DEEPEND

DEEP PELAGIC NEKTON DYNAMICS OF THE GULF OF MEXICO

Cruise Report

R/V *Point Sur* cruise DP08

Photo credit: 2022 DEEPEND/Rosanna Boyle

25 July – 7 August 2022

DEEPEND DP08 Cruise Participants on the R/V *Point Sur*

From top—down, left to right: Gray Lawson, Dan Hahn, Kevin Boswell, Tamara Frank, Heather Judkins, Lisa Rose-Mann, Heather Bracken-Grissom, Pedro Peres, Haley Glasmann, Jon Moore, Hannah Johnson, April Cook, Rosanna Milligan, Tracey Sutton, Danté Fenolio

Report of
DEEPEND Cruise DP08
25 July – 07 August 2022; USM R/V *Point Sur*, Gulfport, MS
Chief Scientist: Tracey Sutton

This report was prepared by: Tracey Sutton, Heather Bracken-Grissom, Kevin Boswell, April Cook, Danté Fenolio, Tammy Frank, Haley Glasmann, Hannah Johnson, Matthew Johnston, Heather Judkins, Gray Lawson, Jon Moore, Isabel Romero, Lisa Rose-Mann, and Michael Vecchione

A DEEPEND (Deep Pelagic Nekton Dynamics)
Consortium Report

Available online from the DEEPEND website,
www.deependconsortium.org

Acknowledgements

This was the eighth DEEPEND cruise in the Gulf of Mexico. The success of this cruise was due to the outstanding efforts of the Captain and Crew of the R/V *Point Sur*, LUMCON Marine Operations, the University of Southern Mississippi Department of Marine Science, Okeanus Science and Technology, Sea-Gear Corporation, the San Antonio Zoo, and all members of the science party. This cruise was supported by the National Oceanic and Atmospheric Administration's RESTORE Science Program under award NA19NOS4510193 to Nova Southeastern University.

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1 Purpose of Cruise

The DEEPEND Consortium is an ocean realm field project supported by the RESTORE Science Program. The focus of the DEEPEND Consortium is to develop a quantitative, taxonomically comprehensive assessment of the deep-pelagic faunal assemblages of the northern Gulf of Mexico (GoM hereafter), investigate longer-term consequences of the Deepwater Horizon oil spill (DWHOS) on these assemblages, and translate knowledge learned into resource management efforts. The project goals of this eighth cruise included: 1) quantitative assessment of deep-pelagic nekton (fishes, macrocrustaceans, and cephalopods) assemblage structure, abundance, and distribution; 2) quantitative acoustic profiling of the fine- and mesoscale distributions of oceanic nekton; 3) collect nekton samples for genomics/genetic analyses; 4) collect *in situ* biophysical oceanographic data for community analyses; 5) conduct a pilot study on water filtration for environmental DNA; and 6) collect photographic and video content for various imaging projects. As with previous DEEPEND cruises, sampling/sensing was conducted aboard the R/V *Point Sur* in the northern GoM using established SEAMAP stations (see Figure 1). Scientific participants on this cruise (see frontispiece) included: expert taxonomists in the major deep-pelagic nekton faunal groups, acousticians, geneticists, technicians, an outreach/imaging specialist, and graduate students. Specimens were identified at sea using traditional taxonomic approaches. After the cruise, molecular analyses and expert taxonomic evaluation and description of any putative new records or undescribed species were completed in association with the DEEPEND Taxonomic Working Groups.

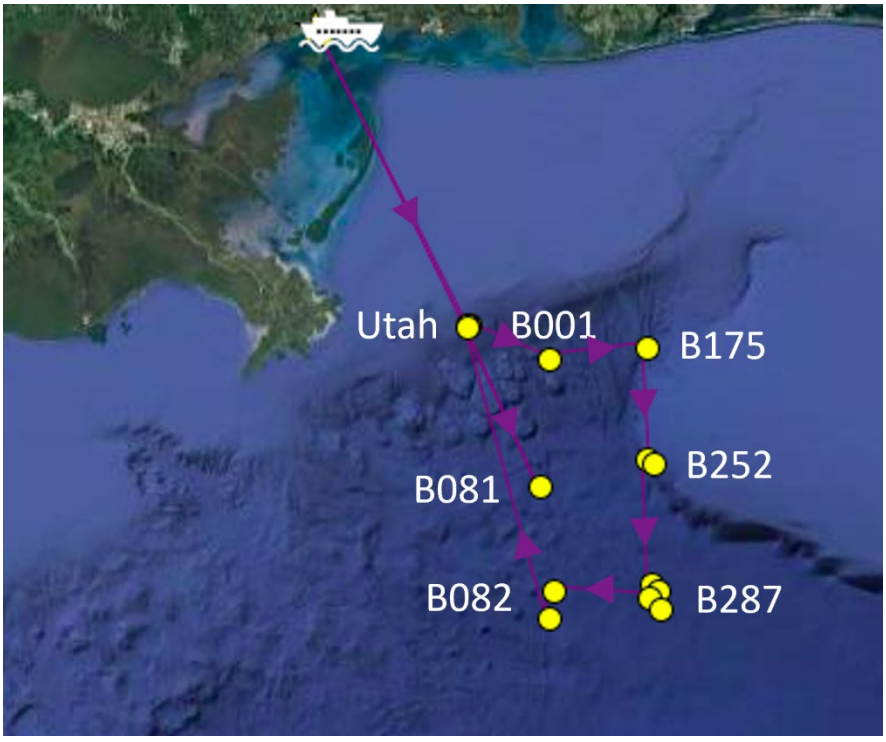

Figure 1. Cruise track of DEEPEND cruise DP08 (27 July - 07 August 2022) relative to seafloor topography.

2 Narrative

Ship's cruise number: PS_23_04_Sutton
DEEPEND cruise number: DP08

All cruise activity times are presented as 24-h clock notation in Central Daylight Time (UTC – 5 h). A map of standardized station names and station order is presented in Figure 1. The naming conventions for trawl samples remained the same as those used in DP01, DP02, DP03 DP04, DP05, DP06, and DP07:
Example: DP08-27JUL22-MOC10-B250N-221-N0.
Key: Cruise No. – Date – Gear Type - SEAMAP station code + (N = night, D = day) - Trawl No. - Net No.

Trawl numbers are cumulatively increased across all sampling years and are not restarted each cruise.

27 July 2022: We left Gulfport at 0011 and arrived at Station UTAH (29°53.511'N, 88°44.655'W) at 0404 and lowered the acoustic transducer. A CTD cast (CTD_225) of Station UTAH during the day was conducted at 1014 to a maximum depth of 334 m. A second CTD cast (CTD_226) of Station UTAH at night was conducted at 1943 to a maximum depth of 342 m. The MOCNESS gear was deployed at 2106 for Trawl 221 at station UTAH and fished to a maximum depth of 450 m. The MOC-10 conducted a tow-yo trawl (Figure 2). The lanyard on net 4 broke which prematurely released net 5 which fished most of the downcast. Nets 4 and 5 will be added together and be considered "meat" samples.

28 July 2022: The MOC-10 gear was recovered from station UTAH at 0153. The codends were emptied and processing began. The ship transited to station B001 (29°03.178N, 88°10.080W). A daytime CTD cast (CTD_227) of station B001 was conducted at 0636 to a maximum depth of 1400 m. The CTD touched the bottom and caused a discrepancy between the acoustic and pressure sensors so only the downcast will be utilized. The MOC-10 gear was deployed at 0912 (Trawl 222) and recovered onto deck at 1456. The nets were fished to a maximum depth of 1639 m. We stayed at station B001 to repeat these tows and conducted a nighttime CTD cast (CTD_228) at 1903 to a maximum depth of 1480 m. A discrepancy appeared in the acoustic depth and pressure depth. The ship sounder 3.5 was used to confirm the CTD pressure as correct. The MOC-10 gear was deployed (Trawl 223) at 2102 and fished to a maximum depth of 1644 m. All nets 1-5 fished standardized depth bins (net 0: 0-1500 m, net 1: 1500-1200 m, net 2: 1200-1000 m, net 3: 1000-600 m, net 4: 600-200 m, net 5: 200-0 m).

29 July 2022: The MOC-10 gear (Trawl 223) was retrieved at 0255. The acoustic transducer pole was pulled out of the water and the ship transited to station B175 (29°00.175N, 87°31.131W). The transducer was lowered at 0546. We conducted a daytime CTD cast (CTD_229) of station B175 at 0652 to a depth of 1555 m. The MOC-10 gear was deployed (Trawl 224) at 0902. Nets 0-5 were fished to a maximum depth of 1503 m. The MOC-10 was recovered at 1456. We stayed at station B175 to repeat these tows. A nighttime CTD cast (CTD_230) was conducted at 1729 to a depth of 1500 m with a Wideband Autonomous Transceiver (WBAT) attached. The MOC-10 gear was deployed at station B175 (Trawl 225) at 2103 to a maximum depth of 1504 m. Winch issues occurred at the start of the downcast on net 0 and net 1 did not respond. Net 1 and net 2 catches were combined and considered "non-standard" samples.

30 July 2022: The MOC-10 gear (Trawl 225) was retrieved from station B175 at 0253. The ship transited to station B252 (28°31.506N, 87°29.1755W). A daytime CTD cast (CTD_231) was conducted at 0613 to a maximum depth of 1500 m with the WBAT attached. The MOCNESS was deployed for a daytime trawl (226) at station B252 at 0911. The MOC-10 fished to a maximum depth of 1501 m and was retrieved at 1459. *Avocettina sp.* was stuck in net 3 and did not make it into the codend. We stayed at station B175 to repeat these tows. A nighttime CTD cast (CTD_232) was conducted at 1746 to a maximum depth of 1500m with the WBAT attached. The MOCNESS was deployed at station B252 for a night trawl (227) at 2100 and fished to a depth of 1505 m.

31 July 2022: The MOC-10 trawl (227) was retrieved from station B252 and brought onboard at 0250. The acoustic transducer pole was pulled out of the water and the ship transited to station B287 (29°00.175N, 87°31.131W). The acoustic transducer pole was deployed at 0613 and a daytime CTD cast (CTD_233) was conducted at 0644 to a maximum depth of 1500 m. The MOCNESS was deployed for a daytime trawl (228) at 0906 and fished to a depth of 1510 m. The MOCNESS was retrieved at 1522 and brought on deck. The MOC-10 gear experienced winch issues and fished at the surface for an extended period of time while the winch was being fixed. Therefore, net 5 was labeled as a MEAT tow. We stayed at station B287 to repeat these trawls. A nighttime CTD (CTD_234) was deployed at 1802 and was pulled back on deck at 2049. The MOCNESS (Trawl 229) was deployed at 2059 and trawled to a maximum depth of 1501 m.

01 August 2022: The MOC-10 gear (Trawl 229) was retrieved and brought onboard at 0255 for processing. The ship stayed at station B287 to repeat the tows with the dark nets instead of the white nets. A daytime CTD was conducted (CTD_235) at 0543 to a maximum depth of 1500 m before being pulled on deck at 0852. The MOCNESS was deployed at 1101 for a daytime trawl (230) to a depth of 1003 m at station B287 and then retrieved at 1500. The ship stayed at station B287 to repeat the trawls with the dark nets for a nighttime trawl. The nighttime CTD was conducted (CTD_236) at 1738 and pulled on deck at 2045. The MOC-10 gear was experiencing hydraulic failure on the A-frame and was delayed in its deployment. The MOCNESS (Trawl 231) was deployed at 2330 and trawled to a maximum depth of 1004 m.

