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First HOV *Alvin* study of the pelagic environment at Hydrographer Canyon (NW Atlantic)

Amanda N. Netburn[a,*], Joanna D. Kinsey[b], Stephanie L. Bush[c,d], Anni Djurhuus[e], Julianne Fernandez[f], Colleen L. Hoffman[g], Doreen McVeigh[b], Katrina I. Twing[h], Laura Bagge[i]

[a] NOAA Office of Ocean Exploration and Research, Silver Spring, MD 20910, USA
[b] Department of Marine, Earth, and Atmospheric Sciences, North Carolina State University, Raleigh, NC 27695, USA
[c] Monterey Bay Aquarium, Monterey, CA, 93940, USA
[d] Monterey Bay Aquarium Research Institute, Moss Landing, CA, 95039, USA
[e] College of Marine Science, University of South Florida, St. Petersburg, FL, 33701, USA
[f] Department of Geology, University of Cincinnati, Cincinnati, OH 45211, USA
[g] Department of Earth Science, University of Minnesota-Twin Cities, Minneapolis, MN 55455, USA
[h] Department of Biology, University of Utah, Salt Lake City, UT, 84112, USA
[i] Biology Department, Duke University, Durham, NC 27708, USA

ABSTRACT

Continental slope canyons off the United States Atlantic coast remain poorly studied, and in particular, the distributions of pelagic organisms in waters overlying these unique environments are not well documented. During the Early Career Scientist Deep Submergence Training cruise, AT36-EAGER, the distribution of organisms in the water column overlying Hydrographer Canyon, which cuts through the northwestern Atlantic continental margin, was investigated through daytime midwater observations using HOV *Alvin* (AD4831) at three depths. Mixed swarms of krill and *Themisto* sp. amphipods were observed at all depths surveyed. Observations centered at 250 m were also dominated by chaetognaths, copepods, and *Phronima* sp. amphipods, while at 500 and 750 m, the assemblages were dominated by the fishes in the families Paralepididae, Nemichthyidae, and Mytophidae. Additionally, measurements of methane, nitrous oxide, optical properties (absorbance and fluorescence), dissolved organic carbon, and base-extracted particulate organic carbon were made to better characterize the hydrography and biogeochemistry over Hydrographer Canyon. This study was aided by the use of telepresence to communicate between ship and shore-based researchers, and the expedition marks the first use of SMS messaging to communicate between the submersible and the ship. This study demonstrates the capabilities and utility of using *Alvin* for conducting water column science.

1. Introduction

Despite the proximity of the United States northeastern continental margin to some of the most populated regions in the country, it remains a poorly surveyed environment. There are over 70 canyons between Cape Hatteras, NC and the US-Canadian border. Initial investigations into currents and sediment transport in canyons along the shelf-break of this region took place in the 1970s (Keller and Shephard, 1978, Keller et al., 1979) and in the early 1980s, the Shelf Edge Exchange Processes (SEEP) study examined the fate of particulate matter from the shelf to the open ocean (e.g., Walsh et al., 1988, Biscaye et al., 1994). More recently (early 2010s), a multi-year partnership and related efforts by NOAA, USGS, and BOEM, as well as their state, regional, and academic partners, have provided opportunities to conduct detailed mapping and ROV surveys of the canyons along the US East Coast. This work has led to astounding new discoveries, including the high diversity of deep-sea corals (Quattrini et al., 2015) and the unexpected presence of hundreds of methane seeps along the passive margin between Cape Hatteras and Georges Bank (Skarke et al., 2014). Submarine canyons can be hotspots of productivity and biodiversity in the ocean, and provide essential habitats to a range of benthic, demersal, and pelagic organisms (Fernandez-Arcaya, 2017), including targeted fisheries species such as American lobster, tilefish, and hake (Cooper et al., 1987; Stevenson et al., 2004). However, canyon ecosystems are threatened by a number of anthropogenic factors, including fishing, pollution from various sources such as litter, sewage, and chemical waste, ocean warming, and

* Corresponding author.
E-mail address: amanda.netburn@noaa.gov (A.N. Netburn).

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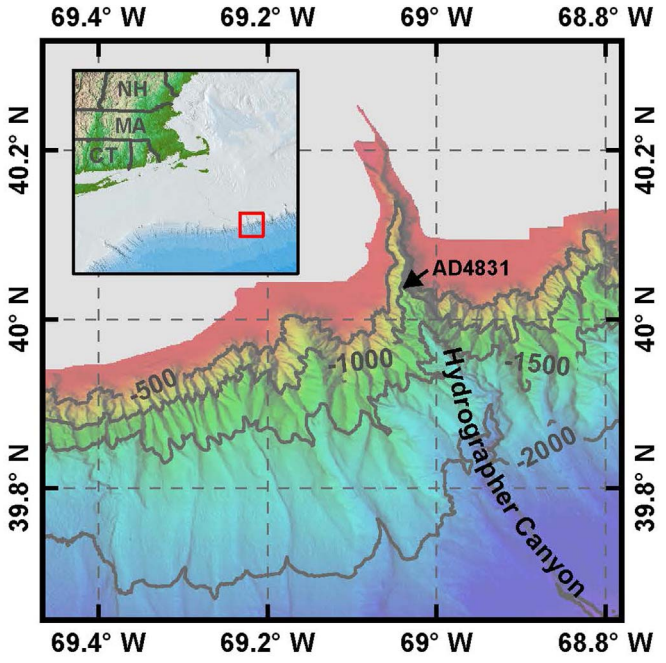

Fig. 1. Map of Hydrographer Canyon. AD4831 took place at 40.0444°N, 69.0388°W, with a bottom depth of 950 m.

ocean acidification (Ramirez-Llodra, 2011, Levin and Lebris, 2015). In recognition of the value of canyon environments, the first Atlantic marine national monument, the Northeast Canyons and Seamounts Marine National Monument, located off New England, was designated by President Obama in 2016.

