ADEON Recovery/Deployment Cruise Report #EN615 - RV Endeavor

06 – 25 June 2018
San Juan, Puerto Rico to Narragansett, RI

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Cruise Summary

The objectives for this cruise were to recover bottom landers at seven sites (Figure 1) along the shelfbreak (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 8 km by 8 km) multi-frequency acoustic surveys at each site (Figure 2). All cruise objectives were completed safely. In addition, 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. Water samples were also collected by a UNH undergraduate in support of her capstone paper. We were fortunate to have good weather for much of the cruise which allowed us to complete additional net tows, CTD, and fine-scale acoustic surveys at some sites (Table 1). 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 EN615 research cruise. We were able to accomplish additional sampling at the VAC and HAT sites.

Site	**Lander Recovered and 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	2	1	2	Yes (2)	Yes	Yes	Yes
HAT	Yes	2	1	2	Yes (2)	Yes	Yes	Yes
WIL	Yes	2	1	2	Yes	Yes	Yes	Yes
SAV	Yes	1	1	1	Yes	Yes	Yes	Yes
BLE	Yes	2	1	1	Yes	Yes	Yes	Yes
JAX	Yes	2	1	1	Yes	Yes	Yes	Yes
CHB	Yes	2	1	1	Yes	Yes	Yes	Yes
Cruise Total	7	13	7	10	9			

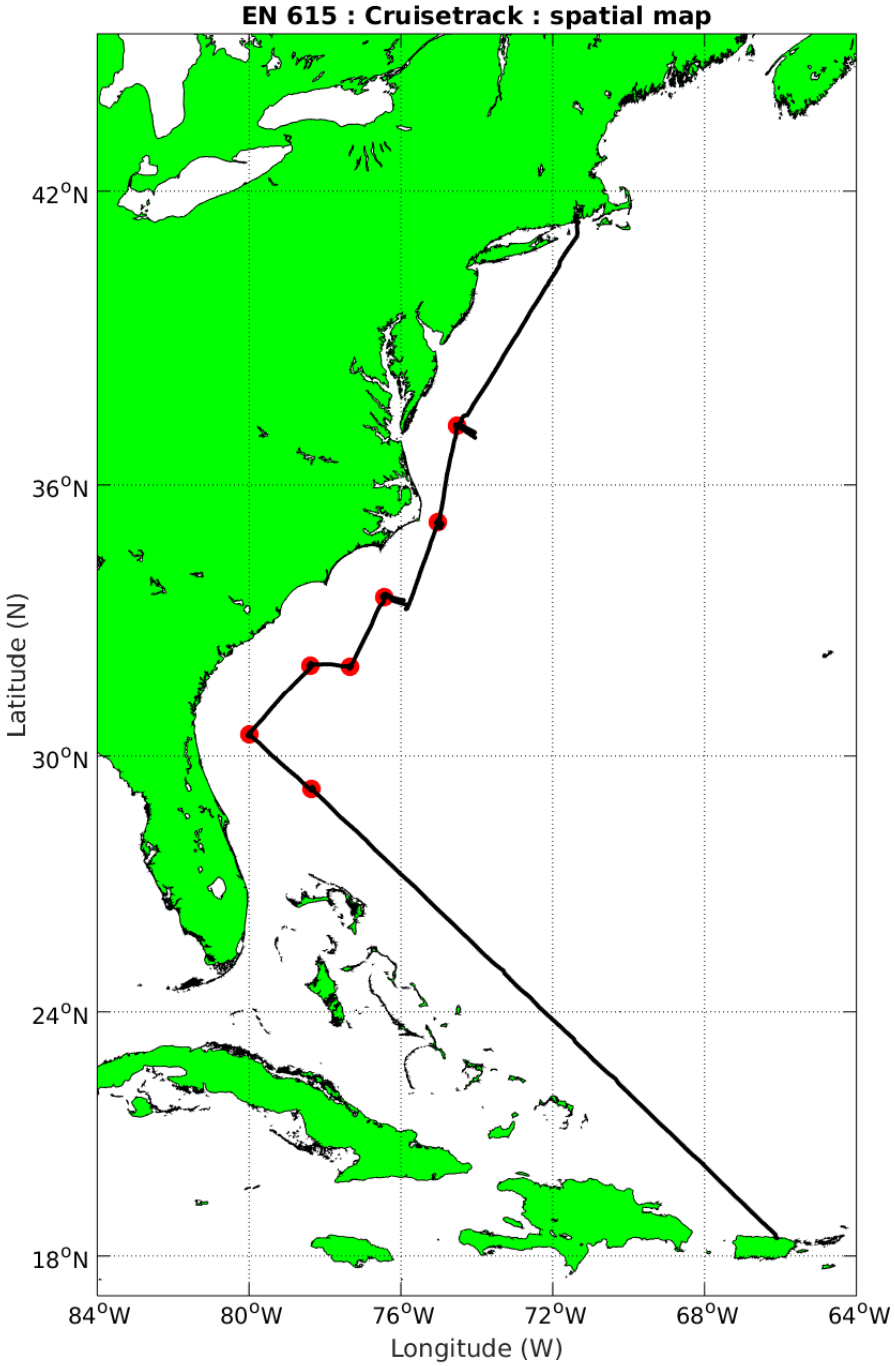

Figure 1. Cruise track for RV Endeavor Cruise #EN615 from 06 – 25 June 2018. Site locations are highlighted by red circles.

