North Pond 2017 Expedition Report

R/V Atlantis Expedition AT39-01 with the ROV Jason-II

Chief scientist: Geoff Wheat, wheat@mbari.org

Expedition Information:

Pre-mobilization in Woods Hole MA, September 28-October 1, 2017
Departure: October 2, 2017
Arrival: November 2, 2017 – Bridgeport, Barbados
Demobilization: November 3, 2017

Field work supported by:

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C-DEBI Bioinformatician Ben Tully and C-DEBI postdoctoral fellow Jackie Goordial,
Others support from C-DEBI included a Senior Scientist research award that supported
Tim D'Angelo, Rose Jones, and Elizabeth Trembath-Reichert

Project Co-PIs:

K. Becker, P. Girguis, J. Huber, B. Orcutt, S. Shah, G. Steward, and C. G. Wheat[1]

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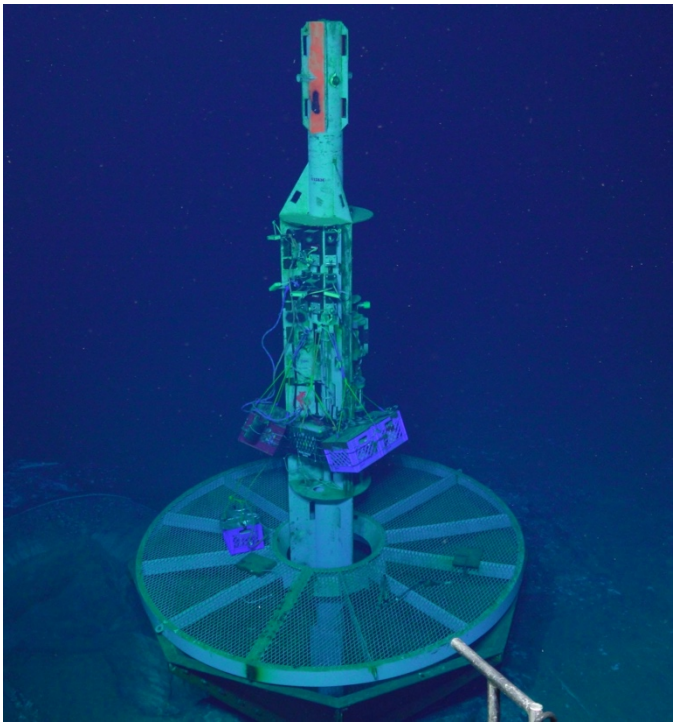
Preface



Hole U1383C with OsmoSamplers that were deployed in 2014. These samplers populate each of the zones with two samplers in zone 1 (deep). These OsmoSamplers were recovered on J2-1032.

View from the back of the new control van.

North Pond Expedition 2017

Summary

AT39-01-24 was the third expedition to service the borehole observatories in North Pond after the drilling expedition (IODP Exp. 336) in the fall of 2011. The first expedition occurred in the spring of 2012 during which borehole fluids were sampled for biogeochemical and microbial content and a CORK-Lite was installed. Borehole fluids were again collected in the spring of 2014. The goal of this, the final, expedition was to sample borehole (formation) fluids prior to the removal of the downhole stings, which included fluid samplers, sensor and experiments, and after the boreholes were sealed. In addition we planned to conduct a tracer test, measure the rate of fluid flow from the boreholes, filter large (hundreds of liters) amounts of formation fluid for shore-based virus and organic carbon studies, and measure heat flow to constrain models of subsurface hydrologic flow.

During AT39-01 we conducted 13 remotely operated vehicle (ROV) dives with Jason II. The initial focus for the dives was to collect pristine fluids from the formation at two of the boreholes from four umbilicals that reach distinct horizons within the formation (U1382A and U1383C) (J2-1024 to J2-1029). The middle set of dives focused on the recovery of downhole stings (J2-1030 to J2-1033). The later dives (J2-1034 to J2-1036) focused on sealing boreholes and conducting high priority science.

We had many setbacks during the expedition. A list of these setbacks was submitted to NSF and WHOI. The primary problem was an issue with the ROV's tether. The tether was re-terminated three times at sea and an additional time ashore. This and other problems resulted in multiple adjustments to the operational schedule.

In the end we collected fluid sand in-situ particles from each of the four umbilicals prior to the removal of the borehole seals and downhole strings. We recovered the entire downhole string at U1383B and recovered elements of the strings from U1382A and U1383C. In both cases of partial recovery, the problem was corrosion of the stainless steel strength members. Interestingly, the same materials have been used in a dozen boreholes, most/all of which are anoxic, without incident. The North Pond boreholes should be oxic, thus stainless steel should not corrode, whereas it should have corroded in the dozen other anoxic boreholes. The difference must be the contrast between the fiberglass and mild steel casings. A fourth recovery was not attempted because of the lack of time and the inability to use the heavy lift capabilities of Jason II.

Other accomplishments included a shortened tracer test, testing a novel borehole flow meter, sealing each of the three boreholes, collecting fluids after the boreholes were sealed, conducting a transect of heat flow measurement, and completing 12 hydrocasts – one of which lasted about 30 hours so that bottom seawater could be filtered for 24 hours.

This expedition included participates from three funded NSF awards. As a result the ship was loaded with graduate students (5), postdoctoral fellows (6), and one person who was going to start her first faculty position when she returned home. For most of these young investigators it

was their first experience with Jason II and working far from shore. Nevertheless, they were very efficient and adapted sample processing efforts as time and materials required.

In addition, Beth Orcutt led the adopt-a-microbe program targeting Girl Scouts from the state of Maine. She had help from many of the participants. Beth also led social media announcements of discovery, again with aid from many of the younger scientist.

AT39-01 was a group effort, one that could not have been completed without the skilled support from the Atlantis and Jason II crews. We are indebted to all of their assistance.

At 39-01
This report is displayed in the following order:

Data, Laboratory, and Sample Summaries

Personnel and Affiliations

R/V *Atlantis* Voyage #39-01

Science party
Dr. Charles Geoffrey Wheat, University of Alaska, Fairbanks – chief scientist
Dr. Keir Becker, University of Miami – RSMAS
Mr. William Billings, University of Miami, RSMAS
Mr. Timothy D'Angelo, Bigelow Laboratory for Ocean Sciences
Mr. Trevor Fournier, University of Alaska, Fairbanks
Dr. Kelle Freel, University of Hawaii at Manoa
Dr. Jacqueline Goordial, Bigelow Laboratory for Ocean Sciences
Ms. Anne Hartwell, University of Alaska, Fairbanks
Dr. Rose Jones, Bigelow Laboratory for Ocean Sciences
Ms. Megan Mullis, Texas A&M University
Dr. Olivia Nigro, University of Hawaii at Manoa
Dr. Beth Orcutt, Bigelow Laboratory for Ocean Sciences
Dr. Grieg Steward, University of Hawaii at Manoa
Ms. Clarisse Sullivan, University of Hawaii at Manoa
Dr. Elizabeth Trembath-Reichert, Woods Hole Oceanographic Institution
Dr. Benjamin Tully, University of Southern California
Dr. Tess Weathers, University of Santa Cruz
Ms. Kristin Yoshimura, University of Delaware

Jason party
Mr. Alberto Collasius, Woods Hole Oceanographic Institution – Expedition Leader
Mr. Jefferson Grau, Woods Hole Oceanographic Institution
Mr. James Varnum, Woods Hole Oceanographic Institution
Mr. Drew Bewley, Woods Hole Oceanographic Institution
Mr. Korey Verhein, Woods Hole Oceanographic Institution
Mr. Christopher Judge, Woods Hole Oceanographic Institution
Mr. Scott Hansen, Woods Hole Oceanographic Institution
Mr. Scott McCue, Woods Hole Oceanographic Institution
Mr. Nile Akel Kevis-Stirling, Woods Hole Oceanographic Institution
Mr. Richard Sanger, Woods Hole Oceanographic Institution
Ms. Catherine Graver, Woods Hole Oceanographic Institution – SSSG
Mr. David Sims, Woods Hole Oceanographic Institution – SSSG

Ship's crew
Master RV *Atlantis*: AD Colburn (final cruise!)
Chief Mate – Jennifer Hickey
Second Mate – Max Kantor
Third Mate – Kenny Beaver
COMET – Jim Panter
Bosun – Pat Hennessy
SSSG: Dave Sims, Catie Graver
Able-Bodied Seaman – Jerry Graham, Patrick Neumann, Ronnie Whims

Ordinary Seaman – Patrick Porter, Brian Perkins
Chief Engineer – Chris Morgan
First Engineer – JT Walsh
Second Engineer – Alex Deveaux
Third Engineer – Bill Robinson
Electrician – Troy Pew
Oilers – Mike Spruill, Corey Lawton
Wiper/OS – Clindor Cacho
Steward: Carl Wood
Cook: Liz Zacharias
Mess Attendant: Tanzania Edwards

Valve Orientation on North Pond CORKs

CORK-Lite at Hole U1383B– If the Jannasch valve is open (vertical) then turn valve counter clockwise to close (horizontal). The valve on the cap (top plug) should be closed (horizontal). If the valve on the cap is in the closed position then to open the valve turn it counter clockwise (vertical).

CORKs at Holes U1382A and U1383C:
– In the MBIO and Chem bays, if a Jannasch valve is open (vertical), then turn valve counter clockwise to close (horizontal).
– In the Pressure bays, if on formation then turn counter clockwise for hydrostatic. Note the extra metal on one end of the valve handle. This piece of metal points to the direction of the valve. If the valve is on hydrostatic (valve is in a vertical position and the piece of metal is on the top), then turn 180 degrees clockwise to the formation (valve is in a vertical position and the piece of metal is on the bottom).

North Pond 2017:
Daily Operational Summaries
(GMT is Local time +4 Hours)

There were no time zone changes during the expedition.
A broad summary of operations is provided in Table 1.

Monday October 2, 2017

We left port at 9 AM (local) to the sound of canons fired from the bow to celebrate the Captain's (A.D.) retirement.

Tuesday October 3, 2017

We continued the transit at about 10.5 knots over the Gulf Stream. The lower speed was a consequence of the weight on the fantail. We stopped at 17:30 to test the Jason winch, which had problems during the last expedition, but repairs were completed before our expedition. The test ended at 23:30 and we continued the transit.

Wednesday October 4, 2017

We continued the transit at about 10.5 knots. Beth presented an overview of the work that has and is being done at North Pond. We crossed the Gulf Stream in the morning and plugged forward. Our current ETA is Monday October 9 at 10:00.

Thursday October 5, 2017

We continued the transit at about 10.5 knots. Ben presented his recent work in North Pond. Our current ETA is Monday October 9 at noon.

Friday October 6, 2017

We woke to a small electrical malfunction in the ice maker in the main laboratory which was quickly contained by the ship's crew. We continued the transit at about 10.5 knots. Keir presented an overview of the decade of work that has been completed in North Pond. Our current ETA is Monday October 9 at noon.

Saturday October 7, 2017

We continued the transit at about 9.5-10.5 knots.

Sunday October 8, 2017

We continued the transit at about 9.5-10.5 knots. Our current ETA is Monday October 9 at 19:00.

Monday October 9, 2017 (local)

We continued the transit at about 9.5-10.5 knots. We reached our dive position about 17:00 (21:00 GMT) and were in the water at 18:15 (22:15 GMT) starting J2-1024.