02 August 2022: The MOCNESS (Trawl 231) was retrieved and brought on deck at 0314. The ship transited to station B082 while processing occurred. The acoustic transducer was pulled back on deck at 0830. The ship arrived at station B082 at 0956 (28°00.023N, 87°58.144W). The transducer pole was deployed at 1000 and a daytime CTD (CTD_237) was conducted at 1010 to maximum depth of 1500 m. The CTD was pulled on deck at 1107 and the MOCNESS with the dark nets (Trawl 232) was deployed at 1125. A storm rolled through the area and lasted for about 30 min during the duration the MOCNESS was in the water. The MOCNESS was pulled back on deck at 1530. Another nighttime CTD deployment began at 1856 (CTD_238) without the WBAT. The CTD reached a maximum depth of 1500 m before being pulled back onto deck at 2000. A nighttime MOCNESS (Trawl 233) was deployed at 2300 with dark nets.

03 August 2022: The MOC-10 gear (Trawl 233) was retrieved at 0259. Net 3's lanyard parted, and the net misfired. Thus, Net 3 samples were categorized as MEAT samples. A daytime CTD cast (CTD_239) was conducted to a depth of 1500 m at 0441 and brought back on deck at 0833. The MOCNESS dark nets were switched back to the white nets for the remainder of the cruise. The MOCNESS was deployed for a daytime trawl (Trawl 234) at 0925. Nets 1-5 fished to a depth of 1502

m and the gear was retrieved at 1517. The ship traveled back to port to manage a medical issue on board. The ship stayed in port until the next afternoon.

04 August 2022: The ship left port at 1444 and traveled to station UTAH. The ship arrived at UTAH at 2315 (29°08.199N, 88°22.968W). The MOC-10 gear (Trawl 235) was deployed at 2326 for a tow-yo trawl (Figure 3). The sampling depth scheme was altered to sample as follows: 0-150 m (net 0), 150-100 m (net 1), 100-60 m (net 2), 60-40 m (net 3), 40-20 m (net 4), and 20-0 m (net 5). This trawl will be considered quantitative, non-standard which means these trawl samples will need to be binned together for comparison to typical quantitative, standard trawl samples.

05 August 2022: The MOCNESS was brought on deck at 0256 and fished to a maximum depth of 150 m (Trawl 235). The acoustic transducer pole was brought up on deck at 0310 and secured for travel. The ship transited to station B081 and arrived at 0733 (28°30.498N, 88°01.187W). Upon arrival, the acoustic transducer pole was deployed and a daytime CTD (CTD_240) was conducted at 0740. The CTD sampled to a maximum of 1500 m and was pulled back on deck at 0844. The MOCNESS was deployed at 0901 (Trawl 236) and fished to a maximum depth of 1501 m until it was brough back on deck at 1507. The CTD (CTD_241) was deployed at 1730 and sampled to a maximum depth of 1500 m. The CTD was brought back on deck at 2049 and the MOCNESS was deployed at 2100 (Trawl 237).

06 August 2022: The MOC-10 gear (Trawl 237) was retrieved at 0250 and fished to a maximum depth of 1507 m. The acoustic transducer pole was back on deck at 0307 and the ship transited to station UTAH at 0312. The ship arrived at station UTAH at 0640 and the acoustic transducer pole was deployed (29°04.143N, 88°22.733W). A CTD deployment began at 0708 (CTD_242) and sampled to a maximum depth of 389 m. The CTD was brought back on deck at 0855. The MOCNESS was deployed at 1010 (Trawl 238) and fished to a maximum depth of 501 m. Trawl 238 was conducted to replicate the tow-yo Trawl 219 conducted during DP07 (Figure 4). The MOC-10 gear was retrieved at 1518. A final CTD deployment occurred at 1944 and sampled to a maximum depth of 340 m. The WBAT was calibrated before deployment and used 38.1 mm and 22 mm WC spheres. The CTD was retrieved at 2106 and the last MOCNESS deployment began at 2200 (Trawl 239).

07 August 2022: The MOC-10 gear (Trawl 239) was retrieved at 0256 and fished to a maximum depth of 506 m. The acoustic transducer pole was back on deck at 0307 and secured. The ship headed back to port at 0313 and arrived for the end of DP08 cruise at 1333.

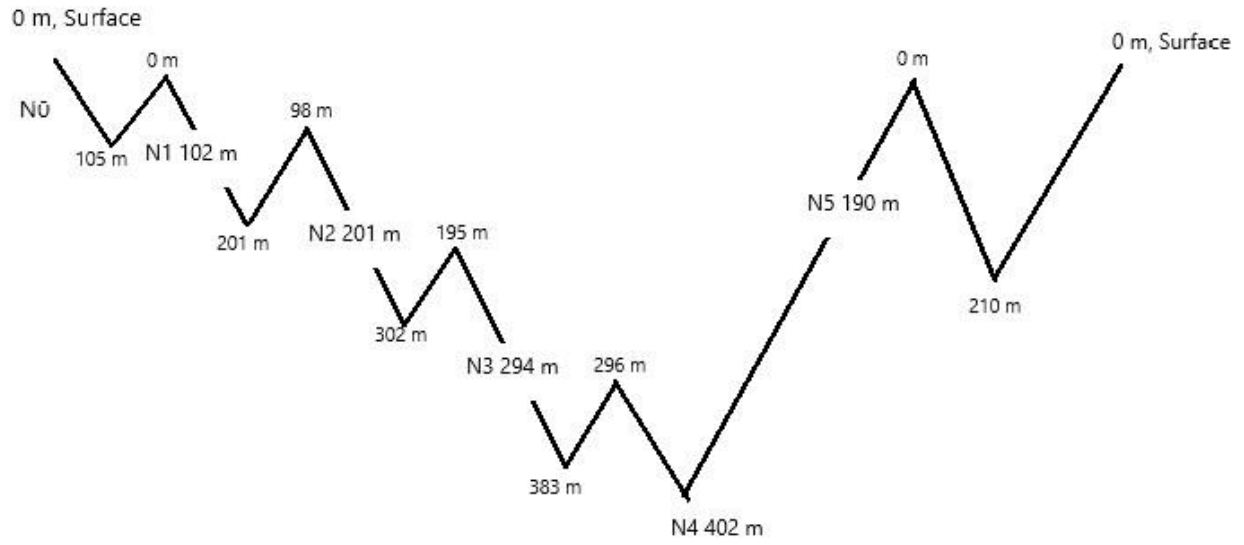

Figure 2. "Tow-yo" sampling profile for Trawl 221 at station Utah.

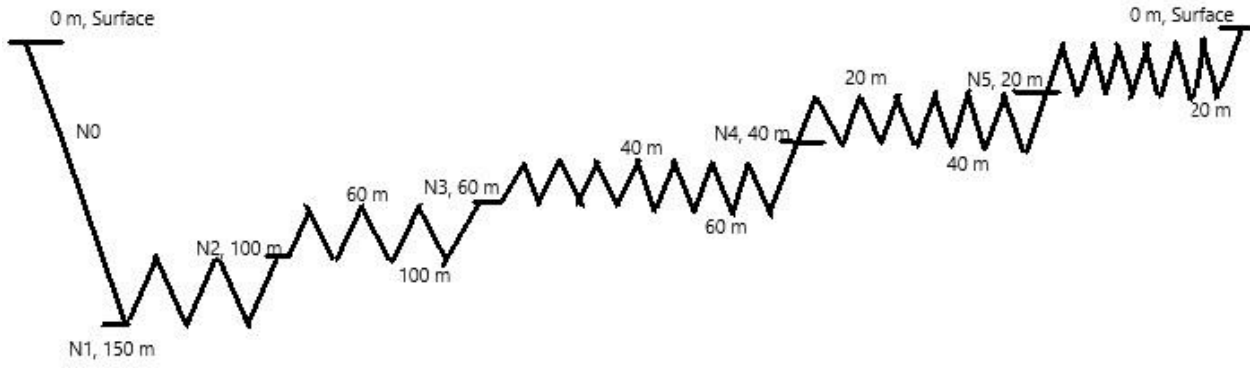

Figure 3. "Tow-yo" sampling profile for Trawl 235 at station Utah.

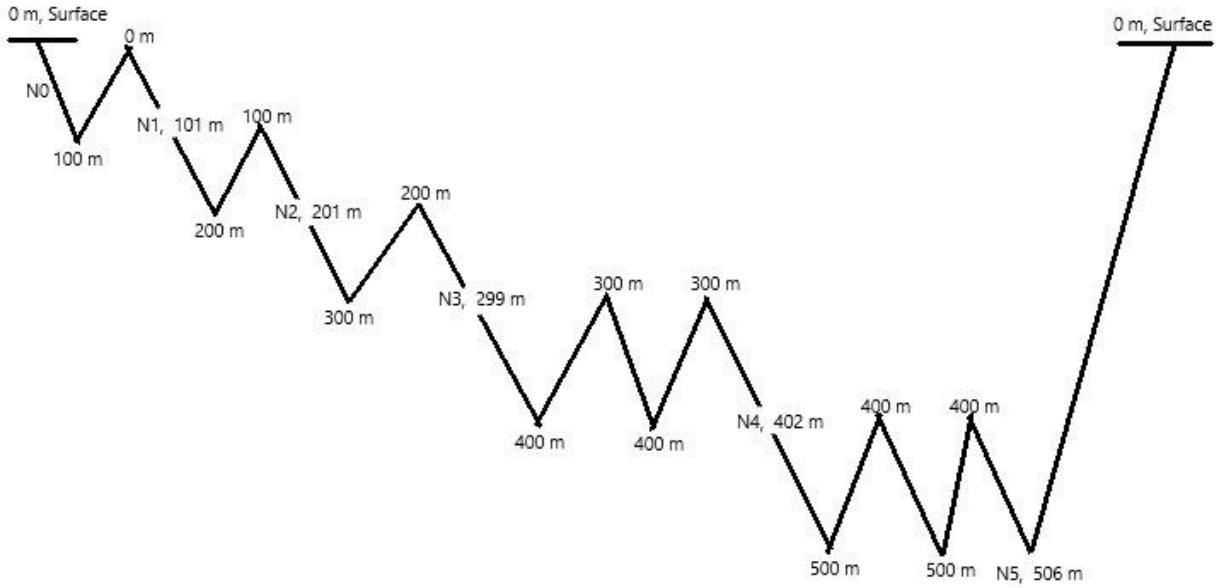

Figure 4. "Tow-yo" sampling profile for Trawls 239 and 328 at station Utah

3 Operations and Protocols

3.1 Midwater Trawling

Midwater trawling was conducted using a 10-m^2 mouth area MOCNESS (MOC-10 hereafter) unit (Figure 5), leased from OKEANUS Science and Technology (Houma, LA), rigged with six 3-mm mesh nets manufactured for DEEPEND by Sea-Gear Corporation (Melbourne, FL). Each net was fitted with a removable PVC cod end (Figure 6), numbered consecutively to correlate with depth sampled. Sampling was conducted to 1500 m, bottom depth allowing. The first net (Net 0) was fished from the surface to 1500 m, Net 1 from 1500 to 1200 m, Net 2 from 1200 to 1000 m, Net 3 from 1000 to 600 m, Net 4 from 600 to 200 m, and Net 5 from 200 m to the surface (Figure 6). This was the same depth scheme used during the NOAA NRDA Offshore Nekton Sampling and Analysis Program.