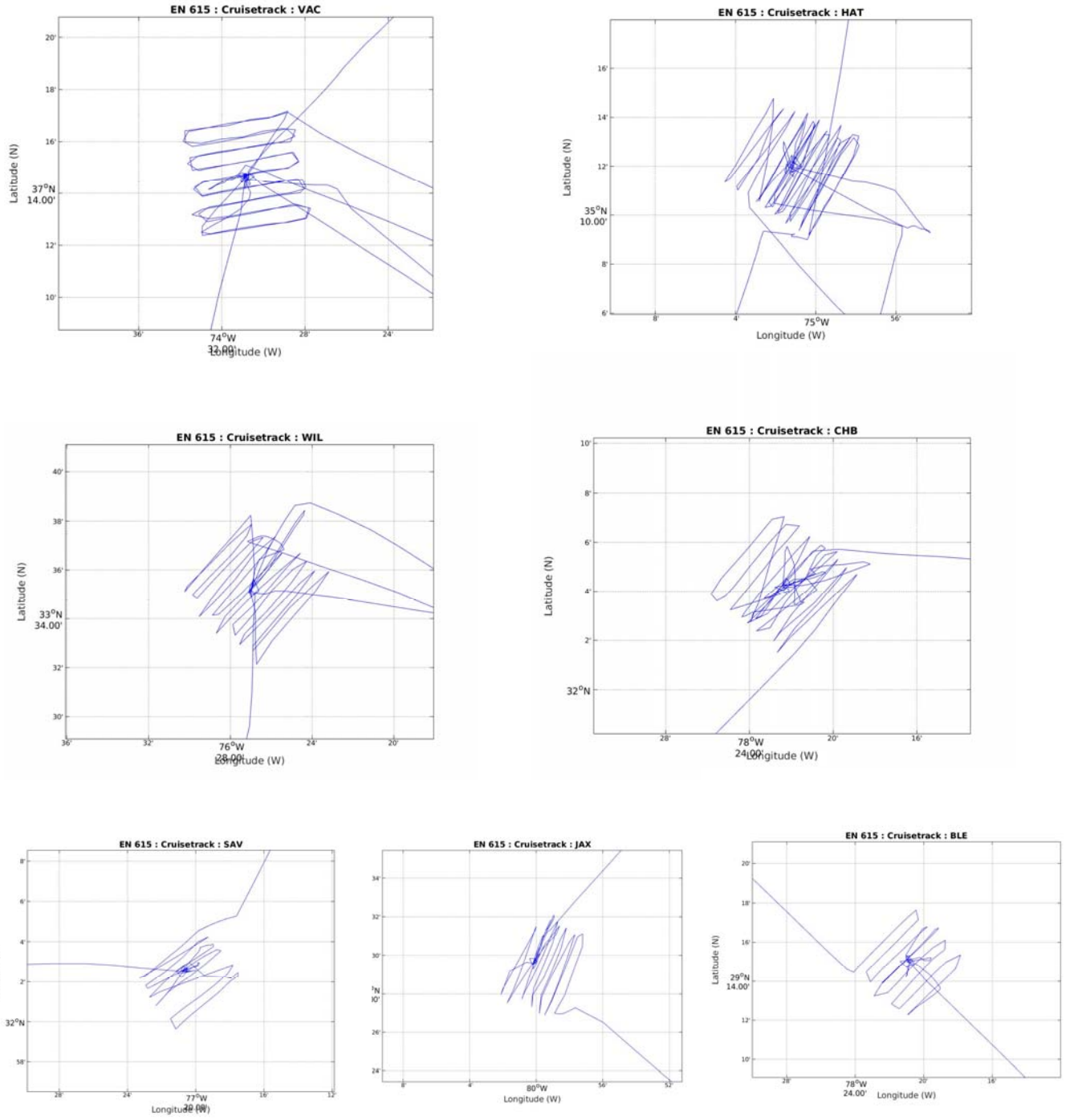

Figure 2. Cruise track for the ship at each site during RV Endeavor Cruise #EN615.

Bottom 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 appropriate, the lander was released from the anchor. Once the lander was spotted on the surface, the vessel approached the lander on the starboard side aft and was hooked via a snap hook line that went to the A-frame of the vessel and to the capstan. The lander was then brought on board through the A-frame and placed on a new anchor.

At three of the seven stations (JAX, HAT and VAC), the AZFP with transducers and VEMCO V2R2 receivers were dismounted from the lander for new batteries and data download. They were then installed on the new lander to be deployed again at the same site.

Lander deployment was performed via the A-frame, capstan, 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. Much of the hardware showing corrosion was either replaced or cleaned (Figure 3). New anodes (Figure 4) were added to the hydrophone cages and uprights, and a new anchor, AMAR, and acoustic releases were installed. The visual flasher, satellite beacon, and MicroCAT CTDs were refurbished at sea and replaced on each lander once the data had been downloaded and the batteries replaced.

The mounting position of the beacon had to be modified at each site due to the placement of the water sensor within the beacon housing to assist in triggering the surface response. Several of the hydrophones also needed to be swapped out because of decreased system gain values. (Figure 5)

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	N29 15.069	W78 21.007	10-Jun-2018	17:04	N29 15.021	W78 21.007	867
JAX	N30 29.581	W80 00.172	12-Jun-2018	17:23	N30 29.693	W80 00.139	318
CHB	N32 04.230	W78 22.444	13-Jun-2018	17:11	N32 04.279	W78 22.396	404
SAV	N32 02.523	W77 20.857	14-Jun-2018	21:55	N32 02.626	W77 20.755	792
WIL	N33 35.164	W76 27.045	15-Jun-2018	22:33	N33 35.278	W76 26.954	456
HAT	N35 11.987	W75 01.225	18-Jun-2018	17:15	N35 11.959	W75 01.218	294
VAC	N37 14.762	W74 30.862	20-Jun-2018	17:23	N37 14.754	W74 30.855	212

Figure 3. Hardware corrosion on lander (photo by Joe Warren).

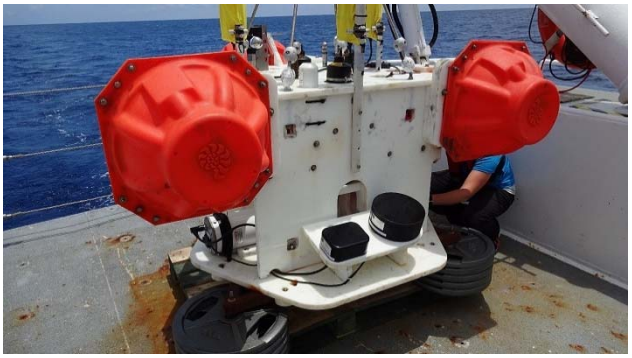

Figure 4. Lander post-refurbishment, showing replaced hardware and anodes (photo by Jennifer Miksis-Olds).

Figure 5. Modified beacon mount and added anodes (photo by Carmen Lawrence).

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 5 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 130 on-effort MMO hours were logged (Table 3). Most of the marine mammals sighted during this cruise were small to medium odontocetes, as the larger baleen whales have likely migrated out of the area north for the season. Species confirmed were bottlenose dolphin, rough-toothed dolphin, common dolphin (Figure 6), Atlantic white-sided dolphin, and pilot whales.

Figure 6. Common dolphin sighted within 25 m of R/V Endeavor (Photo credit S. Velez).

Table 3. MMO Effort Log for EN615 ADEON Cruise 2.

Date	Local Time Start	Local Time End	Hours Effort	Site
6/6/2018				Transit
6/7/2018				Transit
6/8/2018				Transit
6/9/2018				Transit
6/10/2018	6:08	20:00	13:52	BLE
6/11/2019	17:58	20:00	2:02	JAX
6/12/2019	5:58	15:07	9:09	JAX
6/13/2019	5:58	20:02	14:04	CHB
6/14/2019	5:52	8:57	3:05	CHB
6/14/2019	10:24	20:01	9:37	SAV
6/15/2019	5:59	7:15	1:16	SAV
6/15/2019	14:43	20:00	5:17	WIL
6/16/2019	6:00	20:02	14:02	WIL
6/17/2019	6:00	8:47	2:47	WIL
6/17/2019	18:20	19:59	1:39	HAT
6/18/2019	6:00	19:59	13:59	HAT
6/19/2019	5:59	17:01	11:02	HAT
6/20/2019	6:01	19:59	13:58	VAC
6/21/2019	5:55	19:59	14:04	VAC
6/22/2019	6:00	6:45	0:45	VAC
6/23/2019				Transit
6/24/2019				Transit

Multiple Frequency Acoustic Echosounder Data – Joe Warren

The RV Endeavor (unlike the previous ADEON cruise aboard the RV Armstrong) does not contain any hull-mounted echosounders. Therefore a pole-mount (Figure 7) was deployed on the starboard side of the ship (roughly even with the door to the main lab) with an 8 ft vertical arm at the end of which were 38, 70, 120, and 200 kHz transducers. The pole could be raised and lowered by a 3 person team when conditions (sea-state, vessel speed needs) warranted. Surveys running in the direction of sea state and current could be conducted at speeds of 6-7 kts (through the water), however vessel speeds needed to be slower (3-4 kts through the water) when running into currents or sea state or when sea state was elevated. The primary issues with the pole mount were: the loss of one orange fender (belonging to the ship) which was swept away by a wave), an inboard-outboard wiggle in the vertical pole when conditions were rough, and wave forces hitting the raised pole during high speed transits. Installation was done under ideal conditions, while disassembly was conducted in somewhat poorer conditions (rain, increased seas). Both installation and disassembly required additional personnel and the use of the ship knuckle crane.

