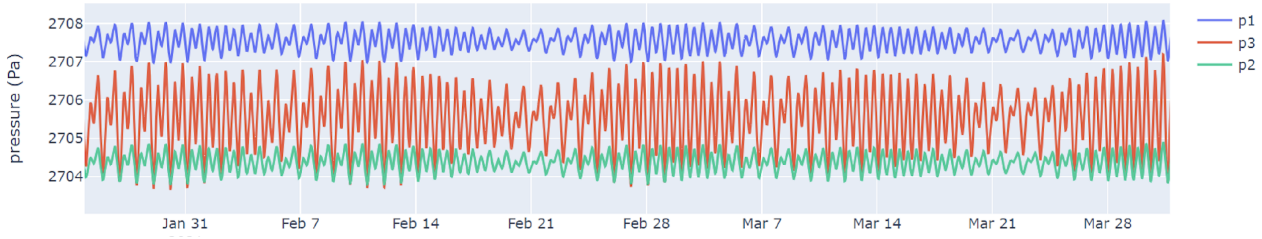


Figure 8. Snapshot of converted pressure data downloaded from Site U1362A

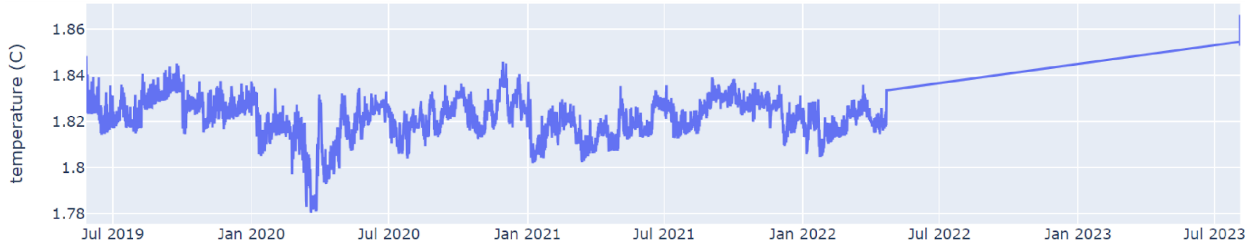


Figure 9. Snapshot of converted temperature data downloaded from Site U1362A

Crustal fluid and gas chemistry summary

Summary by Stephanie Carr, Huei-Ting (Tina) Lin and Serhat Sevgen

The ridge flank basaltic crustal environment is an energy and nutrient scarce environment with dissolved hydrogen concentrations at the low micro to sub-micromolar level (0.03–1.8 µmol/Kg) and phosphate concentrations below 0.1 µM. Dissolved methane has been measured at between 1 and 36 µmol/Kg range and with isotopic compositions suggesting abiotic and biotic origin[1] (Fig. 1). The abiotic origin is supported by observed mantle degassing of helium-3 that likely emit along with abiogenic methane from the interior early. The presence of up to 18 mM sulfate might have inhibit the growth and activity of methanogens in this environment, making the biogenic source of methane in this environment extremely interesting. Geochemical objectives of this cruise continue our longitudinal geochemical study of the crustal aquifer (concentrations of major ions and nutrients) and to deeper explore methane and phosphorus cycling in this environment through the use of isotopic measurements ($\boldsymbol{\delta}^{13}$C-CH_4, $\boldsymbol{\delta}$D-CH_4, D_2O, CH_4-clump isotopes[2], $\boldsymbol{\delta}^{18}$O-PO_4).

General crustal fluid geochemistry

To provide a comprehensive view of the methane biogeochemistry in the basaltic environment, we collected samples for the dissolved methane concentration, bulk stable carbon and deuterium isotope of methane, deuterium isotope of water, for methane clumped isotope measurements (Table 18). Small amounts of fluids were also collected for peripheral geochemistry. All crustal fluid samples were collected as quickly as possible after ROV recovery under N_2 atmosphere (Figure10). DOC, inorganic nutrients, H_2S, and major ions will be analyzed at the National Taiwan University using a TOC analyzer, photometric method, or an ion chromatography.

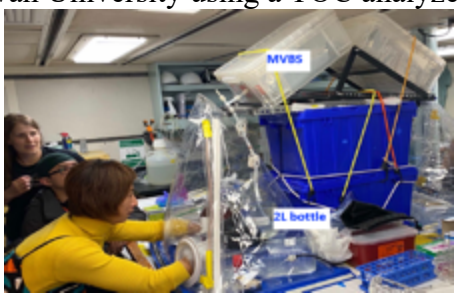


Figure 10. Gravity filled 2L bottle from the medium volume bag sampler (MVBS) inside a N_2

Figure 11 Shipboard GC analysis of extracted CH_4 concentration in a serum bottle.

[1] Lin, H.-T., Hsieh, C.-C., Cowen, J.P., and Rappé, M.S., 2015. Data report: dissolved and particulate organic carbon in the deep sediments of IODP Site U1363 near Grizzly Bare seamount. In Fisher, A.T., Tsuji, T., Petronotis, K., and the Expedition 327 Scientists, Proc. IODP, 327: Tokyo (Integrated Ocean Drilling Program Management International, Inc.).

[2] Young ED. **2019**. A two-dimensional perspective on CH_4 isotope clumping: distinguishing process from source. In *Deep Carbon: Past to Present*, ed. BN Orcutt, I Daniel, R Dasgupta, pp. 388–414. Cambridge, UK: Cambridge Univ. Press

Onboard methane concentrations

Triplicate 20 mL glass vials were gravity filled without headspace. A headspace of nitrogen was displaced into the vials. Samples were sonicated upside for more than 95 mins at room temperature. Concentrations were measured using an SRI 310C Gas Chromatograph equipped with FID and methanizer within two hours (Figure 11). One ml of the headspace was injected and separated on a 3" mol sieve 5A column with column temperature held at 40ºC for 2 mins, ramped to 50ºC at 2.5ºC min-1 and held at 45ºC for 2 min, then ramped to 150ºC at 10ºC min^{-1}. Methane contents were calibrated versus a standard curve made from Restek gas standards #501824 standard $PV = nRT$ equation formulations. Then headspace methane was converted to the dissolved methane concentrations. The dissolved methane concentrations of the basement fluids varied at different sampling sites and depths (Table 2). Highest dissolved methane concentrations were observed at CORK 1362A deep bio-line, consistent with our previous observations between 2011-2014. Lowest methane observed for CORK 1362A shallow stainless steel (ss) line sample.

Shore-based methane isotope measurements

Triplicates of fluid samples were gravity filled into 12 mL Exetainers© without head space and preserved with 80 µL of HCl to bring down pH <2. The samples were stored at room temperature in the dark for ^{13}C and deuterium isotopes of the bulk dissolved methane, which will be analyzed at the paid Stable Isotope Facility (SIF) at UC Davis.

Shore-based water deuterium isotope measurements

Triplicates of fluid samples were gravity filled into 12 mL Exetainers© without head space, stored at room temperature in the dark. The analysis will be done at the SIF, UC Davis.

Shore-based clumped isotope methane measurements

Basement fluid samples were gravity filled with approximately 1700 mL of crustal fluid in large 2 L bottles under a nitrogen atmosphere. Bottles were sonicated upside down at room temperature for more than 95 minutes. Headspace was transferred to pre-evacuated thick blue stoppers crimped sealed serum vials (450 mL and 220 mL). Concentrations of the methane in the serum bottles were measured by subsampling 1 mL of the gas with a gastight glass syringe injecting into the GC (method describe above). It appears that enough methane was collected for clumped isotope analysis. The headspace methane will further be purified and analyzed at UCLA.

Shore-based oxygen isotope measurements in phosphate

The fluids were collected by Large Volume Bag Samplers (LVBS) in collaboration with Drs. Rappé, Nigro and Carr and Niskin bottles (by CTD and ROV Jason) where appropriate. Samples collected include crustal fluids from U1362A (both shallow and deep), U1362B, a mixture of seawater and crustal fluid collected with the Niskin bottle fired by Jason near CORK U1301A, and background bottom seawater samples from the two CTD casts (see Table 1). Ten mL of filtered

(either 0.45, 0.2 or 0.01 μm) subsamples were prepared as replicates and diluted with 18 mΩ-cm deionized water for anion analysis upon retrieval of the samples on ROV Jason or CTD. Additionally, half of the diluted replicates was treated with 0.15 mL 37% trace metal grade HCl for cation analysis. No pre-treatment was applied to the rest of the filtered samples for phosphate analysis. All samples stored in the cold room at 4 °C. All collected samples will be analyzed by analytical geochemistry techniques in University of Calgary, Canada.

Table 18. Sample inventory for dissolved methane biogeochemical research and peripheral measurements of fluid chemistry.

Parameters to be analyzed	Volume (mL)	Vial type	Preservation	J2-1508 1362B-Bio LVBS*	J2-1509 1362A-deep Bio MVBS	J2-1509 1362A-deep Bio LVBS	J2-1510 1362A-shallow SS LVBS	J2-1510 1362B-Bio MVBS
				Number of replicates collected				
$\delta^{13}C\text{-}CH_4$	12	Exertainer	6N HCl 80μL	3	3	0	3	3
$\delta D\text{-}CH_4$	12	Exertainer	6N HCl 80μL	3	3	0	3	3
D_2O	12	Exertainer	No	3	3	0	3	3
CH_4 for GC	20	Serum vial	No	3	3	0	3	3
CH_4-clump	1700	2L glass	No	0	5	0	0	5
DOC/TN	40	Combusted glass	6N HCl 160μL	2	0	2	2	0
Nutrients	100	HDPE	6N HCl 160μL	2	0	2	2	0
Major ion	2	Centrifuge vial	No	1	1	1	1	1
H_2S	20	Serum vial	No	0	0	2	0	0
H_2S	12	Exertainer	No	0	0	0	2	0

*Vials for methane (CH_4) analyses were purged with N_2 during sampling.

