ADEON 4 Recovery/Deployment Cruise Report AR040 – R/V Armstrong 22 Oct – 06 Nov 2019 Woods Hole, MA to Woods Hole, MA

Photo credits: Joe Warren

Chief Scientist Joseph Warren, Jennifer Miksis-Olds, Anthony Lyons, Carmen Lawrence, Brandyn Lucca, Hannah Blair, Hilary Kates Varghese, Aaron Aunins, Jacob Norry, Cassidy Bell, Katelyn Castler, Wendy Klepperer, Emmanuelle Cook, Dylan Wilford, Mackenzie Meier

Cruise Summary

The objectives for this cruise were to recover bottom landers at seven sites (Figure 1) along the shelf break (depths ranging from 200 – 900 m roughly), redeploy a bottom lander at each site after downloading its data, collect CTD profiles to characterize hydrographic conditions at the sites, conduct net sampling to collect biological specimens at each site, and conduct fine-scale (roughly 10 km by 10 km) multi-frequency acoustic surveys at each site (Figure 2). We were able to complete the lander turnarounds at five locations (VAC, HAT, WIL, SAV, and CHB) successfully, but two landers (JAX, BLE) were not recovered (Table 1). Neither lander surfaced after the acoustic releases were tripped even though the acoustic releases were communicating fine with the ship. Additional efforts were made at these two sites to grapple for the landers (1.5 days at BLE and 1 day at JAX) to dislodge or "hook" them with a trawl cable and clump weight, but none of those efforts were successful. A spare lander was deployed at the BLE site. In addition, at several ADEON sites we collected animal specimens from net tows for collaborators associated with the DEEP SEARCH project as well as collecting water samples for eDNA analysis for DEEP SEARCH and other collaborators. On our return leg to Woods Hole, we made a slight detour to retrieve a disabled WHOI-operated OOI glider that needed to be recovered. We appreciate the excellent work of the ship's Captain and crew (in all aspects on the boat) in helping us to accomplish our cruise objectives.

Table 1. Summary of sampling that occurred at each site location during the AR040 research cruise.

Site	**Lander Recovered / Deployed**	**CTD casts**	**Ring net tows**	**IKMT net tows**	**Finescale Acoustic Survey**	**Water Samples Taken for DEEP SEARCH**	**eDNA samples collected**	**Fish specimens preserved**
VAC	Yes / Yes	3	1	5	Yes (3)	Yes	Yes	Yes
HAT	Yes / Yes	2	1	3	Yes (3)	Yes	No	Yes
WIL	Yes / Yes	1	1	2	Yes (2)	Yes	No	Yes
SAV	Yes / Yes	1	2	2	Yes	Yes	No	Yes
BLE	No / Yes	1	1	2	Yes	Yes	Yes	Yes
JAX	No / No	1	1	2	Yes (2)	Yes	Yes	Yes
CHB	Yes / Yes	1	1	1	Yes	Yes	No	Yes
Cruise Total	5 / 6	13	8	17	13			