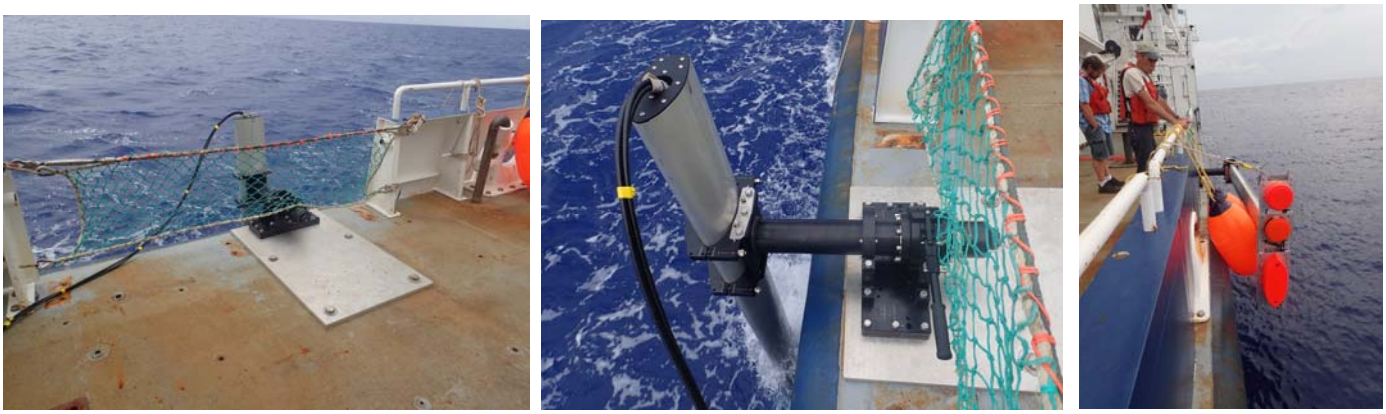

Figure 7. To install the pole-mount system on the ship, a bulwark was removed, and a 4ft x ~2.5ft x 1" aluminum plate was bolted to the deck bolt grid. The pole-mount apparatus was attached to the aluminum plate with the pole in the horizontal position. (Photos by Joe Warren)

Three fishery echosounders (Figure 8) were used during this cruise: an ES60 GPT (Simrad) with a 120kHz-7C transducer; a WBT mini (Simrad) with a 38kHz-18 200kHz-18 transducer; a WBT (Simrad) with a 70kHz-18CD transducer. The 70kHz and 120kHz transducers are 4 quadrant split-beam transducer, the 38 kHz transducer is a 3 triad split-beam transducer, and the 200kHz is a single-beam transducer. The broadband systems (38, 70, and 200 kHz) were run in narrowband (CW) mode the majority of the time, although at least one survey transect at each site was run with those systems in broadband mode.

A calibration was conducted on 17 June on all transducers at the WIL site. With good sea state and several people with long arms and a willingness to lie down on the deck holding a fishing pole, we were able to get good coverage on all quadrants on all transducers.

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 8. Example echograms from the ES60 at 120kHz (left) and the EK80 at 38, 70, and 200 kHz (right). (Photo by Joe Warren)

At each survey site, a fine-scale acoustic grid (Figure 9) was conducted at a speed of 4-5 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.

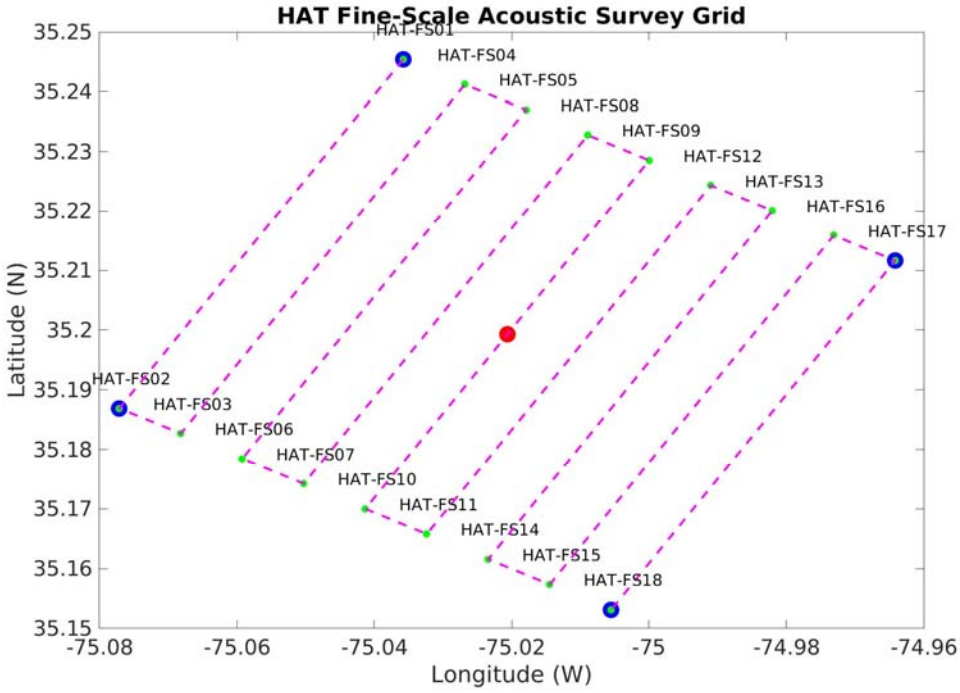

Figure 9. The planned grid for the fine-scale acoustic survey at the HAT site. The red dot at the center represents the location of the bottom lander. The survey grid covers an area roughly 8 km by 8 km. Due to limited night-time (as this cruise was during the summer) some lines were skipped in order to complete the grid before the sun rose.

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 10). 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.

A larger net (5 m2 Isaacs-Kidd Midwater Trawl) was also deployed at each site (Figure 10), 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 echosounder. Additional tows were conducted to sample the deep (> 750 m) scattering layers and to collect specimens from the mesopelagic region.

Figure 10. Bongo net (left) being deployed for a vertical cast to a depth of 100 m. The Isaacs Kidd Midwater Trawl (right) being deployed. Wire-out speeds were 20 – 40 meters per second, and haul-back speeds were 10 to 30 meters per second. Tow depths ranged from ~150 m to 1300 m.

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 11), individual preservation, or for collaborators with the DEEP SEARCH project for stable isotope and DNA analysis.

Figure 11. Some of the animals collected by net tows during the EN615 cruise.

Density Contrast Measurements – Hannah Blair

The density contrast (g) of an organism, or the ratio of animal density to the surrounding seawater, is an important acoustic material property. Density contrast values are major components of acoustic scattering models, which allow for estimating zooplankton abundances from active acoustic data. We conducted shipboard density contrast measurements on zooplankton collected by net tows throughout the EN615 cruise.