Table 19. Shipboard measurements of dissolved methane concentrations in basaltic fluids.

Sample ID	Dive #	CORK	Horrizon	FDL material	CH_4 (µmol/L)
1362B_Bio_LVBS	J2-1508	1362B	-	PVDF	13.9
1362B_Bio_LVBS	J2-1508	1362B	-	PVDF	12.2
1362B_Bio_LVBS	J2-1508	1362B	-	PVDF	13.2
1362A_Bio_LVBS	J2-1509	1362A	Deep	PVDF	46.6
1362A_Bio_LVBS	J2-1509	1362A	Deep	PVDF	76.1
1362A_Bio_LVBS	J2-1509	1362A	Deep	PVDF	66.2
1362A_Bio_MVBS	J2-1509	1362A	Deep	PVDF	64.0
1362A_Bio_MVBS	J2-1509	1362A	Deep	PVDF	69.0
1362B_Bio_MVBS	J2-1510	1362B	-	PVDF	9.2
1362B_Bio_MVBS	J2-1510	1362B	-	PVDF	4.4
1362B_Bio_MVBS	J2-1510	1362B	-	PVDF	4.7
1362A_SS_LVBS	J2-1510	1362A	Shallow	SS	2.3
1362A_SS_LVBS	J2-1510	1362A	Shallow	SS	1.9
1362A_SS_LVBS	J2-1510	1362A	Shallow	SS	1.4

Table 20. Sample inventory for oxygen isotopes of phosphate

Sampling Date	Sampling Location	Dive #	Sampled by	Filtration	Sample Volume
08/01/2023	U1362A Background Seawater	U1362A_CTD01	CTD	0.45 µm	1 liter
08/02/2023	U1362B Crustal Fluid	J2-1508	LVBS in ROV Jason	0.2 µm	5 liters
08/04/2023	U1362A Deep Horizon Crustal Fluid	J2-1509	LVBS in ROV Jason	0.2 µm	1 liter
08/04/2023	U1362A Deep Horizon Crustal Fluid	J2-1509	LVBS in ROV Jason	0.01 µm	10 liters
08/06/2023	U1362A Shallow Horizon Crustal Fluid	J2-1509	LVBS in ROV Jason	0.2 µm	1 liter
08/06/2023	U1362A Shallow Horizon Crustal Fluid	J2-1509	LVBS in ROV Jason	0.01 µm	10 liters
08/06/2023	U1301A Crustal Fluid and Seawater mixture	J2-1510	Niskin in ROV Jason	0.45 µm	2 liters
08/06/2023	U1362B Background Seawater	U1362B_CTD02	CTD	0.45 µm	1 liter

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

Summary by Michael 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- and lab-based processing via active water pumping using the Mobile Pumping System (MPS), or through passive filtering via the *in situ* tangential flow viral sampler. Active pumping was 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) to collect raw crustal fluid for downstream analyses, as well as for pumping fluid through filters attached to the submersible to collect microbial biomass on the seafloor. As described elsewhere (viral *in situ* filtration unit sections), microbial and viral biomass was collected *in situ* using a passive *in situ* viral filtration sampler led by Co-PI Nigro. The passive *in situ* filtration samplers were deployed and recovered via the elevator. Raw fluids served four primary purposes: (i) inoculum for enrichment and microbial isolation experiments, (ii) filtration for microbial nucleic acids, (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, (iv) material for assessment of methane in crustal fluids. In addition to these primary uses, crustal fluids were also distributed to multiple collaborators.

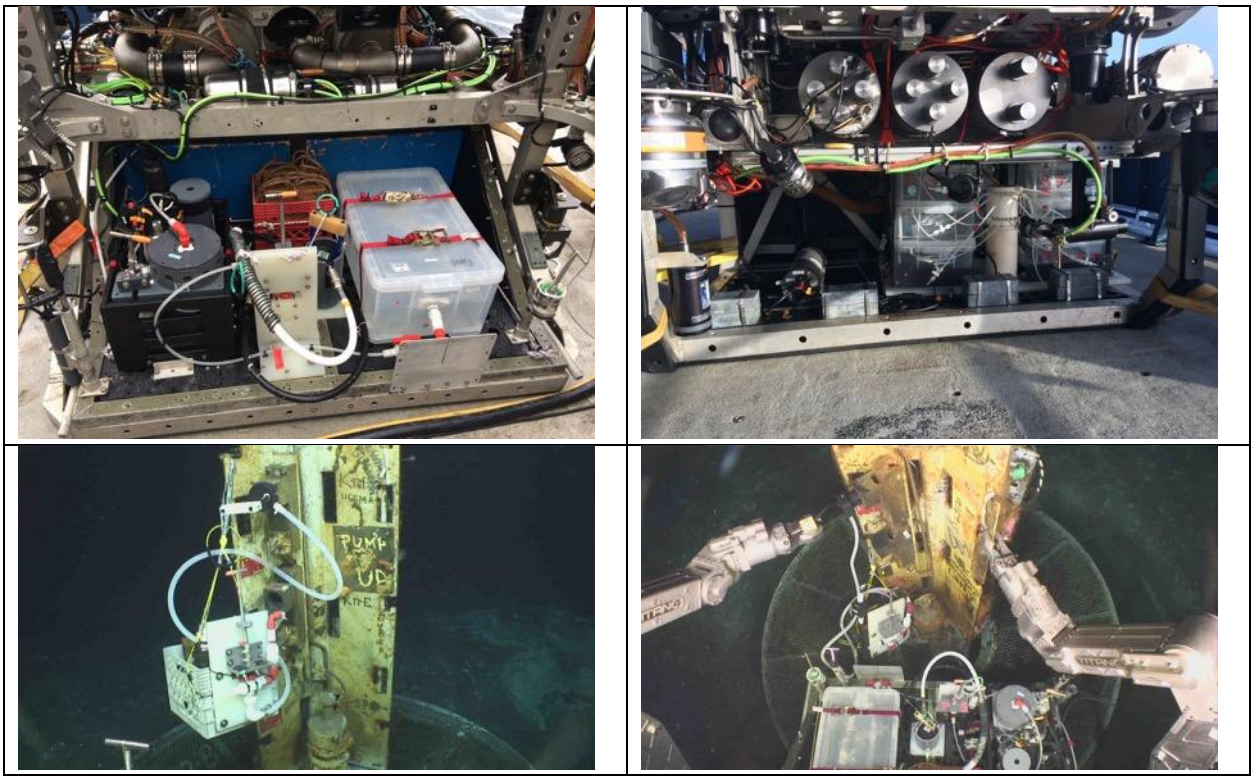


Figure 12. Mobile Pumping System (MPS) mounted on the front of Jason (top left photo, left side) along with the Large Volume Bag Sampler (LVBS, top left photo, right side), and the Medium Volume Bag Sampler (MVBS) and McLane manifold unit mounted in the back of Jason (top right photo). Photos on the bottom row are examples of the In Situ Filtration Units connected to the CORK fluid horizons with Jannasch connectors.

On dives J2-1508, J2-1509, and J2-1510, these systems collecting several hundreds of liters of fluids from each CORK horizon, and passed hundreds of liters of fluids through the passive filters *in situ*. The three passive viral *in situ* filtration systems were recovered on J2-1511. On the MPS, flow rates were maintained at ~0.5 L min^{-1} when pumping from CORK PTFE fluid delivery lines, achieving fluid temperatures of ~12°C and O_2 of less than 1 μM during active sampling. The multiple active pump In Situ Microbial Filtration samplers achieved hundreds of liters of fluid filtered *in situ*.

Overall, cruise objectives were met in recovering fluids and filters from each of the target CORK access points (U1362A shallow horizon, U1362A deep horizon, and U1362B single horizon) on three dives during the cruise for examining spatial heterogeneity in the crustal fluid deep biosphere.

Table 21. Crustal fluids sampled with the MPS during cruise TN-421

Dive	**J2-1508**[1]	**J2-1509**		**J2-1510**	
CORK horizon	U1362B_bio	U1362A_SS_ shallow	U1362A_bio_ deep	U1362A_SS_ shallow	U1362B_bio
In situ McLane 0.2μm filter	44 L	161 L	167 L	--	--
LVBS	68 L	--	67 L	70 L	--
MVBS	--	--	102 L	55 L	11 L

[1]Dive numbers refer to deployment.

Summary of culture experiments from crustal fluids

Summary by Michael Rappé

Collaborators Carr, Nigro, Rappé, and team members Freel, Brown, Daisernia, Mochimaru, Hunckler, Ramfelt, Spotkeff led efforts in establishing enrichment cultures from crustal fluids of the crustal aquifer. Raw crustal fluid was subsampled from MVBS bags and anoxically transferred into serum vials with stoppers, which were previously filled with of variety of anaerobic growth media prepared prior to the expedition. Vials were incubated shipboard at 65ºC and monitored by optical density. At the end of the cruise, enrichment replicates were divided and shipped to Hartwick College (Carr, Brown, and Daisernia), and the University of Hawai‘i (Nigro, Mochimaru, Hunckler, Freel and Rappé) for further shore-based monitoring and subsampling.

Table 22. Inventory of enrichment media, targets and replicates.