Each station was sampled at least twice, with one deployment centered at solar noon (1000 h -1600 h) and one centered at midnight (2200 h – 0400 h). During a portion of the cruise, the standard white nets were switched with 3-mm mesh dark nets at stations B287 and B082 (Trawls 230, 231, 232, 233). Sampling with the dark nets occurred at the same solar noon and midnight deployments as the white nets. After trawl 233 was completed, the dark nets were switched with white nets and trawl sampling continued with white nets for the remainder of the cruise. Ship's speed was kept minimal, between 1 and 2.5 kn. Winch deployment and retrieval speeds (non-zero) ranged from 5-25 m min^{-1}, with 15 m min^{-1} typical. The MOCNESS operator stayed in constant radio contact with the winch operator to keep the MOCNESS frame at an optimal angle (between 35-50°).

Figure 5. 10-m² MOCNESS (MOC-10) unit being retrieved (left) and codends being retrieved (right) on the R/V *Point Sur* during DEEPEND cruise DP06. Photo: DEEPEND 2018/Danté Fenolio.

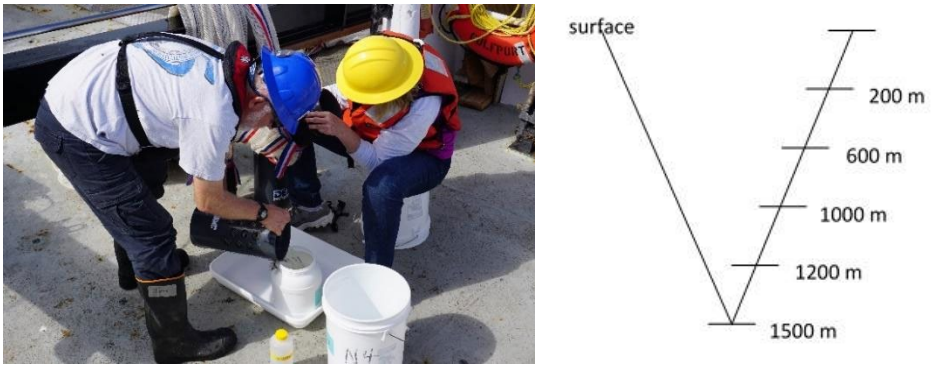

Figure 6. (Left) MOC-10 codend being collected into a cold-water bucket, and (right) depth sampling scheme.

3.2 Permitting

An E5 categorical exclusion from the National Environmental Policy Act was granted to this project on April 1, 2020. A Letter of Acknowledgement was received from NOAA Fisheries on July 8, 2020, acknowledging the proposed work and providing an exemption from the use of a turtle excluder device. The National Marine Fisheries Service Highly Migratory Species Management Division issued a Scientific Research Permit (HMS-SRP-22-39) allowing the collection of 70 unspecified tunas and 70 unspecified billfish species. All field protocols, fish handling and preservation, and removal of fish tissues were conducted in compliance with Nova Southeastern University IACUC protocol (Protocol #2020.01.TS3-A2 DEEPEND RESTORE midwater trawling) for the study of vertebrates and adhered to the USA legal requirements.

3.3 Hydroacoustics

Multi-frequency (18, 38, 70, 120, and 200 kHz) acoustic data were collected continuously during all MOC-10 deployments, CTD casts, bio-optical profiler casts, and while in transit between stations via a pole-mounted transducer (when possible, Figure 7). Mechanical and electrical noise associated with operating the MOC-10 reduced the effective range of each echosounder. The 38, 70, 120, and

200 kHz echosounders collected meaningful data at depths of approximately 1500 m, 400 m, 100 m, and 75 m respectively. An 18 kHz EK80 echosounder was used to characterize the entire water column (< 3000 m). The echosounders were calibrated using tungsten and copper spheres at sea following standardized procedures (e.g., Foote et al. 1987).

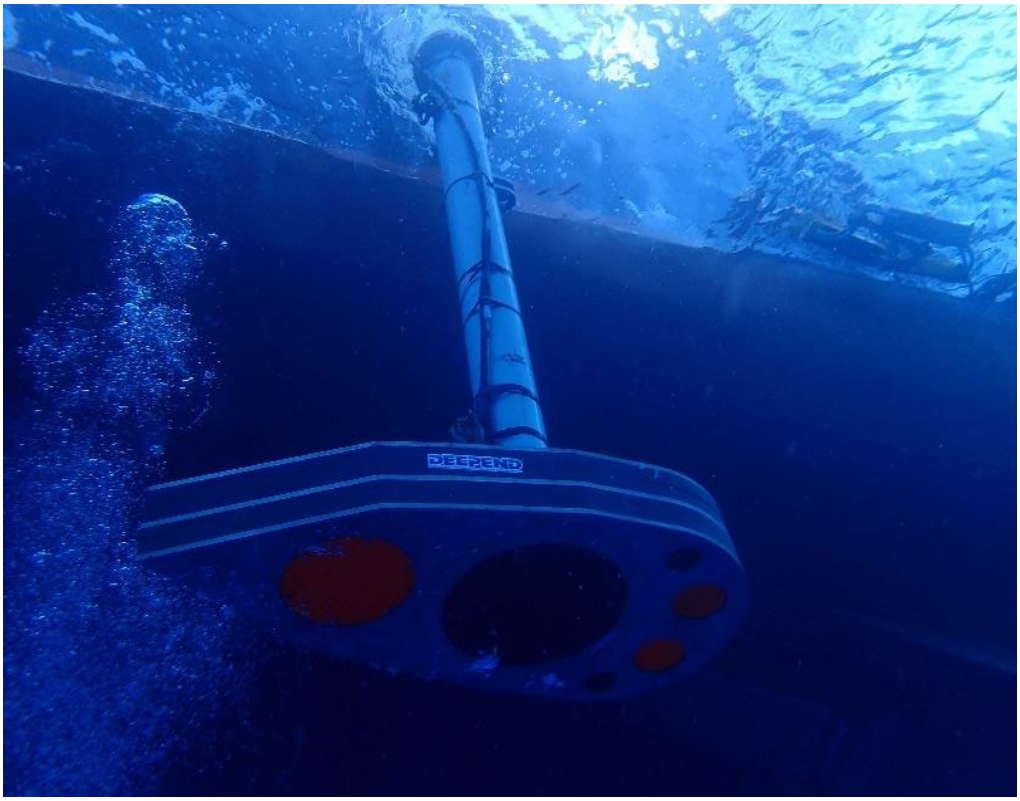

Figure 7. Hydroacoustics transducer in sensing mode (underwater) on the R/V *Point Sur.*

3.4 CTD Profiling

Twenty CTD profiles were conducted using the ship's CTD rosette (Figure 8) at nine different station locations. The WBAT was attached to the CTD (Figure 8) for some of the deployments which altered the typical speed of deployment and/or recovery. The average maximum depth of deployment was 1500m, as the WBAT is not rated to go beyond that depth threshold. A full water column profile was done first in all CTD casts, then the WBAT was held at different depth strata within the deep scattering layer to investigate fine-scale organism scattering and capture the full migration, these casts typically lasted between 3 and 5 hours, operating a 38 and 200 kHz in wideband (FM).

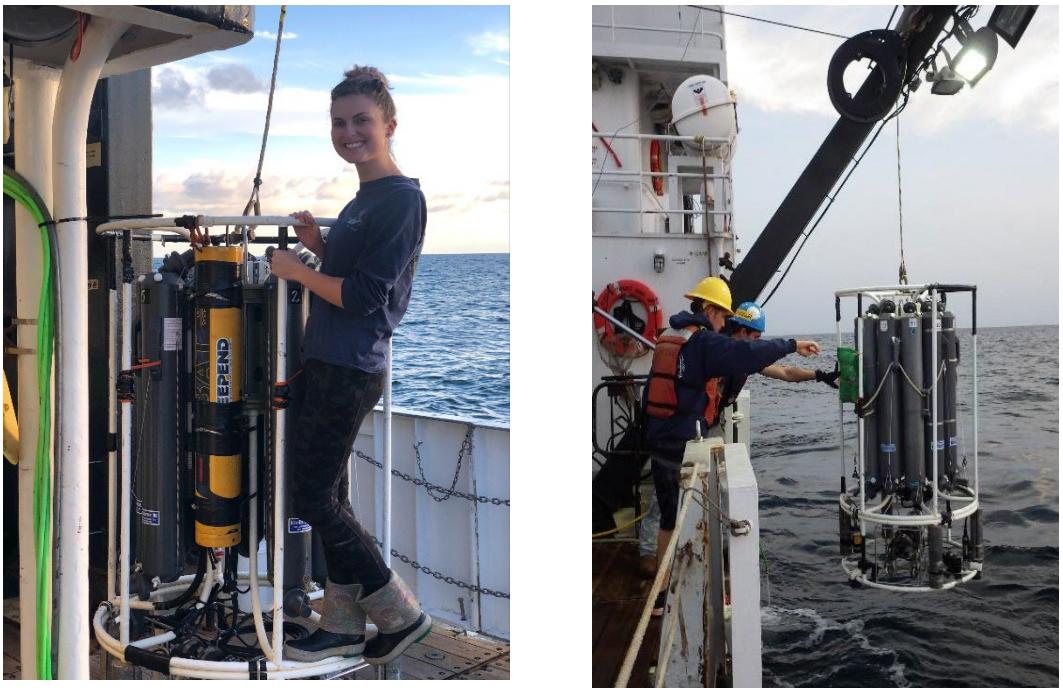

Figure 8. (Left) CTD rosette showing yellow WBAT mounted and (right) R/V *Point Sur* CTD rosette deployment.

3.5 Water Collection

Seawater was collected to perform an environmental DNA (eDNA) pilot study. Seawater was collected via CTD-mounted Niskin bottles (twelve 12-L bottles, Figure 8) at station B287N at night on August 1, 2022. Niskin bottles were fired at 450m depth. Water from CTD rosette Niskin bottles was then drawn into sterilized 1-L Nalgene plastic bottles. In the ship's lab, sterilized forceps were used to place PALL GN-6 0.45-micron filters onto a filtration rig. Seawater was filtered with a MilliporeSigma 1.3 cfm/25.5" Hg-60psi/115V vacuum pump. Filters were used to filter 5 replicates of 1-L, 5-L, 10-L, and one negative control then stored at -20C for future processing.

3.6 Sampling on Station

Sampling and sensing operations on station were organized around daytime and nighttime MOC-10 trawling, with these centered on solar noon and midnight, respectively. Each deployment took approximately 6 h. MOC-10 sample processing occurred between MOC-10 deployments, as were CTD casts. Transit to the next station generally occurred during the morning interval after day and night MOC-10 deployments at each station. Acoustic profiling was conducted during all hours except during transits when the transducer boom was raised.