Our shipboard method for measuring density required seawater of known salinity and vegetable glycerin. As soon as possible following each tow, animals were placed in a known volume of seawater in a clear beaker, so that they sank to the bottom. A 50:50 solution of glycerin to seawater was added until the animal floated neutrally-buoyant within the fluid mixture. If the animal floated all the way to the surface of the mixture, more seawater was added until the animal sunk again and neutral buoyancy was achieved (Figure 12). When each animal was neutrally buoyant, the temperature of the solution was recorded and each animal was photographed with a ruler (Figure 13).

Figure 12. A neutrally-buoyant crab megalops.

Figure 13. Three *Maurolicus weitzmani* fish measured for lengths.

Following each bongo and IKMT tow, the zooplankton sample was inspected and animals were separated by taxonomic type. Animals from the sample were selected for g-measurement if five to ten animals of a type were present and more than 20 individuals of that type were not already measured. Certain types of animals were excluded as they are too dense to be measured with this technique, including pteropods.

A total of 263 animals were measured for density contrast (Figure 14). Animal types measured included fish, amphipods, mollusks, ctenophores, jellyfish, chaetognaths, stomatopods, euphausiids, and decapods.

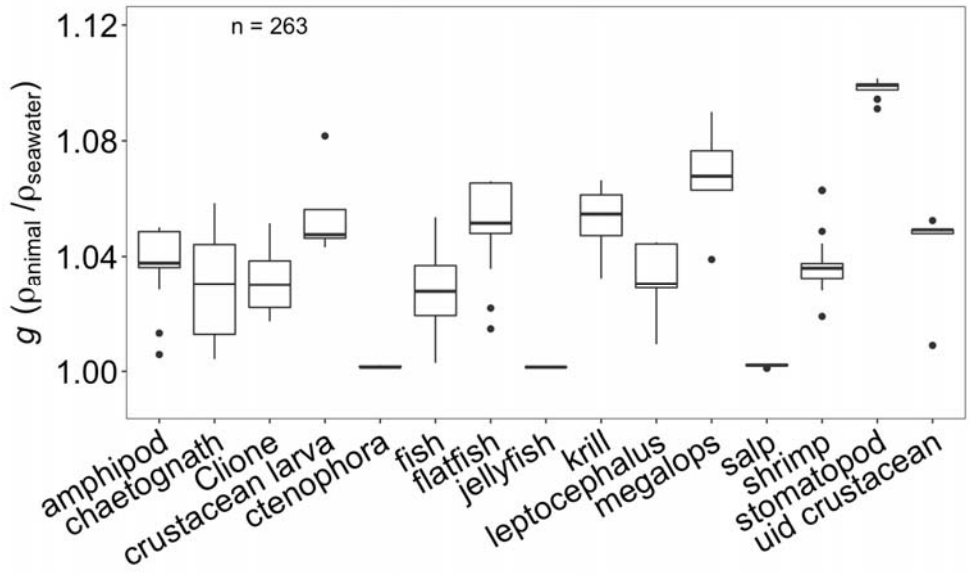

Figure 14. Density contrast (g) values varied both within and among different taxa collected from net tows. The minimum g value was 1.001, and the maximum was 1.102.

Copepod Species Identification – Cassandra Fries

On this cruise, copepods collected in the nets were identified. Samples were collected mostly from bongo net tows, and some larger copepods were found in the IKMT samples. Copepods were separated out and photographed. The photographs were more helpful than the microscope for identifying, as it had a better magnification and a more in-depth view of appendages. About 5 different copepods were identified, and more were found but were not able to be identified. Included are photos of a *Candacia* sp. copepod found, and *Rhincalanus* spp. Copepods (Figures 15 and 16). Additional investigation needs to be done to be able to narrow down the identification to exact species.

Figure 15: Rhincalanus spp. From HAT site, cast B-06.

Figure 16: *Candacia* sp. From WIL site, cast B-05. Spine on the genital segment means male.

Euphausiid and Shrimp Identification – Peter Larios

Sampling an IKMT net taking place primarily at night was used to collect shrimp and euphausiid species which were identified to different families including: Solenoceridae, Aristeida, and Pandalidae. While genera identified include Haliporus, Plesionika, and Peisos. One krill species was identified, species indicum of the genus Stylocheiron.

Figure 17: Specimen of the family Anchistiodidae collected at station 6.

Figure 18. Specimen of the genus Haliporus collected at a deep tow location at station five.

Biological Sample Collections for collaborators with the DEEP SEARCH project - Sebastian Velez

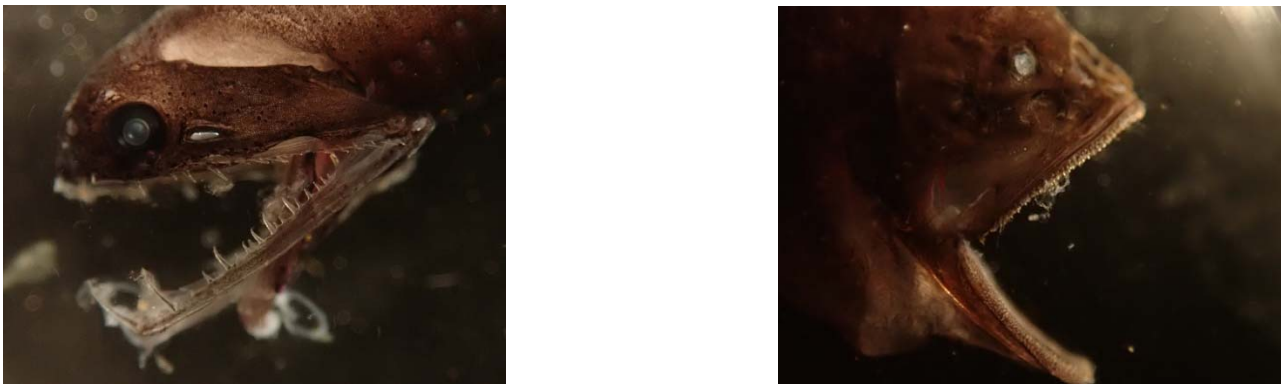

Figure 19: On the left we have a *Photostomias guerni* (left) from family Malacosteidae and a whalefish on the right (Family: Cetomimidae). These images represent the only individuals of these families caught onboard this research cruise. (Photo Credit: Sebastian Velez)

A series of biological samples were collected from this research cruise for the DEEP SEARCH NOPP. Tissue samples were collected and stored in 95% Ethanol for DNA Analysis from larger specimens while whole frozen individuals (both invertebrates and vertebrates) were collected for future isotope analysis and DNA analysis. A total of 128 samples for DNA analysis were collected from a variety of different specimens, the majority of which were whole frozen for future analyses (Table 4). Animals were collected for future isotope analyses whenever 10 individuals of a single species were found within the IKMT trawl. A total of 160 individual animals were collected that met these criteria (Table 4).

It should be noted that the biodiversity of these hauls was much higher than what is represented in these collections. We were limited in our sampling by the number of individuals within a single species per haul. The condition of many of the animals upon retrieval of the IKMT net would range from pristine to unrecognizable depending on the length of time the net was in the water and the organism itself. As a result, identification of species was made difficult with those organisms who deteriorated within the net.