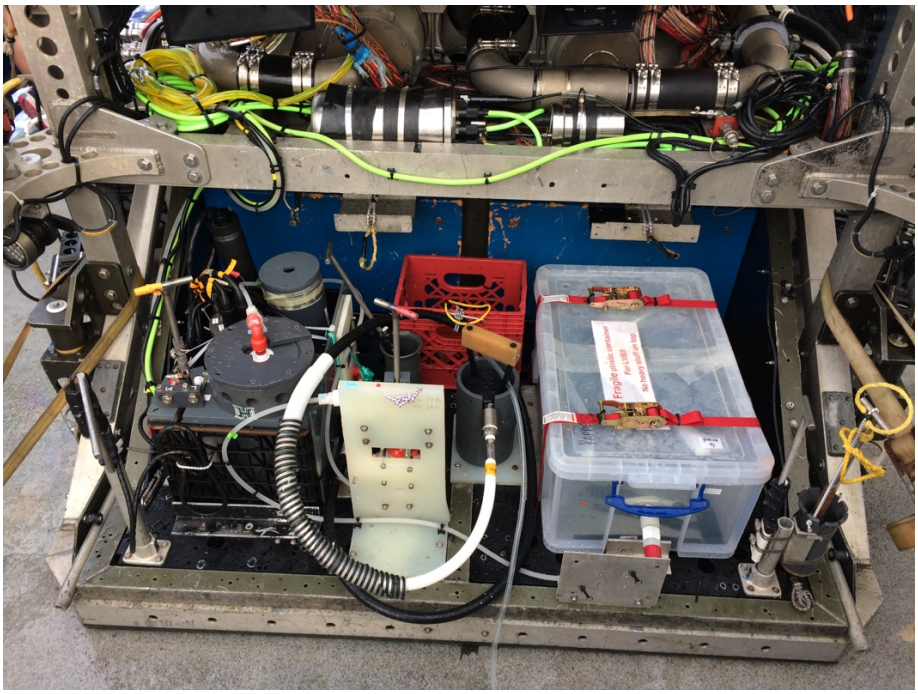
J2-1024 operations

U1383B *Jason II* conducted 360 of wellhead. All valves remain as expected. A little more white mater on the Jannasch handle for the pressure sensor. The pressure valve was removed and the valve closed (1:43 GMT).

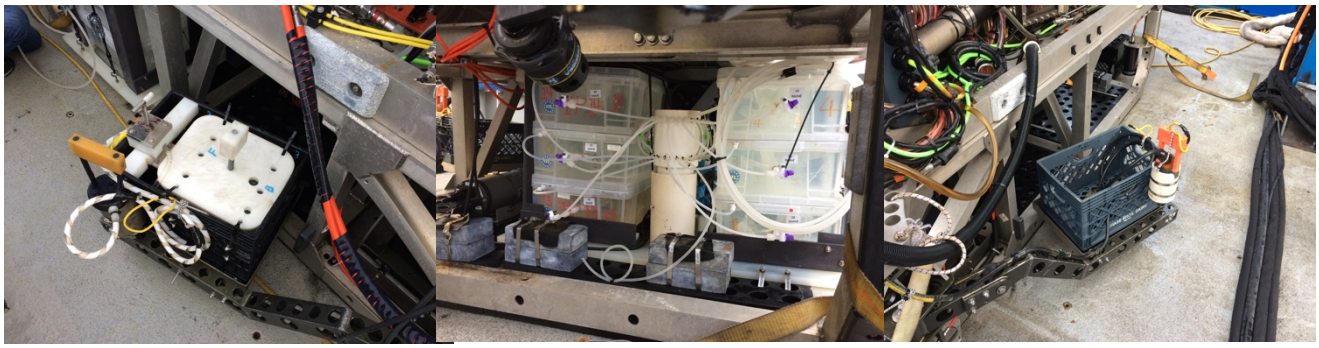
U1383C *Jason II* conducted a 360 survey of the wellhead. All valves remain as expected. There is sediment in the top of the top plug. There is no indication of flow from the OsmoSamplers. Each of the three pressure valves were turned to hydrostatic.

U1383B The Cs delivery system was attached to the wellhead via a Jannasch valve and the CsCl solution was injected over a period of about 15 minutes. The amount of Cs solution that was delivered was approximately 3.9 gallons (~15.6 L). The total bag volume was 5.3 gallons (21 L). The bag was purchased from REI. The Cs solution included 6 kg of CsCl diluted in 4 gallons of water. Then two containers of table salt were added (each 737 g [1 lb. 10 oz.]) and the bag filled with water (5.3 gallons [21 L]). This solution is slightly over saturated. The net CsCl+NaCl solution is 352 ppt (CsCl alone is 283 ppt). The Cs concentration in the solution that was injected was 1.6 mol Cs/kg. Given a total borehole volume of 11,254 kg, the Cs concentration in the borehole if well mixed would be 2.3 mmol Cs/kg. Seawater has a Cs concentration of about 2.1 nmol/kg, six orders of magnitude lower. The Cs delivery system was put away. The port manipulator hooked up the ODI pressure connector; however, an oil leak occurred in the port manipulator. This aborted the dive. The dive was aborted around 23:00 (2:00 GMT) and Jason II began its ascent. *Jason II* was on deck around 1:30 October 10 (5:30 GMT).

There were three problems with the vehicle. The oil leak in the manipulator was a result of a bolt that was not tightened. The cable to the vehicle was “un-winding”. The cable was taken off the vehicle and spun 4 times. The cable was then taped and armored. Lastly the crane “relaxed” during the dive. The Chief Engineer was going to take a look at the locking valve on the crane. Note that the crane relaxing was a problem throughout the expedition.



Front of basket (Dives J2-1024 thru 1028).



Starboard swing arm with Cs delivery system (J2-1024), back of Jason II with water sampling capabilities (J2-1024 thru 1029), and port swing arm with ODI connector for pressure downloads (J2-1024 thru 1029).

Tuesday October 10, 2017 (local)

J2-1025 operations

Jason II was in the water at 10:25 (14:25 GMT).

U1383C Valves in the pressure bay were left open during the previous dive and were turned clockwise 180 degrees to close.

U1383B Pressure data were downloaded and the intake was left on the ROV platform. Initial assessment of the data shows that U1383B is UNDER-pressured by a kPa or two.

U1383C Pressure data were downloaded. An initial assessment of the data shows that all ports at U1383C are OVER-pressured. A hydraulic leak in the port manipulator was observed. This leak occurred at the joint. This aborted the dive and *Jason I*I was off bottom an on the ship shortly after 18:00 (00:00 GMT).

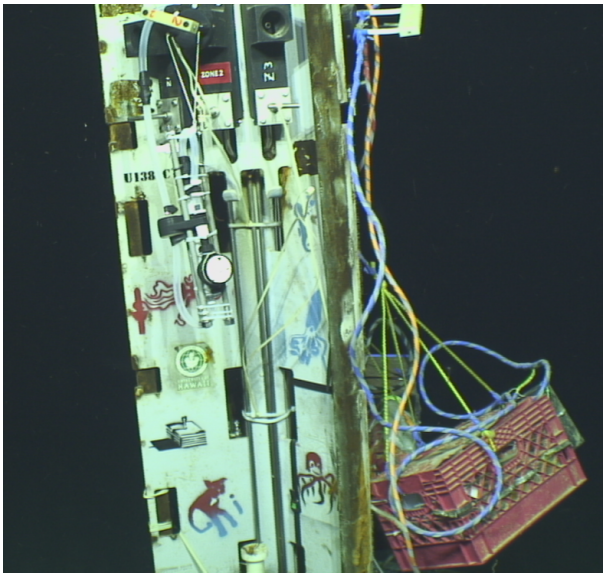
Once on deck the port manipulator was removed and a brand-new manipulator was put in its place.

J2-1026 operations

Jason II was in the water ~21:00 (1:00 GMT) and on bottom at 0:15 (4:25 GMT)

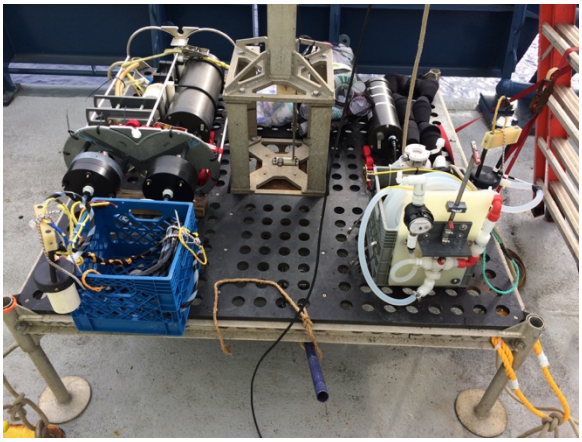
Wednesday October 11, 2017 (local)

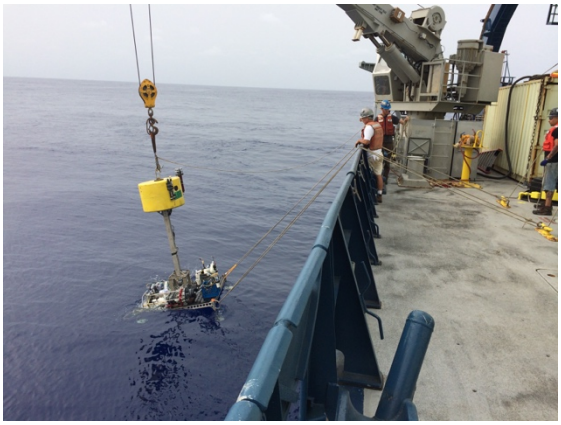
U1383C The two bolts at the top of the wellhead were turned clockwise using the nut driver. One turned 3 revolutions to a stop (orange line pained on the wellhead). The other turned 3.5 revolutions to a stop (no line or paint). We looked at the mechanical drawings for the bolts. The bolts were cut to a specific length. If the top plug cannot be removed, then we will release the nuts one turn. We connected the mobile pumping system (MPS) to the wellhead using a Jannasch handle in MBIO bay zone 1 (deep) lower valve. The umbilical was purged for 30 minutes before filling the large bag, the other bags, and filters. Lastly, Grieg's passive flow sampler was put on the wellhead at the end of the dive in the middle zone (MBIO bay zone 2) after first being attempted to be placed in zone 1 to check flow rates. However, the flow meters on Grieg's samplers did not work. Nevertheless, the sampler was left on zone 2 (lower) valve. It appears as though the dissolved oxygen is about 200 micromolar in the deep zone but there is some change with time and values that dipped lower. The large volume bag sampler may have captured fluids that were still in the umbilical. The rest of the samplers should be pristine formation fluids. We will have to look at the dissolve oxygen record. It also is taking longer to pump that expected (about twice the amount of time). The dive was ended as planned at 8:00 (12:00 GMT).



Grieg's passive sampler was deployed on U1383C in MBIO bay zone 2 on the lower valve. The red basket is an OsmoSampler that was deployed in 2014 and will be recovered on a later dive.

At the end of J2-1026, an elevator was deployed at 15:00 (19 GMT). We placed a WHOI LVP McLane sampler (100 lbs, borrowed by Sunita Shah) and Grieg's sampler (45 lbs) on the elevator. We also placed four bags of cups in various bags. The elevator was dropped at U1382A and landed about 100 m from the wellhead.





The elevator was deployed with a McLane sampler (left) and Grieg's sampler (right) at 1382A. The elevator used a foam float. The elevator ahs a capacity of about 170 lbs. The float has a buoyancy of about 350 lbs but no one was sure of this number. The elevator sank at a rate of about 30 meters per minute.