Media ID	**Target**	**Inoculation source**				**# of vials**
		CORK	Horizon	Dive	Source	
M1 M2, M3, M4, M5	Methanomicrobia archaea, Hydrothermae bacteria Anaerolineales, Archaeoglobus, Nitrospirae, Thermococcus	U1362B U1362A U1362A	Bio line Deep Shallow	1508 1509 1510	LVBS MVBS MVBS	135
M6, M7, D6, D7	Desulfarculaceae, Archaeoglobus	U1362B U1362A U1362A	Bio line Deep Shallow	1508 1509 1510	LVBS MVBS MVBS	72
M8, D8	Aminicentantes	U1362B U1362A U1362A	Bio line Deep Shallow	1508 1509 1510	LVBS MVBS MVBS	33
M9, D9	Use short chain fatty acids as a carbon source	U1362B U1362A U1362A	Bio line Deep Shallow	1508 1509 1510	LVBS MVBS MVBS	36
M10	Bathyarchaeota	U1362B U1362A U1362A	Bio line Deep Shallow	1508 1509 1510	LVBS MVBS MVBS	24
M11	Use Archaeoglobus detritus as carbon source	U1362B U1362A U1362A	Bio line Deep Shallow	1508 1509 1510	LVBS MVBS MVBS	24

Summary of passive *In Situ* Viral Filtration sampler

Summary by Olivia Nigro

Collaborators Nigro and Rappe led efforts sampling fluid from CORK wellheads U1362A and U1362B. Two *In situ* viral filtration samplers were suspended to wellhead U1362A and one to U1362B using the ROV Jason. Deployments and recovery of the samplers was done using the elevator. Samplers filtered crustal fluid through a 0.2 µm filter followed by either a 30kDa or 100kDa Millipore filter. This provided both microbial and viral biomass on separate filters for downstream processing. Expected volume for the filters during their attachment was 1l/50 min based on previous dives. The deployment of these sampler *in situ* is critical since these volumes cannot feasibly be transported to the surface for filtering.

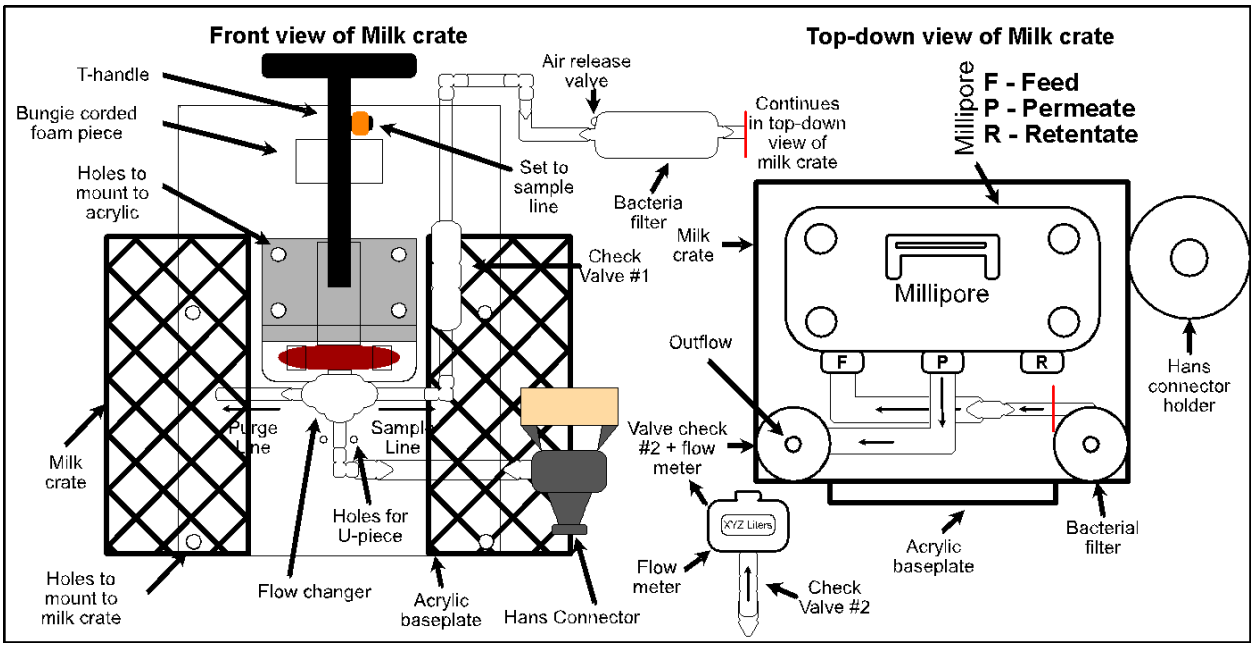


Figure 13. Diagram showing key components of passive In Situ viral filtration sampler.

Passive samplers were successfully suspended on wellheads during two separate dives. Passive samplers #2 and #3 were deployed to wellheads U1362B and U1362A “deep” respectively during dive J2-1509. Sampler #1 was attached to wellhead U1362A during dive J2-1510. All three samplers were recovered during dive J2-1511.

Issues with deployment and fluid flow meant that lower than expected amounts of volume passed through each of the sampler systems. Deployment issues and subsequent repair of samplers following dives J2-1507 and J2-1509 meant that some samplers were not attached to wellheads for as long as was initial anticipated. Lower than expected fluid flow at 1362B also meant that volume amounts were below expectations.

Table 23. Crustal fluid volume amounts filtered during cruise TN-421

Deployment Dive	J2-1509		J2-1510
Cork Horizon	U1362B	U1362A_deep	U1362_shallow
Sampler	#3	#2	#1
Time Start	03/08/2023 11:47	03/08/2023 13:07	05/08/2023 18:30
Time End	06/08/2023 17:26	06/08/2023 15:51	06/08/2023 16:24
Time Elapsed	77 hrs 39 minutes	74 hrs 44 minutes	21 hrs 54 minutes
Flow Meter Start (L)	16486	2523	2471
Flow meter end (L)	19393	4424	2529
Volume filtered (L)	2907	1901	58

Activity Experiments with Redox Sensor Green

Summary by Tim D'Angelo

Pristine crustal fluids were subsampled to perform experiments utilizing the redox active dye Redox Sensor Green® (RSG) (Invitrogen). This compound fluoresces after being reduced by enzymes in the electron transport chain, which can be measured by a flow cytometer. Laboratory studies have demonstrated that the amount of fluorescence of cells that uptake RSG is correlated to their metabolic rate, and thus can be used as a proxy for metabolic activity[1]. Four experimental conditions and one control were constructed to stimulate microbial activity with carbon sources or additional electron acceptors (Table 24).

Amendment solutions were prepared in the laboratory prior to the cruise. All carbon sources and/or electron acceptors were added to Artificial Cork Fluid media consisting of 1L of deionized tap water with: 24.48 g/L NaCl, 6.11 g/L $CaCl_2$, 2.56 g/L Na_2SO_4, 0.41 g/L $MgCl_2$, 0.52 g/L KCl, and 0.12 g/L Na_2SiO_3. The pH was adjusted to 8.3. The stock solutions for the stimulation experiments were prepared so that the final concentration of the experiments (1:10 dilution of stock solutions) are as listed in the top row of Table 24. All stock solutions were autoclaved prior to allocation. The autoclaved solutions were left in an anaerobic COY Chamber for 24 hours to degas oxygen prior to aliquoting two replicates of 2 ml per solution in to 30 ml glass vials and sealed with a rubber septa and crimp. All glassware used had previously been baked at 450°C for 2 hours.

Crustal fluids were subsampled from the MVBS in the Bio Lab inside a nitrogen purged glove bag. Approximately 500 ml of crustal fluid was aliquoted in to a baked Pyrex bottle with an autoclaved rubber septa cap. A PreSens® oxygen measuring optode system was used to measure the dissolved oxygen in the fluids. Nitrogen was bubbled through the fluids until oxygen concentrations were ~2 µM (Table 24). The bottle was transferred in to a second glove bag with the experimental vials preloaded with stock solutions (two replicates per condition and control, 10 vials total). Eighteen ml of crustal fluid was transferred in to the experimental vials using a 20 ml syringe and a 21g needle, bringing the total volume in the vials to 20 ml. The vials were taken out of the glove bag and incubated for 24 hours in an incubator set to 65°C.

After the 24-hour incubation the experimental vials were removed from the 65°C incubator and brought back in to a glove bag. The bag was purged five times with nitrogen gas. The vials were decrimped and 6.4 ml aliquots of the augmented crustal fluids were pipetted in to three 15 ml falcon tubes (1. RSG incubation, 2. RSG incubation with formaldehyde fixation and 3. unstained). The tubes for RSG incubation with fixation had 640 µl of 37% formaldehyde added prior to being brought in to the bag. The RSG incubation and the RSG incubation with fixation

[1] Munson-McGee, J.H., Lindsay, M.R., Sintes, E. *et al.* Decoupling of respiration rates and abundance in marine prokaryoplankton. *Nature* **612**, 764–770 (2022). https://doi.org/10.1038/s41586-022-05505-3

had 6.4 µl of RSG added after crustal fluids were aliquoted. All tubes were tightened tightly and brought out of the glove bag and placed back in to the 65°C incubator. The samples were incubated for 30 – 60 minutes. The unfixed RSG incubations were incubated the longest and were inside the 65°C incubator for 45 – 60 minutes. The incubations were deconstructed by removing them from the 65°C incubator and quickly adding 640 ml of 10x glyTE to the 15 ml tube and then inverting the tube several times to mix. Six 1 ml replicates were pipetted from the 15 ml tubes into six cryovials and immediately placed in a -80°C freezer.

Table 24: Details of Samples used for Redox Sensor Green Activity Assays

Horizon	Dive	Oxygen initial µM	Oxygen after bubbling µM	Control	Nitrate 0.1 mM	Ferrihydrite 0.1 g/L	Casmino Acids 10 µM	Acetate 0.04 mM
U1362A Deep MVBS Bag 21	J2-1509	15.4	2	2 x ACF	2 x	2 x	2 x	2 x
U1362A Shallow MVBS Bag 19	J2-1510	26	2	2 x ACF	2 x	2 x	2 x	2 x
U1362B	J2-1510	15	2.1	2 x ACF	2 x	2 x	2 x	2 x

Summary of sediment sampling for thermophilic spores

Summary by Anirban Chakraborty

As a companion project to the primary funded projects, collaborators from Idaho State University 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, sediment samples and bottom seawater were collected opportunistically to evaluate the hypothesis that thermospores are inhabitants of deep hydrothermal fluids and are distributed to nearby seawater and sediments when these fluids are ejected into the ocean. The sediment and water samples from this expedition will be utilized to conduct incubation experiments to resuscitate viable endospores at various temperatures.