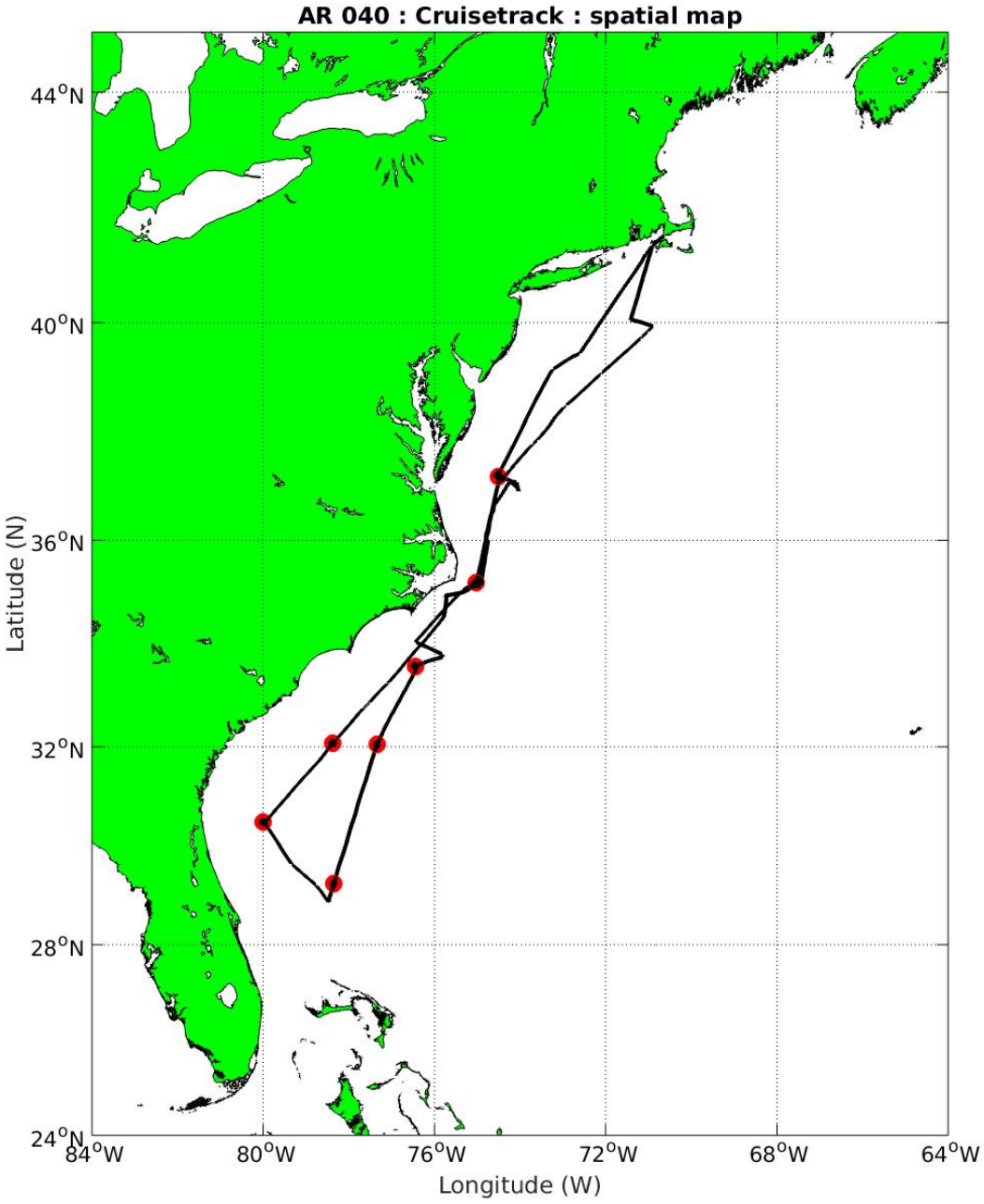

Figure 1. Cruise track for R/V Armstrong Cruise #AR040 from 22 Oct – 6 Nov 2019. Site locations are highlighted by red circles.

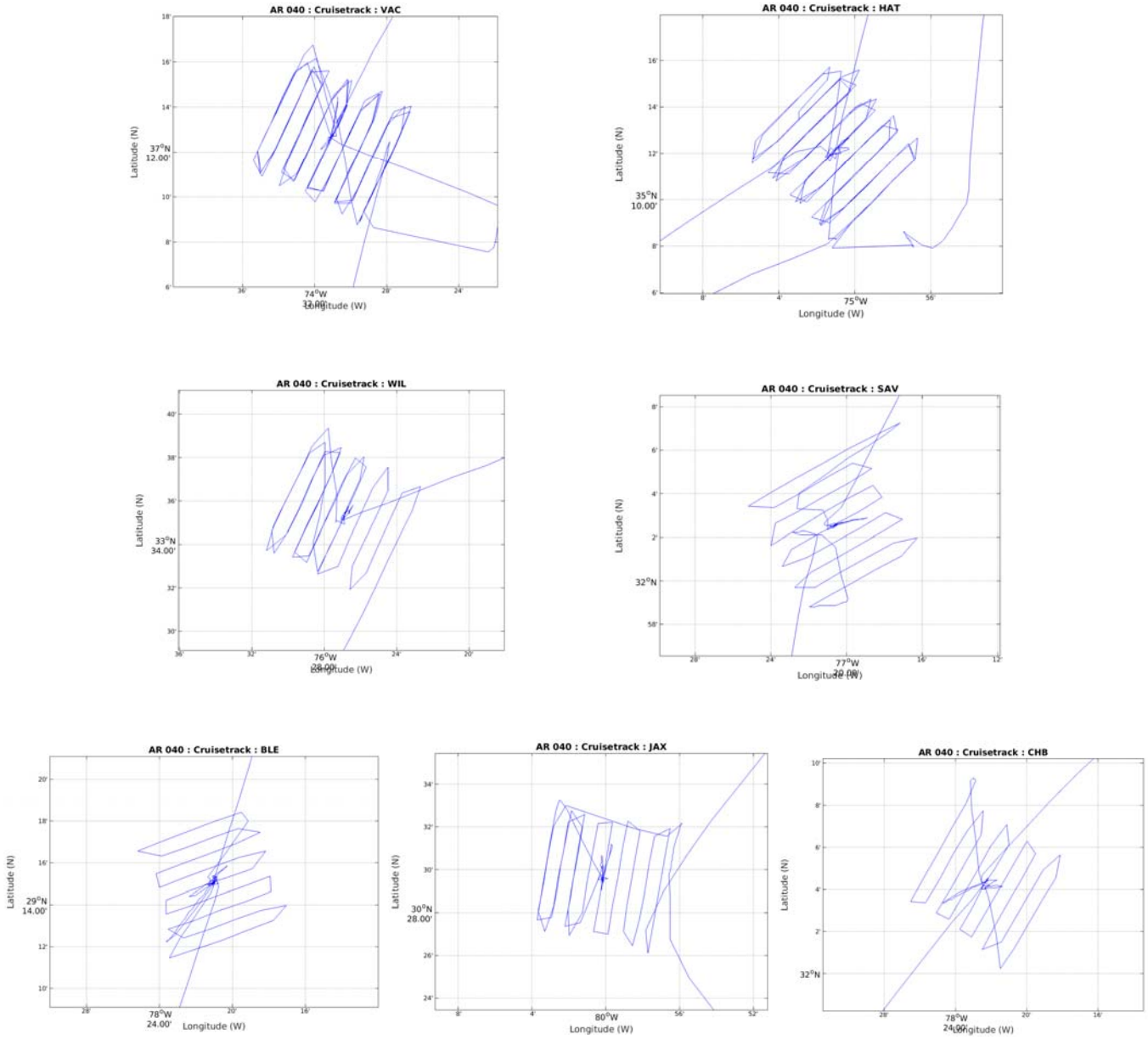

Figure 2. Cruise track for the ship at each site during R/V Armstrong Cruise AR040.

Lander Deployment, Retrieval, and Refurbishment – Carmen Lawrence

Upon arrival to each station, the lander was first communicated with via the acoustic releases and range was established. If the range was acceptable, the landers were released from the anchor. Once the lander was spotted on the surface, the vessel approached the lander on the starboard side and was hooked via a snap hook line that went to the J-frame. The lander was then brought on board over the starboard side and placed on a new anchor.

At VAC, as previous cruises have had interactions with fishing activities, the deployment site was moved approximately 2 nautical miles to the south of the original deployment location, in consultation with the local fishing fleet. At two of the seven stations (HAT and VAC), the AZFP with transducers and the VEMCO receivers were dismounted from the recovered lander for new batteries and data download. They were then installed on the refurbished lander to be deployed again at the same site.