J2-1027 operations

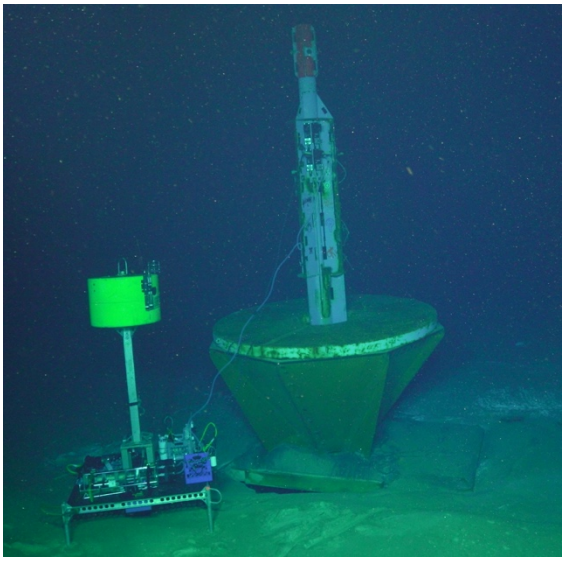
U1382A *Jason II* was in the water at 16:00 (20:00 GMT) heading for 1382A. The first operation was to turn the pressure valve to the formation.

395A Jason II surveyed the re-entry cone, the 5-inch pipe at the bottom and umbilicals that remain in the base of the re-entry cone. It appears that a heading of 287 is about the best heading to get a fishing tool down the hole. From this heading you can see the ROV platform in the sediment

U1382A Back at 1382A the pressure valve was returned to the formation. The bolts on the top of the CORK were turned clockwise (to tighten) 3.25 and 3.75 turns with the manipulator (not the nut driver). Pressure data were downloaded. The elevator was retrieved. The elevator was about 100 m from the wellhead and was positioned next to the CORK.

Thursday October 12, 2017 (local)

U1382A Pressure data were downloaded. Water sampling began with a 30-minute purge of MBIO bay Zone 2 lower port followed by filling up the large bag, the bags in the back and the filters (ended at 06:00 [10:00 GMT]). The zone 2 lower port was closed and the zone 1 lower port was opened. The Jannasch handle was moved to zone 1 and the line was pumped for 30 minutes. The valve was closed. The elevator was adjusted slightly to place the samplers closer to the wellhead. The elevator McLane sampler was connected to the zone 2 lower port (25-foot length of tubing). Grieg's handle was connected to the zone 1 lower valve (20-foot length of tubing). This operation ended at 7:40 (11:40 GMT). We surveyed Grieg's sampler to see if there was detectable flow. The flow meter on Grieg's system did not show any passive flow through the filters. The dive ended. *Jason II* was recovered around 12:00 (16:00 GMT).



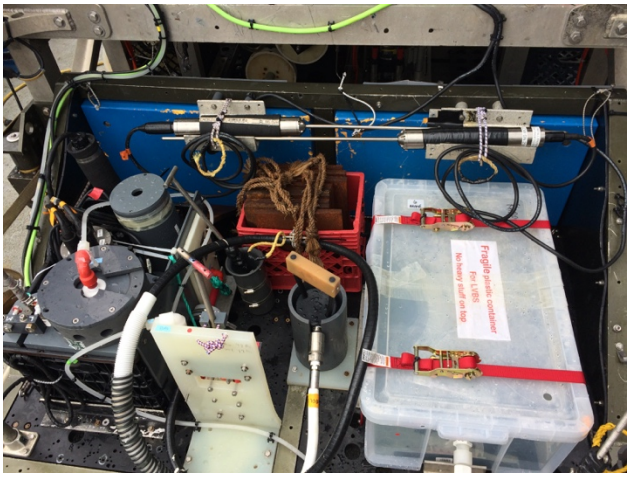
The elevator was positioned next to the wellhead (U1382A) with both sample ports attached to the wellhead.

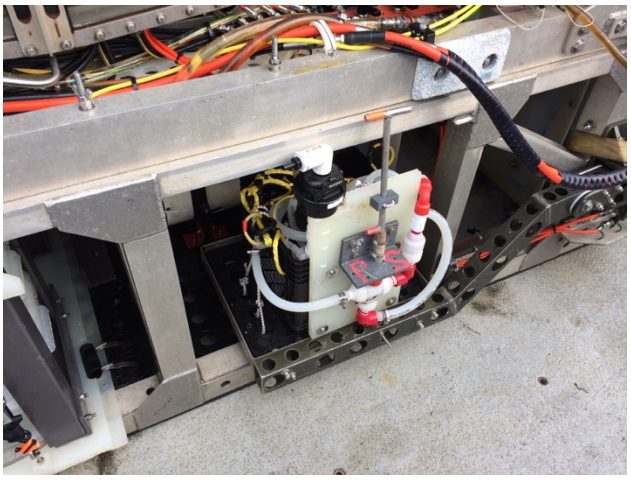
J2-1028 operations

U1383C This dive was dedicated to the collection of water, the deployment of one of Grieg's passive flow samplers, and testing a new heat flow probe. The dive started at 16:00 (20:00 GMT). On the seafloor the first operation was to purge MBIO Bay zone 1 (deep horizon) lower valve for 30 minutes at ~2l/min. The valve was closed, then one of Grieg's samplers (in a milk crate) was connected and the valve was opened. Next, the fluid sampler was attached to the zone 3 (shallow horizon) lower valve and purged for 30 minutes at 2 l/min. The large volume bag, smaller bags, and filters were filled as prescribed.

Friday October 13, 2017 (local)

U1383C After the last filter we pulled in bottom water over the dissolved oxygen probe to measure the concentration in bottom seawater. It took almost an hour for it to reach an asymptote. The DO was ~214 micromolar in zone 3 and ~273 in bottom water. The DO measured with the Jason DO probe in bottom water was 280 (or so). The pumps were turned off, we drove about 20 m, and we began a test of the two heat flow probes, a new one from WHOI (9 thermistors) and an old ALVIN-style (5 thermistors) heat flow probe from UCSC. Both were inserted multiple times about 1 meter apart. Responses were slightly different due to the construction. Further analysis will assess the usefulness of the new system. *Jason II* was recovered at 12:00 (16:00 GMT). At some point during the dive we noted "water" (which turned out to be oil) in the 4K camera. The camera was immediately turned off and the pan and tilt was secured facing downward so that the "water "would not affect the electronics. At the surface it was determined that oil had leaked in from the connector.





The front of the basket included two heat flow probes (J2-1028 and 1029). One probe was a new prototype and the other was a standard "ALVIN heat flow probe" from UCSC (Tess). Grieg added a passive flow filter and flow totalizer to the starboard swing arm that was placed in the deep zone (1) of U1383C.

After recovery of *Jason II* the two plates that secure the *Jason II* to the winch were compromised. Several of the bolts were sheared off. The *Jason II* team fixed the plate, delaying the launch for 3 hours.

At 16:00 (20:00 GMT) *Jason II* was put into the water for dive J2-1029. As soon as they turned on the power there was a minor ground fault in the mobile pumping system, then a major ground fault in the system. The vehicle was recovered. All of the connectors for the MPS were checked and the major ground fault failure was determined to be a board in the detection system. This board was replaced. The vehicle was powered up and monitored for 30 minutes to assess additional ground fault potential. At 20:00 (0:00 GMT) *Jason II* was lowered into the water and floats were attached. A hydraulic leak in Jason's crane was detected and recovery operations occurred. This little leak turned into a river during recovery. The *Atlantis* crew did a great job in containing the oil and fixing the crane.

Saturday October 14, 2017 (local)

The slip ring in the crane malfunctioned. The solution was to bypass the slip ring. To bypass the slip ring the cable to the vehicle had to be terminated and reconnected after a pull test.

While the crane and the tether were worked on, the ship's crew conducted a test of the CTD's level wind by sending the CTD to ~4000 m and running the system up and down. The test started around 8:00 (12:00 GMT) and ended around 11:10 (15:10 GMT). Then we went back down to ~9 m off the bottom and conducted the first science CTD (CTD 1). Bottles were tripped at multiple depths. The CTD was on deck ~14:30 (19:00 GMT).

At 16:00 (20:00 GMT) we started to put *Jason II* over the side. *Jason II* got over the side, then there was a ground fault and one of the valves in the MPS was disconnected. *Jason II* was placed back on the ship. The water sampler was fixed by drilling a hole in the box. The ground fault detector was fixed by changing out a board, again. At 17:30 (21:30 GMT) they began a 30-minute test with a new board in the rack.

J2-1029 operations

U1383C This dive was dedicated to the collection of water. The dive started at 19:30 (23:30 GMT). The dive started by turning valves to close off existing passive samplers on the wellhead so that they would not be impacted by pumping operations. The large volume bag sampler was filled after purging the deep horizon through the MBIO Bay upper valve in zone 1 (deep).

Sunday October 15, 2017 (local)

U1383C Then the sample intake was moved to zone 2 (middle) prior to closing the valve to zone 1 upper valve. The valve in zone 1 lower was opened (clockwise) to Grieg's passive sampler. The smaller bags were filled after purging zone 2 (medium) upper valve. The pumps were then directed to the filters. After sampling the upper valve in zone 2 (middle) was closed and the lower valve was opened. The gauge on the passive sampler that is

attached to zone 1 lower valve showed a flow of 2299 (last two digits are in the red) and it was turning to 2300 as we watched it for 20 seconds. A single dive weight and a double dive weight were placed on the ROV platform. Now there are two single and one double dive weights on the platform. The dive ended around 8:00 (12:00 GMT).

During the morning and early afternoon the crew swapped the sleds. They took off the sampling sled and attached the pulling sled. It took 20 minutes to take off the old sled and place it on the deck and 20 minutes to move the new sled into place and lower *Jason II* on top of the sled. The time in the swap was a result of connecting and disconnecting science systems.

J2-1030 operations

U1383C Operations began with the deployment of Jason II at 20:00 (0:00 GMT). On the seafloor we closed the two valves that contained Grieg's samples.

Monday October 16, 2017 (local)

U1383C We tried to recover an OsmoSampler but the line was too long and decided that it would be best to be recover it with an elevator. The top plug was cleaned out with the brush. There was a lot of material in the top plug. The 2-inch RS pulling tool (14 lbs in water with shackles and a pair ring) was installed. There was a weak link that would break at 5800 lbs. An attempt was made to pull the top plug out with the manipulators to show that the latch was loose, but that didn't work. We hooked up the vehicle and used the vehicle thrust to pull the top plug out. We sat in auto XY, heading, and depth while the ship moved over the vehicle, then began to pull slowly out of the well. There was only one tension spike during the pullout but never above the maximum tension. Once out of the wellhead the vehicle was stopped and allowed to spin for 2 hours. The vehicle then continued to the surface and arrived on deck at 4:30 (8:30 GMT). While we pulled up the string we got to the first dissolved oxygen sensor. It was missing, as was everything else below. It looks like the metal corroded and the weakest part was the frame around the dissolved oxygen sensor. The downhole string was on board by 5:30 (9:30 GMT). In summary, the OsmoSamplers (OS) collected from Hole U1383C were the Acid, BOSS, Standard, MBIO and Enrich OS from the upper horizon only, and we lost the upper section Gas OS and the entire sets from the middle and lower horizons. Of special note, coil 3 of 1383C upper standard OsmoSampler was backwards with respect to intake and outflow.

The top end of the "cage" that surrounded the RBR dissolved oxygen sensor.

Table 1. Hole U1383C OsmoSampler Recovery Pump Information

Name	# of Membranes	Salinity in H2O Reservoir (per mil)
Acid Sample Pump	8	10
Acid Delivery Pump	1	12
BOSS Sample Pump	8	0
BOSS Delivery Pump	1	0
Enrich Sample Pump	8	27
Enrich delivery pump	1	3
MBIO top pump	8	24
MBIO Lower Pump	8	40
Standard Sample Pump	8	15

We recovered one miniature temperature recorder with this system.