Hanako Mochimaru's group (The National Institute of Advanced Industrial Science and Technology, Japan) also collected sediment samples for use in lab cultivation of uncultured microbial lineages.

Sediment push coring

Dive J2-1509; August 03, 2023

Six 25-cm deep sediment push cores were collected from two locations (three cores per location) 5 m apart and 50 m west of CORK U1362B. Chakraborty's group (Idaho State Univ.) sampled five cores and Mochimaru's group sampled a single core.

Dive J2-1510; August 05, 2023

Nine 25-cm deep sediment push cores were collected following a transect from three locations (three cores per location), each 50 m apart, west of CORK U1301A. Chakraborty's group (Idaho State Univ.) sampled all nine cores.

Core processing and sample storage

The Chakraborty group sampled cores at 5-cm depth intervals and stored at 4°C in sterile WhirlPak bags. In addition to bulk sediment, triplicate aliquots of 1.5-ml sediment were frozen at -20°C for DNA-based community assessment. Mochimaru sampled core at 5-cm intervals for

DNA and used sediment from depth interval 20-25 cm bsf for enrichment inoculations. Mochimaru DNA samples were frozen at -80˚C and inoculations were stored at RT.

Seawater collection; Dive J2-1510; August 05, 2023

Two 5 L Niskins were deployed to collect seawater mixed with continuously leaking crustal fluids from the base of CORK 1301A. Water samples were collected in sterile 1 L Nalgene bottles and stored at 4°C. Chakraborty's group sampled ~8L and Serhat Sevgen (Ben Tutolo's group; Univ. Calgary) sampled ~2L of water.

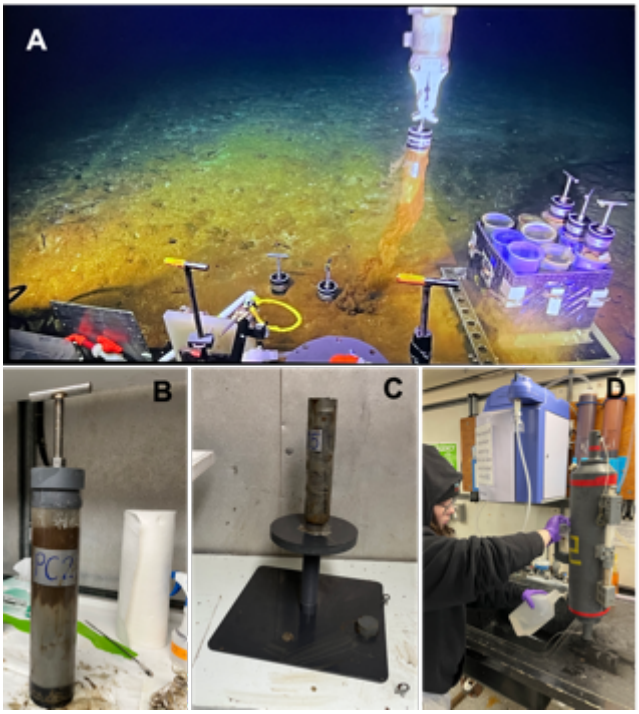


Figure 14: (A) Sediment push core collection near borehole U1362B during Dive J2-1509; (B) a 25-cm deep sediment core being prepared for processing; (C) Sediment core being sectioned at defined depth intervals; (D) Seawater mixed with crustal fluid collected during Dive J2-1510 being sampled from 5-L Niskin bottle.

CTD casts and sample collection summary

Summary by Anirban Chakraborty

The University of Washington's 24x 10L bottle CTD Niskin rosette sampler was used during periods of opportunity to collect water column samples for various groups. User groups included the Rappé/Nigro/Carr team, Ben Tutolo (Univ. Calgary), Nagissa Mahmoudi (McGill Univ.) and Huei-Ting (Tina) Lin. Science party members participated in the operation of the CTD system (e.g., cocking of sampling bottles, firing of niskins) after appropriate training from R/V Thompson's SSSG. The CTD sampler was deployed and recovered by R/V Thompson's SSSG and the crew. The system consists of a custom-built SBE 9plus Seabird instrument package operating with SBE11plus Firmware Version ">= 5.0".

Table 25. CTD log of August 1st CTD Cast (all times local, GMT-7)

Cast	**Date**	**Launch time**	**Recovery time**	**Latitude**	**Longitude**
U1362A_CTD01	20230801	12:15	14:15	47.761389	-127.760833
Comments: All sensors worked well for both downcast and upcast. Niskin #11 did not fire.					
Depth (m)	**Bottles**	**Experiments**			
2648 m (bottom)	1 – 12, 13, 14	Rappé / Nigro / Carr group sampled ~120L from the bottom of the water column as control for crustal fluid experiments.			
2648 m (bottom)	13	Huei-Ting (Tina) Lin sampled ~6L from the bottom of the water column for water chemistry analysis.			
2648 m (bottom)	14	Serhat Sevgen (Ben Tutolo's group) sampled ~1L from the bottom of the water column for water chemistry analysis.			
2500 m	15, 16, 17	30L water was sampled for collaborator Nagissa Mahmoudi.			
1000 m, 550 m, 250 m, 150 m, 60 m, 20 m, Surface	18-24	Huei-Ting (Tina) Lin sampled ~6L from seven additional water column depths for water chemistry analysis.			

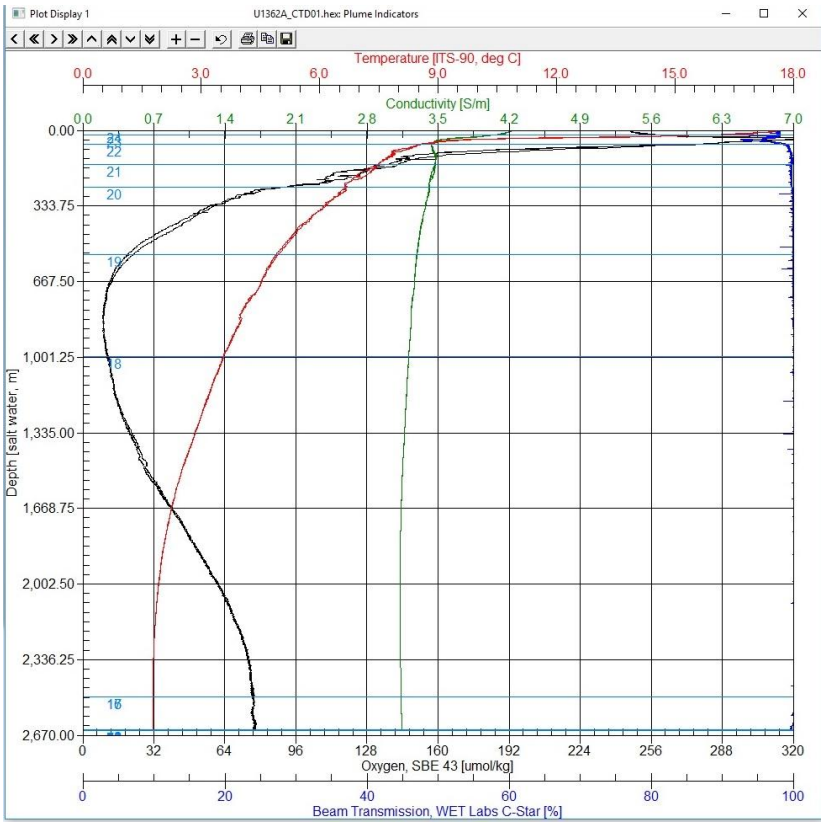


Figure 15. Depth profile of August 1st CTD Cast.

Table 26. CTD log of August 6th CTD Cast (all times local, GMT-7)

Cast	**Date**	**Launch time**	**Recovery time**	**Latitude**	**Longitude**
U1362B_CTD02	20230806	4:02	6:05	47.761666	-127.760555
Comments: All sensors worked well for both downcast and upcast. All niskins fired, no issues with sampling.					
Depth (m)	**Bottles**	**Experiments**			
2648 m (bottom)	1	Serhat Sevgen (Ben Tutolo's group) sampled ~1L from the bottom of the water column for water chemistry analysis.			
2000 m, 1500 m, 1250 m, 800 m, 750 m, 700 m, 650 m, 600 m, 500 m, 400 m, 300 m, 200 m	3-24	Huei-Ting (Tina) Lin sampled ~6L from twelve water column depths for water chemistry analysis.			

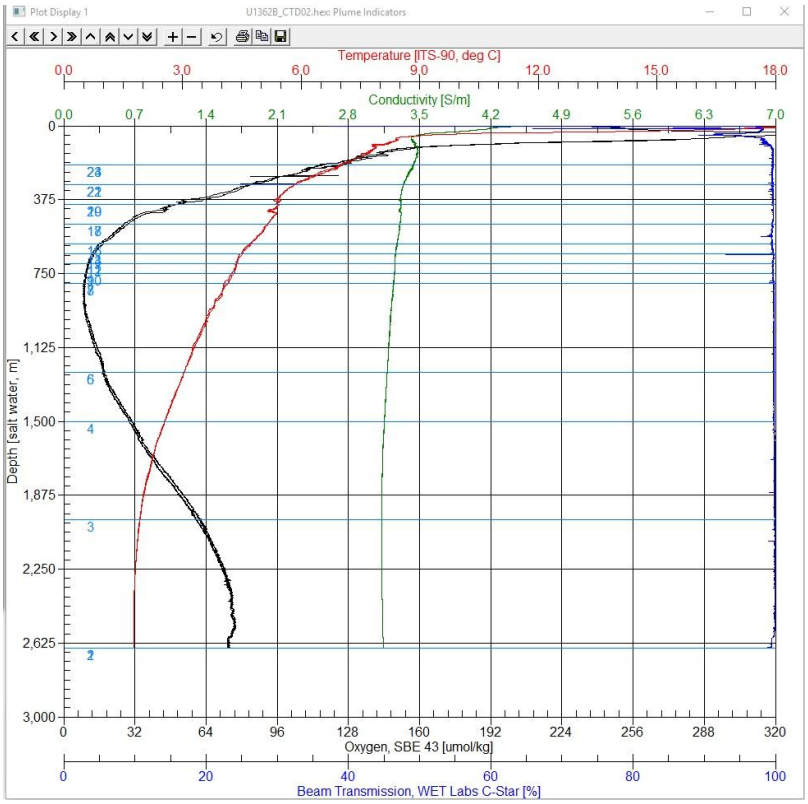


Figure 16. Depth profile of August 6th CTD Cast.