Lander deployment was performed via the A-frame, winch, and quick release. The lander was lifted and suspended over the aft of the vessel and then lowered into the water. Once the lander was fully submerged, the quick release was triggered, and the lander dropped. For each station, range measurements were taken at multiple locations around the lander to triangulate a more precise lander position on the seafloor (Table 2).

Between stations, each lander was refurbished for redeployment. New anodes were added to the hydrophone cages and uprights, the new anchor was installed, and a new AMAR and new battery pack were installed. Much of the hardware showing corrosion was either replaced or cleaned. The MMF flasher, PORT LF acoustic releases, Kilo beacon, and the MicroCATs were refurbished at sea and replaced on each lander once the data had been downloaded and the batteries replaced. Several of the hydrophones also needed to be swapped due to decreased system gain values indicated during onboard calibrations.

At BLE, communication was established with both acoustic releases and was released, but the lander did not surface, despite repeated release attempts at various ranges and positions. Approximately 1.5 days were spent attempting to grapple the lander and hopefully dislodge it from whatever was preventing it from surfacing during daylight hours. The lander was re-triangulated to ensure accuracy for grappling. The grappling was unsuccessful, so the spare lander was deployed. The BLE lander was visible on the ship EK80 echosounder system and is marked at 29º 15.0542'N / 078 º 21.0550'W.

Similarly, at JAX, one of the acoustic releases was successfully communicated with and confirmed released, but the lander did not surface. We could not establish communications with the second acoustic release. Again, the lander was re-triangulated and approximately one day was spent grappling. As there was not a second spare lander, no new lander was deployed at this site. Additionally, this site's lander contained an AZFP and VEMCO receiver. The JAX lander was visible on the ship EK80 echosounder system and is marked at 30 º 29.6168 N / 80 º 00.1598 W.

For both the BLE and JAX landers which did not return to the surface, multiple attempts were made using the ship's trawl wire and a clump weight to grapple the lander to try to "hook" it or dislodge it (as one possibility is that sediment had accumulated around the base of the lander preventing it from coming to the surface). A large loop of cable was placed on the seafloor surrounding the lander

location, then the ship would pull the cable in an attempt to drag the cable loop along the bottom as the loop circumference shrank. A variety of different cable/ship orientations were attempted in order to try to recover each lander.

Table 2: Lander deployment locations, depth and estimated bottom locations

Site	Deployment Location		Drop Date	Time (UTC)	Estimated Location		Depth (m)
	Latitude	Longitude			Latitude	Longitude	
BLE	29° 15.069'N	078° 21.022'W	2019-10-28	14:58	29° 15.0539' N	078° 21.0547' W	900
CHB	32° 04.209'N	078° 22.442'W	2019-10-31	09:29	32° 04.2062' N	078° 22.4236' W	415
SAV	32° 02.515'N	077° 20.855'W	2019-10-25	19:58	32° 02.5275' N	077° 20.7537' W	814
WIL	33° 35.100'N	076° 27.035'W	2019-10-24	16:37	33° 35.2176' N	076° 26.9743' W	464
HAT	35° 11.951'N	075° 01.237'W	2019-10-23	17:07	35° 11.9927' N	075° 01.1821' W	294
VAC	37° 12.600'N	074° 31.110'W	2019-10-21	19:15	37° 12.5883' N	074° 31.1151' W	257

Marine Mammal Observers (MMOs) – Jennifer Miksis-Olds

A dedicated team of Marine Mammal Observers (MMOs) conducted visual surveys from the ship during daylight hours while at the seven ADEON lander locations. MMO watch commenced approximately 2 nm from the lander drop location when the ship reduced speed upon approach during daylight hours. These observations were made to provide a record and groundtruth for marine mammals sighted in the area for comparison to the lander passive acoustic datasets. Over the course of the cruise, approximately 76.3 on-effort MMO hours were logged (Table 3). Most of the marine mammals sighted during this cruise were small to medium odontocetes. Species confirmed were bottlenose dolphin common dolphin, Clymene dolphin (Figure 3), and pilot whales.

Table 3. MMO Effort Log for AR040 ADEON Cruise 4.

Date	Local Time Start	Local Time End	Hours Effort	Site
10/20/2019				Transit
10/21/2019	11:55	18:07	6:12	VAC
10/22/2019	17:09	11:24	1:15	HAT
10/23/2019	6:49	14:17	7:28	HAT
10/24/2019	8:37	18:28	9:49	WIL
10/25/2019	13:37	18:40	5:03	SAV
10/26/2019	7:02	9:00	1:22	SAV
10/27/2019	6:40	18:01	10:41	BLE
10/28/2019	6:47	11:55	5:08	BLE
10/29/2019	7:01	18:00	10:59	JAX
10/30/2019	13:58	18:00	3:58	CHB
10/31/2019				Transit
11/1/2019	12:42	18:14	5:32	HAT
11/2/2019	6:44	8:39	1:55	HAT
11/3/2019	7:26	9:09	1:43	VAC
11/3/2019	10:29	18:18	7:49	VAC
11/4/2019	7:06	7:44	:38	VAC
11/5/2019				Transit

Figure 3. Clymene dolphin sighted bow riding off the R/V Armstrong (Photo credit: Anthony Lyons).

Multiple Frequency Acoustic Echosounder Data – Joseph Warren

The R/V Armstrong's EK80 system (Figure 4) was run continuously during the cruise with the 18 and 38 kHz transducers in narrowband (due to ship constraints) and the 70, 120, and 200 kHz transducers operating in broadband mode. Pulse lengths were 1024 microseconds and ping rate was set to maximum, except when in shallow (< 150 m) water or when sea state (and thus data quality) were poor. Ping rate was then set to 0.2 to 1 Hz.