Hydrographer Canyon is located approximately 160 km south-southeast of Nantucket and intersects the continental shelf, connecting shallow shelf waters to the bathyal plain (Fig. 1). Depths range from 580 m at the upper reaches to 1423 m at the shelf break (Quattrini et al., 2015). Currents in Hydrographer Canyon generally follow semidiurnal, along-canyon tidal periodicity; in addition, internal waves may advance currents up-canyon (Keller and Shepard, 1978). Current speeds are typically 20–40 cm s^{-1}, but bottom velocities can reach 53 cm s^{-1} and up to 70–75 cm s^{-1} in the upper portions of the canyon, resulting in erosion (Keller and Shepard, 1978). The shelf waters in this area undergo seasonal stratification due to reduced wind forcing in the spring and increased solar insolation (Biscaye et al., 1994). Shelf waters are typically cooler than slope waters, because they originate from the southward flow of cold Scotian Shelf waters from the Labrador Sea (Stevenson et al., 2004; Flagg et al., 2006), while slope waters are derived from the northward flow of warm Gulf Stream water. Abrupt changes in salinity and temperature between these two water masses occur at the shelf-slope front. However, direct and indirect influence of the Gulf Stream can modify the water flow near the edge of the continental shelf and initiate exchange between shelf and slope water masses (e.g., Gawarkiewicz et al., 2001, 2012,; Hare et al., 2002). These water masses, along with freshwater input from several large rivers in the Middle Atlantic Bight and the Gulf of Maine (Bisagni, 2016), can transport organic matter and nutrients from both continental and oceanic waters to support the high biodiversity and biomass found in and around canyons (Quattrini et al., 2015). Additionally, the Gulf Stream can transport larvae spawned along the southeast US continental shelf to the Middle Atlantic Bight (Hare et al., 2002), which may be important to the distribution and abundance of fish and other organisms in this area.

The steep bathymetry of canyons makes traditional net sampling for pelagic organisms challenging beyond depths of ~200 m, and submersible technologies provide a mechanism to study fine-scale distributions of organisms and make targeted collections in the water column. While trawl sampling is biased toward hard-bodied animals, submersible observations may be biased by avoidance and attraction of animals due to the lights, sound, and motion of the vehicles (Backus, 1968, Widder et al., 2005). The seafloor at Hydrographer Canyon was first surveyed by submersible in 1973 as part of an investigation into submarine currents and sedimentation (Keller and Shepard, 1978), and more recently during two remotely-operated vehicle (ROV) dives conducted in 2013 to characterize the benthic fauna (Quattrini et al., 2015). The Johnson-Sea-Link submersible was used to study bioluminescence, distributions, and vertical migrations of pelagic organisms to depths of ~300 m in the nearby Gulf of Maine (Widder et al., 1992, Frank and Widder, 1997, 2002). A water column acoustics survey conducted at the shallower portion (~430 m water depth) of Hydrographer Canyon using the Johnson-Sea-Link submersible revealed high densities of krill living near the seafloor, with a subset of the krill community conducting a vertical migration at night (Greene et al., 1988). We know of no efforts to survey the full water column at this site for biological or physicochemical characteristics.

In this paper, we present the first visual site characterization of the water column overlying the deepest portion of Hydrographer Canyon. This work was also the first test of the midwater capabilities of HOV *Alvin* following its major rebuild of 2010–2013. In the midwater, we conducted video surveys of the vertical zonation of organisms in relation to environmental parameters, and collected animals for further observations onboard and in shore-based labs. A CTD rosette was deployed following the *Alvin* dive to provide a physical and chemical characterization of the water column using sensor data (e.g., temperature, oxygen) and discrete water samples. Discrete depth measurements focused on determining dissolved and base-extracted carbon concentrations and optical measurements (absorbance and fluorescence) to examine the influences of primary production and terrestrial input to the water column at Hydrographer Canyon. Dissolved methane (CH_4) and nitrous oxide (N_2O) concentrations were measured to characterize these gases throughout the water column to investigate any potential influence of nearby CH_4 seepage. In areas that lack denitrification such as the northwest Atlantic, N_2O is inversely related to the consumption of O_2 (nitrification) (Cohen and Gordon, 1979; Walter et al., 2006), therefore N_2O concentrations can be an indicator of biological activity throughout the water column (Yoshinari, 1976).

Telepresence capabilities on this cruise allowed for collaboration between onboard and land-based scientists to hold joint planning meetings, confer on protocols and dive plans, and transmit data to shore for analysis. A novel SMS messaging system was used to transmit information between the submersible and the ship.

2. Methods

The Early Career Scientist Deep Submergence Training cruise, AT36-EAGER, took place aboard the RV *Atlantis* on two legs, from 28 Jul - 02 Aug and 02 - 07 Aug 2016 (A. Skarke, A. Dekas, Chief Scientists). The overall goals of this cruise were to provide graduate students, postdoctoral researchers, and early-career scientists with operational training using deep submersible assets and telepresence technology to communicate from seafloor to ship to shore. Objectives included both benthic (McVeigh et al., in preparation) and pelagic characterizations. During the cruise, half of the scientific team was on the ship and half was stationed at the Inner Space Center (ISC) at the University of Rhode Island Graduate School of Oceanography. The shore- and ship-based teams switched locations between the two cruise legs.

2.1. Visual surveys and zooplankton collection

Alvin conducted its first water column dive at Hydrographer Canyon (Fig. 1, 40.0444°N, 69.0388°W), AD4831, on 03 Aug 2016. Total water depth was 996 m. The 3-person (pilot plus two observers) submersible

was equipped with its standard complement of lights, cameras, and sensors (http://www.whoi.edu/main/-alvin), in addition to a slurp sampler with five 3-L chambers for zooplankton collections. Both the midwater and benthic habitats were surveyed during the same dive, with ~150 min allocated for midwater exploration. On descent, *Alvin* hovered at three depths - 250, 500, and 750 m (+/- 25 m) - to observe the diversity and vertical distribution of macroorganisms, and to collect representative specimens. *Alvin* lights were off during the initial descent to 250 m, and once they were turned on at that depth they remained on for the remainder of the survey. The pilot was able to hover within 1 m of the target depth through a combination of mechanisms. Neutral buoyancy was achieved by first dropping two steel descent weights to slow *Alvin*'s initial descent. Next, a variable ballast system was used to pump salt water into or out of titanium spheres in order to fine-tune buoyancy and achieve neutral trim. Finally, vertical lift thrusters could be used to change the desired depth as required by the scientists.