4 Sample Processing Protocol

4.1 Nekton, Micronekton, and Macroplankton Samples

Upon MOC-10 recovery, individual nets were washed down with seawater to assure all collected organisms were concentrated in the cod ends. Cod ends were disconnected from the net one at a time and the contents were poured/washed into 6-L wide-mouth containers filled with pre-chilled seawater. Each container was numbered to correspond with the net from which samples were collected.

These containers were taken inside the ship's lab and stored cold in a refrigeration unit pending processing. Only one sample was processed at a time to prevent cross-sample mixing. "Net 0" (0-1500 m oblique) samples were generally processed first except in cases where live animals suitable for imaging were collected, in which case these samples were processed first. Afterwards, samples were processed in numerical order.

Processing involved the identification, enumeration, weighing (when possible) and measurement of all fish, macrocrustacean, and cephalopod specimens. Once a sample was completely subsampled, then the entire remaining sample was fixed in 10% buffered formalin (v/v formalin:seawater). A running tally was kept of specimens collected for all analyses. In the individual project reports that follow, only data for those portions of samples that were taken for genetic or biochemical analyses are included. The remaining data will be presented after the complete laboratory sample work-up.
Tissues or whole samples were taken of each taxon according to a pre-determined protocol. Sample processing for genetic analyses was as follows:

1) fishes were preserved whole, or the lateral muscle tissue was dissected from the specimens' right side and then stored in 99% non-denatured alcohol.
2) macrocrustacean whole specimens were stored in 99% non-denatured alcohol, RNALater, and/or were frozen.
3) pteropods and heteropods were stored whole in 70% ethanol; and
4) cephalopod tissue samples were stored in RNALater.

Fish specimens from which tissue was taken for genetic analysis (i.e., vouchers) were individually marked with a paired tag matching that of the tissue sample and fixed in formalin.

For polycyclic aromatic hydrocarbon (PAH) analyses, whole specimens and/or tissue samples were frozen at -80°C. Prior to PAH sample collection, reusable 20-ml VOA vials were washed with water and detergent, rinsed three times with deionized water then combusted in an oven at 450°C for 4-5 hours. Aluminum foil was combusted as well in an oven at 450°C for 4-5 hours and used to cover the inside of each VOA vial plastic cap. Samples were deposited in each vial and then frozen. Prior to lipid extraction (i.e., PAHs) samples will be freeze-dried. Lipid extraction of freeze-dried samples will be conducted under high temperature (100°C) and pressure (1500 psi) with a solvent mixture 7:3 v:v cyclohexane : dichloromethane using an Accelerated Solvent Extraction system (ASE 2001, Dionex) following modified EPA methods.

4.2 Water Column Structure at the Stations

Detailed hydrographic analyses are currently ongoing, but the predominant mesoscale oceanographic features during DEEPEND cruise DP08 was a stable cyclonic eddy northwest and adjacent to a large anticyclonic Loop Current eddy (LCE). Both were located in the center of the DEEPEND station grid (Figure 9).

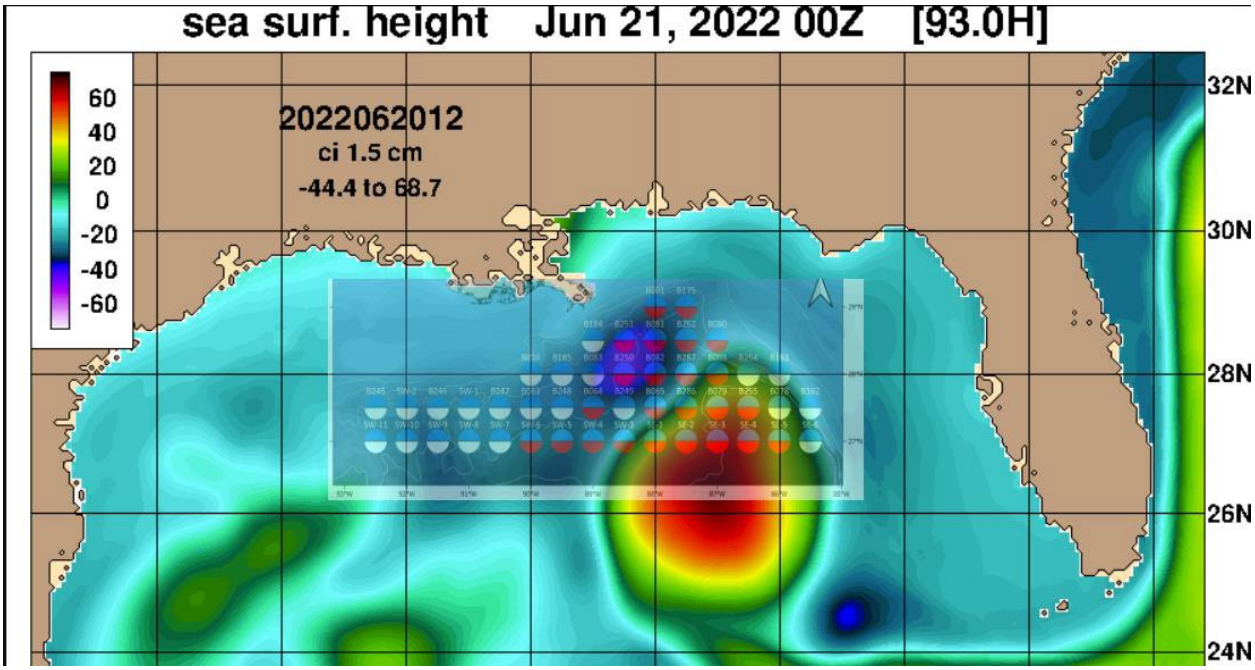

Figure 9. Mesoscale oceanographic features during DEEPEND cruise DP08 as inferred from satellite-sensed sea surface height. High values (warm colors) indicate sea surface elevation, indicative of anticyclonic rotation (Loop Current Eddy) while cool colors indicate sea surface depression, indicative of cyclonic rotation.

Hydrographic structure at depth via analysis of CTD sensor data for each station is presented in Figure 10 - Figure 18. The influence of the Mississippi River plume on near-surface waters (~20 m depth) was evident in all but the most southeastern stations of the DP08 cruise. Depths of the chlorophyll maximum varied from < 10 m to > 70 m, and stations with chlorophyll maximum depths < 40 m were influenced by the Mississippi River plume.

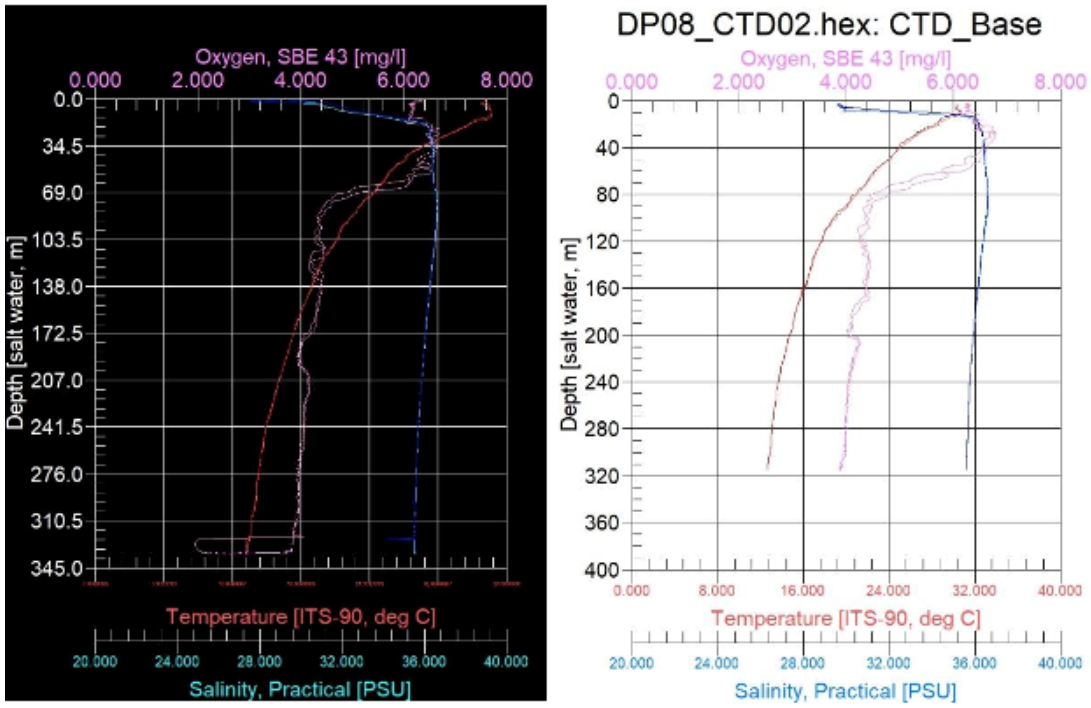

Figure 10. CTD temperature, salinity, and oxygen data from cast CTD_225 (left) and CTD_226 (right) at station UTAH.

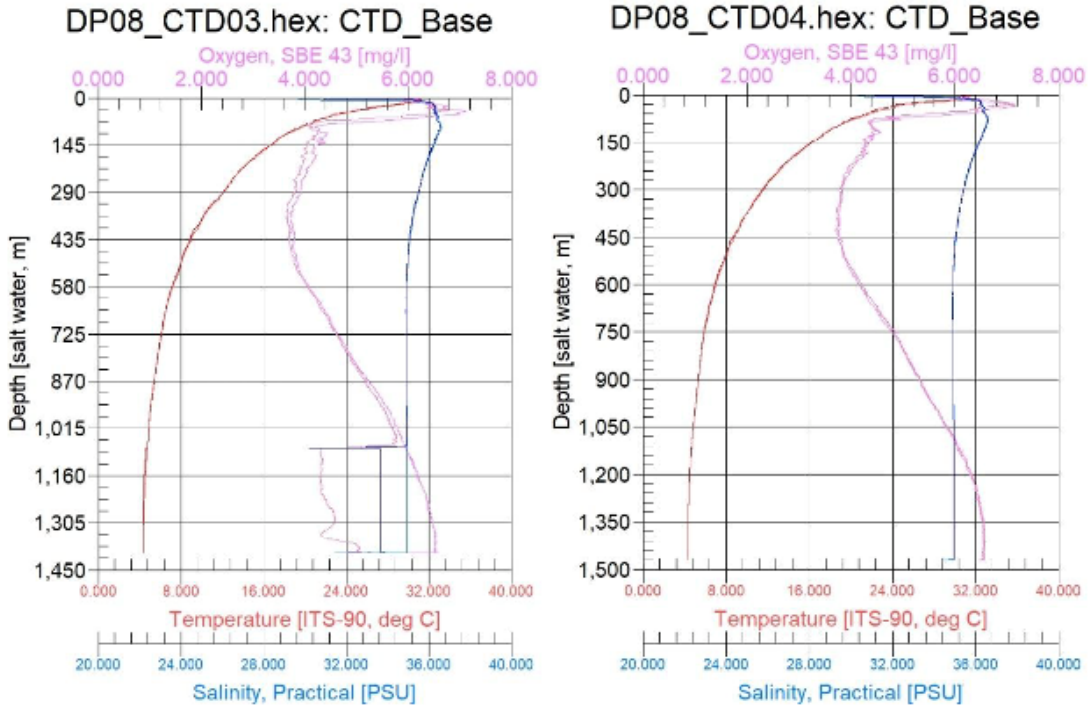

Figure 11. CTD temperature, salinity, and oxygen data from cast CTD_227 (left) and CTD_228 (right) at station B001.