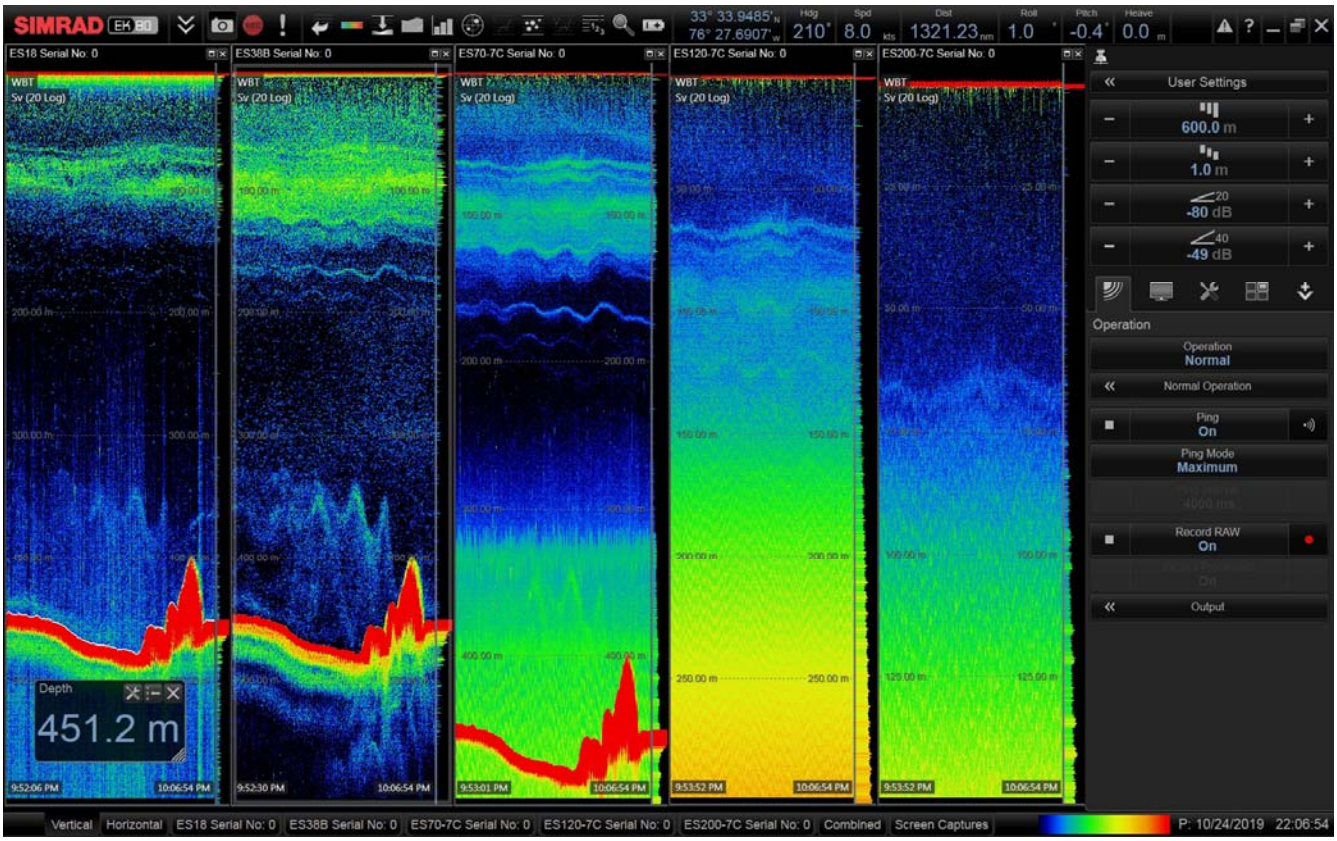

Figure 4. Example echogram from the ship's EK80 showing internal waves which were commonly observed during the cruise as the ship transited across the continental shelf.

At each survey site, a fine-scale acoustic grid (Figure 5) was conducted at a speed of 8 kn. Survey lines were adjusted for the direction of the sea state. At a few sites, the survey grid was run multiple times, either during the day and then the night, or separated by several days or weeks.