The failure of recovering the entire instrument string means that in all likelihood the two deep landing seats are in their original locations, closing off the middle and lower sections. What remains in the upper section of the borehole (from the plug upwards) is 35.5 m of spectra rope, a copper OsmoSampler (3 coils 2.63 m long) and the actual RBR probe. These materials are likely just above the landing seat at 145.7 mbsf. Thus when one removes the top plug the top interval of the borehole is accessed via the perforations in the casing. This depth interval is similar to that at Hole U1383B.

Upon recovery, the umbilical on Jason II was unraveled. There were some strands that were broken. The tether was re-terminated and tested. Effort was directed to deploying Medea at the end of the tether. However, after further consideration and multiple hours this idea was canceled because of the difficulty in getting the system in the water. The weather also picked up so we did not dive. Instead we conducted the second hydrocast. The CTD started at 13:00 (17:00 GMT) and ended at 16:15 (20:15 GMT). The CTD was a complete hydrocast with bottles tripped at multiple depths for Kristin.

Tuesday October 17, 2017 (local)

We started the day waiting on the weather. The weather forecast was calling for winds off ~20 knots and seas of 7-9. The seas are a bit confused. We conducted our third CTD (for Grieg) starting at 13:00 (17:00 GMT) and finished at 16:15 (20:15 GMT). We looked at the weather before dinner but it was still too poor to dive. Instead we conducted our fourth CTD. The CTD started at 18:30 (22:30 GMT) and ended about 3.5 hours later. This hydrocast was for Kristin.

Wednesday October 18, 2017 (local)

We waited on weather. The winds died but the swell remained although the weather seemed fine. At 13:30 (17:30 GMT) we conducted our fifth hydrocast. This hydrocast provided water for Kristin and Ben.

Thursday October 19, 2017 (local)

At 5:00 (9:00 GMT) the decision was made to dive.

J2-1031 operations

U1383C Operations began with the deployment of the flow meter in the top of the CORK by 10:15 (14:15 GMT). *Jason II* then turned the valves to Grieg's two experiments in the MBIO bay to the open position. The flow totalizer on the system attached to zone 1 (deep) read 2287, less than the last reading of 2299. This is interesting because the valve to the umbilical has been closed. Around 10:25 we began the 6-kilometer trek to hole 395A.

395A We arrived at Hole 395A around 17:30 (21:30 GMT). All of the pilots looked at the problem and discussed the issues of getting the fishing tool into the 5-inch casing. We then went to U1382A.

U1382A First *Jason II* uncoupled the two connectors attached to the Shah McLane and Grieg's pumping systems on the elevator. The valves were closed and the elevator was released. The elevator reached the surface at 20:45 (0:45 GMT). The elevator was retrieved at 21:25 (1:25 GMT) and the vehicle returned to Hole U1382A. The top plug was clean (no brushing required). We latched in with the pulling tool and began pullout around 23:00 (3:00 GMT). Jason II was stopped at 4100 m and allowed to spin freely.

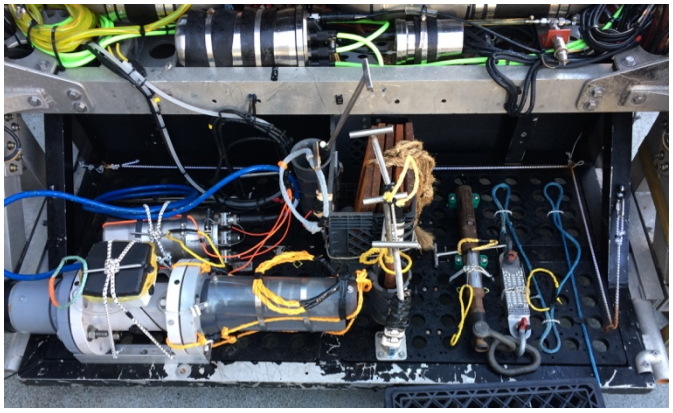
Friday October 20, 2017 (local)

U1382A The recovery of Jason II began at 4:00 (8:00 GMT) and was on deck after a dead vehicle recovery. The issue was a massive tether failure just above the first float. This was by far the most mangled the cable has been. This is of concern. When the vehicle was allowed to rotate at depth it turned 33 times. Then at 450 m the vehicle was allowed to rotate and it rotated about 11 times in the opposite direction. In the end it was a massive mess. The downhole string also was a mess. The landing seat plug, which is different from the top plug, was recovered without an o-ring. After the landing seat plug was a length of spectra and the first OsmoSampler (MBIO). The next OsmoSampler was an Enrichment OS; however, the ½" stainless steel rod that was down the middle of the sampler was completely corroded. This means that all of the materials below the pump on the enrichment experiment is at the bottom of the hole in the fill. The casing should be open all the way to open hole.

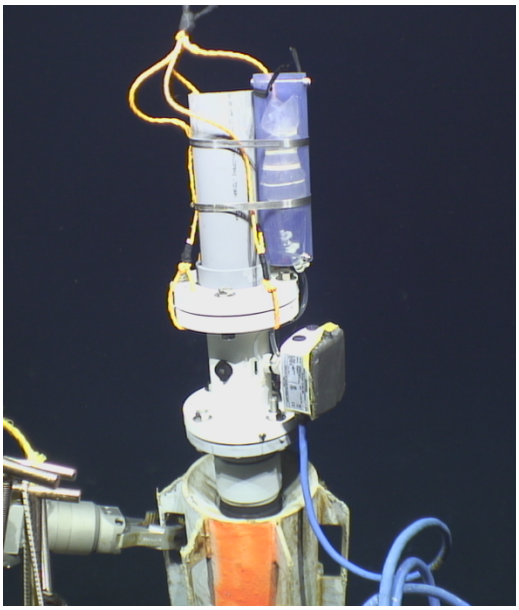
Table 2. Hole U138A OsmoSampler Recovery Pump Information

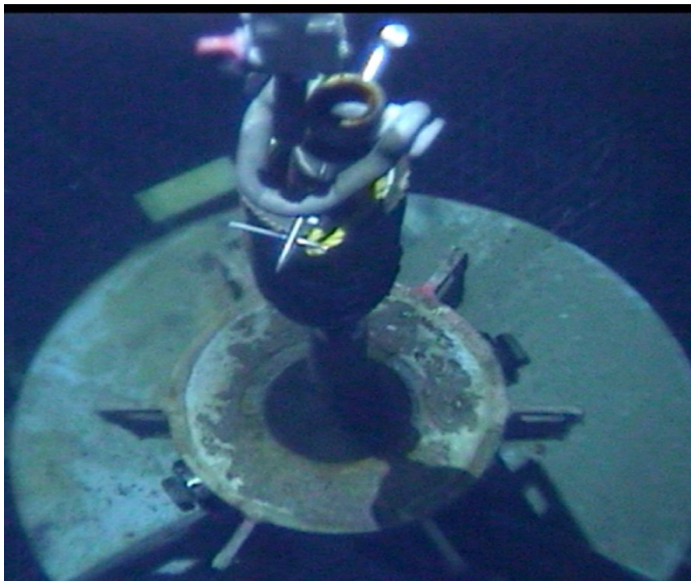
Name	# of Membranes	Salinity in H2O Reservoir (per mil)
MBIO Top Sample Pump	8	0
MBIO Lower Sample Pump	8	0

But wait, there is more bad news. It appears that Grieg's system that was attached to the umbilical on U1382A pumped for 12 seconds and failed. Kristin's pumps that were attached to the umbilical on U1382A worked. The internal pump registered 3600 L but we know this is an overestimate because the system does not pump as recorded at depth. Thus, this is an overestimate. The pump also failed before the 24-hour operational period. Another problem is that other gauges recorded two different values that didn't add to the total flow meter. One filtered ripped, but there was material on the filters. On the positive side, we recovered a miniature temperature recorder that was part of the MBIO OsmoSampler.



The front basket of Dive J2-1031 with the flow meter, pulling tool and weak link.



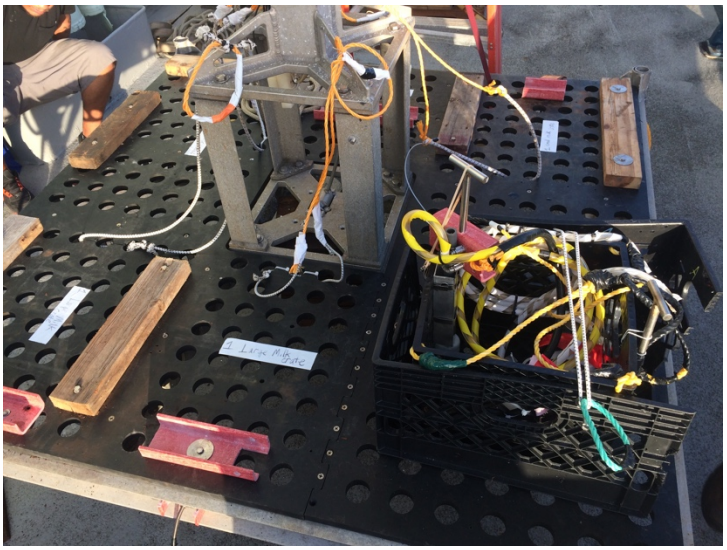


The flow meter was position in the top of U1383C. The top plug is removed from the borehole during retrieval.

This 0.5-inch stainless steel rod corroded through. It was the top of the Enrichment OsmoSampler.

Shortly after 6:15 (10:15 GMT) we conducted our sixth CTD. This deep hydrocast provided water for Grieg, Kristin and Ben.

A short-term OsmoSampler using an RO CA (47 mm) membrane was fabricated with a single Teflon coil. It was placed on the elevator along with space for all of the items on the wellhead at U1383C. The elevator was launched at 16:00 (20:00 GMT).



Elevator with RO, short-term OsmoSampler in a black milk crate.

J2-1032 operations

U1383C The pulling sled basket was modified from the last dive to include a large milk crate with 4 squeezers. After landing and moving the elevator close to the wellhead, we closed valves on the wellhead and transferred the RO, short-term OsmoSampler to the wellhead and four OsmoSampler packages in milk crates and two of Grieg's passive flow samplers to the elevator. The elevator was sent to the surface while *Jason II* remained at depth.

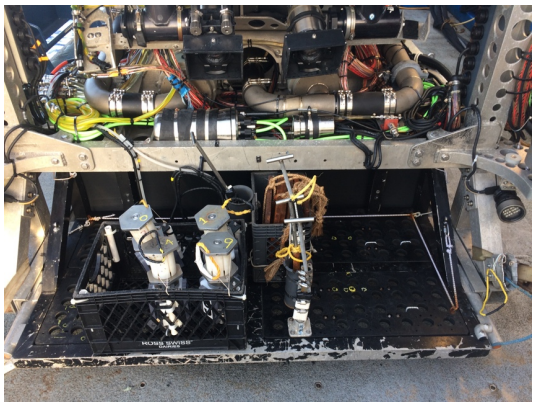
Saturday October 21, 2017 (local)

At 0:25 (4:25 GMT) the elevator was on deck. The OsmoSampler packages and Grieg's samplers were removed. The elevator was then dismantled and made into a system of two floats and a weight stack. The floats had a buoyancy of ~750 lbs. The float package was launched near U1383B.

U1383B At 4:00 (8:00 GMT) Jason was recovering from a squall with 40-knot winds that moved the ship. The float package was acquired and moved next to the wellhead.

U1383C Jason II moved to U1383C to test the squeezer samplers. None of the three fired. We did not attempt the fourth. The RO OsmoSampler was deployed by moving it from the ROV platform to the wellhead. The intake was placed within the flow meter. The end of the intake is a ½ PVC tube. It should not influence the flow meter.