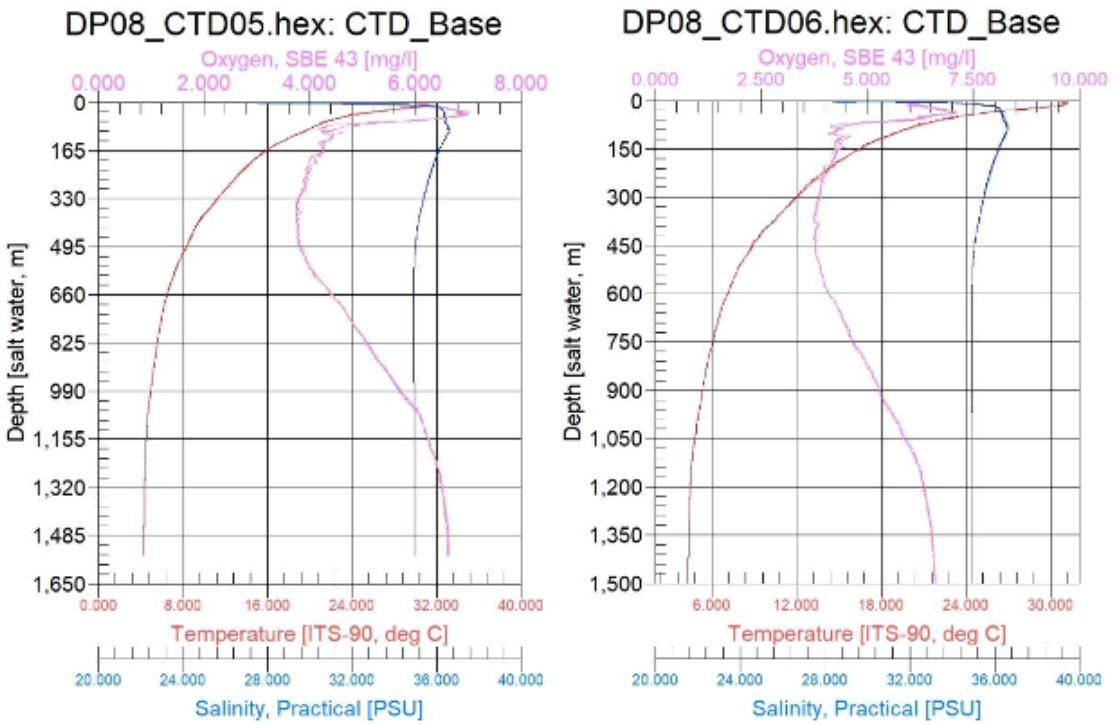

Figure 12. CTD temperature, salinity, and oxygen data from cast CTD_229 (left) and CTD_230 (right) at station B175.

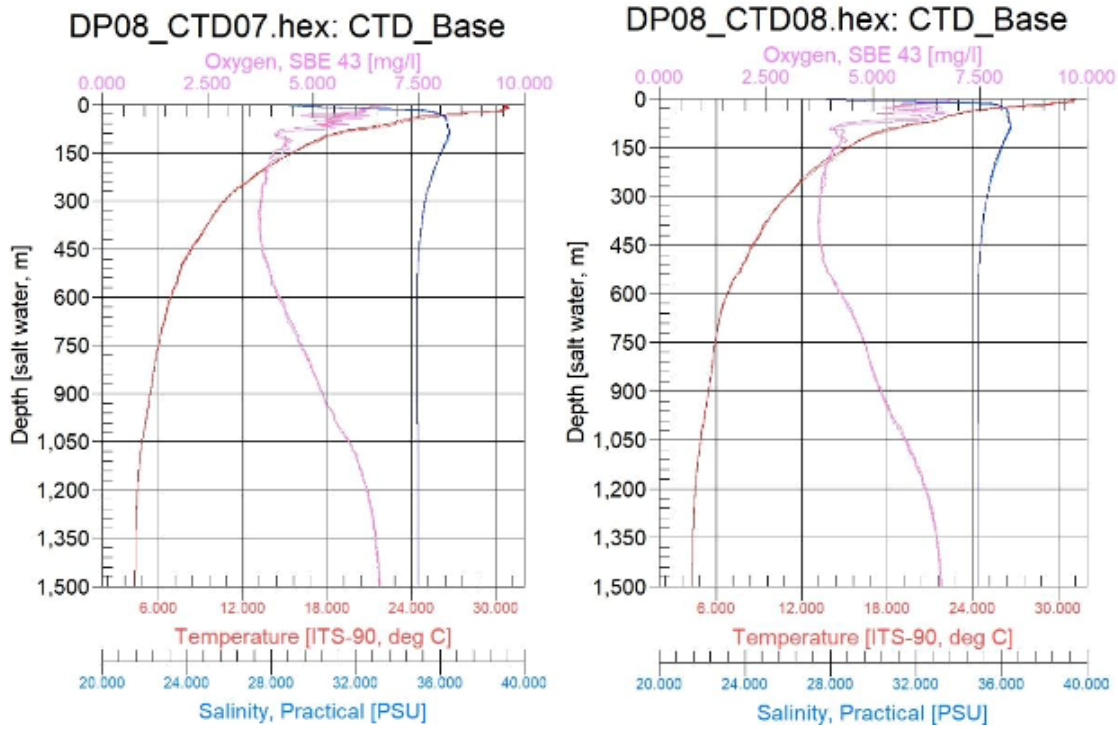

Figure 13. CTD temperature, salinity, and oxygen data from cast CTD_231 (left) and CTD_232 (right) at station B252.

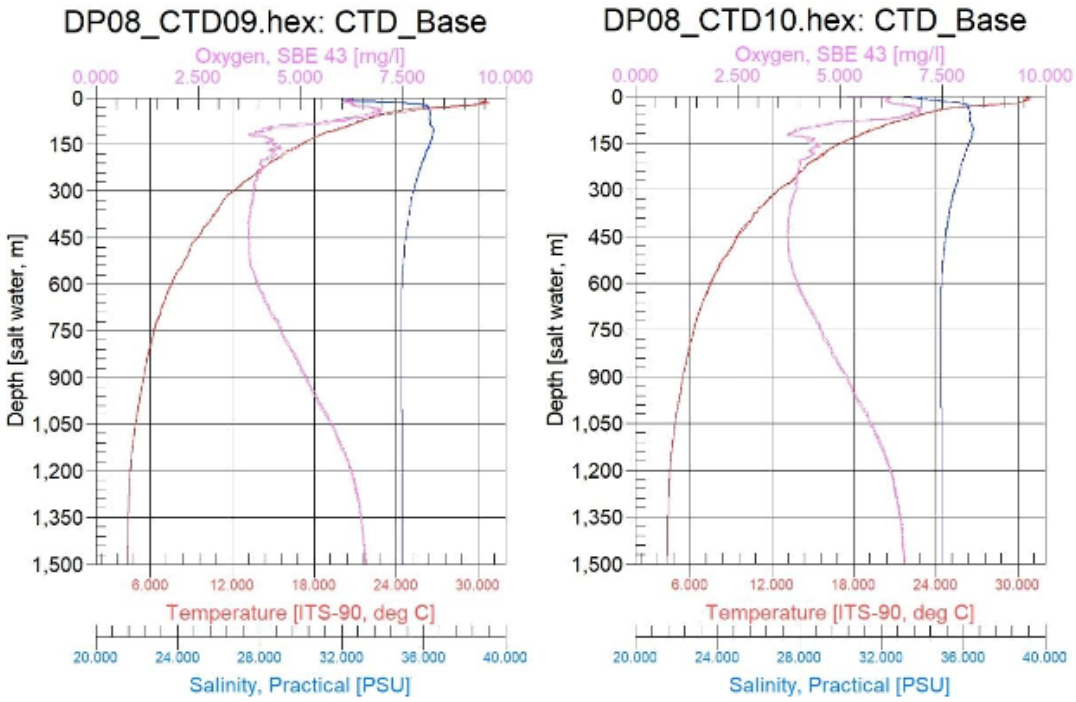

Figure 14. CTD temperature, salinity, and oxygen data from cast CTD_233 (left) and CTD_234 (right) at station B287.

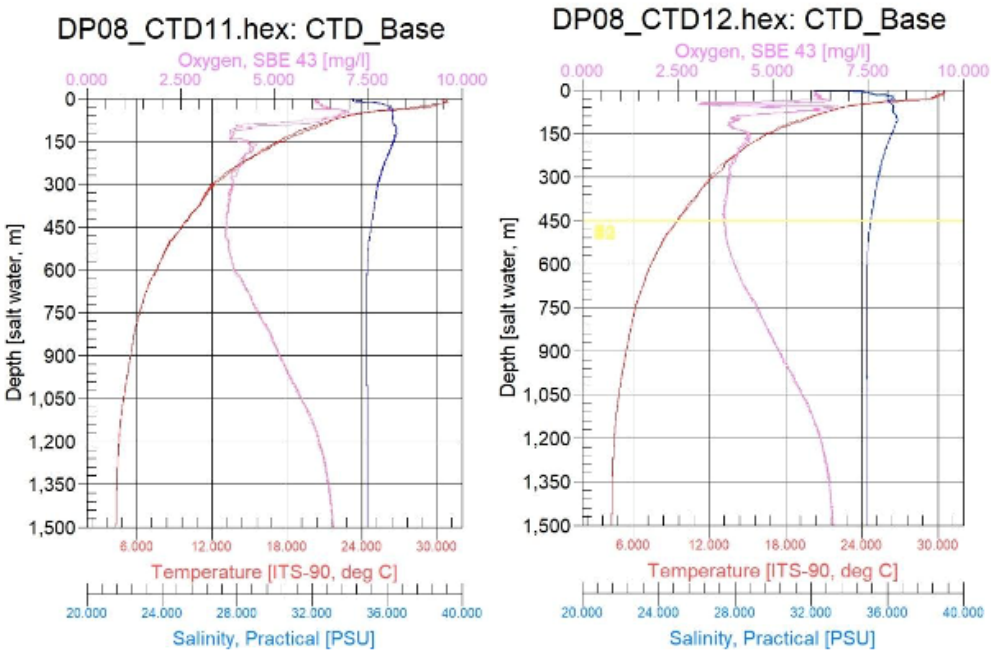

Figure 15. CTD temperature, salinity, and oxygen data from cast CTD_235 (left) and CTD_236 (right) at station B287.