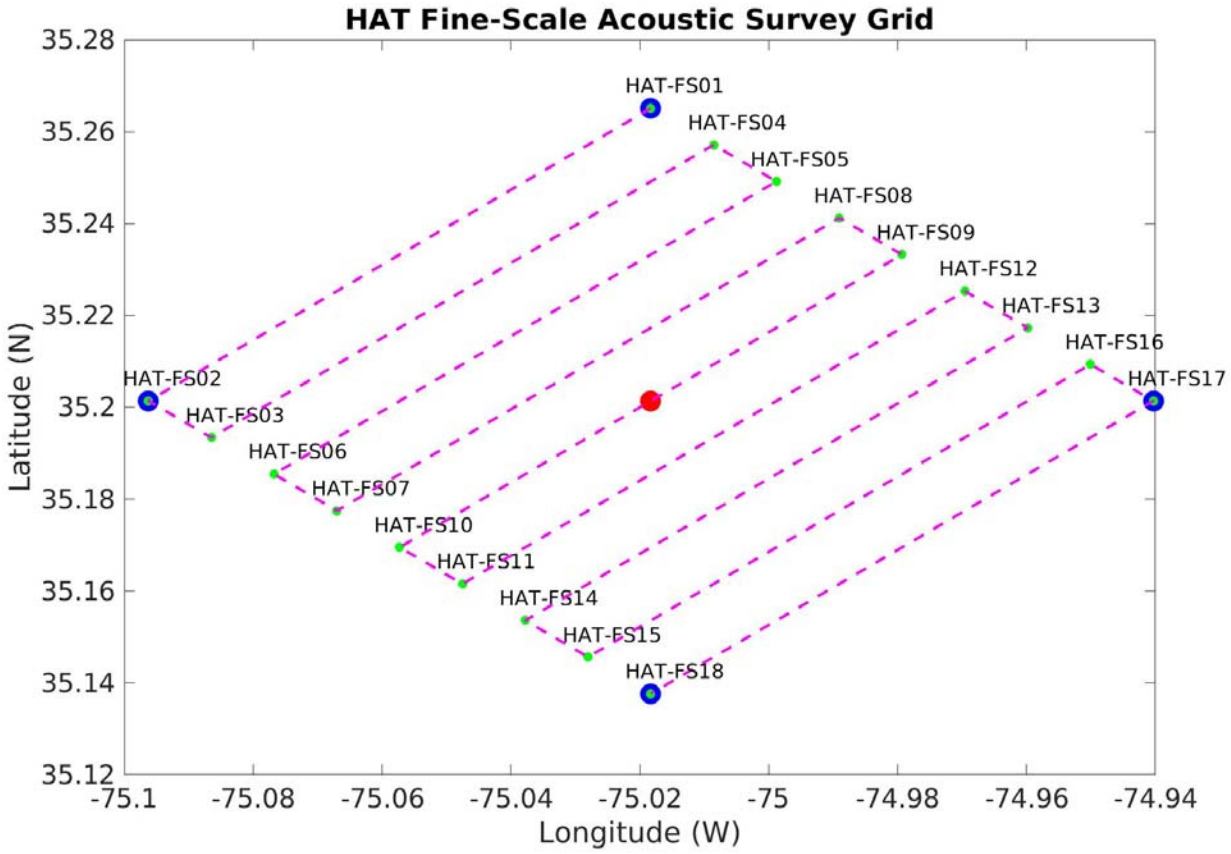

Figure 5. The grid acoustically surveyed at the HAT site. The red dot at the center represents the location of the bottom lander. The survey grid covers an area roughly 10 km by 10 km.

Net Tow Sampling – Joseph Warren

Biological specimens were collected at each site using two different nets. A 60 cm diameter, ring-net BONGO pair (one with 1000 um mesh, the other with 333 um mesh) was deployed at each site (roughly at the lander location) with a vertical cast to 100 m (Figure 6). Actual net depths may be slightly less than the wire out due to surface currents causing the tow wire to be slightly off-vertical. Zooplankton and larval nekton collected in the ring net were preserved in buffered formalin solution for post-cruise identification and enumeration. Unique or interesting specimens from these tows were occasionally photographed or preserved individually.

Figure 6. Bongo net being deployed on deck (left) for a vertical cast (right) (Photo credit: Joe Warren).

A larger net (5 m2 Isaacs-Kidd Midwater Trawl) was also deployed at each site (Figure 7), typically multiple times per site. One tow was done at the lander location and was targeted to sample the scattering layers observed in the water column on the ship's echosounder. Additional tows were conducted to sample the deep (> 750 m) scattering layers and to collect specimens from the mesopelagic region.

Figure 7. The Isaacs Kidd Midwater Trawl being recovered. Wire-out speeds were 10 – 30 meters per second, and haul-back speeds were 10 to 30 meters per second. Tow depths ranged from 150 m to 1800 m (Photo credit: Joe Warren).

Animals from these net tows were preserved in formalin solution for post-cruise identification and enumeration. Selected individual animals were removed (noted on the tow data sheet) for photography (Figure 8), individual preservation, or for collaborators with the DEEP SEARCH project for stable isotope and DNA analysis.

Figure 8. Some of the animals collected by net tows during the cruise (Photo credits: Joe Warren).

Material Property Measurements of Net-collected Animals – Hannah Blair

The density contrast (g) of an organism, or the ratio of animal density versus the surrounding seawater, is an important acoustic material property. Density contrast values are important components of acoustic scattering models, which allow for estimating zooplankton abundances from active acoustic data. Shipboard density contrast measurements were conducted on zooplankton collected by net trawls. Zooplankton taxa that composed a large proportion of each trawl sample were targeted for taking density measurements. Measurements were conducted as soon after trawls were completed as was feasible.

Measurements were conducted using the titration method. In this method, animals are placed in a beaker with a known volume of seawater. Small known volumes of a solution of 50:50 or 25:75 seawater to glycerin are added until the animal floats up at neutral buoyancy, indicating that the animal's density matches that of the solution. Following each density measurement, all measured animals were photographed with a ruler to acquire animal lengths (Figure 9). Many of these animals were then frozen so that their masses may be measured after the cruise's conclusion.

Density measurements were made on a total of 215 animals (Figure 10). Taxa measured included amphipods, decapod shrimp, euphausiids(krill), myctophid fish, *Cyclothone* fish, and elopomorph fish larvae (leptocephali).

Figure 9. Animals are photographed with a ruler alongside their ID numbers to match up g-measurements with animal length (Photo credit: Hannah Blair).