The encountered fishes on this cruise included members of the following taxonomic groups: Acanthuridae, Antennariidae, Bramidae, Caproidae, Carangidae, Cetomimidae, Chauliodontidae, Echeneidae, Eurypharyngidae, Evermanellidae, Exocoetidae, Gadiformes, Gempylidae, Gonostomatidae, Holocentridae, Labridae, Malacosteidae, Melamphaeidae, Monacanthidae, Myctophidae, Nomeidae, Paralepididae, Percophidae, Phosichthyidae, Pomacanthidae, Pleuronectiformes, Priacanthidae, Scombridae, Scorpaenidae, Serranidae, Sternoptychidae, Tetraodontidae, and Triglidae. Taxonomic groups that were found at each net tow site are provided in Tables 5-14.

Figure 20. Flatfish larvae were a common occurrence in our hauls onboard the *R/V Endeavor*. Here we have a larval *Chasconopsetta danae*, also known as the Angry Pelican Flounder. (Photo Credit: Sebastian Velez)

Table 4: Tissue and isotope samples from the ADEON research cruise aboard the *R/V Endeavor.*

Lowest Taxonomic Identification	**Common Name**	**Number of Isotope Samples**	**Number of DNA Samples**
Amphipods	-	10	2
Anthias nicholsi	Yellowfin Bass	-	1
Antigonia capros	Deepbody Boarfish	-	1
Argyropelecus sp.	Hatchetfish	-	2
Bembrops sp.	Flathead	-	2
Bonapartia pedaliota	-	-	1
Caranx sp.	Jack	--	1
Chauliodus sp.	Viperfish	-	1
Clione sp.	Sea Angel	-	1
Copepods	-	-	3
Cyclothone sp.	Bristlemouth	30	3
Gempylidae	Snake mackerals	-	1
Krill	-	30	10
Leptocephali	-	-	7
Maurolicus weitzmani	Weitzman's Pearlside	10	3
Melamphaeidae	Slimeheads	-	2
Myctophidae	Lanternfishes	20	4
Ostracod	-	-	1
Paralepididae	Deepsea Lizardfish	-	4
Phronema sp.	-	10	1
Pleuronectiformes	Flatfish	-	9
Pteropods	-	-	19
Salps	-	10	2
Scombridae	-	-	3
Scorpaenidae	Scorpionfish	-	1
Shrimps	-	40	31
Sigmops elongatum	-	-	1

Squids	-	-	3
Stephanolepis hispidus	Planehead Filefish	-	1
Sternoptyx sp.	Hatchetfish	-	3
Stomatopod	-	-	2
Tetraodontidae	Pufferfish	-	1
Urophycis sp.	Hake	-	1

Table 5. Fish biodiversity at the Blake Escarpment site.

Site: Blake Escarpment	
Lowest Taxonomic Identification	**Common Names**
Sphoeroides maculatus	Northern puffer
Ichthyococcus sp.	-
Malamphaeidae	Slimeheads

Table 6. Fish biodiversity at the Jacksonville site.

Site: Jacksonville	
Lowest Taxonomic Identification	**Common Names**
Antigonia capros	Deepbody Boarfish
Holocentridae	Squirrelfish
Scombridae	-
Caranx sp.	Jack
Psenes cyanophrys	Freckled driftfish
Nesiarchus nasutus	Black gemfish
Coryphaena hippurus	Mahi mahi
Priacanthus arenatus	Bigeye
Prestigenys alta	Short Bigeye
Scorpaenidae	Scorpionfish
Remora osteochir	Marlin Sucker
Acanthurus sp.	Surgeonfish
Centropyge argi	Angelfish
Gempylidae	Snake Mackerals
Bothus sp.	Flatfish
Sigmops elongatum	-

Figure 21. Although deep-sea fishes were common in our tows we also had a solid representation of many epipelagic species. Here we have a tilefish (*Caulolatilus sp.*) and pompano (*Trachinotus sp.*), both of which are important for recreational and commercial fisheries. (Photo Credit: Sebastian Velez)

Table 7. Fish biodiversity at the Charleston Bump site.

Site: Charleston Bump	
Lowest Taxonomic Identification	**Common Names**
Prionotus sp.	Triglidae
Cyclopsetta fimbriata	Spotfin Flounder
Scombridae	-
Myctophidae	Lanternfishes
Selene sp.	Lookdown
Trachinotus sp.	Pompano
Caranx sp.	Jack
Caulolatilus sp.	Tilefish
Vinciguerria sp.	-

Table 8. Fish biodiversity at the Savannah Deep site.

Site: Savannah Deep	
Lowest Taxonomic Identification	**Common Names**
Bembrops gobioides	Triglidae
Argyropelecus hemigymnus	Short Silver Hatchetfish
Cheilopogon sp.	Flying Fish
Halichoeres sp.	Wrasse

Table 9. Fish biodiversity at the Wilmington site.

Site: Wilmington	
Lowest Taxonomic Identification	**Common Names**
Selene vomer	Lookdown
Scombridae	-
Bembrops sp.	Flathead
Urophycis sp.	Hake
Cyclothone sp.	Bristlemouth
Bothus sp.	Flatfish

Anthias nicholsi	Yellowfin Bass
Hemanthias vivanus	Red Barbier
Chascanopsetta danae	Angry Pelican Flounder

Table 10. Fish biodiversity at the Wilmington site's second tow.

Site: Wilmington 2	
Lowest Taxonomic Identification	**Common Names**
Vallenciennellus tripunctulatus	-
Eurypharynx pelecanoides	Gulper eel
Argyropelecus sp.	Hatchetfish
Sternoptyx sp.	Hatchetfish
Cyclothone sp.	Bristlemouth
Trichopsetta ventralis	Sash Flounder
Pterycombus brama	Atlantic Fanfish
Centropyge argi	Angelfish
Antenariidae	Frogfish
Gempylidae	Snake Mackerals
Myctophidae	Lanternfishes
Bonapartia pedaliota	-
Vinciguerria sp.	-
Sigmops elongatum	-
Chauliodus sp.	Viperfish

Table 11. Fish biodiversity at the Hatteras site.

Site: Hatteras	
Lowest Taxonomic Identification	**Common Names**
Maurolicus weitzmani	Weitzman's Pearlside
Syacium papillosum	Dusky Flounder
Scorpaenidae	Scorpionfish
Anthias nicholsi	Yellowfin Bass
Stephanolepis hispidus	Planehead Filefish
Halichoeres sp.	Wrasse
Caranx sp.	Jack

Table 12 Fish biodiversity at the Hatteras site's second tow.

Site: Hatteras 2	
Lowest Taxonomic Identification	**Common Names**
Stephanolepis hispidus	Planehead Filefish
Anthias nicholsi	Yellowfin Bass
Scorpaenidae	Scorpionfish
Paralepididae	Deepsea Lizardfishes
Sternoptyx sp.	Hatchetfish
Carangidae	-
Scopelogadus sp.	Slimehead
Bothus sp.	Flatfish

Melamphaeidae	Slimeheads
Cyclothone sp.	Bristlemouth

Figure 22. These are the light emitting organs (photophores) of Weitzman's Pearlside (*Maurolicus weitzmani*) used to break up its silhouette and stay hidden from predators down below. (Photo Credit: Sebastian Velez)

Table 13. Fish biodiversity at the Virginia Canyon site.