At each depth, we searched for organisms of interest to collect for species identifications and lab-based observations of optical properties of the animals. When a target was identified, the submersible was held in position while a manipulator arm directed the suction hose toward the organism to transfer the sample into one of the collection chambers. Time spent at each depth varied depending on time spent observing and collecting target organisms. For the semi-quantitative analysis of organismal distributions, video data were analyzed from only the starboard recorder after the cruise. Each video file was annotated using the Monterey Bay Aquarium Research Institute (MBARI)'s Video Annotation Reference System (VARS) (Schlining and Jacobsen-Stout, 2006), with organisms identified to the lowest taxonomic level possible. Zooplankton collections allowed for more precise organism identifications using morphological features, which were then used to inform video analysis. While the pilot was able to maintain a precise depth, we binned observations across a 50 m depth range (target depth ± 25 m) to balance the dual objectives of collecting specimens and documenting species distributions during the limited time allocated for the midwater portion of the dive. Lighting, camera angle, zoom, and vehicle speed were not consistent throughout the dive, so no survey volume was estimated. To make comparisons between the three depth strata, given that survey times varied between depths, observations were normalized as percent of total animals encountered.

2.2. Physical and chemical characterization

2.2.1. CTD cast

Following the Hydrographer Canyon midwater survey with *Alvin*, a hydrocast was conducted at the same location to characterize physical and chemical parameters of the water column and to collect seawater for subsequent analyses. The primary sensors were the Sea-Bird Electronic SBE conductivity, temperature, and depth (CTD) sensors, along with fluorescence and oxygen sensors. Raw data (conductivity, temperature, and oxygen) from the CTD downcast were quality checked with the R package 'oce' (Kelley, 2014), by trimming, removing large spikes, and smoothing using a moving 5-point average. The smoothed and trimmed data were binned into depth averages of 2-m intervals and plotted (Fig. 4).

Twenty-four 12-L Niskin bottles were used to collect seawater at the following depths: ~15 m above the seafloor (981 m), midway between the seafloor and thermocline (700 m), in the thermocline (301 m), in the middle of the mixed layer (100 m), at the chlorophyll maximum (27.2 m), and at the surface (5.7 m; Table 2). These six depths were selected to characterize the physical and chemical environment of the water column, and were consistent with experiments conducted at other stations. These data were collected for ancillary projects, and thus the water sampling depths are not aligned with the midwater observation depths from *Alvin*, which were selected to evenly sample the water column. Nonetheless, the Niskin bottle measurements provide environmental context valuable for interpreting species distributions. For each sample depth, water was filtered using gentle vacuum through pre-combusted 0.7 µm glass fiber filters. The filtrate and particulate samples were stored at −20 °C until analyzed at North Carolina State University for absorbance and fluorescence optical measurements and carbon concentrations of the dissolved and base-extracted particulate organic matter fractions (CDOM and BEPOM, respectively). Samples for dissolved methane and nitrous oxide concentration measurements were collected at four of the six depths (5.7, 100, 700, and 981 m). The dissolved gas samples were collected by overflowing borosilicate serum vials ten times. The samples were fixed with saturated mercury chloride and crimped closed (Townsend-Small et al., 2016). Samples were stored at 4 °C until analyzed at the University of Cincinnati.

2.3. Absorbance and fluorescence

Absorbance and fluorescence of CDOM and base-extracted particulate filters (Brym et al., 2014), were measured to provide information regarding the sources, in situ processing, and chemical properties of the dissolved and particulate fractions. Absorbance (200–800 nm) was measured on a Varian 300 UV spectrophotometer. Fluorescence was measured on a Varian Eclipse spectrofluorometer with excitation (EX) from 240 to 450 nm at 5 nm intervals and emission (Em) from 300 to 600 nm every 2 nm. Blank-corrected absorbance values were converted to Napierian absorption coefficients (a_λ) as described in Osburn et al. (2012). Excitation and emission corrections were applied and inner-filter effects were corrected following Tucker et al. (1992) for fluorescence measurements. Final fluorescence values were calibrated in quinine sulfate units (QSU, where 1 QSU = 1 ppb quinine sulfate) (Lawaetz and Stedmon, 2009) and corrected for extraction volumes when appropriate. Fluorescence results were visualized as excitation-emission matrices (EEMs) contour plots.

2.4. Carbon concentrations

Organic carbon concentrations in filtrate and BEPOM samples were measured on an OI Analytical 1030D TOC analyzer in combustion mode (Lalonde et al., 2014) or wet oxidation mode (Osburn and St. Jean, 2007), respectively. Prior to analysis, all samples were acidified with 85% phosphoric acid (H_3PO_4) to pH 2 and sparged with ultrahigh purity argon. Dissolved organic carbon (DOC) concentrations and base-extracted particulate organic carbon (BEPOC) were blank-corrected using ultrapure water and calibrated with caffeine standards and Hansell deep sea reference (DSR) water. Final BEPOC concentrations were corrected for extraction and initial filtration volumes. Carbon concentrations provide information regarding the organic carbon content of the dissolved and BEPOM fractions.

2.5. Methane and nitrous oxide concentrations

Methane (CH_4) and nitrous oxide (N_2O) were extracted from each water sample at room temperature using the methods of Ioffe and Vitenberg (1984) and Townsend-Small et al. (2016). The water samples were agitated to release the dissolved gases and then transferred into glass vials containing desiccant beads. The stored gases were analyzed using a Shimadzu Scientific Instruments GC-2014 Greenhouse Gas Analyzer equipped with a flame ionization detector (FID) and an electron capture detector (ECD). The original dissolved gas concentrations in the water samples were determined with the temperature-specific Bunsen solubility Coefficients (Yamamoto et al., 1976; Beaulieu et al., 2014) using the measured headspace gas concentrations. Equilibrium dissolved gas concentrations were calculated using water temperature, barometric pressure, and global average atmospheric concentrations for each gas derived during the month of sampling from the NOAA Mauna Loa Observatory.