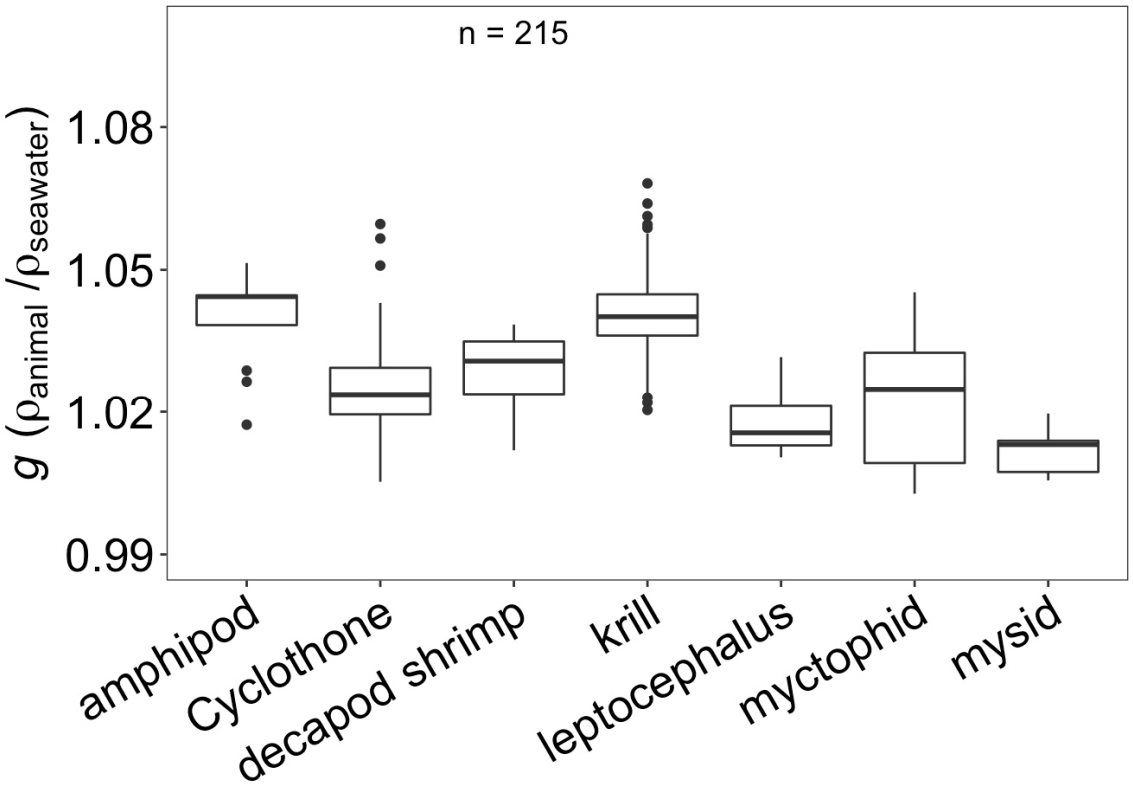

Figure 10. Of the 215 g-measurements taken, 119 were krill. Three genera of krill (Thysanopoda, Nematoscelis, and Stylocheiron) made up a large proportion of several of the Isaacs Kidd Midwater Trawl (IKMT) samples, and were therefore frequently targeted for g-measurements.

Individual Organism Target Strength Measurements – Brandyn Lucca

Target strength (TS, dB re: 1 m^2) measurements were made on 31 tethered animals in a 44 gallon aquarium (Figure 11) including: myctophids, *Cyclothone spp*., *Melanostomiidae spp.*, *Gonadostomatidae spp.*, and *Genpylidae spp.* from IKMT tows I-05 (24 October 2019), I-12 (30 October 2019), I-15 (03 November 2019), and I-17 (04 November 2019). These measurements were made at both discrete (38, 50, 120, 150, and 200 kHz with frequency modulation) and wideband (35-73 and 130-210) frequencies. These measurements will be used to validate theoretical backscattering models that are parameterized using a variety of measurements collected from animals, including length, shape, body density, and body sound speed. Of all the animals captured, photographs of 638 animals were taken (Figure 12) that will provide shape and length information for krill (*Thysanopoda spp.*, *Nematoscelis spp.*, *Stylocheiron spp.*), shrimp (*Haliporus spp.*, several unidentified species), hatchetfish, viperfish, dragonfish, pearlsides, myctophids, flatfish, bristlemouths, slimeheads, glass eels (i.e., *Leptocephali*), mysids, pteropods, squid, and copepods. The majority of these photographed animals were frozen for future measurements, such as measuring their mass and CT scans to collect swimbladder information. Sound speed measurements, which measure the sound speed contrast (*h*) between an animal and its surrounding medium, were made on krill captured from IKMT tow I-17 on 04 November 2019. A total of 5 measurements were made on a mixed assemblage of *Nematoscelis spp.* and *Thysanopoda spp.*

Figure 11. Example of a tethered myctophid (AN# 347) collected on 03 November 2019 from an IKMT tow (I15) (Photo credit: Brandyn Lucca).

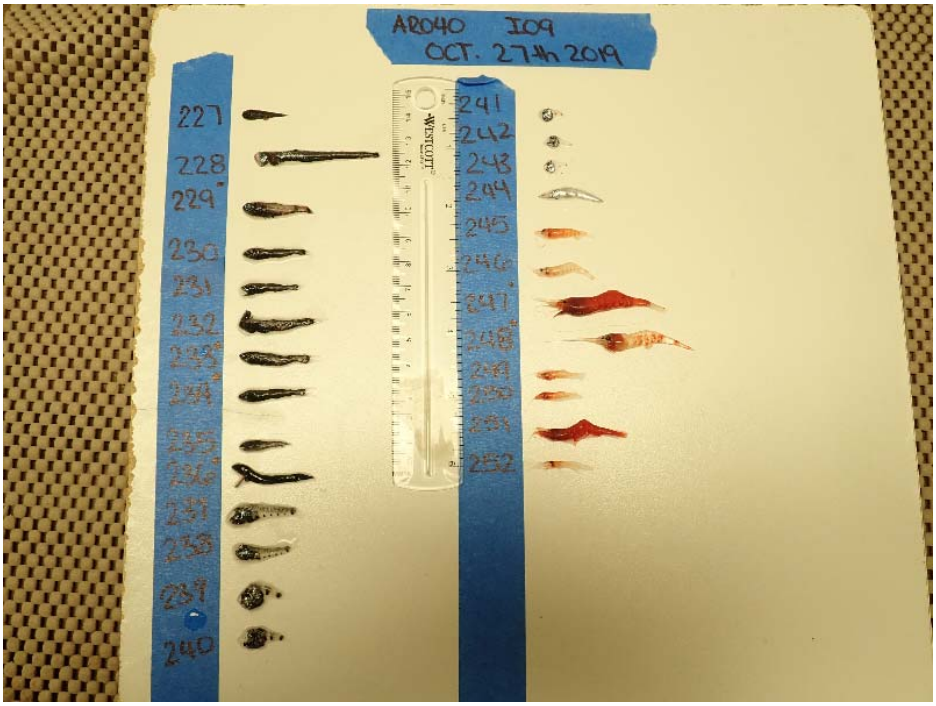

Figure 12. An overview photograph of some of the animals collected from an IKMT tow (I09) on 27 October 2019 (Photo credit: Brandyn Lucca).