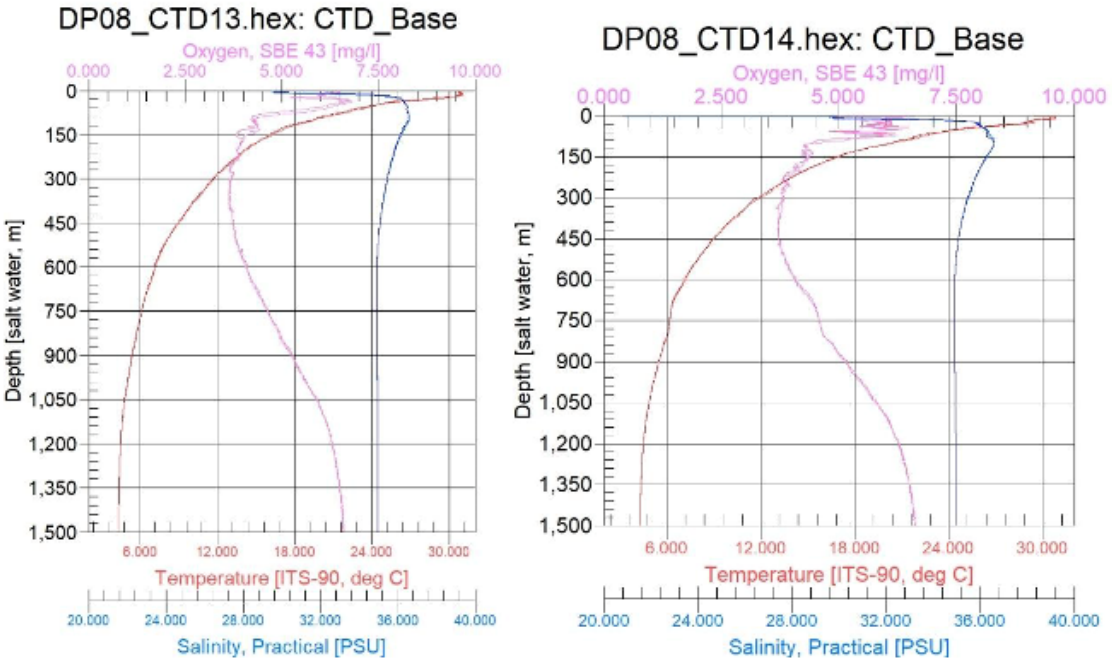

Figure 16. CTD temperature, salinity, and oxygen data from cast CTD_237 (left) and CTD_238 (right) at station B082.

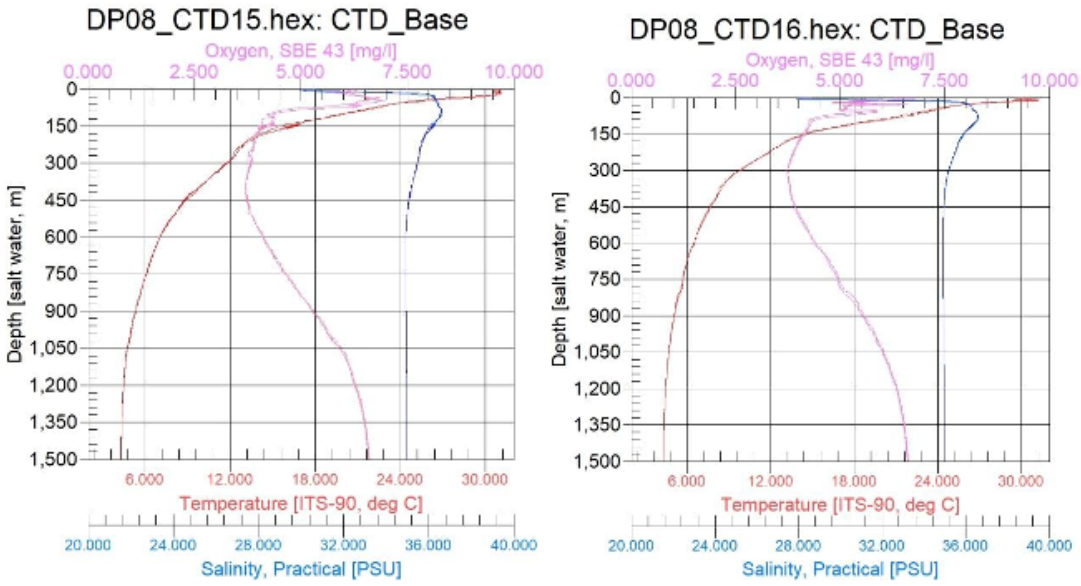

Figure 17. CTD temperature, salinity, and oxygen data from cast CTD_239 (left) at station B082 and CTD_240 (right) at station B081.

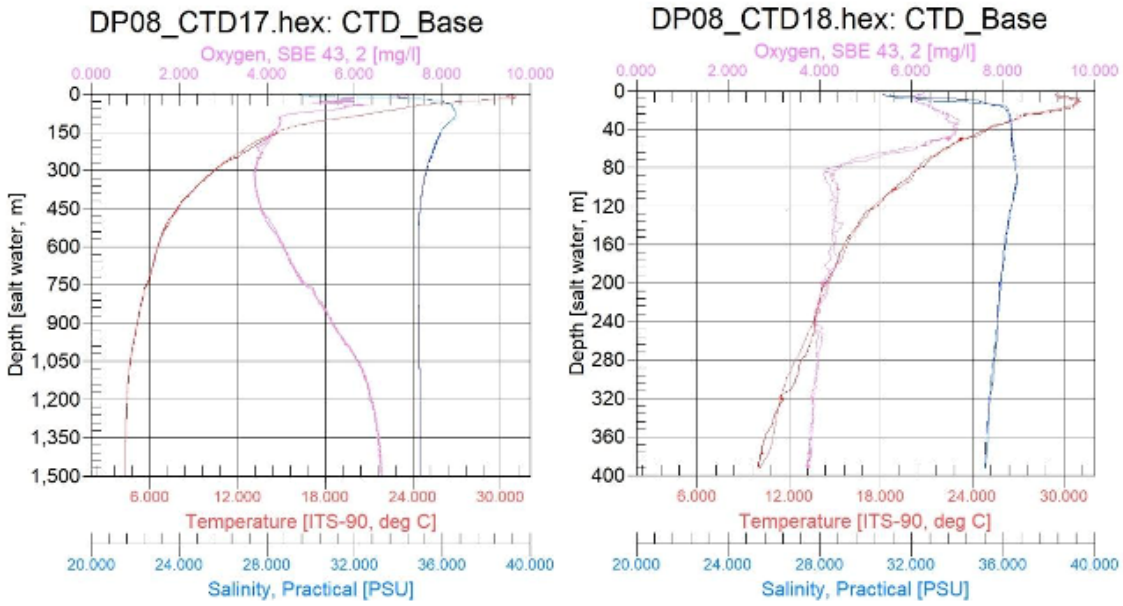

Figure 18. CTD temperature, salinity, and oxygen data from cast CTD_241 (left) at station B081 and CTD_242 (right) at station UTAH.

5 Individual Project Reports

5.1 MOCNESS Sampling

A total of 106 trawl samples were collected during 19 deployments (Table 1; Figure 19). Of these, 63 samples were considered 'quantitative,' having met the criteria of: 1) proper opening and closing at prescribed depths; 2) proper flowmeter (volume) readings; 3) proper net behavior (mouth angle, net speed) during deployment; and 4) no signs of mechanical failure (tears, holes). These samples combined for a cumulative total of ~2 million cubic meters of water filtered. There were 17 "Net 0" samples that fished from the surface to max depth, which we classified as "non-standard," though flow data were taken. There were 6 "MEAT" samples (non-quantitative) and 20 "tow-yo" samples (Figs. 2-4) taken. The remaining samples fished non-standard depth strata, had flow meter validation errors, or suffered mechanical problems. Specimens for genetic and biochemical analyses (see 5.3-5.6) were taken from all trawls.

Table 1. MOC-10 trawl deployment times and locations during DP08.

Trawl Number	Station ID	Sample Date	Tow Start Time (CDT)	Start Latitude	Start Longitude	Tow End Time (CDT)	End Latitude	End Longitude
221	Utah	27Jul22	21:06	29.13	-88.37	01:53	29.07	88.02
222	B001	28Jul22	09:12	28.98	-87.84	14:56	28.85	87.93
223	B001	28Jul22	21:02	28.96	-87.98	02:55	28.83	87.91
224	B175	29Jul22	09:02	29.03	-87.51	14:56	28.89	87.55
225	B175	29Jul22	21:03	29.01	-88.49	02:53	28.87	87.51
226	B252	30Jul22	09:11	28.54	-87.48	14:59	28.41	87.51
227	B252	30Jul22	21:00	28.56	-87.52	02:50	28.43	87.44
228	B287	31Jul22	09:06	28.03	-87.49	15:22	27.92	87.35

229	B287	31Jul22	20:59	28.00	-87.46	02:55	27.84	87.52
230	B287	01Aug22	11:01	27.93	-87.45	15:00	27.98	87.55
231	B287	01Aug22	23:30	28.97	-87.50	03:14	27.88	87.47
232	B082	02Aug22	11:25	28.00	-87.96	15:30	27.98	88.02
233	B082	02Aug22	23:00	27.88	-87.98	02:59	27.94	88.06
234	B082	03Aug22	09:25	27.99	-87.95	15:17	28.03	88.08
235	Utah	04Aug22	23:26	29.13	-88.38	02:56	29.13	88.28
236	B081	05Aug22	09:01	28.51	-88.02	15:07	28.47	88.16
237	B081	05Aug22	21:00	28.45	-88.029	02:50	28.49	88.15
238	Utah	06Aug22	10:10	29.12	-88.38	15:18	28.99	88.38
239	Utah	06Aug22	22:00	29.12	-88.38	02:56	28.96	88.38

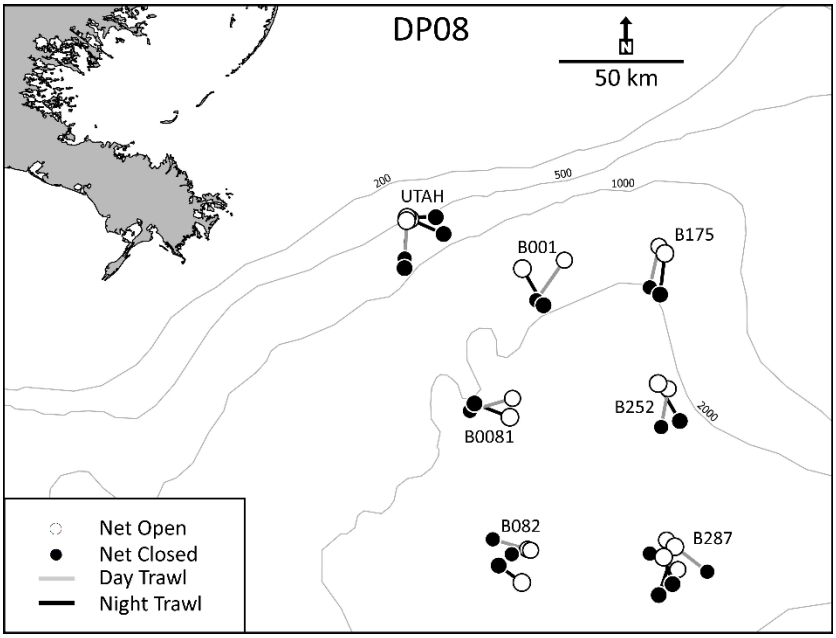

Figure 19. DEEPEND cruise DP08 MOC-10 trawl locations and trajectories, labeled by station number. Day samples are indicated by the light line, while night samples have dark lines.