U1383B The top cap of the wellhead was spun 12 times counter clockwise. The hook was removed from the float stack and attached to the top of the cap. The weights were cut and the floats took off. We watched the wellhead and saw the OsmoSampler package get pulled from the borehole. The ascent of the float-OsmoSampler Package is about 60 m/min and arrived on the surface around 7:15 (11:15 GMT). Jason II was recovered then the ship was maneuvered to recover the float-OsmoSampler Package. Some issues were noted with respect to the tether upon recovery. The upper float with the instrument string was recovered around 9:00 (13:00 GMT); however, only the top float was recovered. The remainder was gone. The rope that connected the two floats was frayed by rubbing at the bottom of the float where the rope emerged. Thus the sampler and another float reside on the seafloor.



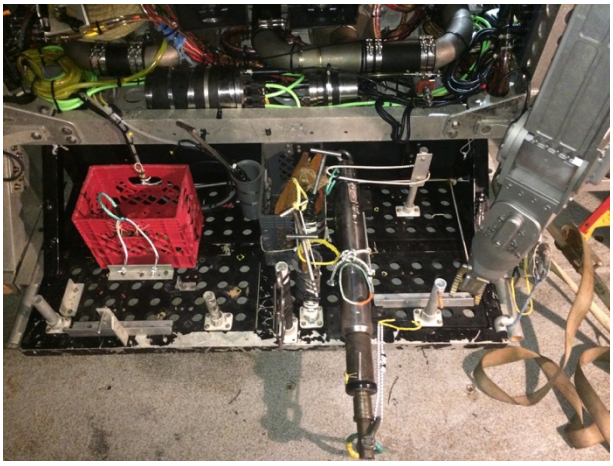
The front of the basket prior to dive J2-1032.

Table 3. Osmotic Pump Information for Wellhead OsmoSamplers.

OsmoSampler package	# membranes	Salinity in H2O Reservoir (per mil)
U1382A wellhead green handle (zone 1 shallow)		
Orcutt FLOCS sample pump	13	45
JP FLOCS sample pump	13	35
Acid sample pump	13	71
Acid delivery pump	2	5
U1383C wellhead blue handle (zone 1 deep)		
Orcutt FLOCS sample pump	13	35
JP FLOCS sample pump	13	33
Acid sample pump	13	46
Acid delivery pump	2	6
U1383C wellhead black handle (zone 2 middle)		
Orcutt FLOCS sample pump	12	28
JP FLOCS sample pump	12	33
Acid sample pump	13	24
Acid delivery pump	2	11
U1383C wellhead red handle (zone 3 shallow)		
Orcutt FLOCS sample pump	10	100+
JP FLOCS sample pump	10	100+
Acid sample pump	12	100+
Acid delivery pump	2	0
U1383C wellhead orange handle (zone 1 deep)		
JP FLOCS bottom water sample pump	12	33
JP FLOCS sample pump	13	100+
Standard sample pump	8	15

Prior to J2-1033 the Jason II group removed the tether and attached it to a 300-lb. The tether was lowered to 4400 m and recovered.

J2-1033 operations

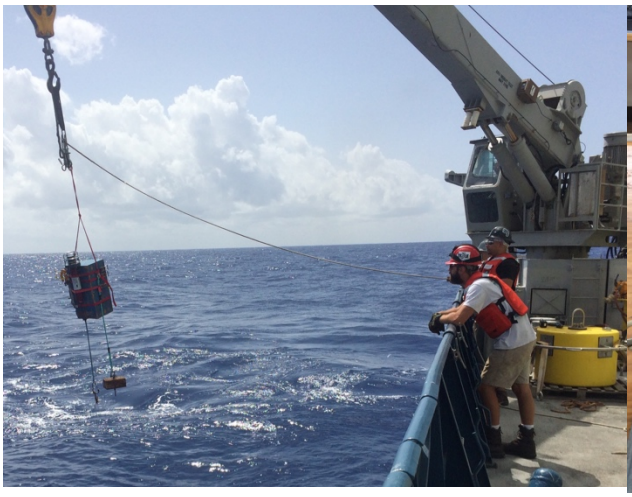


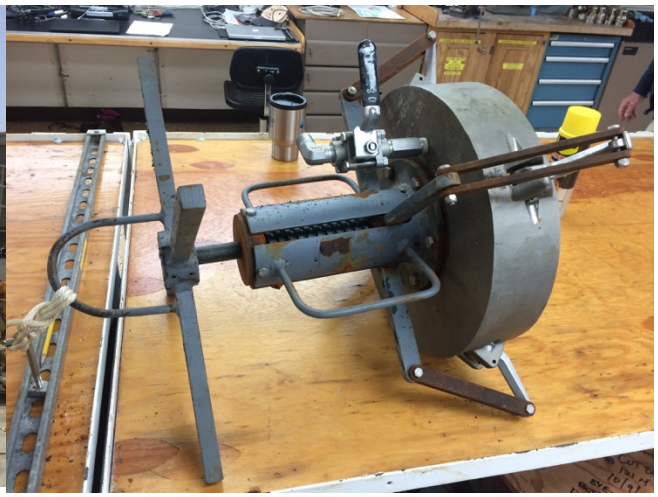
The front of the basket prior to dive J2-1033. It includes the top plug for 1383C and an Otis running tool.

U1383C The dive started at U1383C to recover all of the remaining instruments and to seal the wellhead. Jason II was in the water at 20:15 (0:15) and began the descent. Once on the bottom the bolts were loosen by turning them counter clockwise 3.5 times, the fast-flow OsmoSampler was recovered, and the flow meter was recovered.

Sunday October 22, 2017 (local)

U1383C Lastly the top plug was inserted and the running tool was retrieved. *Jason II* then began a survey starting at the site where the float first arrived on the surface to the location where it was retrieved. Jason II then turned 180 degrees and headed back. The path back was offset 100 m to the north. Near the end of the line (~100 m north of the location where the float surfaced) the OsmoSampler, sinker bar, and float were observed. A survey showed that the line to the OsmoSampler directly under the float was chafed. This caused concern. We made up a float package with a transponder to by-pass this line and provide a little more buoyancy so that as the package floated to the surface it would make it at a reasonable rate. The float package consisted of two float blocks, a transponder, weights (6 ALVIN weights [~15.5 lbs each]), and two hooks. The float package was released around 10:40 (14:40 GMT). At the seafloor the float pack was attached to the downhole package and released from the seafloor at 13:15 (17:15 GMT). The sampler and top hat were almost perfectly balanced by the float pack and extra flotation (~300 for the float pack and 50 for the extra flotation) once the sinker bar at the bottom of the instrument string was cut off with a knife. This conclusion is because even with the added float pack (with weights released) the system was not buoyant. Jason II was recovered around 16:00 (20 GMT) and the floats and OsmoSampler package from U1383B was recovered about 17:15 (21:15 GMT) after a very nice sunset and with all sampling systems intact. In addition to this good luck, the flow meter worked and recorded data.





The float package is sent over the side to rescue the downhole sampler from U1383B. The cap on the CORK-Lite is cleaned and ready for deployment. The cap weighs 84 lbs in air.

Table 4. Hole U1383B CORK-Lite OsmoSampler Recovery Pump Information

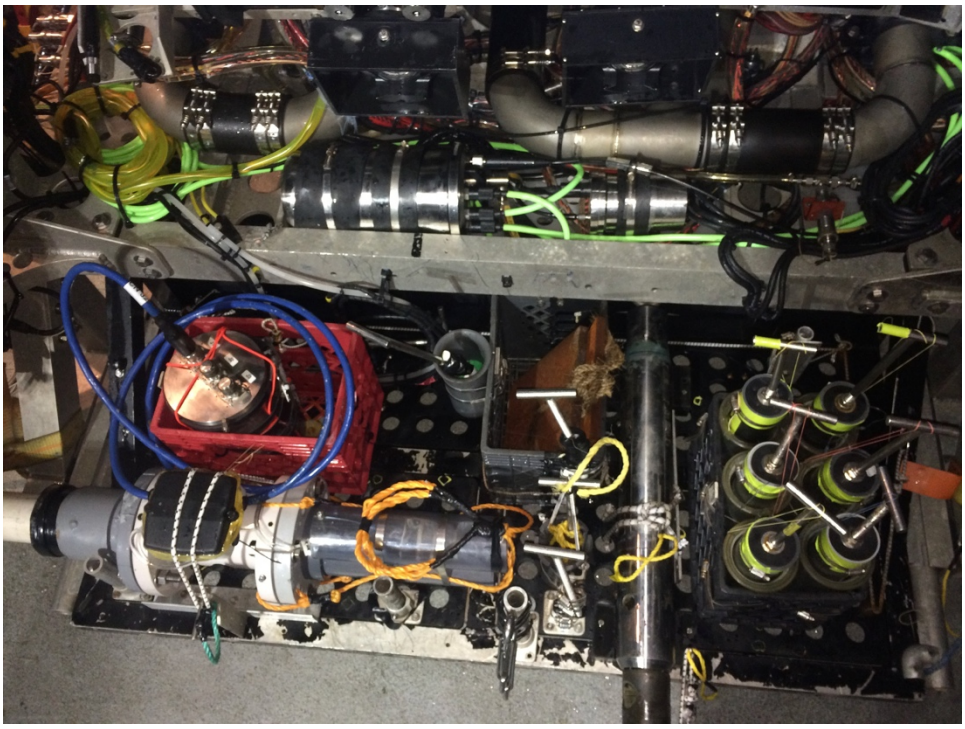
OsmoSampler package	# membranes	Salinity in H2O Reservoir (per mil)
Acid sample pump	8	0
Acid delivery pump	2	0
BOSS sample pump	8	0
BOSS delivery pump	2	0
Enrich sample pump	8	0
Enrich delivery pump	2	0
gas sample pump	8	0
MBIO 1 sample pump	8	0
MBIO 2 sample pump	8	0
Standard sample pump	8	0

J2-1034 operations

1382A Jason II was deployed at 20:00 (0:00 GMT). Once on the bottom the bolts were loosen 3.5 turns and the flow meter was deployed in the top of the CORK at 23:25 (3:15 GMT). The sampler was set to start at 0:00.

Monday October 23, 2017 (local)

1382A At 3:00 (7:00 GMT) the flow meter was removed. The top plug and Otis tool were deployed and the Otis tool was removed and placed in the red milk crate along with the pull pin. The valve in the Chem bay with the Green OsmoSampler was closed. A survey of all of the valves indicated that all valves were closed. The OsmoSampler was removed and *Jason II* did a 360 view of the wellhead looking at the valves in each bay. At 3:45 (7:45 GMT) we headed north 100 meters to collect push cores. Along the way (about 50 meters from U1382A we came across a hole from previous drilling. We settled another 50 m north and collected 6 push cores. The vehicle was retrieved to be on deck at 8:20 (12:20 GMT).



Jason II basket prior to dive J2-1034.

We started our seventh CTD at 9:00 (13:00 GMT). The sled swap started with some work prior to moving the sleds at 10:30. The operation of moving the sleds was completed before lunch. Then the Jason II group began connecting components of the sample sled.

J2-1035 operations

U1383B Jason II was launched around 20:00 (0:00 GMT). The first operation was to attach the cap on U1383B. Then the valve on the cap was turned 12 times clockwise to close. The valve on the cap was closed (from vertical to horizontal – a clockwise move). Then the intake for the pressure sensor was inserted and the valve on the Jannasch connector opened. Finally there was a 360-degree survey of the well head to make sure all of the

valves were closed with the exception of the one that was attached to the pressure port. Jason II then moved to U1383C.