Biological Sampling for DEEP SEARCH Collaborators – Jacob Norry

Across all sites: Representatives from Gonostomatidae, Sternoptychidae, Myctophidae, and Pleuronectidae were collected at most sites. These fishes are expected as they are representative of mesopelagic Atlantic. Sampling in the vicinity of the Gulf Stream resulted in the collection of some tropical fishes. Acanthuridae larvae were present at most sites. As broadcast spawners, eggs and larvae are easily transported by currents. Pleuronectiformes larvae were also collected in high frequencies across most sites and have been preserved for later identification to lower taxonomic levels. Cyclothone and Myctophidae were the most abundant and predictable groups across all sites.

Points for consideration and special cases: Long silvery fishes originally identified at the first four sites as Trichiuridae (Cuttlasfish) need to be compared to members of Gempylidae and have ID's validated. The larval Ogcocephalidae collected at HAT appears to most closely match illustrations of an undescribed genus and species previously collected in North Carolina localities. This larva has been fixed and preserved in 95% ethanol to leave later genetic analysis as a possibility. A tissue sample was taken from a juvenile *Anoplogaster sp*. to help provide data toward validating the difference between *A.cortuna* and *A.brachycera.* Collected specimen of *Sigmops elongatus* were frequently at the higher end of their size range. This could have interesting implications for available nutrients and/or *Sigmops elongatus* realized niche at the ADEON research sites.

Specimens collected for members of the Sutton lab included an Anoplogaster tissue sample (brought back to Florida on the plane by Jacob Norry).

224	10/24/2019	I-05	WIL	Anaplogaster	N-EtOH	Tissue sample in 5ml Vial	1199m

The remaining samples (Table 4) were saved in separate redline bags with either “For Sutton Lab at NSU” (frozen) or “For Jake for clear and staining” (1 eel preserved in formalin collected from one of the landers) written on them.

Table 4. Specimens from IKMT net hauls frozen for later analysis in the Sutton Lab at Nova Southeastern University.

Sample #	Date	Trawl #	Location/Trawl site	**Field ID**	**Frozen (Y/N)**	**Comments**	**trawl max depth**
213	10/24/2019	I-05	WIL	Stomiidae	y	IKMT, not sure if s.affinis, but allowed to take	1199m
219	10/24/2019	I-05	WIL	Chauliodus sp.	y	IKMT, hard to tell if 8 or 9 photophors before dorsal origin	1199m
220	10/24/2019	I-05	WIL	Melamphaidae	y	IKMT	1199m
221	10/24/2019	I-05	WIL	Melamphaidae	y	IKMT	1199m
227	10/27/2019	I-09	BLE	Melamphaidae	y	IKMT	752m
228	10/27/2019	I-09	BLE	Chauliodus sp.	y	IKMT	752m
293	10/28/2019	I-10	BLE	Chauliodus sp.	y	IKMT,max wire out 500.3m. wire angle :45	N/A
294	10/28/2019	I-10	BLE	Chauliodus sp.	y	IKMT,max wire out 500.3m. wire angle :45	N/A
295	10/28/2019	I-10	BLE	Chauliodus sp.	y	IKMT,max wire out 500.3m. wire angle :45	N/A
296	10/28/2019	I-10	BLE	Stomiidae	y	IKMT,max wire out 500.3m. wire angle :45	N/A
441	11/3/2019	I-15	VAC	Melamphaidae	y	IKMT	1569m
442	11/3/2019	I-15	VAC	Melamphaidae	y	IKMT	1569m
443	11/3/2019	I-15	VAC	Omosudis lowii	y	IKMT	1569m
536	11/4/2019	I-17	VAC	Chauliodus sp.	Y	IKMT, Believed to be Chauliodus Sloani, but need to validate	1351m

Water Sampling – Dr. Aaron Aunins (USGS)

A CTD equipped with a 24 Niskin bottle rosette was deployed at each ADEON location to obtain the sound velocity profile in support of the acoustic propagation portion of the study. In addition, water samples were collected for ADEON's sister project, DEEP SEARCH. Dr. Aaron Aunins, a biologist in the laboratory of Dr. Cheryl Morrison at the USGS Leetown Science Center, participated in the ADEON Research Cruise AR040 for the purpose of collecting samples of water from within deep scattering layers (DSLs) and other portions of the water column for environmental DNA analyses. Cheryl Morrison is a member of the collaborative DEEP SEARCH project, which is a multi-year and multi-agency study designed to increase both biological and geological knowledge about cold-water coral, canyon, and cold-seep habitats along the mid-Atlantic US coast. Some of the ADEON and DEEP SEARCH monitoring sites are within close proximity to each other, making data collection at these sites beneficial for objectives and data-sharing across both projects.

In total, 10 CTD casts were completed across 7 ADEON sites. Target water layers at each site were next to the bottom, 50 m below the DSL, within the DSL, 50 m above the DSL, and 5 m below the surface. Water samples were also collected at the deep scattering layer and surface for an environmental DNA (eDNA) study being conducted by Dr. Alison Watts (UNH). In total,127 liters of water were sampled: 109 liters for DEEP SEARCH and 18 for eDNA. At least three Niskin bottles were closed at each depth enabling three replicate samples to be collected. Upon retrieval of the CTD, one liter of water was collected from each of the three bottles as follows: 1) gloved hands were used to open the knurled air release knob at the top of the Niskin bottle; 2) the CTD spigot was opened for approximately five seconds to purge any water within the spigot; 3) a 1.3 L sterile Whirl-pak bag was used to collect approximately one liter of water from each respective Niskin bottle and then closed (Figure 13); 4) water samples were placed into a 4 ˚C walk-in refrigerator for storage until filtering. Water collection from the CTD took approximately 20 minutes for 15 one liter samples. Most water samples were filtered within 3 hours of collection. Filtering was accomplished by using a peristaltic pump to push 1 liter of water through a 0.2 micron Sterivex filter capsule (Figure 14). New tubing and fittings were used for each sample to help minimize the chances of sample contamination. Sterivex filters were placed in small whirl pack bags and frozen in a -20 ˚C freezer.