5.2 Faunal Accounts

5.2.1 Crustacea.

A total of 7693 nektonic crustaceans were sampled, sorted, and preserved from nets 0-5 in various fixatives (EtOH, formalin, or frozen). Of these, analysis is currently ongoing, with 1374 identified to species level and preserved in 99% EtOH for studies of population connectivity and/or barcoding so far. Additionally, 25 of these individuals were identified to species-level and frozen for PAH analysis.

The remaining crustaceans were identified to genus and stored in 10% formalin for species identification back in the shoreside laboratory. All copepods, amphipods and isopods were collected and stored in formalin for other investigators who may want them.

5.2.2 Mollusca.

A total of 123 individual cephalopods were collected with 26 species represented from 14 families. A total of 2243 pteropods (at least 5 genera) and 155 heteropods from six species were collected. Forty-seven cephalopods were frozen at -80°C as part of the ongoing cephalopod stomach content metabarcoding project led by H. Judkins. Samples not used for the metabarcoding project were preserved in 95% ethanol for species identification and future genetic work at Dr. Judkins' laboratory at the University of South Florida St. Petersburg. Nine cephalopods were frozen at -80°C for future contamination studies.

5.2.3 Fishes.

A total of 12,244 fish specimens were collected from a minimum of 276 species. Analysis is currently ongoing.

5.3 Genetic/Genomic Analyses

5.3.1 Genetic diversity and connectivity.

More than 533 individual crustaceans and fishes were collected for genetic diversity and connectivity studies. All animals were preserved in EtOH or RNAlater. The primary aim of the genetic diversity and connectivity objective was to collect crustaceans (Table 2) and fish (Table 3) indicator species for downstream population genomic analyses. The target was 20+ individuals per species.

Table 2. Crustacean species collected during DP08 for population genetics.

Species	**N**
Acanthephyra purpurea	24
Acanthephyra stylorostratis	34
Eucopia sculpticauda	Many
Sergestes armatus	5
Sergestes vigilax	15
Sergia grandis	6
Sergia regalis	1
Sergia robusta	11
Sergia splendens	25
Sergia tenuiremis	20
Plesionika richardii	8
Systellaspis debilis	26

Table 3. Fish species collected during DP08 for population genetics.

Species	**N**
Argyropelecus aculeatus	50
Bregmaceros atlanticus	22
Ceratoscopelus warmingii	47
Diaphus dumerilii	49
Lepidophanes guentheri	44
Notolychnus valdiviae	54

Sternoptyx diaphana	64
Sternoptyx pseudobscura	28

5.3.2 Deep-sea crustaceans and fish barcoding.

New species of crustaceans and fishes encountered during DP08 were collected and subsampled for COI DNA barcoding. Some of these individuals will be DNA barcoded for the 16S gene as well to be included in future publications. Table 4 lists the species which were collected for DNA barcoding.

Table 4. Crustaceans and fishes collected during DP08 for DNA barcoding.

Species	**N**
Anguilliformes	1
Apogon	1
Ariosoma	1
Ariosoma coquettei	2
Aristostomias tittmanni	1
Aristostomias xenostoma	1
Astronesthes niger	1
Astronesthes similus	2
Ataxolepis apus	1
Balistes capriscus	2
Barbourisia rufa	1
Bassozetus	2
Bathylaco nigricans	1
Canthigaster	2
Cetomimus	1
Chaetodon	1
Chaunax	1
Chiasmodon braueri	1
Coelorinchus	1
Diaphus bertelseni	1
Diaphus dumerilii	2
Diaphus taaningi	1
Dicrolene	1
Dolichopteryx longipes	1
Dysomma anguillare	2
Echiodon dawsoni	1
Ectreposebastes imus	1
Eustomias brevibarbatus	1
Foetorepus	2
Gigantura chuni	1

Gordiichthys randalli	1
Gymnothorax	2
Gymnothorax sp. JAM5	2
Heteroconger luteolus	1
Howella	6
Howella atlantica	1
Ipnops murrayi	1
Labichthys carinatus	1
Lophiodes reticulatus	1
Melamphaes longivelis	2
Mentodus longirostris	1
Muraenidae	2
Nettenchelys	4
Oneirodes	2
Oneirodidae	1
Ophichthinae	2
Physiculus kaupi	1
Polymixia	2
Pontinus rathbuni	1
Poromitra "Gibbsi" Keene undescribed JM1	2
Pristipomoides	1
Rhynchactis leptonema	1
Rondeletia loricata	1
Ruvettus pretiosus	1
Scopeloberyx opisthopterus	4
Scopeloberyx robustus	6
Scopeloberyx rubriventer	2
Scopelogadus	2
Scorpaenidae	3
Serranidae	1
Sphyraena guachancho	1
Synagrops bellus	2
Talismania	1
Xenomystax congroides	1
Xenophthalmichthys danae	1
Yarrella blackfordi	1

5.3.3 Crustacean and fish transcriptomics and genomics.

A total of 249 specimens were collected for transcriptomics and genomic studies (Tables 5-6). Specimens were stored in liquid nitrogen to allow full genome sequencing in the future. At least 3-10 individuals of each species were collected as part of this initiative.

Table 5. Crustaceans collected for transcriptomics and genomic studies during DP08.

Species	N
Acanthephyra purpurea	6
Acanthephyra stylorostrata	3
Acanthephyra stylorostrata	4
Gardinerosergia splendens	29
Phorcosergia grandis	3
Plesionika richardii	6
Robustosergia regalis	1
Robustosergia robusta	4
Sergia tenuiremis	4
Systellaspis debilis	6

Table 6. Fish taxa collected for transcriptomics and genomic studies during DP08.

Species	N
Argyropelecus aculeatus	8
Bregmaceros sp.	6
Ceratoscopelus warmingii	6
Chauliodus sloani	5
Cyclothone acclinidens	29
Cyclothone alba	10
Cyclothone braueri	16
Cyclothone obscura	16
Cyclothone pseudopallida	15
Diaphus mollis	4
Diaphus roei	5
Echiostoma barbatum	3
Lepidophanes guentheri	6
Maurolicus weitzmani	1
Notolychnus valdivae	4
Photostomias guernei	2
Sternoptyx diaphana	8
Sternoptyx pseudobscura	7
Valenciennullus tripunctulatus	32

5.4 Ultra-black coloration study.

Table 7 lists the fish species that were collected for a research project investigating the ultra-black color of their skin. Three individuals per species were collected and preserved in RNAlater and in paraformaldehyde for histology. This project is in collaboration with Sonke Johnsen at Duke University.

Table 7. Fish taxa collected for the ultra-black experiment during the DP08 cruise.

Species	N
Bolinichthys photothorax	2
Echiostoma barbatum	2
Eustomias hypopsilus	2
Lampadena luminosa	4
Lepidophanes guentheri	2
Photostomias guernei	1
Sigmops bathyphilus	1
Sigmops elongatus	6

5.5 Photophore pattern study.

As part of a PhD project in the Bracken-Grissom lab, photophore patterns of deep-sea shrimp were documented for use in a guide to identify shrimps while at sea. Table 8 lists the shrimp species that were targeted as part of this effort. At least 3 to 5 specimens were targeted per species and preserved in glycerol for downstream spatial analysis of photophore pattern and photography.

Table 8. Shrimp taxa collected for the photophore pattern experiment during the DP08 cruise.

Species	N
Acanthephyra acutifrons	*2*
Allosergestes pectinatus	*13*
Allosergestes sargassi	*12*
Bentheuphausia amblyops	*3*
Challengerosergia hansjacobi	*21*
Challengerosergia talismani	*6*
Deosergestes corniculum	*3*
Deosergestes henseni	*8*
Deosergestes paraseminudus	*7*
Eucopia sculpticauda	*1*
Gardinerosergia splendens	*15*
Gennadas spp.	*13*
Hymenodora gracilis	*1*
Notostomus elegans	*1*
Oplophorus gracilirostris	*6*
Parasergestes armatus	4
Parasergestes vigilax	2
Phorcosergia grandis	4
Plesionika richardii	1
Polychelidae	2
Robustosergia regalis	4
Robustosergia robusta	7

Sergestes atlanticus	8
Sergestes edwardsii	4
Sergia tenuiremis	2
Stenopodidean larvae	1
Stylopandalus richardii	4
Systellaspis cristata	1
Systellaspis debilis	9

5.6 Polycyclic aromatic hydrocarbon (PAH) analysis

5.6.1 Crustaceans.

A total of 23 specimens from at least five species were frozen whole for immediate or future PAH analysis. These included *Acanthephyra purpurea* (n = 2), *Acanthephyra stylorostratis* (n = 2), *Nematoselis* spp. (n = 4), *Oplophorus gracilirostris* (n = 2), and *Systellaspis debilis* (n = 2).

5.6.2 Cephalopods and Other Pelagic Mollusca.

A total of nine cephalopod specimens from four species were collected for immediate or future PAH analysis, including *Abralia redfieldi* (n = 3), *Onychoteuthis banksii* (n = 1), *Vampyroteuthis infernalis* (n = 3), and *Japetella diaphana* (n = 2). Additionally, 21 heteropods from two species, including *Pterotrachea coronata* (n = 9) and *Pterotrachea scutata* (n = 12), and 54 pteropods from four species, including *Clio* sp. (n=23), *Diacria trispinosa* (n=5), *Peracle bispinosa* (n=12), Pteropoda (n=14) were frozen for PAH analysis. All specimens were frozen as whole bodies.

5.6.3 Fishes.

A total of 442 organ/tissue samples were collected from 17 species of fishes for immediate or future PAH analysis (Table 9). Large fish specimens were dissected at sea and organs/tissues kept separate (guts, liver, muscle, skin, ovaries). Other fish specimens were frozen as whole bodies.

Table 9. Fish specimens collected for PAH analysis on DEEPEND cruise DP08.

Species	**N**
Anoplogaster cornuta	2
Argyropelecus aculeatus	27
Argyropelecus hemigymnus	56
Ceratoscopelus warmingii	24
Chauliodus sloani	20
Cyclothone alba	9
Cyclothone braueri	1
Cyclothone obscura	72
Cyclothone pallida	37
Diaphus dumerilii	23
Diaphus mollis	17
Lampanyctus alatus	26
Lepidophanes guentheri	20

Pyrosoma atlanticum	34
Sigmops elongatus	30
Sternoptyx diaphana	26
Sternoptyx pseudobscura	18

5.6.4 Gelatinous Zooplankton.

A total of 60 gelatinous zooplankters from three species were collected for immediate or future PAH analysis, including *Atolla sp.* (n = 19), *Aurelia sp.* (n = 20), and *Periphylla periphylla* (n = 21). Specimens were frozen whole.