U1383C After arriving at U1383C the connector to the UH fluid system was inserted into MBIO bay zone 1 lower valve. The valve was opened and the line was purged before taking samples. Samples were collected from each of the three horizons after purging the line at each horizon. Once fluid sampling was completed (~10:30 (14:30 GMT) *Jason II* conducted a 360-degree survey to confirm that all of the valves in the MBIO and Chem bays were closed and the pressure valves were open to the formation. After the survey, *Jason II* started the move to U1382A, 6 kilometers away, requiring about 7 hours to reach.

The front porch prior to dive J2-1035.

Tuesday October 24, 2017 (local)

U1382A *Jason II* reached the wellhead around17:45 (21:45 GMT) and opened the MBIO bay lower valve to zone 1. The umbilical was purged collecting two water samples (small bags) and one filter. Upon completion the vehicle was recovered. During the ascent the UH pump and dissolved oxygen probe were left on to acquire data that would be compare with the data to the sensor on *Jason II*. Note that there was an offset in the bottom water readings (270 micromolar for the UH system and 280 micromolar for the *Jason II* system). This offset appears to be consistent with other dives.

Wednesday October 25, 2017 (local)

We started our eighth CTD at 0:00 (4:00 GMT), with bottles removed from the rosette to mount the Shah LVP McLane pumps. This was a 12-hour near the bottom CTD for Kristin. She set the start time for the McLane pumps to begin to filter bottom water at 2:00 (6:00 GMT). At the same time the Jason team began another re-termination of the tether. The fibers were connected

shortly after breakfast and a pull test was conducted after breakfast. Also after breakfast the basket was changed to focus on heat flow, push cores, and squeezers. Jason II was ready for operation later in the day but Tito decided that the weather was coming up and that he would delay operations at least until breakfast. Kristin's 12 hour CTD was retrieved around 16:00 (20:00 GMT). After dinner we conducted our ninth science CTD. This one had the flow meter on it and we used the CTD to calibrate the flow meter. The CTD was in the water at 18:35 (22:35 GMT) and out of the water around 20:35 (00:35 GMT).

Thursday October 26, 2017 (local)

The forecast was for high swell throughout the day. While it is a bit higher, it is by no means no dive weather. It would be an ALVIN day. However, Tito decided that we should sit this one out. Jason II is prepared with the exception of adding push cores and squeezers to the vehicle. Instead we conducted a test of the trawl winch in the morning. After lunch we started the Grieg CTD marathon. The first (tenth science CTD) was in the water around 13:00 (17:00 GMT). The second (eleventh science CTD) was in the water about 19:00 (23:00 GMT).

Friday October 27, 2017 (local)

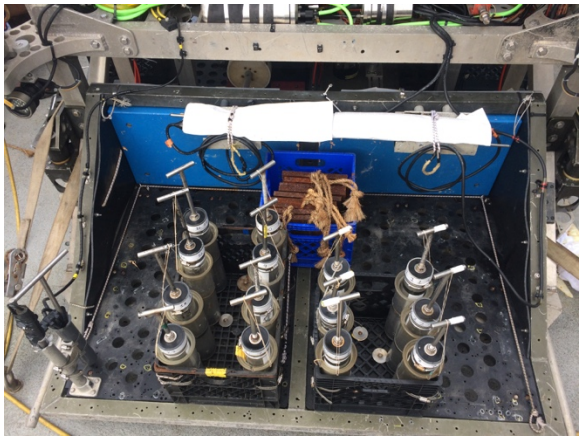
In the early hours of the night we conducted our twelfth CTD. We were on station at Marker BD to the north of Site U1384 by 6:00 and set for launch. It is a fabulous day with winds of 8 to 10 knots.

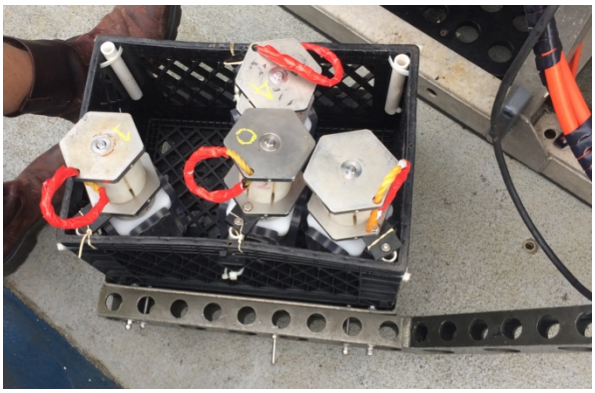
J2-1036 operations

High Heat flow site: The OK was given and Jason II was lowered over the side at 8:00 (12:00 GMT). We reached the bottom and navigated to the Marker BD that Korey made in 2012. It sits in a pile of rocks on a slope next to a chute. We conducted a variety of measurements. The vehicle heading was 170 during this work with the rock on the port side. We made measurements along the rocks about a meter from the rock-sediment interface. Three measurements were made with both probes. Then two measurements were made 5 and ~13 m to the starboard side of the vehicle by crabbing the vehicle and maintain the heading. At the furthest measurement, we collected three push cores (1-3) (background) with a thermal gradient of ~0.1 degree per meter, still higher than the basin below. We went back to the top of the chute for a measurement. Around 15:30 (19:30 GMT) we were back at the marker, deploying heat flow probes. The highest thermal gradient measured was about 0.8 degrees per meter. We collected three push cores (4-6) at this site. We then maneuvered down and around the outcrop and then back on top of the outcrop. We deployed a marker in honor of Akel's childrens' birthdays. By 19:00 (23:00 GMT) we began a survey to the southeast heading 130, stopping to make heat flow measurements as we went at places near rock outcrops. Surprisingly there were elevated thermal gradients along this ridge ~0.4 degrees per meter.

Saturday October 28, 2017 (local)

High Heat Flow Site: We located several outcrops and made measurements near them. We also found a v-shaped channel, which we sampled in two places. We made it to the bottom of the hill and made a heat flow measurement. Then we headed NNE 400 m, made a measurement and went uphill at a heading of 310. Along the way we made more heat flow measurements and collected several push cores for Grieg. We tested the squeezer samplers, two of the four triggered. The dive ended and we were on the deck at 11:00 (15:00 GMT) to begin the transit to Barbados.





The front basket and the starboard swing arm before dive J2-1036.

Sunday October 29, 2017 (local)

We continued the transit to Barbados and packed.

Monday October 30, 2017 (local)

We continued the transit to Barbados. Packing continued. Experiments were ongoing.

Tuesday October 31, 2017 (local)

We continued the transit to Barbados. Packing continued. Experiments were ongoing.

Wednesday November 1, 2017 (local)

We reached port and off-loaded samples and gear.

Table 5. Broad overview of operations completed during the North Pond Expedition in 2017 (AT39-01).

Operation	395A no wellhead	U1382A 1 horizon	U1383B CORK-Lite	U1383C 3 horizons	Discharge area
download pressure data	na	**YES**	**YES**	**YES**	na
recover downhole strings	**NO**	**YES**	**YES**	**YES**	na
sample fluids from umbilical	na	**YES**	na	**YES**	na
sample fluids from outflow	na	**NO**	na	**NO**	na
3-day fluid samplers	na	**NO**	na	**YES**	na
elevator	na	**YES**	na	**NO**	na
flow meter	na	**YES (1)**	na	**YES (1)**	na
Cs input - Gravity	na	na	**YES**	na	na
push cores	na	**YES**	na	na	**YES**
heat flow	na	**YES**	na	na	**YES**
Ship - CTD	na	**YES**	na	**YES**	na

Data, Laboratory, and Sample summaries

CORK Pressure Data Downloads

A primary objective of the NSF-funded Jason dives on AT39-01 was downloading long-term formation and seafloor pressure data from three CORKs in North Pond. These include two CORK-II hydrological observatories installed in fall 2011 in Holes U1382A and U1383C during IODP Expedition 336 (Edwards et al., 2012) and a "CORK-Lite" observatory installed in spring 2012 in Hole U1383B during MSM-20-5 (Wheat et al., 2012). All the downloads were conducted successfully, and the data are of excellent quality. In addition, hydrostatic calibrations were successfully conducted at all three CORK installations, to correct for slight offsets in the formation gauge calibrations. After the downloads, the memories of all three loggers were cleared, their clocks were reset to UTC, and their sampling intervals were changed from 2 min to 20 min because there are no concrete plans to revisit them in the future. Two weeks later during the cruise, second download was also conducted at Hole U1383C after conclusion of a flowmeter deployment.

Post-cruise processing of pressure data from all three CORKs will allow accurate determination of in-situ formation pressure state and response to tidal loading. Preliminary results confirm the indications from the data files downloaded in 2012 and 2014 that (1) all three holes are sealed and (2) that the formation zones sampled in Holes U1382A and U1383C are slightly positively pressured, whereas the shallower zone sampled in Hole U1383B is slightly negatively pressured (Becker et al., 2012).

References

Becker, K., Villinger, H.W., Davis, E.E., and IODP Exp. 336 Scientists, 2012, OS24B-02: Initial Pressure Data from the IODP Expedition 336 CORKs at North Pond, Fall 2012 AGU Meeting, EOS, Trans. AGU.

Edwards, K.E., Wheat, C.G., Orcutt, B.N., Hulme, S., Becker, K., Jannasch, H., Haddad, A., Pettigrew, T., Rhinehart, W., Grigar, K., Bach, W., Kirkwood, W., and Klaus, A., 2012, Design and deployment of borehole observatories and experiments during IODP Expedition 336, Mid-Atlantic Ridge flank at North Pond, in Edwards, K.J., Bach, W., Klaus, A., and the Expedition 336 Scientists, Proc. IODP, 336: Tokyo (IODP-MI), doi: 10.2204/iodp.proc.336.109.2012.