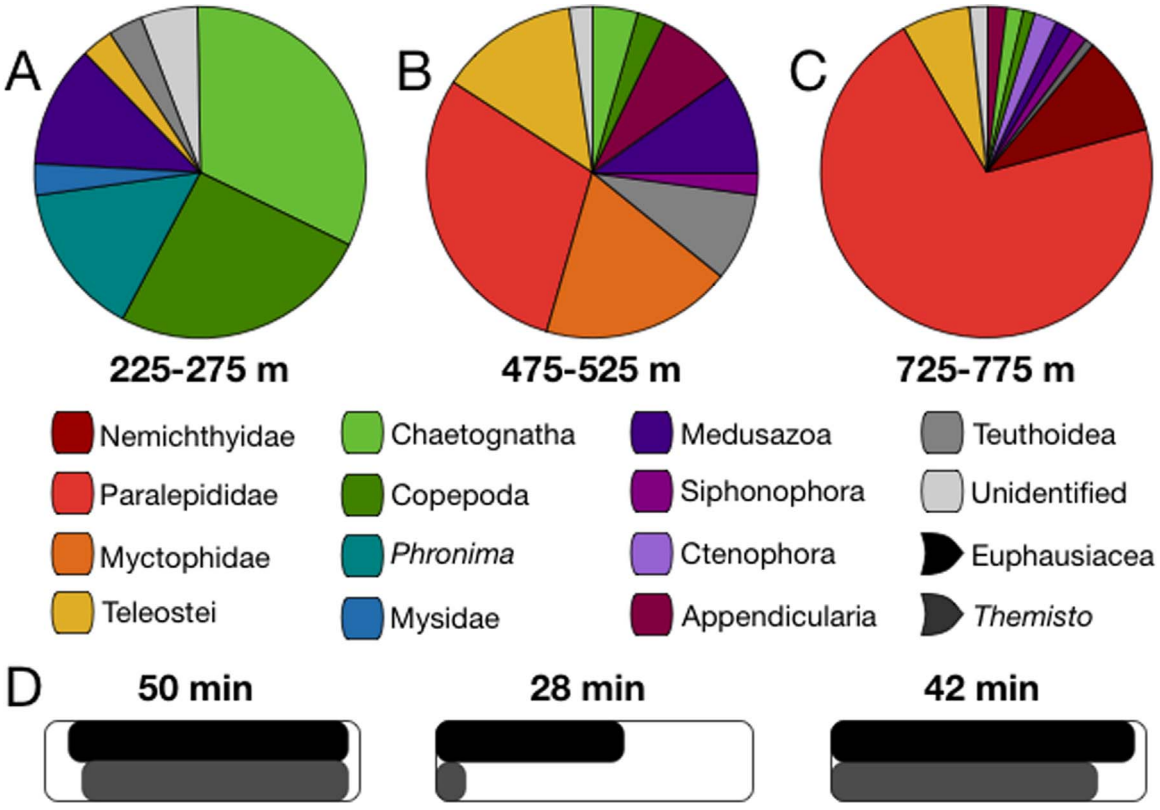

Fig. 2. Relative abundance of organisms observed at (A) 225–275 m, (B) 475–525 m, and (C) (c) 725–775 m. (D) Presence/absence of Euphausiacea and *Themisto* sp. (Amphipoda) during each of the observation periods. White rectangles represent the time span of the observation (duration in minutes above each), with black (Euphausiacea) and dark grey (*Themisto* sp.) showing the time during which these taxa were observed.

2.6. Telepresence

Telepresence is the live broadcast of data and information to individuals or groups not physically present to allow for remote participation in an expedition. The Inner Space Center (ISC) at the University of Rhode Island Graduate School of Oceanography enables shore-based participants to engage in ship-based ocean research, exploration, and education in real time through telepresence communications (Van Dover et al., 2012; Marlow et al., 2017). Aboard the R/V *Atlantis*, telepresence operations were overseen by a marine electronics expert and a videography intern from Duke University, while shore-based operations were managed by the ISC staff. The ship- and shore-based support teams facilitated meetings among early career scientists to plan *Alvin* dives, CTD deployments, and processing of samples collected for scientists onshore.

The *Alvin* and *R/V Atlantis* each have an acoustic modem with specialized hull transducers to transmit and receive digital information acoustically (Gallimore et al., 2010). This newly developed acoustic communication system (ACOMMS) and software allow for the transmission of images (progressive jpeg) of a moderate resolution and SMS messages (text) between the submersible and the ship to rapidly transmit scientific observations and other information, which can then be relayed to shore through telepresence. This new capability allows the shipboard science party and the onshore team to both be involved in real-time decision-making regarding sample collection and operations from the submersible. This system was tested during AT36-EAGER, and the first successful transmission of the SMS capabilities of the ACOMMS system occurred during dive AD4831.

3. Results

3.1. Visual surveys and zooplankton collection

Zooplankton distributions were assessed using 120 min of video collected by *Alvin* - 50 min at 250 (± 25) m, 28 min at 500 (± 25) m, and 42 min at 750 (± 25) m. Euphausiacea (krill) and the swarming hyperiid amphipod *Themisto* sp. (Vinogradov, 1999; Vinogradov et al., 1982) were the most abundant taxa observed at all three depth ranges, and the presence/absence of these two taxa were plotted separately from all other observed organisms (Fig. 2). Most occurrences consisted of mixed-taxa swarms ranging from two to hundreds of individuals. Both taxa were present at all depth ranges during the observational periods, but they were the least abundant at 500 m (Fig. 2d).