Summary of OsmoSampler deployments on TN-421

Summary Beth Orcutt, 6 August 2023

Two OsmoSampler experiments were assembled on TN-421 for deployment at the Holes U1362A and U1362B CORK wellheads, based on the principles outlined in Wheat at el. 2011[1]. The purpose of these deployments is to collect crustal fluid samples from these sites for major/minor/trace element and dissolved gas concentration baselines to inform the SolidCarbon project and partners from Ocean Networks Canada. The experiments were assembled by Beth Orcutt (Bigelow Laboratory for Ocean Sciences, USA) with assistance from Hannah Robutka (University of Victoria, Canada) and Serhat Sevgen (University of Calgary, Canada). OsmoSampler components were provided by Beth Orcutt and Geoff Wheat (University of Alaska Fairbanks). These systems are expected to sample crustal fluids continuously for years until recovery at an undetermined time in the future.

Each OsmoSampler consisted of two sampling systems, based on the principles outlined in Wheat at el. 2011. One system was the "regular" OsmoSampler, consisting of two coils of Teflon tubing attached to a 4-membrane OsmoPump; these are for measuring major/minor/trace element concentrations. The Teflon coils were flushed with 10% vol:vol concentrated hydrochloric acid prior to filling with ultrapure 18.2 Ω water. As spare parts were used, the Teflon coils varied in length from ~150-300m. The second system was the "gas" OsmoSampler, consisting of one or two coils of copper tubing attached to a 4-membrane pump; these are for measuring unsaturated dissolved gas concentrations. The copper coils were filled with ultrapure water. Each coil had approximately 300m of tubing; the "Green OsmoSampler" had two copper coils and the "Blue OsmoSampler" has one. Based on OsmoPump membrane properties[2], the 4-membrane pumps are expected to sample at ~0.2 mL d^{-1}. As each meter of tubing contains approximately 1mL of fluid, the OsmoSamplers with 300-600m of tubing should enable uninterrupted sampling for 1,500-3,000 days (4-8 years).

Each OsmoSampler experiment was assembled in a 6-gallon milkcrate. The intakes of each OsmoSampler were inserted into 0.5" OD plastic tubing to slowly sip on crustal fluids freely venting at liters/second velocities from the CORK observatories. Several feet of 0.5" tubing was connected to a "Jannasch connector" that was plugged into a specific valve on the CORK wellheads. The Jannasch connector was attached to a holster mounted on the side of the milkcrate with a bungee cord. A bridle of yellow polypropylene rope was attached to the

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[1] Wheat, C.G., Jannasch, H.W., Kastner, M., Hulme, S., Cowen, J., Edwards, K.J., Orcutt, B.N., and Glazer, B., 2011. Fluid sampling from oceanic borehole observatories: design and methods for CORK activities (1990–2010). *In* Fisher, A.T., Tsuji, T., Petronotis, K., and the Expedition 327 Scientists, *Proc. IODP,* 327: Tokyo (Integrated Ocean Drilling Program Management International, Inc.). doi:10.2204/iodp.proc.327.109.2011

[2] Jannasch et al. 2004. Continuous chemical monitoring with osmotically pumped water samples: OsmoSampler design and applications. Limnology and Oceanography: Methods 2: 102-113.

milkcrate to allowing ROV manipulation and hanging on the CORK wellheads. Two 2” metal bolts were screwed into the short sides of the milkcrates for anchoring the milkcrates with bungee cords for deployment. Colored duct tape covered the tops of the milkcrates to minimize movement of the tubing connectors and/or interference from the ROV manipulator.

Both OsmoSamplers were deployed by elevator at the beginning of ROV Jason dive J2-1509 on 3 August 2023. They sat on the seafloor until deployed on CORK wellheads during dive J2-1510 on 5 August 2023. The “Green OsmoSampler” was deployed at Hole U1362A connected to the lower valve #1 in the CORK Osmo Bay, which was sampling the deep horizon. Not that installation on the deep horizon was an accident (the intended valve was 2 or 3 for the shallow horizon), and was intended to be corrected on dive J2-1511. However, J2-1511 was cut short for mechanical issues and the correction could not be made. The Blue OsmoSampler was deployed at Hole U1362B connected to upper valve #1 in the CORK Osmo Bay to sample the shallow basement horizon.

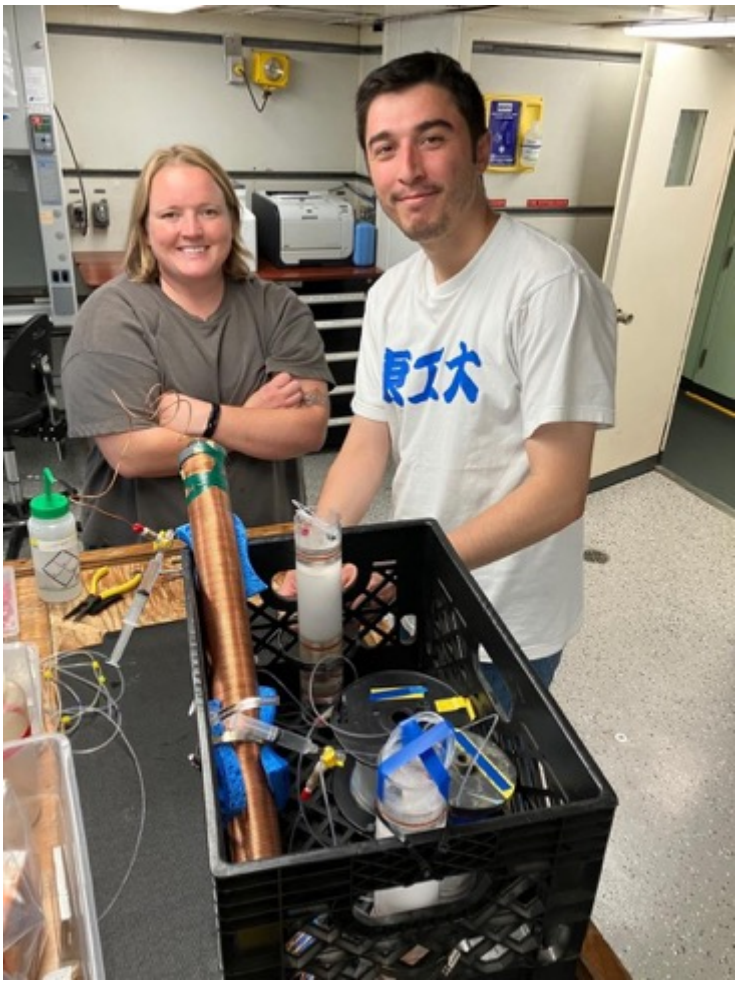


Figure 17. Hannah Robutka and Serhat Sevgen helping with assembly of the OsmoSamplers. Inside the milkcrate can be seen one copper coil, two spools of Teflon tubing, and two 4-membrane OsmoPumps.

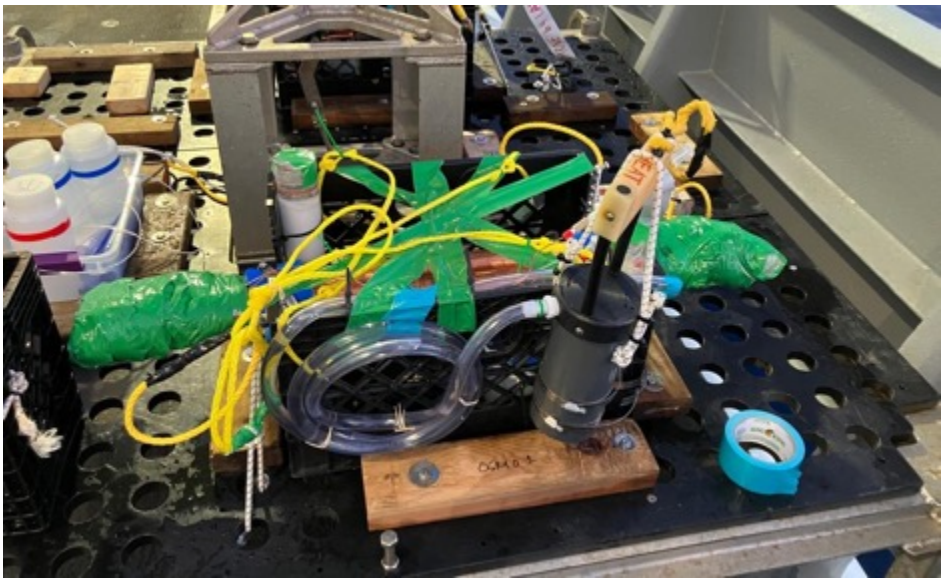


Figure 18. View of the assembled Green OsmoSampler attached to the elevator. Wooden blocks were used to create a cradle for the milkcrate, with bungee cord tie points on either of the shorter sides of the milkcrate. Prior to elevator deployment, the intakes of the OsmoSamplers were connected to syringes filled with ultrapure water; these were removed immediately prior to deployment.