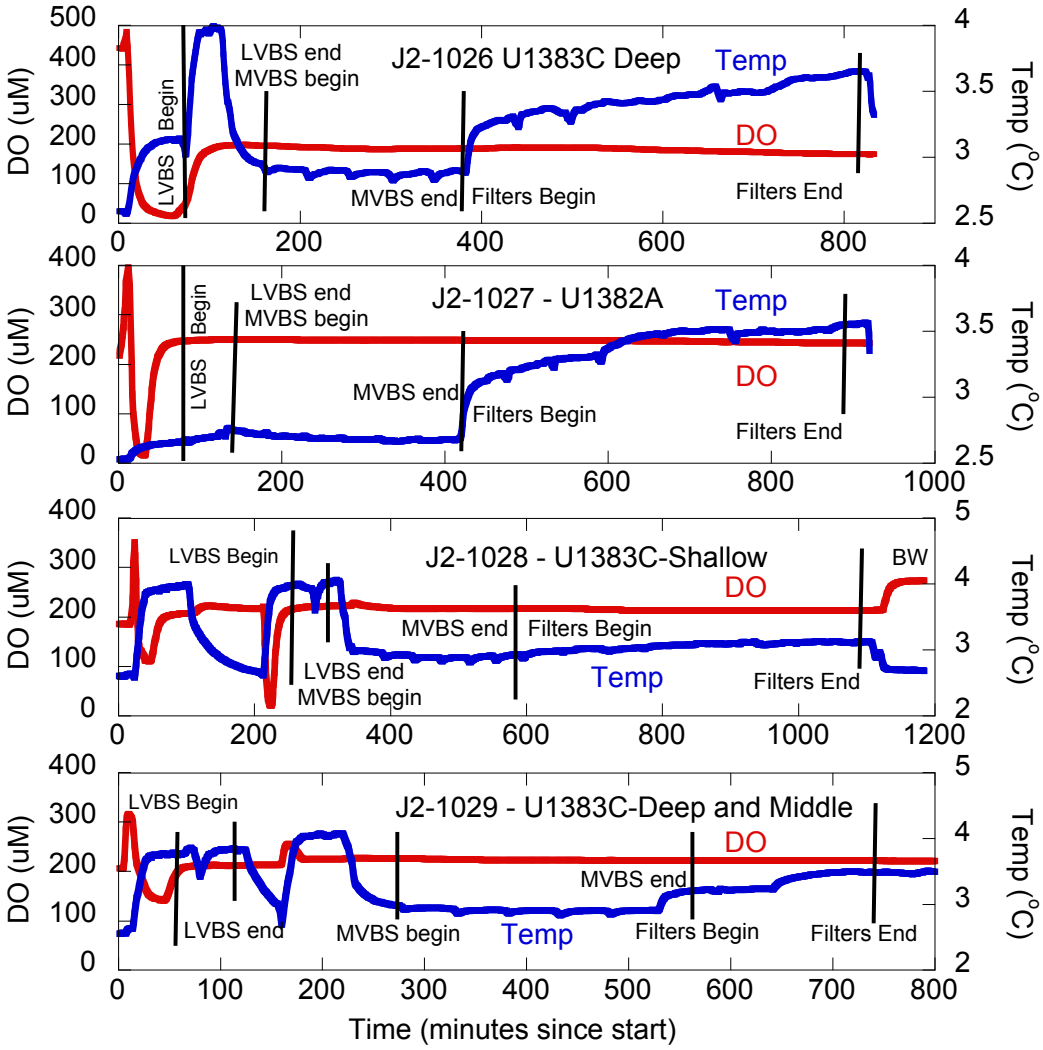
Wheat, C.G., Edwards, K.J., Pettigrew, T., Jannasch, H.W., Becker, K., Davis, E.E., Villinger, H., and Bach, W., 2012, CORK-Lite: Bringing legacy boreholes back to life, Sci. Drilling, 14, 39-43, doi: 10.2204/iodp.sd.14.05.2012.

Table 2. Summary of AT3901 North Pond CORK Pressure Data Downloads. Times in UTC.

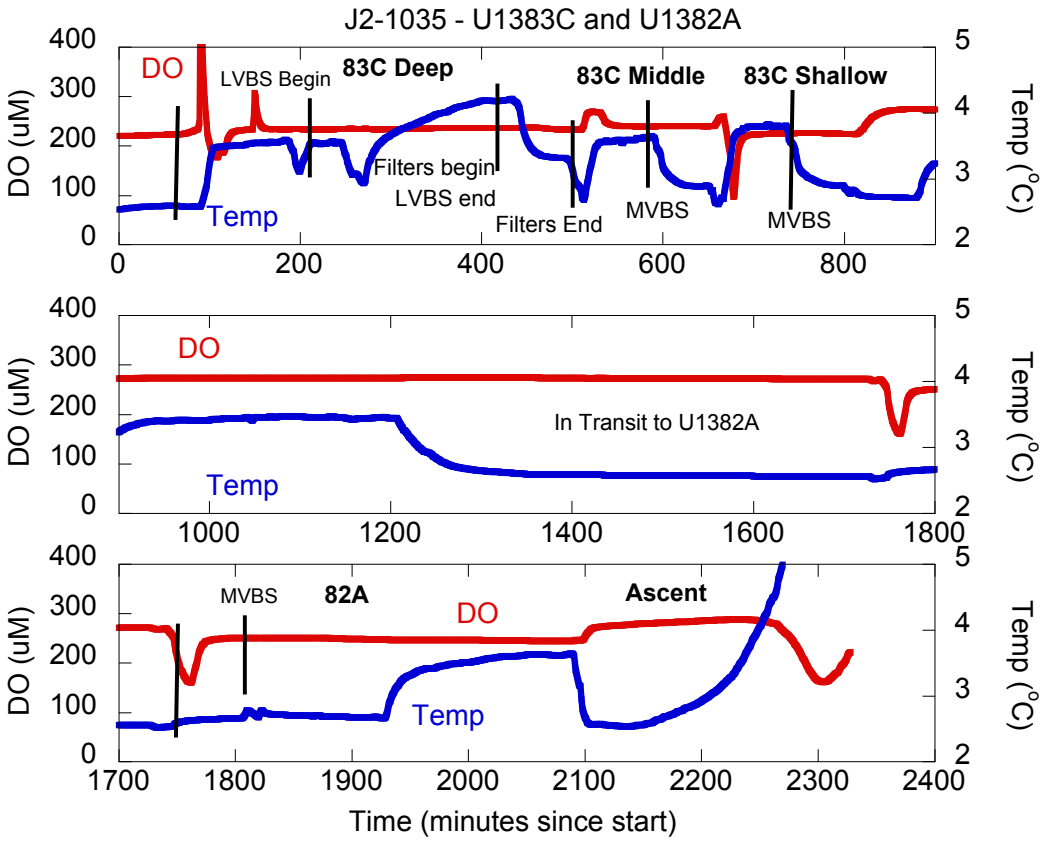
CORK	U1382A	U1383C	U1383B (CORK Lite)
Installation Date	11 Oct 2011	6 Nov 2011	29 April 2012
# Formation Gauges	1	3	1
Sampling Interval	2 min through Oct 2017, now 20 min	2 min through Oct 2017, now 20 min	2 min through Oct 2017, now 20 min
2012 Hydrostatic Calibration	22 Apr 15:52-1644	20 Apr 15:30-16:20	23 Apr 2012 19:20 – 29 Apr 2012 15:08
2014 Hydrostatic Calibration	5 Apr 12:45-13:14	31 Mar 12:37-13:20	None
2017 Hydrostatic Calibration	11 Oct 2017 23:14 – 23:42	10 Oct 2017 02:06 - 17:24	10 Oct 2017 01:50 – 17:30
AT 39-01 2017 Download Data Time Range	6 Apr 2014 12:06 – 12 Oct 2017 00:16	1 Apr 2014 13:17 – 10 Oct 2017 18:26	01 Apr 2014 14:18 – 10 Oct 2017 17:30
Clock Check	12 Oct 2017, 00:22:00, logger 14.7 s ahead	10 Oct 2017, 18:27:24, logger 526.2 s ahead	10 Oct 2017, 17:32:34, logger 345.5 s ahead
Previous Clock Sync to UTC	6 April 2014, 12:04	1 April 2014, 13:20	1 April 2014, 14:17
Clock Reset and Memory Clear	12 Oct 2017, 02:02	10 Oct 2017, 20:29	10 Oct 2017, 19:12
Second AT39-01 Download Data Time Range	n/a	10 Oct 2017 19:49 – 24 Oct 2017 00:20	n/a

CORK dissolved oxygen and temperature during MPS operations

The UH fluid sampling system (MPS) recorded a variety of data including dissolved oxygen, temperature, and pump amperage and rotation. The two plots below highlight the dissolved oxygen and temperature data from the five dives that included the UH fluid collection system. LVBS – Large Volume Bag Sampler (50 L max), MVBS – Medium Volume Bag Sampler (15 L max).



During dive J2-1035 the system was left on during ascent so that the DO record could be compared with the record from the CTD on *Jason II* and the optode on the CTD. During this dive, all four horizons from the two wellheads (U1383C and U1382A) were sampled.



On the basis of these data the following dissolved oxygen and temperature values were estimated. Note that as pumping continues at a specific horizon, the concentration of DO decreases. Values were chosen when the LVBS was in use, prior to the steady drop in concentration. Note that on J2-1035 we sampled all of the horizons again with different results. These results suggest that much of the flow in basement is centered at the depth of the middle umbilical. These data have to be examined closer. Note that the measured bottom water with the UH system was 10 uM lower than the CTD on *Jason II*. These data need to be adjusted to the ship's CTD or *Jason II*'s CTD.

				J2-1035	J2-1035
	Dive	DO uM	Temp Deg C	DO uM	Temp Deg C
U1383C Deep	J2-1026	190	>3.98	234	>4.19
U1383C Middle	J2-1029	213	>3.83	240	>3.6
U1383C Shallow	J2-1028	217	>4.05	225	~3.42
U1382A	J2-1027	250	>2.75	251	>3.63
Bottom Water	J2-1028	273	<2.69	275	2.57

CORK downhole and wellhead OsmoSampler recovery summaries

The following OsmoSampler packages from the CORK downhole instrument strings and wellheads were collected and processed for geochemistry (Wheat) and MBIO (Orcutt).

Downhole OsmoSampler summaries:

Hole	**U1383C**	**U1382A**	**U1383B**
Dive	J2-1030	J2-1031	J2-1033
Issues	String parted below upper oxygen sensor due to corrosion. No recovery from middle and deep horizons.	String parted below MBIO OS due to corroded steel rod	None
OS Recovered	**Upper/shallow: Acid, BOSS[a], Enrich, MBIO, Standard**	**MBIO**	**Acid, BOSS[a], Enrich, Gas[b], MBIOx2, Standard**
OS Lost	Upper/shallow: Gas, All OS from middle and deeper horizons	Acid, BOSS, Enrich, Standard, Gas	None
MBIO OS comments	Sample coils pumped all of the way through, salty pump reservoirs	Intake coils very rusty, FW in half of last coil and pump reservoirs.	Did not overpump
ENRICH OS comments	Sample coils pumped all of the way through, delivery coil did not overpump, delivery intake coil did not overpump, salty pump reservoirs	n.a.	Did not overpump
Rock DNA samples	64	32	96
Filter DNA samples	100	49	97
PFA samples	20	10	30
BONCAT samples	64	30	72
PSTAT samples	28	14	42
Major/minor ion samples	198	97	188
NUTZ samples	2	2	6

a: BOSS coils given to Girguis lab for DOC measurement, b: Gas coils given to Girguis lab for DIC measurement, but the membranes in these OS coils were weird.

Wellhead OsmoSampler summaries:

Hole	**U1383C**	**U1383C**	**U1383C**	**U1383C**	**U1382A**
Color	**Blue**	**Black**	**Red**	**Orange**	**Green**
Horizon	**Zone 1 Deep**	**Zone 2 Middle**	**Zone 3 Shallow**	**Zone 3 Shallow**	**Zone 1 Shallow**
Dive	J2-1032	J2-1032	J2-1032	J2-1032	J2-1034
OS Recovered	Orcutt FLOCS, JP FLOCS, Acid	Orcutt FLOCS, JP FLOCS, Acid	Orcutt FLOCS, JP FLOCS, Acid	JP FLOCS BW, JP FLOCS	Orcutt FLOCS, JP FLOCS, Acid
Orcutt FLOCS comments	none	none	No salt in pump	n.a.	Over-pumped
JP FLOCS comments	Over-pumped	Over-pumped, Sulfidic smell	Orcutt FLOCS did not over-pump, JP FLOCS did	BW FLOCS did not pump, distilled water in FLOCS and coils; **Consider to be control**.	Over-pumped
Rock DNA samples	3	3	3	4	3
Filter DNA samples	8	4	5	2	4
PFA samples	2	2	2	4	2
BONCAT samples	3	3	3	4	3
PSTAT samples	5	5	5	8	5
Major/minor ion samples	35	42	33	26	45
NUTZ samples	5	0	1	0	0

CTD Operations

Table AT39-01 CTD locations. The first CTD cast was for mechanical purposes to ensure the level mechanism on the winch was working properly.