For non-swarming organisms, the 250 m observations were dominated by invertebrates, comprised of 33% Chaetognatha (arrow worms, n = 11), 24% Copepoda (n = 8), 15% *Phronima* sp. (hyperiid amphipod, n = 5), and 12% Medusozoa (Hydrozoa and Scyphozoa jellyfish, n = 4) (Fig. 2a, Table 1). Other observations at this depth included Mysidae (opossum shrimp, 3%, n = 1), Teleostei (fish, 3%, n = 1), Teuthoidea (squid, 3%, n = 1), and unidentified organisms (i.e. too small, fast, far away, or out of focus to identify, 6%, n = 2). At 500 m, fishes accounted for 63% (n = 60) of the organisms, of which 48% (n = 29) were Paralepididae (barracudina), 30% (n = 18) Myctophidae (lanternfish), and 22% (n = 13) unidentified Teleostei (Fig. 2b, Table 1). We observed 8% (n = 8) each of Ctenophora (comb jellies), Medusazoa, and Teuthoidea at this middle depth range, 5% (n = 5) Chaetognatha, 3% (n = 3) Copepoda, and 2% (n = 2) each of

Table 1
Organisms observed at each depth range, with count, percent of total observations, and further taxonomic classification when known.

Depth (m)	Taxon	Count	% Total	Further Taxonomic ID
225–275	Chaetognatha	11	33.0	
	Copepoda	8	24.2	
	Phronima sp.	5	15.2	
	Mysidae	1	3.0	
	Medusazoa	4	12.1	3 Scyphozoa
	Teleostei	1	3.0	
	Teuthoidea	1	3.0	
	Unidentified organism	2	6.1	
	Total	33		
475–525	Chaetognatha	5	5.2	
	Copepoda	3	3.1	
	Ctenophora	8	8.3	8 Lobata
	Medusazoa	8	8.3	1 Hydrozoa, 1 Narcomedusae, 1 *Poralia* sp., 2 Rhopalonematidae, 1 *Solmundella bitentatulata*
	Siphonophora	2	2.1	2 Calycophorae
	Teuthoidea	8	8.3	1 Gonatidae
	Myctophidae	18	18.8	
	Paralepididae	29	30.2	
	Teleostei	13	13.5	1 *Cyclothone* sp., 1 Bathylagidae, 1 Gonostomatidae
	Unidentified organism	2	2.1	
		96		
725–775	Appendicularia	7	2.0	
	Chaetognatha	6	1.7	
	Copepoda	4	1.1	
	Ctenophora	8	2.2	1 Ctenophora, 3 Cydippida, 4 Lobata
	Medusazoa	6	1.7	1 Coronatae, 2 Hydrozoa, 2 Scyphozoa, 1 *Solmundella bitentacultata*
	Siphonophora	6	1.7	1 Calycophorae, 4 Physonectae
	Teuthoidea	2	0.6	
	Nemichthyidae	35	9.8	
	Paralepididae	253	71.1	At least 2 species
	Teleostei	23	6.5	3 *Cyclothone* sp., 2 Myctophidae, 1 Stomiidae, 2 Sternoptychidae
	Unidentified organism	6	1.7	
	Total	356		

Siphonophora and unidentified organisms. Finally, the 750 m observations were dominated by Paralepididae (71%, n = 253) and Nemichthydae (snipe eels, 10%, n = 35; Fig. 2c, Table 1). Teleostei (7%, n = 23) were the third most abundant group at this depth, and we also observed < 5% each of Appendicularia, Chaetognatha, Copepoda, Ctenophora, Medusazoa, Siphonophora, Teuthoidea, and unidentified organisms.

Two *Phronima* sp. that were parasitizing salp barrels were collected near 250 m. At 500 m, we collected one juvenile gonatid squid and 12 hyperiid amphipods (*Themisto* sp.). Twelve *Themisto* sp., one physonectid siphonophore (which broke apart during collection and therefore could not be further identified), and one juvenile polychelid lobster were collected around 750 m (Fig. 3). The collection of the polychelid was not captured in the starboard camera, so is not accounted for in Table 1 and Fig. 2. All *Themisto* sp. collected were part of mixed crustacean swarms that were intentionally targeted. Unfortunately, no Euphausiacea were collected, so we were unable to identify the krill to genus or species.

3.2. Physical and chemical characterization

3.2.1. CTD cast

Temperature was highest at the surface (24 °C) and rapidly decreased in the upper 300 m to 8 °C (Fig. 4, Table 2). Chlorophyll fluorescence and oxygen concentration had subsurface maxima (~2.6 mg m^{-3} and ~6.5 ml L^{-1}, respectively) at ~ 20 m, which were correlated with a salinity minimum (33.4 compared with 34.8 at the surface). Though oxygen never reached hypoxic levels (i.e., < 1.4 ml L^{-1}; Hofmann et al., 2011, Vaquer-Sunyer and Duarte, 2008), there was an oxygen minimum (< 4.0 ml L^{-1}) between 120 and 312 m.

3.3. Absorbance and fluorescence

CDOM and BEPOM absorbance and fluorescence were measured at all six discrete depths to gain information regarding the chemical properties and potential sources of organic material to Hydrographer Canyon. CDOM absorbance coefficients at 254 nm (CDOM α_{254}) increased non-linearly as a function of α_{254} values for BEPOM (BEPOM α_{254}) (Fig. 5) with the highest α_{254} values occurring at the chlorophyll fluorescence maximum for both CDOM and BEPOM. Fluorescence data for each sample were compiled into excitation-emission matrix (EEM) contour plots (Fig. 6). For the dissolved samples, CDOM fluorescence was dominated by a common humic-like signal, typically attributed to terrestrial runoff to coastal waters (peak A, Ex/Em 260/380–460 nm) (Coble, 1996). Marine/microbial humic-like (peak M, Ex/Em 290–310/370–410 nm) and tryptophan-like (peak T, Ex/Em 275/340 nm) components were the second most prevalent areas of fluorescence depending on the depth, and have been associated with microbial processing and local production (Fellman et al., 2010). The highest intensity CDOM signal occurred at the chlorophyll fluorescence maximum, with low intensity throughout the water column (< 0.9 QSU) (Fig. 4). On the other hand, BEPOM samples contained fluorescence in a "three-peak" pattern. These peaks corresponded to fluorescence in the region of tryptophan (peak T) and in the general region of peaks A and C (Ex/Em 350/420-480). However, the maximum excitation wavelengths for peaks A and C were blue- and red-shifted, respectively, by approximately 10 nm from the central region of these peaks. These shifted peaks share fluorescence characteristics with quinone and NADH compounds (Cory and McKnight, 2005; Brym et al., 2014) though the true chemical identity has yet to be determined (Fig. 4). BEPOM fluorescence intensity was weaker with depth and the three-peak pattern was nearly absent at 300 m and appeared to be replaced by marine/microbial humic-like fluorescence (peak M) at 700 and 981 m.