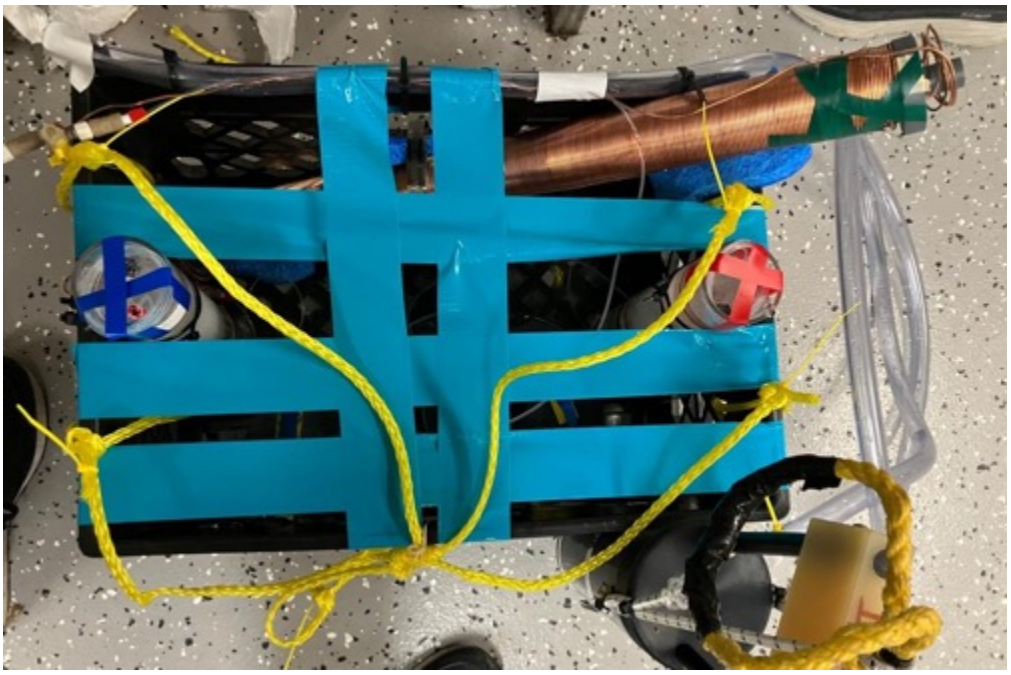


Figure 18. View of the assembled Blue OsmoSampler.

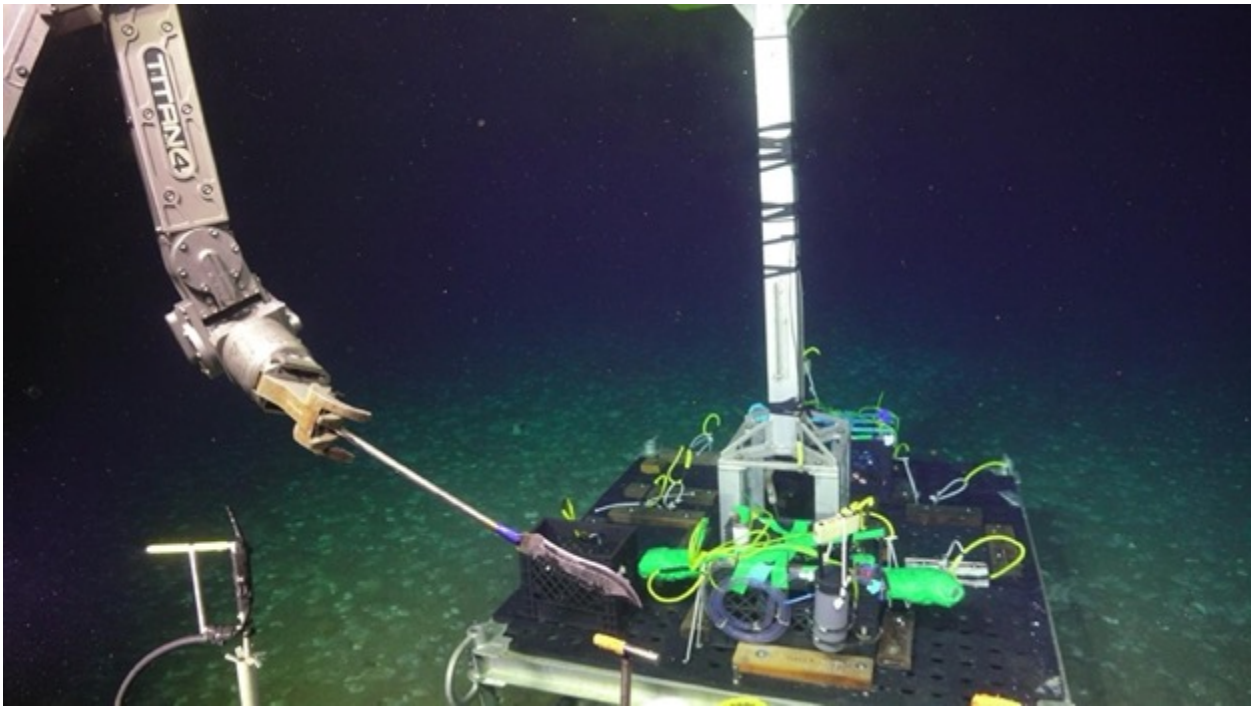


Figure 19. View of the OsmoSamplers on the elevator. A knife was used on the seafloor to poke holes in the plastic tubing around the sampler intakes to allow flow of crustal fluids.

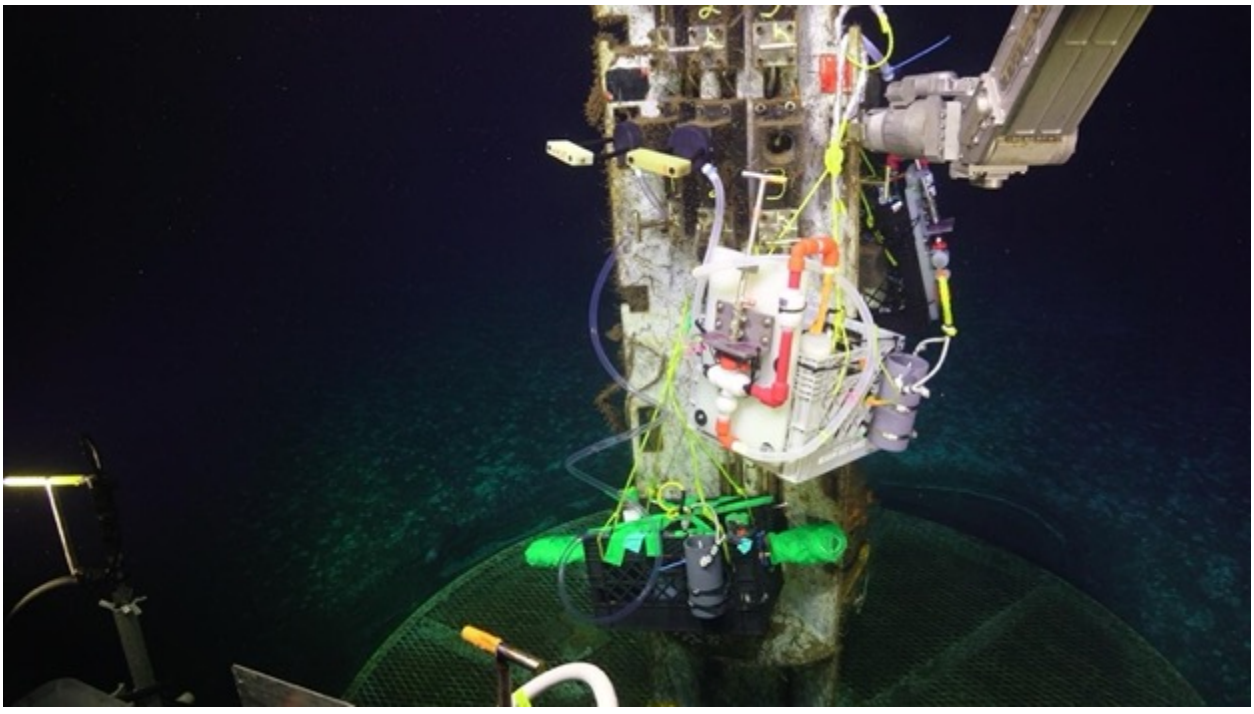


Figure 20. View of the Green OsmoSampler deployed on the Hole U1362A wellhead on lower valve #1. Also in view is a passive flow in situ filter connected to the lower valve #2.

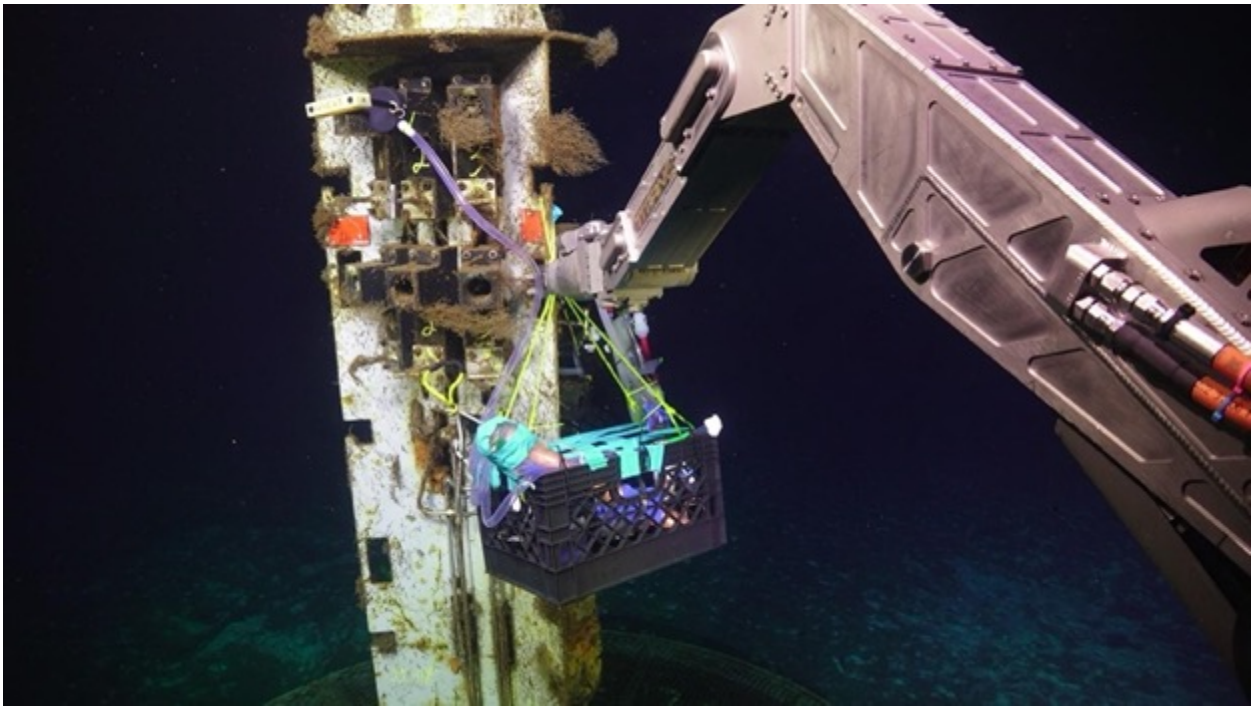


Figure 21. View of the Blue OsmoSampler connected to the Hole U1362B wellhead.