Cast	Date	Latitude (North)	Longitude West
at3901002	14-Oct-17	22 48.60	46 3.62
at3901003	16-Oct-17	22 48.18942	46 3.18888
at3901004*	17-Oct-17	22 48.09657	46 3.16233
at3901005	17-Oct-17	22 48.14688	46 3.13249
at3901006	18-Oct-17	22 48.07639	46 3.17839
at3901007	20-Oct-17	22 48.12962	46 3.16199
at3901008	23-Oct-17	22 45.34065	46 4.89657
at3901009	25-Oct-17	22 45.33436	46 4.98353
at3901010	25-Oct-17	22 45.32	46 4.79
at3901011	26-Oct-17	22 48.122	46 3.150
at3901012	26-Oct-17	22 48.179	46 3.253
at3901013	27-Oct-17	22 48.200	46 3.286

*ended at 22 48.311904N 46 3.15011W

CTD1: Bottle firing sequence for CTD 1.

Bottle #	Depth (m)	Person	Feature
1	4397	Orcutt	Bottom water
2	"	Trembath-Reichert	"
3	"	"	"
4	"	"	"
5	"	"	"
6	"	"	"
7	"	Yoshimura	"
8	"	"	"
9	"	Steward	"
10	3000	Orcutt	Lower bathypelagic
11	"	Steward	"
12 - Beacon	n.a.	n.a.	n.a.
13	2000	Orcutt	Upper bathypelagic
14	"	Steward	"

15	800	Orcutt	Oxygen minimum
16	“	Steward	“
17	300	Steward	?
"	126	Steward	?
19	80	Orcutt	Chlorophyll max
20	80	Steward	“
21	27	Steward	Near surface
22	6	Steward	Surface
23	6	Steward	“
24	6	Steward	“

Organic geochemistry:

We aim to investigate the organic geochemistry of fluids from the subsurface to delineate between autotrophy and heterotrophy in the particulate and free-living fraction of the communities. To accomplish this goal we will determine d13C abundances, C/N ratios, and structure of polar lipid biomarkers to tell us about the metabolisms of organisms living in the crustal fluids at North Pond. We collected 10L of water from each of the three horizons at U1383C and the single horizon at U1382A using the UH MPS ssytem. A bottom water comparison sample was also collected via CTD 10m above the bottom near U1383C. Water collected in this manner was either immediately injected with HgCl for DIC (dissolved inorganic carbon) measurements, filtered for DOC (dissolved inorganic carbon), or frozen at -80C. At each of the 4 subsurface horizones, ~10L was filtered *in situ* to measure POC (particulate organic carbon).

McLane pumps were additionally used for large volume *in situ* pumping and filtration. Crustal fluids from U1382A were pumped for 24 hours using custom filter holders on the McLane pumps. A bottom water comparison was also taken by pumping for 12 hours near U1382A while the pump was attached to the CTD. These large volumes of filtered water will be used for structural and d13C analysis of the polar lipid biomarkers.

Water Column Microbial Ecology

The surface ocean in the Sargasso Sea has been extensively studied, but the deep ocean here has not, nor have the differences between particle-associated and free-living microbial fractions been investigated. Here we sampled the entirety of the water column in the Sargasso Sea at North Pond to study the differences in community composition and core genes with depth and between size fractions, as well as to asses connectivity between depths with emphasis on the plausibility of microbial transport to the deep sea from sinking particles in the water column.

Water samples were collected from 8 depths near U1383C and U1382A CORKs via CTD hydrocasts. These 8 depths represented the surface ocean, DCM, OMZ, reoxygenation zone, and 4 depths in the meso and abyssopelagic, and this sampling scheme was conducted 5 times. These samples were sequentially filtered onto 5 and 0.22 micron mixed cellulose filters to fractionate particulate and free-living fractions and will be used to determine the microbial ecology of particulate and free-living communities throughout the entire water column at North Pond using 16S rRNA gene amplicon analyses and metagenomics.

Huber Lab

The main objectives on this cruise were to (1) collect crustal fluids that were in situ filtered and preserved (with RNALater) to determine and quantify functional repertoire of total active microbial communities in crustal fluids; (2) filter crustal fluids to distinguish particle-attached and free-living communities to determine and quantify functional repertoire of size fractionated microbial communities in crustal fluids; (3) quantify microbial biomass in crustal fluids; (4) preserve crustal fluids for single cell genomic analyses; (5) conduct shipboard stable isotope probing experiments with crustal fluids enriched with labeled DIC to determine which microbes are fixing carbon; (6) estimate potential activity and carbon uptake rates at 1 atm by both autotrophs and heterotrophs in crustal fluids using NanoSIMS; (7) estimate potential activity and carbon uptake rates at 270 atm (~3000 m water depth) by both autotrophs and heterotrophs in crustal fluids using NanoSIMS; (8) collect crustal fluids for microbial enrichment and cultivation; and (9) collect crustal fluids for nitrogen isotopes for Scott Wankel (WHOI). We sampled fluids with the MPS, as well as CTD. A complete sample table is shown below.

	1382A	1383C shallow	1383C middle	1383C deep	Seawater
I. In Situ D/RNA Filter 0.22 um	X	X	X	X	X
II. ShipBoard D/RNA Filter 5um and 0.22 um	X	X	X	X	X
III. Counts	X	X	X	X	X
Single Cell Genomics	X	X	X	X	X
V. RNA Stable Isotope Probing	X	X	X	X	X
VI. NanoSIMS 1 atm	X	X	X	X	X
VII. NanoSIMS, Pressure Vessels	na	na	na	X	na
VIII. Culturing	X	X	X	X	X
IX. N Isotopes	X	X	X	X	X

I. In Situ Filtering for DNA/RNA

To better understand the metabolic potential and gene expression patterns of subseafloor communities, as well as continue our time series of North Pond metagenomes, we collected samples for 'omic analyses from all crustal fluids and seawater. Filter holders containing a 0.2 µm, 47 mm flat filter with ~20 mL RNALater were loaded onto the MPS. For each sample, ~15 L of fluid was pumped through each filter and then the filter was preserved *in situ* with RNALater. Duplicate filters were collected when possible.

II. Filtering for DNA/RNA, Particle Attached and Free-living

To distinguish microbial communities and gene expression profiles from particle-attached and free-living communities, we also filtered crustal fluids and seawater samples for 'omic analyses on deck. Filter holders containing a 5 µm, 47 mm flat filter followed by a 0.2 µm, 47 mm flat filter with ~20 mL RNALater were used, similar to those used in for in situ filtering. 15 liters of fluid was filtered for this analysis. In two cases (1383C deep and 1382A), this was also done on the seafloor with the MPS, after string recovery

III. Preservation of Vent fluids for Total Cell Counts

To quantify microbial biomass using epifluorescent microscopy, crustal fluids and seawater were collected and preserved in scintillation vials with 37% formaldehyde.

IV. Single Cell Genomics

Crustal fluid and seawater was collected for single cell genomics with 100 µL of filter-sterilized GlyTE buffer. Triplicate samples were taken for each sample.

V. RNA Stable Isotope Probing

Fluid was collected to determine who the active autotrophs at each site are using RNA Stable Isotope Probing using 1 liter of crustal fluid or seawater and adding either ^{12}C or ^{13}C sodium bicarbonate. Bottles were incubated at either 4 ºC or 20 ºC and then filtered onto a Sterviex filter at the end of the incubation. The chart below shows the setup and incubation times for all SIP experiments. In all, 72 RNA-SIP experiments were carried out. At 1383C middle, only a 20 ºC experiment was carried out.

	Total Time
4ºC-13DIC-TP1-Rep1	6 days
4ºC-13DIC-TP1-Rep2	6 days
4ºC-12DIC-TP1-Rep1	6 days
4ºC-12DIC-TP1-Rep2	6 days
4ºC-13DIC-TP2-Rep1	12 days
4ºC-13DIC-TP2-Rep2	12 days
4ºC-12DIC-TP2-Rep1	12 days
4ºC-12DIC-TP2-Rep2	12 days
20ºC-13DIC-TP1-Rep1	2 days
20ºC-13DIC-TP1-Rep2	2 days
20ºC-12DIC-TP1-Rep1	2 days
20ºC-12DIC-TP1-Rep2	2 days
20ºC-13DIC-TP2-Rep1	6 days
20ºC-13DIC-TP2-Rep2	6 days
20ºC-12DIC-TP2-Rep1	6 days
20ºC-12DIC-TP2-Rep2	6 days

VI. NanoSIMS, 1 atm

The goal of these experiments was to estimate carbon uptake rates by both autotrophs and heterotrophs, as well as general microbial activity. This was done by adding 13C labeled compounds, together with D2O. Time points were taken at 12 hours, 2 days, and 6 days for all experiments. 1 ml aliquots were taken for geochemistry at time zero (when incubations were first set up) from time point 1 bottles, and then from all bottles before adding fixative. In total, 450! NanoSIMS experiments were carried out.

Condition	13C Source	15N Source	2H Source	Temp	Target Metabolism	Replication	Sample Vol (ml)	Time Points
1	Diatoms	Diatoms	2H2O	4	Complex Heterotrophy	3	50	3
2	Methylamine	Methylamine	2H2O	4	Methylotrophy	3	50	3
3	Diatoms	Diatoms	2H2O	20	Complex Heterotrophy	3	50	3
4	Methylamine	Methylamine	2H2O	20	Methylotrophy	3	50	3
5	Acetate	Ammonium	2H2O	4	Heterotrophy	3	50	3
6	Bicarb	Ammonium	2H2O	4	Autotrophy	3	50	3
7	Acetate	Ammonium	2H2O	20	Heterotrophy	3	50	3
8	Bicarb	Ammonium	2H2O	20	Autotrophy	3	50	3
9	-	-	-	4	No label control	3	50	3
10	-	-	-	20	No label control	3	50	3

VII. NanoSIMS, Pressure Vessels

A subset of 1 atm NanoSIMS conditions were also performed from 1383C deep fluids at 4000 psi in 500 mL volumes at 20°C. These included condition 3 for all three NanoSIMS time points, condition 7 and 8 for time points 2 and 3, and condition 4 for time point 3. A no-label added control was also performed for time point 3. 120 mL of the incubation was preserved for NanoSIMS analysis and the rest was filtered onto a sterivex filter for DNA analysis.

VIII. Cultivation

Crustal fluids and seawater were collected and stored in serum vials for shore-based cultivation.

IX. Nitrogen Isotopes for Scott Wankel

Crustal fluids and seawater were filtered into falcon tubes for shore-based analyses of nitrogen isotopes.

TAMUCC Fungi

Objective:

Collect fluid samples from existing CORKs for enrichments, specifically for fungi. Use media similar in nutrition level to the existing environment to culture fungi. The enrichments will be used for high throughput culturing using FACS.

- Collect fluids for fungal enrichments from each horizon at 1383C and 1382A
- Collect seawater from the bottom of the water column, the oxygen minimum zone, and near surface water.
- Collect sediment cores, as available

Sample Collection

Protocol

1. Prepare media just prior to the dive. Add pre-weighed media to the pre-autoclaved DIW, mix well, and autoclave again.

2. Once fluids are retrieved, calculate how much fluid you have to work with. Our goal is to prepare as many replicate vials as possible on board and bring additional unamended (unaltered) fluid back to the lab.

3. After determining how many samples you will prepare as enrichments, distribute 14 mL of media to _____ vials using sterile technique.

4. Distribute two (2) vials from each media preparation as a Media Blank.

5. Distribute 4 mL of the sample into each replicate vial. Take care to leave cap on the vial until just before adding the sample to prevent contamination. Pipette slowly to avoid splashing. Be careful to not touch anything with the pipette tip, using sterile technique.

6. You may label on the bottle and the cap. Suggested label for vial:
   site-media-rep# date
   1382A-PD-R1 100717