3.4. Carbon concentrations

DOC concentrations were highest in the surface waters (1.11 mg L^{-1}) decreasing to a minimum of 0.70–0.86 mg L^{-1} below the thermocline (Fig. 4, Table 2). BEPOC concentrations (0.007–0.015 mg L^{-1}) were one to two orders of magnitude lower than DOC, with highest concentrations in the chlorophyll fluorescence maximum and just above the seafloor (981 m) (Table 2).

3.5. Methane and nitrous oxide concentrations

The concentration of CH4 was highest (17.87 nML-1) at 700 m, between the thermocline and bottom water, and lowest (14.66 nML-1) near the seafloor(981 m, Fig. 4). CH4 concentration was similar at the surface and within the mixed layer (100 m). N2O concentration was highest (16.54 nML-1) near the seafloor (981 m), while the lowest concentration (9.85 nML-1) occurred in the surface water (Fig. 4).

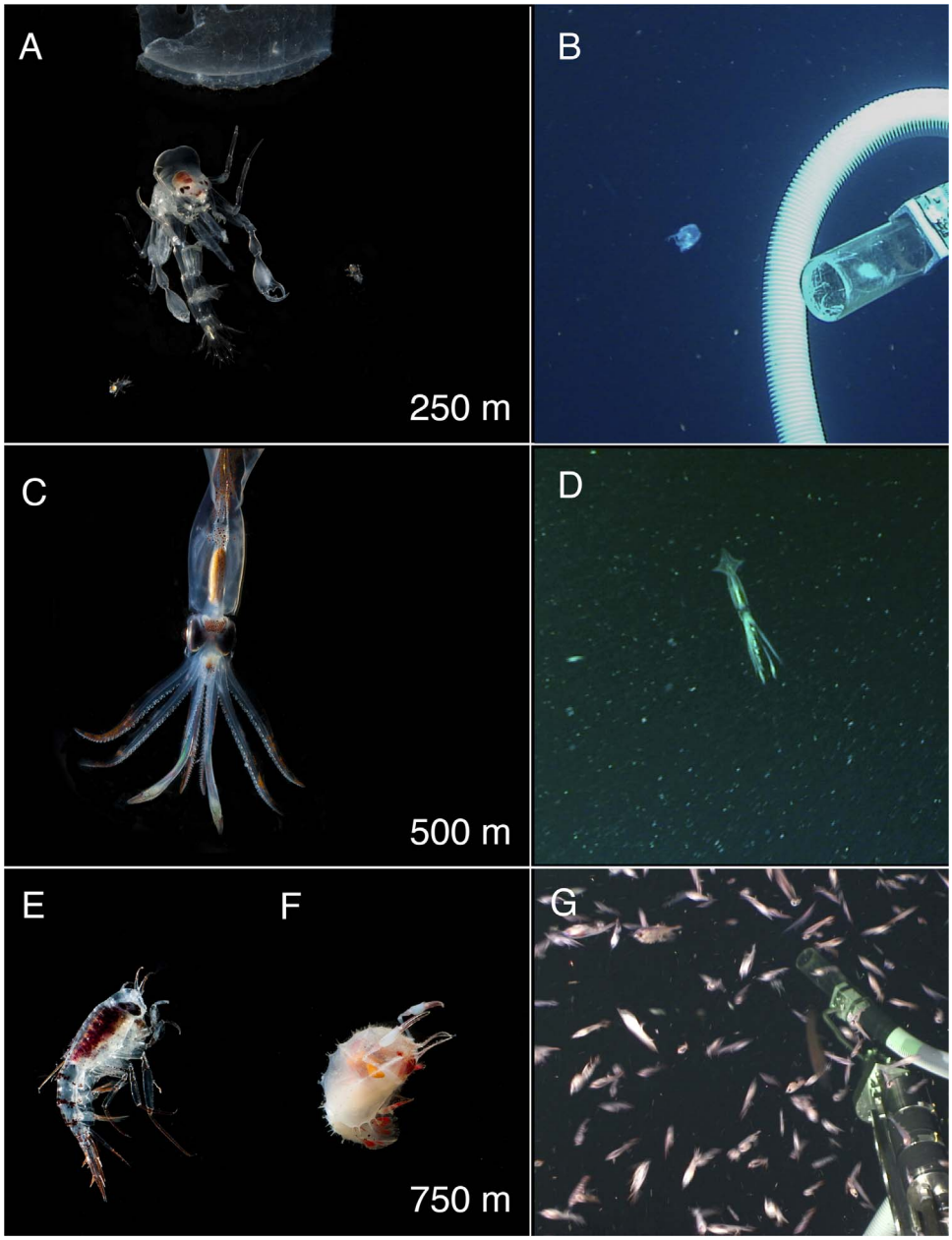

Fig. 3. Animals collected in midwater. Representative photographs of animals collected at following depths: (A-B) 248 m, (C-D) 500 m, (E-G) 738 m. Shipboard photographs of collected organisms (left column) are shown in comparison to *HOV Alvin* video frame grabs (right column). (A-B) Hyperiid amphipod *Phronima* sp. and the salp barrel it was collected in. (C-D) Juvenile gonatid squid. (E) Hyperiid amphipod *Themisto* sp. (F) Juvenile polychelid (blind) lobster. (G) Swarm of *Themisto* sp. and Euphausiacea.

3.6. Telepresence

The ACOMMS system aboard *Alvin* was successfully tested during dive AD4831 at Hydrographer Canyon. Texts were transmitted via an acoustic signal to relay current dive conditions and science reports to shipboard scientists. Ship-to-shore video conferencing and high-bandwidth data transfer allowed immediate transfer of CTD cast data and *Alvin* frame grabs from Hydrographer Canyon from the ship to the shore for processing, allowing shore-based scientists to begin identifying organisms and key features. The success of the *Alvin* dive, CTD cast, and post-dive processing resulted from numerous planning and follow-up conversations facilitated by telepresence using ship-to-shore video conferencing.