Summary of animal observations on TN-421

Summary by Beth Orcutt

Although observation of benthic fauna was not a primary objective of the TN-421 science, we used the Marine Life Field Guide of Ocean Networks Canada, as well as the NOAA Benthic Animal Guide, to categorize observations of animals seen during other science operations. These observations will be shared with partners at the Department of Fisheries and Oceans of the Canadian government to inform benthic ecosystem management for the region, as this is proposed as a new Marine Protected Area. While many animal sightings were logged live during dive operations, the following represents the animal observations that could be verified with a high-quality photo or 4K highlight video taken during the dive that could be used for identification purposes. 4K video was not collected continuously. Scaling lasers were rarely on for these video or photo observations. Asterisks indicates that there is a 4K highlight video available in the data record. Note that no identifiable animal observations were made on J2-1511

Table 29. Identifiable animal observations of J2-1507

Time Stamp **Phylum / Common Name**	**Picture**
2023-08-02T08:21:09.843Z Chordata/ Rattails	
2023-08-02T08:35:50.942Z Mollusca / *Graneledone* octopus	

2023-08-02T08:45:17.916Z *Ctenophora/ Bloody-belly comb jelly	
2023-08-02T08:48:10.606Z Bryozoa/ unknown	

Table 27. Identifiable animal observations of J2-1508

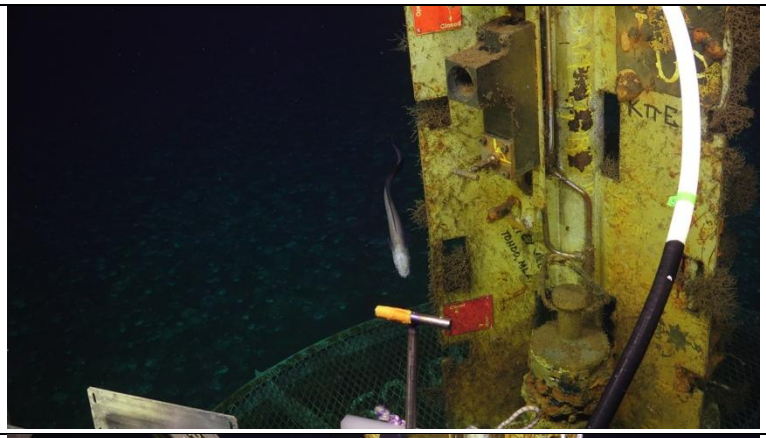
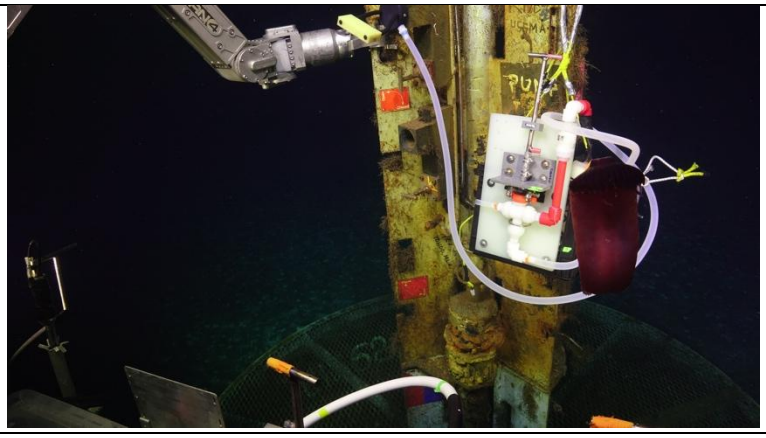
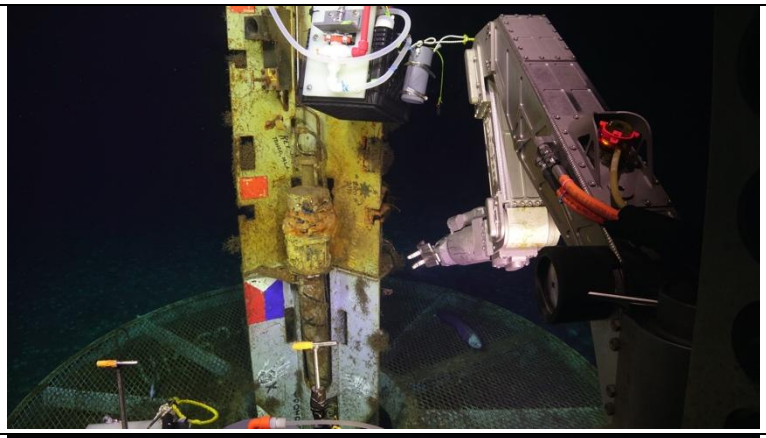
Time Stamp **Phylum / Common Name**	**Picture**
2023-08-02T17:29:22.800Z *Echinodermata / unknown sea cucumber (baby?)	
2023-08-02T18:50:39.418Z *Chordata/ Abyssal snailfish	
2023-08-03T00:20:04.512Z *Echinodermata / unknown sea cucumber	

Table 28. Identifiable animal observations of J2-1509

Time Stamp **Phylum / Common Name**	**Picture**
2023-08-03T20:13:01.515Z *Annelida / scale worm	
2023-08-03T21:05:01.836Z Chordata / abyssal snailfish	
2023-08-03T23:23:26.172Z *Chordata / abyssal snailfish	