Site: Virginia Canyon	
Lowest Taxonomic Identification	**Common Names**
Maurolicus weitzmani	Weitzman's Pearlside
Tetraodontidae	Pufferfish
Myctophidae	Lanternfishes

Table 14. Fish biodiversity at the Virginia Canyon site's second tow.

Site: Virginia Canyon 2	
Lowest Taxonomic Identification	**Common Names**
Cyclothone sp.	Bristlemouth
Paralepididae	Deepsea Lizardfish
Sternoptyx sp.	Hatchetfish
Argyropelecus sp.	Hatchetfish
Maurolicus weitzmani	Weitzman's Pearlside
Myctophidae	Lanternfishes
Gempylidae	Snake Mackerals
Lampadena sp.	Lanternfish
Photostomias guerni	-

Water Collection for DEEP SEARCH Collaborators – Katharine Coykendall

USGS scientists Cheryl Morrison (Leetown Science Center), Amanda Demopoulos (Wetlands and Aquatic Research Center) and Nancy Prouty (Pacific Marine and Coastal Science) are co-project leads of the multi-year, multi-agency DEEP SEARCH project, which aims to increase baseline geological and biological resource information in off shore areas of the mid-Atlantic United States. The first major sampling effort for the project was for April 2018 aboard NOAA's R/V Nancy Foster. However, the boat sustained damage during dry dock which resulted in the cancellation of the entire research cruise. Luckily, the principal investigator, Dr. Jennifer Miksis-Olds and chief scientist Joe Warren of the ADEON project are both collaborators on the DEEP SEARCH project and were able to accommodate me (K Coykendall, USGS Leetown Science Center) to collect samples for the three USGS collaborators mentioned above.

The overall objective for USGS scientists C Morrison and K Coykendall is to characterize the different taxonomic groups from different parts of the water column, with emphasis on the deep scattering layer, and document how that changes both horizontally (within the water column at single sites) and vertically (between sampling sites). Using eDNA techniques, whereby water is collected and filtered, then DNA extracted from the filters, we hope to gain insight into the major taxa found at various depths in the water column and if those groups change in identity and/or proportion with change in depth. Our results will complement the acoustic data collected on this cruise as well. The eDNA we collected from each niskin will most likely be a pool from a wide variety of taxonomic groups. To be able to identify those organisms whose DNA is present in our samples, we need to have a baseline of genetic information from known organisms from the southern mid-Atlantic Bight region. Therefore, our second objective was to obtain samples from the common taxa recovered from the net sampling on this cruise.

To meet the first objective, water was collected from up to five depths at each of the sampling sites – Blake's Escarpment (BLE), Jacksonville (JAX), Charleston Bump (CHB), Savannah (SAV), Wilmington (WIL), Cape Hatteras (HAT), and Virginia Inter-Canyon (VAC). Originally, our team assumed that there were 12 total niskins on the CTD rosette that held 12 liters of water and so planned to sample four depths per sampling site. Once onboard, we learned there were 24, 10L niskins and decided to add a fifth depth per sampling site. Our sampling required three replicates per depth as well, so at least three niskins were fired per depth. The depths sampled were bottom, surface (5m), the deep scattering layer, and 50-100m above and below the deep scattering layer. From each replicate niskin, 1 liter of water was run through a Sterivex filter, then stored the filter at -80C. Three samples were taken from the bottom depth and 3 from the surface from first sample site, BLE. This site is slightly outside the sampling region proposed in the DEEP SEARCH project. Three replicates from 5 depths from the remainder of the sites were obtained, except for the VAC site, where one of the niskins failed to fire, so there were only 2 replicates for that depth. Including the 6 control samples (3 replicates of 1 liter of filtered Milli-Q water pre-sampling and post-sampling), a total of 101 filtered water samples for subsequent eDNA extraction and sequencing.

To meet the second objective, the onboard fish taxonomic expert, Sebastian Velez, set aside representatives of the major taxonomic groups recovered from the IKMT net sampling, provided that it was a duplicate sample. A total of 105 samples were obtained from from 10 IKMT net tows and two Bongo net tows (Table 15). Most of the samples are whole organisms preserved in 95% molecular

grade ethanol. Most of the fish specimens were identified to the lowest possible taxonomic level by Sebastian Velez. Crustaceans (shrimp, krill, copepods, and amphipods) were a dominant taxonomic group recovered in the IKMT net tows. A handful of the shrimp specimens were identified to family, genus, or species by Peter Larios. Additionally, ten specimens of Paracalanus copepods were obtained from the Bongo06 sampling event and identified by Cassandra Fries. For the remainder of the unclassified specimens, once barcoding genes are sequenced, we will cross reference our results with the taxonomic classifications obtained through onshore collaborators of J Warren.

In addition to eDNA samples, water samples were acquired for USGS collaborator N Prouty, who is interested in nutrient content and δ15N NO3 at varying water depths. Not much is known about nitrogen cycling, especially in the deep sea. Capturing the ratio between N-14 (atmospheric nitrogen) and N-15 lends insight into the origin of non-atmospheric nitrogen in the ocean. Two fifty milliliter samples were taken from five depths at six sampling sites (HAT was skipped) for a total of 60 samples. Samples were stored at -80C after sampling.

Water was filtered from five to seven depths for USGS collaborator A Demopoulos to estimate the particulate organic matter at varying water depths with specific interest in how food webs are connected between surface waters and the deep sea and how that varies spatially. This sampling required from 2.7L – 9.3L of water filtered via vacuum pump onto 47 micron pre-weighed filters. Five depths were sampled at the seven sites listed above. In addition, 6 depths were sampled from a site ~30 miles from the Wilmington site, in deeper water (WIL2). Seven depths were sampled from a deeper site near the HAT site as well (HAT2). Filters were stored at -80C. A total of 48 samples were taken.

Table 15. Summary of sample collection for DEEP SEARCH collaborators from USGS.

Sample ID	Station	Sampling Event	Phylum	Subphylum	Class	Order	Family	Genus	Species	Taxa
B01	BLE	Bongo01	Arthropoda	Crustacea	Malacostraca	Euphausiacea				krill
B02	BLE	Bongo01	Arthropoda	Crustacea		Decapoda				shrimp
I01	BLE	IKMT01	Mollusca		Gastropoda					pterapod
I02	BLE	IKMT01	Arthropoda	Crustacea		Decapoda				shrimp
I03	BLE	IKMT01	Arthropoda	Crustacea	Malacostraca	Euphausiacea				krill
I04	BLE	IKMT01	Cnidaria		Hydrozoa	Siphonophorae				siphonophore
I05	JAX	IKMT02	Arthropoda	Crustacea	Malacostraca	Stomatopoda				stomatopod
I06	JAX	IKMT02	Arthropoda	Crustacea	Malacostraca	Amphipoda				amphipod
I07	JAX	IKMT02	Arthropoda	Crustacea						megalops crab larva
I08	JAX	IKMT02	Mollusca		Gastropoda					pterapod curly
I09	JAX	IKMT02	Arthropoda	Crustacea	Malacostraca	Amphipoda				amphipod
I10	JAX	IKMT02	Arthropoda	Crustacea	Malacostraca	Sstomatopoda				
I11	JAX	IKMT02	Arthropoda	Crustacea						lobster-like
I12	JAX	IKMT02	Mollusca		Gastropoda					
I13	JAX	IKMT02	Chordata		Actinopterygii	Pleuronectiformes	bothidae	bothus		flounder
I14	JAX	IKMT02	Chordata		Actinopterygii	Perciformes	caproidae	antigonia	copros	boarfish
I15	CHB	IKMT03	Chordata		Actinopterygii					leptocephali
I16	CHB	IKMT03	Chordata		Actinopterygii					leptocephali
I17	CHB	IKMT03	Chordata		Actinopterygii					leptocephali
I18	CHB	IKMT03	Chordata		Actinopterygii	Pleuronectiformes	bothidae	bothus		flounder
I19	CHB	IKMT03	Chordata		Actinopterygii					leptocephali
I20	CHB	IKMT03	Mollusca		Gastropoda					pterapod
I21	CHB	IKMT03	Mollusca		Gastropoda					pterapod curly
I22	CHB	IKMT03	Ctenaphora							ctenaphore
I23	SAV	IKMT04	Arthropoda	Crustacea		Decapoda				shrimp
I24	SAV	IKMT04	Arthropoda	Crustacea	Malacostraca	Euphausiacea				krill
I25	SAV	IKMT04	Arthropoda	Crustacea		Decapoda				shrimp
I26	SAV	IKMT04	Arthropoda	Crustacea		Decapoda				shrimp
I27	SAV	IKMT04	Chordata		Actinopterygii					leptocephali
I28	SAV	IKMT04	Chordata		Actinopterygii					leptocephali
I29	SAV	IKMT04	Arthropoda	Crustacea						