4. Discussion

4.1. Zooplankton and fish distributions

In addition to the Euphausiacia and *Themisto* sp. that were numerically dominant at all depths, Chaetognatha, Copepoda, and *Phronima* sp. were the most abundant taxa near 250 m, while observations at 500 m and 750 m were comprised mainly of the fish families Paralepididae, Nemichthyidae, and Myctophidae (Fig. 3). The most notable shift in the assemblages was thus from one dominated by small invertebrates at the shallowest depths and by fishes in deeper waters. While the 250 m survey coincided with the area of lowest oxygen (Fig. 4b), the water was still relatively well-oxygenated, with concentrations unlikely to be limiting for most animals (Vaquer-Sunyer and Duarte, 2008). The water at 250 m was, however, warmer and fresher than at the two deeper depths, which may provide a more suitable environment for the dominant small planktonic organisms seen there. Most other factors measured including carbon concentrations, optical properties of CDOM and BEPOM, and dissolved gas concentrations, did not vary considerably between the three sampling depths (Fig. 4, Table 2). It is likely the shift in dominance is due to the generally more lethargic lifestyle of deep-dwelling fishes compared with their shallower counterparts (Childress, 1995), and the fast avoidance response of more active shallow-living fishes. Thus, midwater

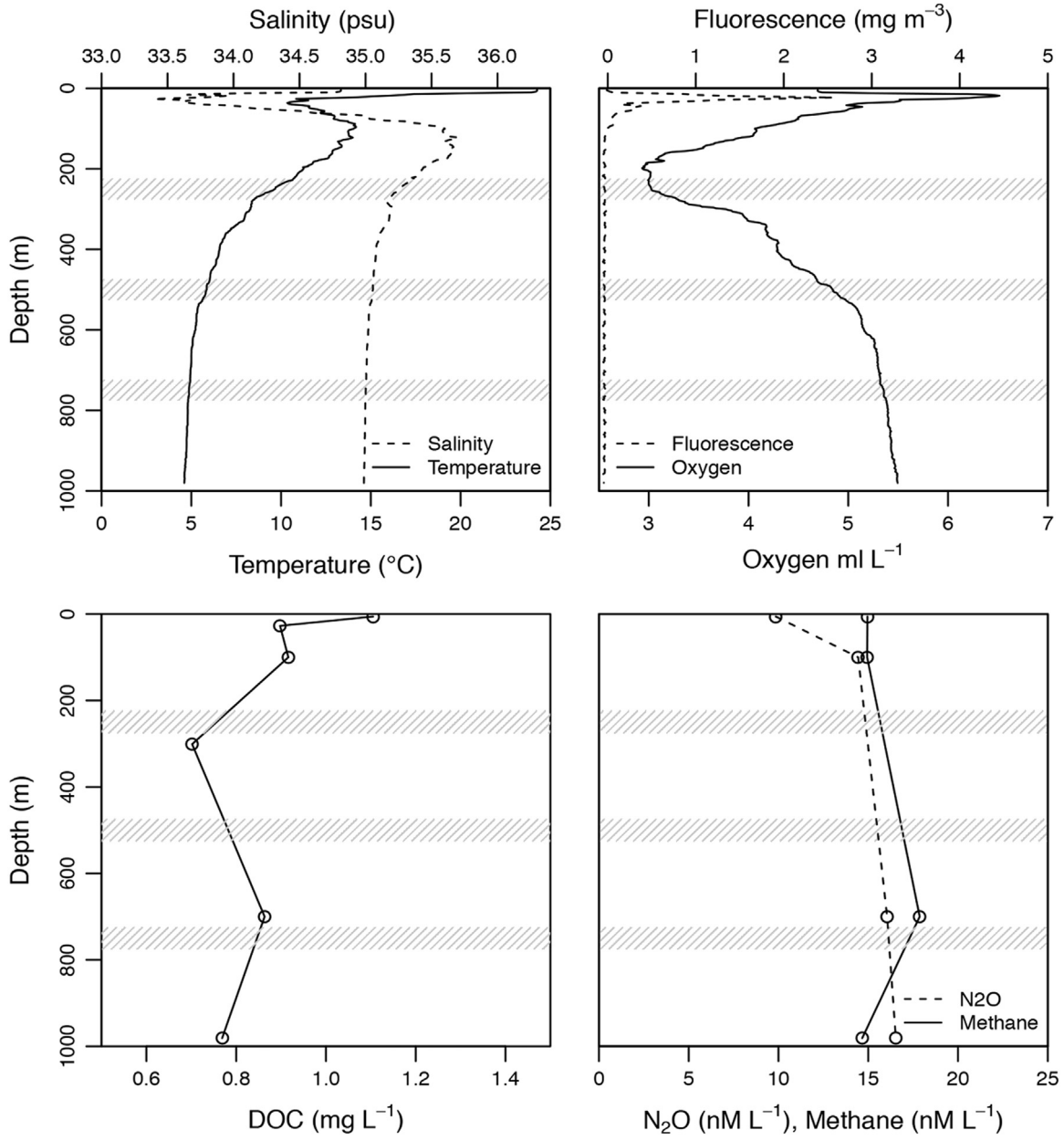

Fig. 4. Depth profiles at Hydrographer Canyon of (A) salinity and temperature, (B) fluorescence and oxygen, (C) dissolved organic carbon (DOC), and (D) nitrous oxide (N_2O) and methane (CH_4). DOC depths also represent depths where base-extracted particulate organic carbon (BEPOC) and dissolved and base-extracted organic matter absorbance and fluorescence were measured. The shaded horizontal bands indicate the depths where visual HOV surveys were conducted at this site.

submersible operations are best-suited to study planktonic organisms and some deep-dwelling fishes.