5.7 Hydroacoustic Data

Over 320 GB of acoustic backscatter data were collected during the DP08 cruise. Five Simrad EK-series splitbeam echosounders (18, 38, 70, 120, and 200kHz) collected data covering 3000 m (18 kHz), 3000 m (38 kHz). ~ 400 m (70 and 120 kHz), and ~100m (200 kHz) of the water column. Both narrowband and wideband (at 18 and 70 kHz) data were collected opportunistically to examine the potential to use frequency spectra to further describe the scattering responses of mesopelagic fauna. Data were collected during day and nighttime MOC10 tows at 7 stations (B175, B252, B287, B082, B081, B001, and UTAH). Passive acoustic surveys were conducted in both continuous wave and wideband form during daytime and nighttime operations to characterize the noise (electrical interference) generated by the ship and associated machinery. Additionally, all five echosounders were calibrated using standard tungsten carbide and copper spheres in both narrowband and wideband (18 and 70 kHz) modes following standardized procedures. Acoustic data have undergone preliminary quality control inspection and are currently being scrutinized and analyzed. The data packaging process is underway from the DP08 data for submission to NECI.

In addition to shipboard echosounders, we collected over 100 GB of wideband (FM) data from a wideband autonomous transceiver (WBAT) that was affixed to the CTD rosette. The WBAT ran continuously during dusk and dawn CTD deployments, capturing an entire water column profile to 1500m depth. The WBAT was held at different depth strata within the deep scattering layer to investigate fine-scale organism scattering and capture the full migration, these casts typically lasted between 3 and 5 hours, operating a 38 and 200 kHz in wideband (FM). These in-layer acoustic measurements were taken during the pre-dusk CTD cast before the scattering layer began its upward nightly migration.

5.8 Physical Oceanographic Data Collected

In situ physical oceanographic data from the CTD (Conductivity-Temperature-Density) rosette casts and the MOCNESS were collected during DP08.

5.8.1 CTD and Water Samples

The CTD was deployed 19 times at seven stations during the DEEPEND DP08 cruise (Table 10). For the eDNA pilot study, water samples were collected at station B287 (night) aiming to investigate community composition variation when filtering different water volumes.

Table 10. CTD rosette deployments during DEEPEND cruise DP08 (* indicates the deployment that collected water for eDNA study).

CTD cast ID	Station	Cast Date	Cast Time (CDT)	Solar Cycle	Lat.	Lon.	Bottom depth (m)
DP08_CTD_225	UTAH	27-Jul-22	10:32	Day	29.07	-88.22	334
DP08_CTD_226	UTAH	28-Jul-22	19:43	Night	29.07	-88.22	322
DP08_CTD_227	B001	28-Jul-22	06:36	Day	29.00	-87.59	1426
DP08_CTD_228	B001	28-Jul-22	19:03	Night	28.58	-87.58	1518
DP08_CTD_229	B175	29-Jul-22	06:52	Day	29.00	-87.31	1599
DP08_CTD_230	B175	29-Jul-22	14:29	Night	29.02	-87.29	1564
DP08_CTD_231	B252	30-Jul-22	06:13	Day	28.31	-87.29	2334
DP08_CTD_232	B252	30-Jul-22	17:46	Night	28.31	-87.29	2207
DP08_CTD_233	B287	31-Jul-22	06:44	Day	28.03	-87.29	2701
DP08_CTD_234	B287	31-Jul-22	18:02	Night	28.00	-87.27	2760
DP08_CTD_235	B287	01-Aug-22	05:43	Day	27.56	-87.33	2775
DP08_CTD_236*	B287	01-Aug-22	17:38	Night	28.00	-87.32	2757
DP08_CTD_237	B082	02-Aug-22	10:10	Day	28.00	-87.59	2494
DP08_CTD_238	B082	02-Aug-22	23:56	Night	27.55	-88.00	2459
DP08_CTD_239	B082	03-Aug-22	09:41	Day	27.59	-88.05	N/A
DP08_CTD_240	B081	05-Aug-22	07:40	Day	28.30	-88.01	2188
DP08_CTD_241	B081	05-Aug-22	17:30	Night	28.28	-88.01	2202
DP08_CTD_242	UTAH	06-Aug-22	07:08	Day	29.04	-88.22	420
DP08_CTD_243	UTAH	06-Aug-22	19:44	Night	29.05	-88.23	360

6 Outreach Activities

6.1 Dr. Danté Fenolio/DEEPEND Photography

Dr. Danté Fenolio, leader of DEEPEND's imaging project, took over 3,500 photos during this cruise. These images will be used for outreach efforts (website, public presentations, media articles, etc.) as well as for scientific purposes (scientific presentations and publications). Examples of his work are shown below in Figures 20 and 21.

Figure 20. A shrimp specimen photographed during the DP08 cruise using a white box technique for the imaging project.

Figure 21. Dorsal and ventral aspects of a juvenile Coffinfish, *Chaunax* sp., in a single composite. Photo 2022, DEEPEND-RESTORE/Danté Fenolio.

6.2 Video Conferencing at Sea

Tracey Sutton and April Cook hosted a video conference with one of NSU University School's Pre-Kindergarten classes. The students were given a virtual tour of the ship, shown some of the animals collected, and were able to ask questions.

6.3 DEEPEND Website

During the cruise, ten blog posts were published on the DEEPEND website along with images of animals, equipment, and the DEEPEND team members. Several posts highlighted the work of DEEPEND graduate students from several different universities who are working towards their M.S. or Ph.D. degrees. Other blogs gave a glimpse of life at sea. Additionally, the ship tracker was updated at each station so the public could follow along the team's cruise through the northern Gulf of Mexico.

Appendix A. Bird, tuna, cetacean, and sea turtle observations

27 Jul 2022
9:36-10:24 AM CDT
N29° 07.789', W88° 22.039'
Green water, mostly cloudy, 80°F
2 Magnificent Frigates (1 juv)
7 Royal Terns
1 Pomarine Jaeger

Midafternoon: Leatherback Turtle spotted at surface by Dan Hahn

5:21 PM CDT
Same station as above
Yellowfin tunas seen jumping after prey while 1 Royal Tern followed the tuna

5:30 PM CDT
N29° 05.598', W88° 24.906'
Green water, mostly cloudy, 80°F
1 Magnificent Frigate harassing a Royal Tern with a fish in its beak

5:39 PM CDT
Same station as above
3 Royal Terns flying by

7:28 PM CDT
N29° 07.454', W88° 24.917'
1 Brown Booby juvenile perched on ship's bow anchor

7:38 PM CDT
Same station as above
Heather Judkins sighted a dark-rumped Leach's Storm Petrel sitting on the water

28 July 2022
6:50 AM CDT
N29° 00.145', W87° 59.812'
Green water, mostly cloudy, light air, swells 1-2 ft
2 Audubon's Shearwater flying by
2 baleen whale blows near the horizon

7:21 AM CDT
Same station as at 6:50 AM
Pod of >15 smaller dolphins with stripes on sides

2:50 PM CDT

N28° 51.134', W87° 56.336'
30°C, partly cloudy, wind 3-4 kn from W, greenish blue water
1 Sandwich Tern circling the ship

3:15 PM CDT
Same station as at 2:50 PM
Pod of dolphins seen
1 Masked Booby juv.

29 July 2022
1:21 PM CDT
1 White Ibis juv enile walking around the back deck

1:44 PM CDT
N28° 54.612', W87° 33.371'
30°C, partly cloudy, wind 9-10 kn from S, greenish blue water
Brown Booby in the midst of a tuna school hitting small fish at the surface, booby briefly pop ped up a little to move to keep up with the tuna and they moved.

6:41 PM CDT
N29° 01.606', W87° 29.387'
27°C, flat seas, sunny, partly cloudy, wind 4 kn from S, greenish blue water
1 Brown Booby juvenile sitting on bow anchor

30 July 2022
6:17 AM CDT
N28° 31.082', W87° 29.874'
27°C, clear, 2-3 ft swells, 3-7 kn from S, greenish blue water
1 Brown Booby juvenile sitting on bow anchor

1:01 PM
N28° 27.554', W87° 29.645'
30°C, partly cloudy, wind 7-10 kn from ENE, greenish blue water
1 White Ibis juv. walking around the back deck
1 Brown Booby juv sitting on bow anchor
1 Sooty Shearwater soaring through wave troughs
1 Laughing Gull juv.

6:50 PM CDT
N28° 32.138', W87° 30.256'
30°C, partly cloudy, wind 9-10 kn, greenish blue water
1 Brown Booby juv sitting on bow anchor
1 White Ibis juv. walking around the back deck

31 July 2022
9:40 AM CDT
N28° 03.717', W87° 29.824'

29°C, cloudy, wind 17-19 kn from SE, greenish blue water
1 White Ibis juv. walking around the ship's deck

10:28 AM CDT
N27° 58.350', W87° 26.124'
30°C, cloudy, wind 13-16 kn from SE, greenish blue water
1 White Ibis juv. walking around the ship's deck
1 Brown Booby juv sitting on bow anchor
2 Masked Booby sitting on the bow

2:47 PM CDT
N27° 56.149', W87° 21.937'
29°C, cloudy, wind 14-17 kn from ESE, greenish blue water
1 White Ibis juv. walking around the ship's deck
3 Brown Booby (2 juv and 1 adult) sitting on bow anchor

1 August 2022
6:04 AM CDT
N27° 57.342', W87° 32.943'
29°C, cloudy, wind 17-19 kn from SE, greenish blue water
1 White Ibis juv. walking around the ship's deck
1 Brown Booby juv sitting on bow anchor

1:02 PM CDT
N27° 58.361', W87° 31.412'
30°C, cloudy, storms all around, wind 12-14 kn from WSW, greenish blue water
1 White Ibis juv. walking around the ship's deck
2 Brown Booby (juv & adult) sitting on bow anchor
1 Laughing Gull adult flying by

7:30 PM CDT
N27° 59.566', W87° 30.084'
29°C, cloudy, wind 3-4 kn from N, blue water
1 White Ibis juv. walking around the ship's deck
3 Brown Booby (2 juv and 1 adult) sitting on bow anchor
1 Masked Booby sitting on the bow
Boobies would swoop down on flyingfishes that launched into the air and the booby would capture the flying fishes in the air

2 August 2022
2:53 PM CDT
N28° 01.230', W88° 02.471'
26°C, thunderstorm nearby, wind 21 kn from NNW, blue water
1 White Ibis juv. walking around the ship's deck
2 Brown Booby juv sitting on bow anchor
2 Cliff Swallows flew by (1 was later found dead on the ship after it collided with the side of the ship)

1 Sandwich Tern flying by

3 August 2022
5:50 AM CDT
N27° 56.669', W88° 04.065'
29°C, thunderstorms on horizon, wind 1 kn from SSE, greenish blue water
1 White Ibis juv. walking around the ship's deck
1 Brown Booby juv sitting on bow anchor

11:00 AM CDT
N27° 59.793', W87° 57.508'
28°C, thunderstorm nearby, wind 17 kn from SE, greenish blue water
1 White Ibis juv. walking around the ship's deck
1 Brown Booby juv sitting on bow anchor

3 PM CDT
Steamed to Gulfport to drop off injured crew to go to hospital

3 August 2022
8:30 AM
Arrived in Gulfport
2:39 PM CDT
Left Gulfport