Figure 13. Aaron Aunins collects water samples into Whirl-Pak bags from a completed CTD cast. These water samples will be filtered to collect environmental DNA (eDNA) (Photo credit: Dylan Wilford).

Figure 14. Wet lab area set up for water filtering. New tubing extends from the water sample within the Whirl-Pak bag in the orange bucket, through a peristaltic pump-head, and through a Sterivex filter attached on the terminal end. New tubing and fittings are used for each sample (Photo credit: Aaron Aunins).

Net-caught Biological Organism Tissue Sampling – Aaron Aunins

In addition to water sampling, taxa were also collected for genetic database reference building. At each of the seven ADEON monitoring sites visited, vertical sampling of the water column with a bongo net, and stepped tows of an IKMT were performed to collect zooplankton samples. Collection of these zooplankton samples in conjunction with echosounder data provide information about what organisms are present in the deep scattering layers. Specific information about the duration of tows, and depths targeted are described elsewhere in the report. A. Aunins opportunistically collected whole organisms from some of these net tows when multiple individuals of a candidate taxonomic group were available. 52 individual whole taxa were placed into individually labeled vials filled with 95% ethanol from across six IKMT tows at six separate sites. In addition, the contents of one 1000 micron bongo net haul were preserved in 95% ethanol when the net folded over itself, rendering the sample useless to the Warren lab for quantitative analyses. All of these preserved samples will be transported to the Leetown Science Center in Kearneysville, WV, where they will be subjected to DNA extraction and sequencing. We aim to sequence the complete mitochondrial genome of each sample, which will aid in the design of better metabarcoding primers, and more genes for robust taxonomic assignment.

Recovery of Adrift Dinghy and WHOI OOI Glider

The ADEON project continued our streak of finding adrift and wayward vessels during our Armstrong cruises (i.e. the autonomous sailboat recovered during AR025). During our transit south from Woods Hole to our first station, the ship noticed a small dinghy that was adrift. We recovered the dinghy (Figure 15) and reported its vessel registration number (from Connecticut) to the Coast Guard who notified the owner. On our return transit from VAC to Woods Hole, the ship was notified that a WHOI-operated OOI glider was in need of pick-up, so we made a small detour from our trackline so that the glider could be recovered (Figure 16) and brought back to WHOI.

Figure 15. The small dinghy that was recovered during our transit to the VAC station (Photo credit: Joe Warren).

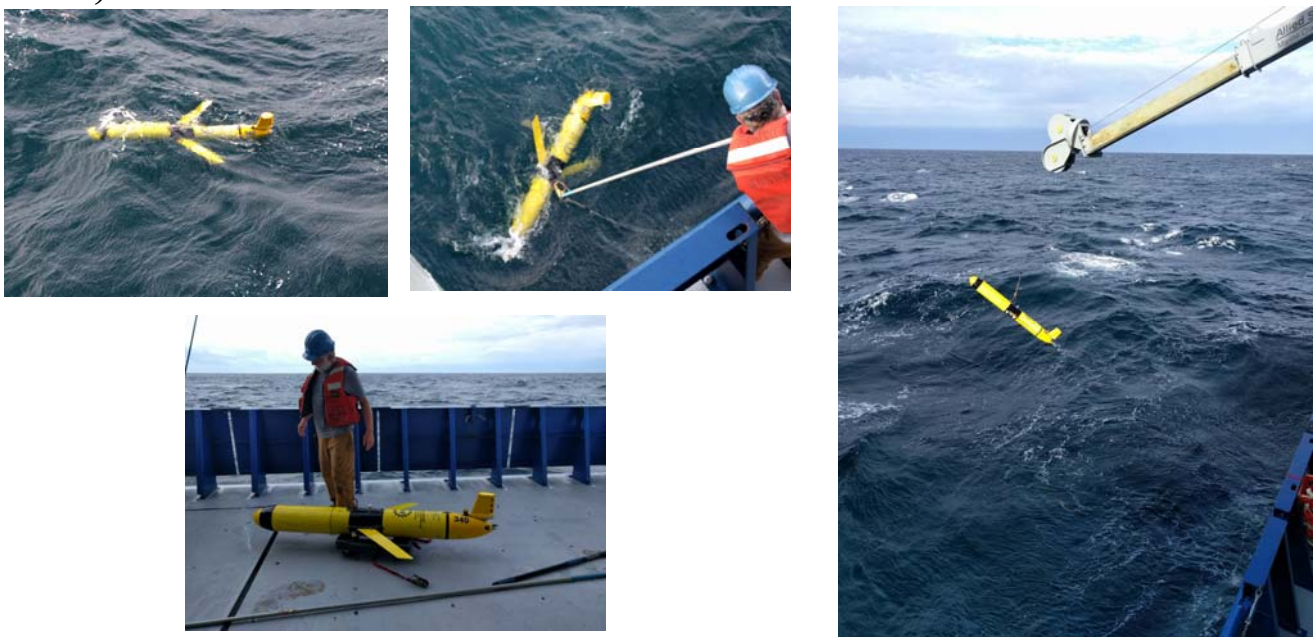

Figure 16. The RV Armstrong crew recovered a WHOI OOI glider that was not operating correctly. The glider was brought aboard the vessel and returned to the WHOI dock for maintenance (Photo credit: Joe Warren).