I30	SAV	IKMT04	Mollusca		Gastropoda						pterapod
I31	SAV	IKMT04	Chordata		Actinopterygii						
I32	SAV	IKMT04	Chordata		Actinopterygii	Pleuronectiformes	bothidae	bothus			flounder
I33	SAV	IKMT04	Chordata		Actinopterygii	Stomiformes	sternoptychidae	argyropelecus	hemigymnus		hatchet fish
I34	WIL	IKMT05	Chordata		Actinopterygii						leptocephali
I35	WIL	IKMT05	Chordata		Actinopterygii	Perciformes	scombridae				
I36	WIL	IKMT05	Chordata		Actinopterygii	Pleuronectiformes	bothidae	bothus			flounder
I37	WIL	IKMT05	Chordata		Actinopterygii	Pleuronectiformes					flatfish
I38	WIL	IKMT05	Arthropoda	Crustacea		Decapoda					shrimp
I39	WIL	IKMT05	Arthropoda	Crustacea	Malacostraca	Euphausiacea					krill
I40	WIL	IKMT05	Mollusca		Gastropoda						pterapod curly
I41	WIL2	IKMT06	Chordata		Actinopterygii						leptocephali
I42	WIL2	IKMT06	Chordata		Actinopterygii						leptocephali
I43	WIL2	IKMT06	Chordata		Actinopterygii		sternoptychidae				hatchetfish
I44	WIL2	IKMT06	Chordata		Actinopterygii		sternoptychidae				hatchetfish
I45	WIL2	IKMT06	Arthropoda	Crustacea		Decapoda					shrimp
I46	WIL2	IKMT06	Arthropoda	Crustacea							
I47	WIL2	IKMT06	Arthropoda	Crustacea	Malacostraca	Euphausiacea					krill
I48	WIL2	IKMT06	Arthropoda	Crustacea	Malacostraca	Euphausiacea					krill
I49	WIL2	IKMT06	Arthropoda	Crustacea	Malacostraca	Euphausiacea					krill
I50	WIL2	IKMT06	Arthropoda	Crustacea		Decapoda					shrimp
I51	WIL2	IKMT06	Chordata		Actinopterygii			cyclothone			bristlemouth
I52	WIL2	IKMT06	Chordata		Actinopterygii	Pleuronectiformes	bothidae	bothus			flounder
I53	WIL2	IKMT06	Mollusca		Gastropoda						pterapod curly
I54	WIL2	IKMT06	Mollusca		Gastropoda						pterapod
I55	WIL2	IKMT06	Arthropoda		Ostracoda						ostracod
I56	HAT2	IKMT08	Chordata		Actinopterygii	Stephanoberyciformes	melamphaidae				
I57	HAT2	IKMT08	Chordata		Actinopterygii			cyclothone			bristlemouth
I58	HAT2	IKMT08	Chordata		Actinopterygii						leptocephali
I59	HAT2	IKMT08	Chordata		Actinopterygii						leptocephali
I60	HAT2	IKMT08	Chordata		Actinopterygii						leptocephali
I61	HAT2	IKMT08	Chordata		Actinopterygii						leptocephali
I62	HAT2	IKMT08	Chordata		Actinopterygii	Aulopiformes	paralepididae				barracudina

I63	HAT2	IKMT08	Chordata		Actinopterygii	Perciformes	serranidae	anthias	nicholsi	yellowfin bass
I64	HAT2	IKMT08	Chordata		Actinopterygii	Tetraodontiformes	monacanthidae	stephanolepis	hispidus	planehead filefish
I65	HAT2	IKMT08	Arthropoda	Crustacea	Hexanauplia					
I66	HAT2	IKMT08	Arthropoda	Crustacea	Hexanauplia					
I67	HAT2	IKMT08	Arthropoda	Crustacea						
I68	HAT2	IKMT08	Arthropoda	Crustacea						
I69	HAT2	IKMT08	Arthropoda	Crustacea						
I70	HAT2	IKMT08	Mollusca		Cephalopoda					big squid
I71	HAT2	IKMT08	Chordata		Actinopterygii	Stephanoberyciformes	melamphaidae	scopelogadus		slimehead
I72	HAT2	IKMT08	Chordata		Actinopterygii					leptocephali
I73	HAT2	IKMT08	Chordata		Actinopterygii					leptocephali
I74	HAT2	IKMT08	Chordata		Actinopterygii	Pleuronectiformes	bothidae	bothus		flounder
I75	HAT2	IKMT08	Chordata		Actinopterygii	Pleuronectiformes				flatfish
I76	HAT2	IKMT08	Chordata		Actinopterygii	Pleuronectiformes				flatfish
I77	HAT2	IKMT08	Arthropoda	Crustacea		Decapoda				shrimp
I78	HAT2	IKMT08	Arthropoda	Crustacea		Decapoda				shrimp
I79	HAT2	IKMT08	Arthropoda	Crustacea		Decapoda				shrimp
I80	HAT2	IKMT08	Arthropoda	Crustacea	Malacostraca	Stomatopoda				
I81	HAT2	IKMT08	Arthropoda	Crustacea		Decapoda				shrimp
I82	HAT2	IKMT08	Arthropoda	Crustacea		Decapoda				shrimp
I83	HAT2	IKMT08	Arthropoda	Crustacea						
I84	HAT2	IKMT08	Mollusca		Gastropoda					pterapod
I85	HAT2	IKMT08	Ctenaphora							
B3	HAT	Bongo06	Arthropoda	Crustacea	Hexanauplia	Calanoida	paracalanidae	paracalanus	sp	10 copepods
I86	VAC	IKMT09	Chordata		Actinopterygii	Stomiiforme		cyclothone		bristlemouth
I87	VAC	IKMT09	Arthropoda	Crustacea	Malacostraca	Euphausiacea		stylocheiron	indicum	krill
I88	VAC	IKMT09	Arthropoda	Crustacea	Malacostraca	Euphausiacea		stylocheiron	indicum	krill
I89	VAC	IKMT09	Chordata		Actinopterygii	Stomiiformes	sternoptychidae	maurolicus	weitzmani	Atlantic pearlside
I90	VAC	IKMT09	Arthropoda		Malacostraca	Amphipoda	phronimidae	phronima		
I91	VAC	IKMT09	mollusca		Gastropoda		Clionidae	Clione		
I92	VAC	IKMT09	Arthropoda		Malacostraca	Amphipoda				
I93	VAC	IKMT09	Arthropoda	Crustacea	Malacostraca	Decapoda	sergestidae	peisos		
I94	VAC	IKMT10	Arthropoda	Crustacea	Malacostraca	Decapoda	sergestidae	peisos		