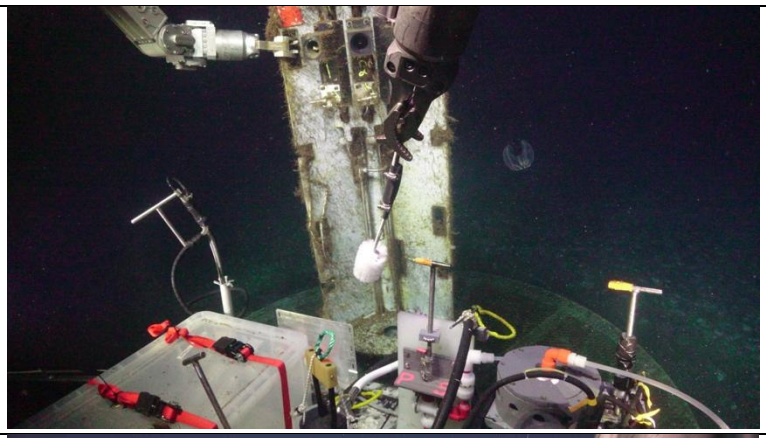
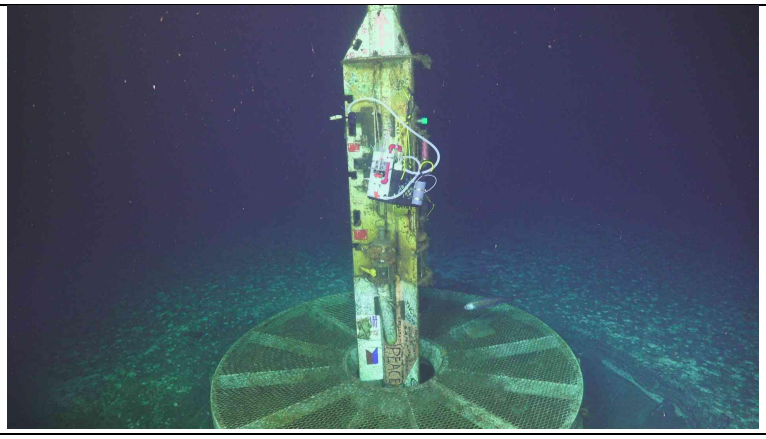
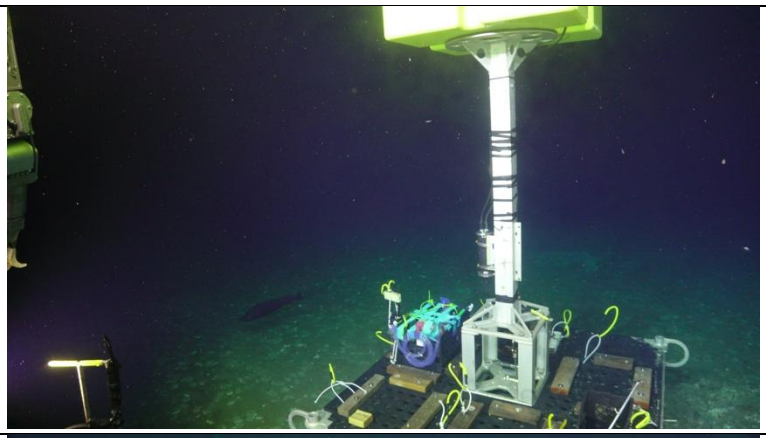
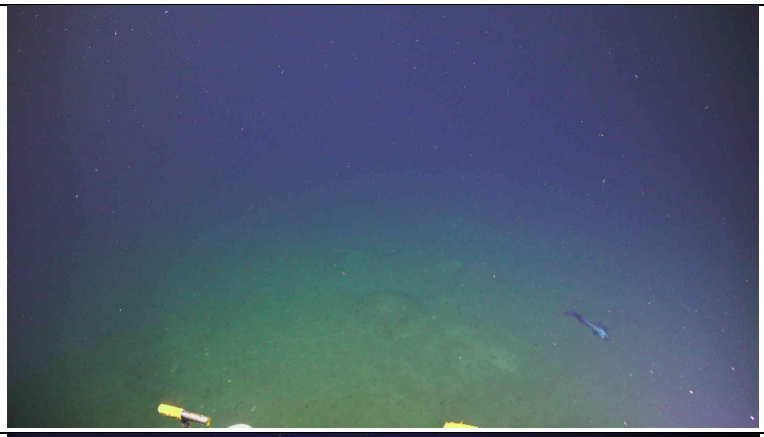
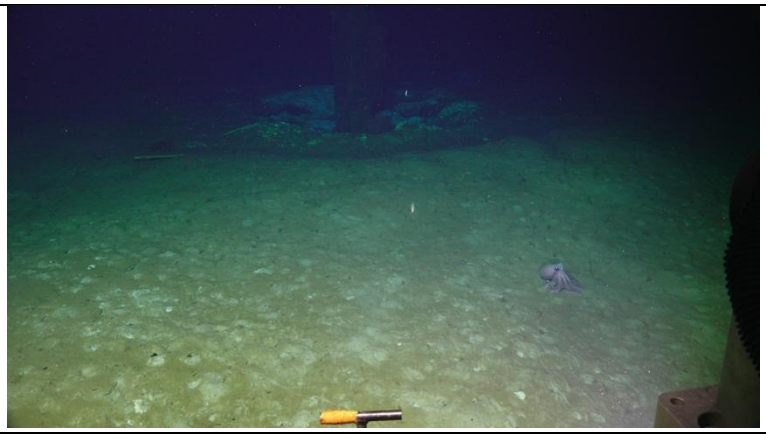
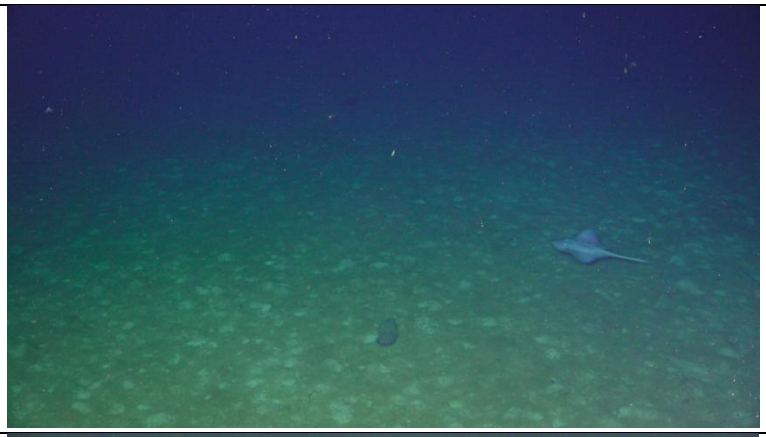
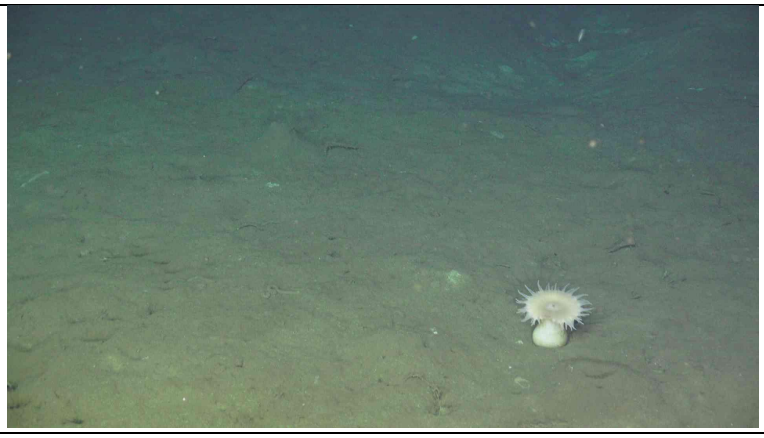
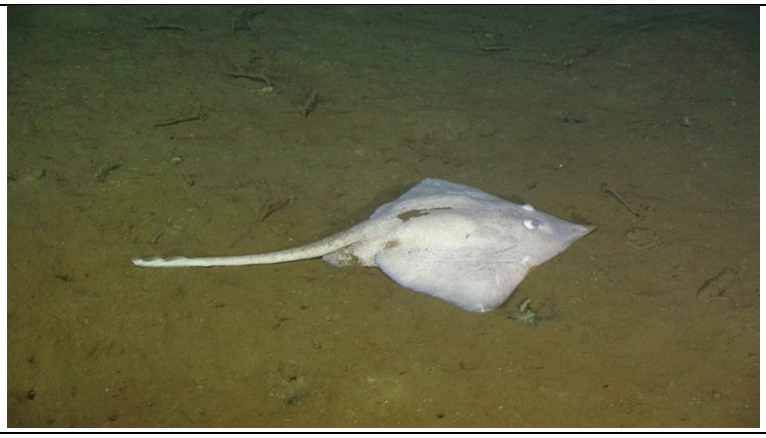
2023-08-04T01:39:34.272Z *Ctenophora / Bolinopsis	
2023-08-04T11:29:14.590Z *Mollusca / *Muusoctopus* (??) octopus	

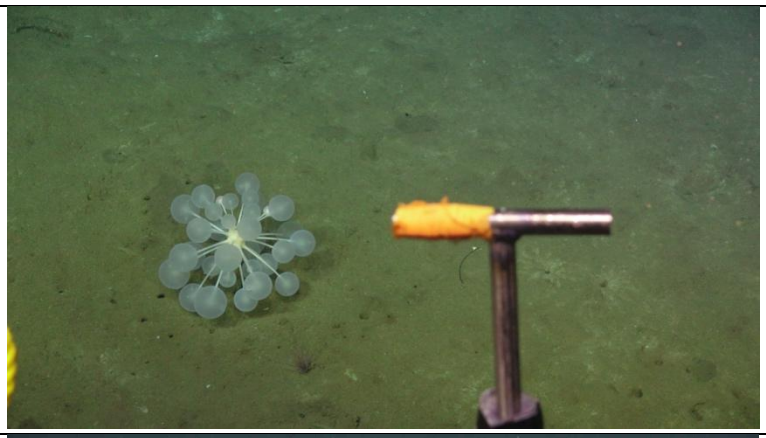
Table 30. Identifiable animal observations of J2-1510

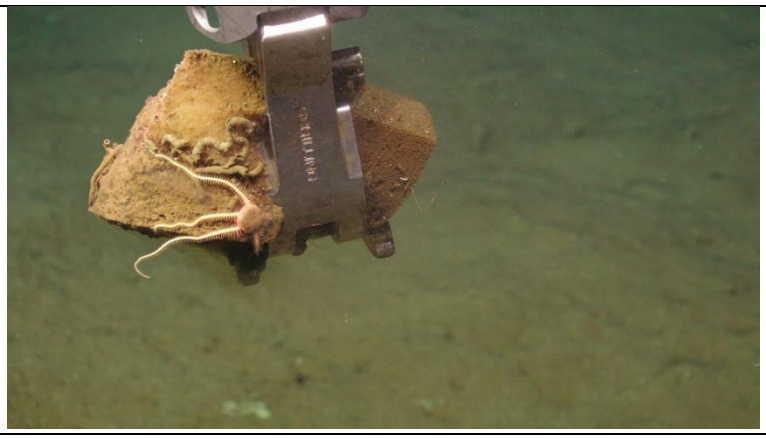
Time Stamp **Phylum / Common Name**	**Picture**
2023-08-05T11:47:16.425Z *Chordata / unknown fish (second photo is of specimen that was accidentally collected in scientific gear – photo by Kelle Freel)	 
2023-08-05T12:39:35.530Z *Cnidaria / unknown jelly	
2023-08-05T13:20:43.076Z *Chordata / unknown snailfish	

2023-08-05T15:01:18.652Z Chordata / Pacific flatnose	
2023-08-05T16:01:25.915Z *Chordata / Rattail Echinodermata / unknown slime star	
2023-08-05T17:09:30.788Z Chordata / Rattail	
2023-08-05T17:20:25.427Z *Mollusca / Graneledone octopus	

2023-08-05T17:25:39.637Z *Mollusca / Graneledone octopus	
2023-08-05T17:27:42.353Z *Mollusca / Graneledone octopus	
2023-08-05T18:02:51.065Z *Chordata / unknown skate	
2023-08-05T19:02:04.044Z Cnidaria / unknown Actinostolidea	

2023-08-05T20:08:45.342Z Chordata / Abyssal snailfish	
2023-08-05T20:12:55.024Z Arthropoda / unknown sea spider	
2023-08-05T20:27:26.953Z *Echinodermata / unknown sea cucumber	
2023-08-05T20:30:02.576Z *Chordata / deep sea skate	

2023-08-05T20:41:00.927Z *Porifera / Chondrocladia ping pong sponge	
2023-08-05T20:57:46.687Z *Arthropoda / unknown crab	
2023-08-06T01:50:27.101Z *Cnidaria / unknown sea pen	
2023-08-06T04:44:04.806Z Echinodermata / unknown sea cucumber	

2023-08-06T04:51:02.256Z Echinodermata / unknown brittle star	

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