I95	VAC	IKMT10	Arthropoda	Crustacea	Malacostraca	Decapoda	pandalidae	plesionika		
I96	VAC	IKMT10	Arthropoda	Crustacea						copepod
I97	VAC	IKMT10	Chordata		Actinopterygii					leptocephali
I98	VAC	IKMT10	Chordata		Actinopterygii	Myctophiformes	Mmyctophidae			lanternfish
I99	VAC	IKMT10	Chordata		Actinopterygii	Aulopiformes	paralepididae			barracudina
I100	VAC	IKMT10	Chordata		Actinopterygii	Myctophiformes	myctophidae	lampadena		lanternfish
I101	VAC	IKMT10	Chordata		Actinopterygii	Stomiiformes	sternoptychidae	maurolicus	weitzmani	Atlantic pearlside
I102	VAC	IKMT10	Chordata		Actinopterygii	Stomiifrmes		cyclothone		bristlemouth
I103	VAC	IKMT10	Arthropoda		Malacostraca	Amphipoda				
I104	VAC	IKMT10	Arthropoda		Malacostraca	Amphipoda	phronimidae	phronima		
I105	VAC	IKMT10	Chordata	Tunicata	Thaliacea	Salpida	salpidae			salp

Enzyme Assays/Samples for Nutrients/Bacterial Cell Counts - Madison Alstede

For my undergraduate senior capstone project, water samples were analyzed through enzyme assays aboard the RV Endeavor, and samples were taken for nutrient analysis. After the cruise, I will be further analyzing the data I collected and interpreting the relation between bioavailable inorganic nutrients and the metabolic rates of heterotrophic microbial communities along the eastern continental shelf.

At each site, water samples were taken from the CTD (Figure 23). Two depths were to be studied; bottom water and the deep chlorophyll maximum. The deep chlorophyll maximum was found from the fluorescence profile of the water column during the downcast of the CTD, and bottom water was taken approximately 10 meters from the seafloor bottom. Two 50 mL filtered water samples were taken for nitrogen and phosphorus analysis from each of the designated depths. The samples were frozen for future analysis. Cryovials were labelled and filled with 1.5 mL of sampled water and also frozen for future cell count analysis. In addition, 200 mL samples from the designated depths were taken to be used in enzyme assays that occurred aboard the ship (Table 16).

Based on the temperature of the sample, two different enzyme assays were set up (Figure 24). The enzyme assay with water from the deep chlorophyll maximum was kept at room temperature and bottom water was kept in the refrigerator. The main focus of the enzyme assays was on nitrogen and phosphorus cleaving enzymes and their growth rate in three time points over a period of 24 hours. Substrates, PO4 and LEU, were used as well as borate buffer and the sampled water. The substrates and the sampled water were combined, and 1 mL of the solution was added to 1 mL of buffer before measuring the fluorescence. The fluorescence and time were recorded and repeated for every cuvette in that time point. Over 24 hours, the water and substrate solution would react with each other, and once added to the buffer, the fluorescence would increase/decrease.

The data from the enzyme assays will be entered into Microsoft Excel and the metabolic rates of the microbial communities at each site will be able to be interpreted more in depth. The filtered, frozen samples taken for nutrient analysis are expected to be analyzed further at the University of New Hampshire under the guidance of Dr. Robert Letscher and the frozen cryovials are expected to be analyzed through flow cytometry for cell counts.

Table 16. The set up of each enzyme assay in table format.

	Control PO4	PO4			Control LEU	LEU		
A	Cuvette 1	Cuvette 1	Cuvette 1	Cuvette 1	Cuvette 1	Cuvette 1	Cuvette 1	Cuvette 1
B	Cuvette 2	Cuvette 2	Cuvette 2	Cuvette 2	Cuvette 2	Cuvette 2	Cuvette 2	Cuvette 2
C	Cuvette 3	Cuvette 3	Cuvette 3	Cuvette 3	Cuvette 3	Cuvette 3	Cuvette 3	Cuvette 3

Figure 23. Taking water samples from the CTD (Photo by Jen Miksis-Olds).

Figure 24. Setting up for the start of the enzyme assays (Photo by Jen Miksis-Olds).

Artist at Sea – Lindsay Olson

Art and science are deeply human endeavors. How scientific research is communicated to the public needs imaginative, creative storytellers who connect scientific discoveries to human experience in exciting ways. By participating in the ADEON Cruise 2, I was inspired to create accessible art that will help others understand the exciting information researchers are discovering by listening in to the ocean soundscape. My goal was to spend time on the cruise participating in the science, learning the basics of ocean acoustics and learning how the field of ocean acoustics is assessing biologic, human, and abiotic sound to study dynamics in the OCS.

Although our society depends on the work of scientific research every day, many remain confused about how science is conducted. When designing my projects, it's important to create art that explains the specific science I am learning and also include the broader context of how science is planned, organized, and executed. Working in close quarters for three weeks, I was able to observe, interview, and be inspired by the work of scientists Dr. Miksis-Olds, Dr. Warren, our excellent ships' crew, and students for the duration of the voyage. Based on these interviews and the hands-on experience of our shift work, I will create colorful, engaging textile art that inspires viewers to take a closer look at ocean acoustics (Figure 25).

Figure 25. Artist-at-sea (and day shift watch-stander) Lindsay Olson laying out fabric samples as she plans her work.

I will base my work on data collected at the acoustically rich Virginia (VAC) site to create two textile art pieces. Using data collected by the lander and the Fine Scale Acoustic Survey (FSAS) equipment, one piece will represent passive acoustics (40"x40") and the other piece active acoustics (40"x60"). The pieces are designed to be light weight and easy to ship.

Once I return to the studio, I will continue learning about ocean acoustics using the Discovery of Sound In The Sea web site (dosits.org), begin work on the background of both pieces, experiment with various materials and techniques, and complete the work over a period of 12-16 months. I will also look for project funding for supplies and outreach travel expenses, research appropriate venues to show the work, arrange for photography of the work, pitch articles to various publications, and book speaking engagements. Dr. Miksis-Olds has invited me to debut the art work at the Ocean Discovery Day event at the University of New Hampshire in September of 2019.

Working aboard the R/V Endeavor has been the trip of a life time and a unique opportunity to experience life and science at sea. This has been among one of my strongest project collaborations to

date and I appreciate the help and support Dr. Jennifer Miksis-Olds and her team have given me. I'm looking forward to the coming months of additional study and art making. The breadth of her vision and her organization of all the moving parts of this complex research will sustain me over the months of work ahead.