The high abundance of krill observed on this *Alvin* dive is typical of shelf breaks, and similar aggregations have been noted around the globe (Mackas et al., 1997, Macaulay et al., 1984). At Hydrographer Canyon, large krill populations have previously been detected using a coupled submersible-acoustic survey with the Johnson-Sea Link submersible (Greene et al., 1988), as well as in net collections at the

Table 2
Hydrographic and chemical data collected at Hydrographer Canyon on 03 August 2016. Methane (CH_4) and nitrous oxide (N_2O) are reported as dissolved gas concentration (nM) per liter of seawater. 'ND' indicates 'not determined'.

Depth (m)	Temp (°C)	Salinity	Fluor. (mg m^{-3})	O_2 (ml L^{-1})	DOC (mg C L^{-1})	BEPOC (mg C L^{-1})	CDOM α_{254} (m^{-1})	BEPOMα_{254} (m^{-1})	CH_4 (nM L^{-1})	N_2O (nM L^{-1})
5.7	24.3	34.8	0.01	4.7	1.11	0.008	1.71	0.015	14.96	9.85
27.2	11.3	33.5	1.88	6.5	0.90	0.014	2.27	0.039	ND	ND
100	14.0	35.6	< 0.01	4.1	0.92	0.009	1.42	0.004	14.94	14.44
301	8.3	35.2	< 0.01	3.8	0.70	0.009	0.97	0.003	ND	ND
700	5.0	35.0	< 0.01	5.3	0.86	0.007	0.97	0.002	17.87	16.05
981	4.6	35.0	< 0.01	5.5	0.77	0.015	1.01	0.003	14.67	16.54

canyon head (Levine, 2014). In bottom depths of ~430 m, Greene et al. (1988) observed a peak in daytime krill abundance at 300–350 m, not far from the 225–275 m survey in our study. They also detected high krill abundances just above the seafloor (Greene et al., 1988). Our survey expands the depth range of visual sampling in the water column overlying Hydrographer Canyon to nearly 1000 m, and demonstrates that krill are found well below common trawl and acoustic sampling depths along the shelf (Levine, 2014). Further, trawl nets are unable to survey near the seafloor due to operational constraints, and observations made from manned and other submersibles could aid in groundtruthing acoustic detections throughout the full water column (Backus et al., 1968). It is important to note that krill and amphipods can be photo-positive, causing aggregations around HOV lights, which could artificially increase the number of organisms observed. Because *Alvin* was illuminating the water consistently from the start and end of this survey, however, this bias can not fully explain the reduced observations of krill and *Themisto* sp. at 500 m compared with 250 and 750 m.

The success of the upgraded *Alvin*'s first foray into midwater exploration demonstrates its extended capabilities for in situ observations. The vehicle was able to maintain predetermined depths at an accuracy of ± 1 m in the water column to make observations and collect specimens for further investigation onboard the ship. The results of this limited study suggest that *Alvin* is a promising tool for surveying and collecting macrofauna in the water column, though more dedicated time and effort should be invested in water column investigations to further test and refine the methods. In the future, adding the capability to image from *Alvin* using low or red-light cameras will decrease disturbance to organisms (Widder et al., 2005), allowing researchers to observe more natural behavior in situ and increase opportunities to image and collect organisms that would otherwise avoid the vehicle.

4.2. Physical and chemical characterization

The Hydrographer Canyon water column depth profiles of salinity, temperature, oxygen, and chlorophyll fluorescence (Fig. 4) were typical of depth profiles in this area (e.g., Biscaye et al., 1994), with a more defined area of decreased oxygen than at other times of the year for this region (Elder and Seibel, 2015) due to stronger seasonal stratification. Overall CDOM fluorescence resembled fluorescent signals commonly found in coastal systems (e.g., Milbrandt et al., 2010) dominated by terrestrial humic-like components. The highest fluorescence intensity occurred in the chlorophyll fluorescence maximum with lower fluorescence in the surface waters due to photobleaching (del Vecchio and Blough, 2002). Below the thermocline, CDOM fluorescence remained low (≤ 0.6 QSU). This pattern is common in the North Atlantic (Jørgensen et al., 2011) but contrasts with other marine environments, such as the northern Pacific Ocean and the Southern Ocean (Yamashita and Tanoue, 2008; Jørgensen et al., 2011). At Hydrographer Canyon, the uniform fluorescence intensities in the deeper waters are likely due to a combination of well-mixed deep water, indicated by uniform dissolved gases (Fig. 4d), and removal of particulate organic matter by zooplankton prior to degradation by microbes, as suggested by low DOC (Fig. 4c) and BEPOC concentrations (Table 2) and relatively high densities of zooplankton in this region as observed from *Alvin*. The slightly elevated BEPOC concentrations near the seafloor were likely due to resuspension of sediments as a result of the high current velocities that occur at Hydrographer Canyon (Keller and Shepard, 1978).

Only a few studies (Osburn et al., 2012; Brym et al., 2014; Ziervogel et al., 2016) have used base-extraction as a way to characterize absorbance and fluorescence of POM, but it may be an important tool to identify plankton- and terrestrially-derived POM precursors to CDOM fluorescence. The BEPOM α_{254} values reported for Hydrographer Canyon follow the same general trend observed in the Gulf of Mexico but with lower values throughout the water column, especially in the surface waters (Brym et al., 2014; Ziervogel et al., 2016). The

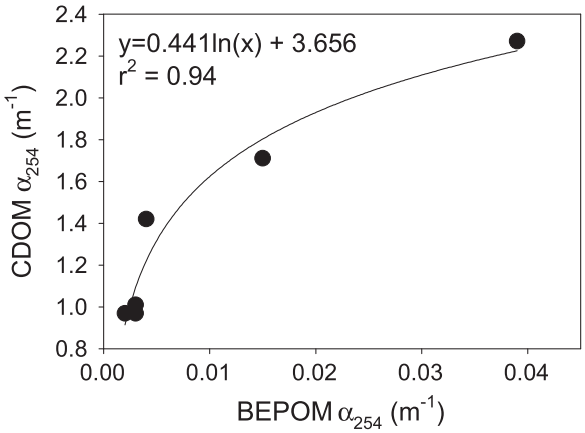

Fig. 5. Natural log relationship between colored dissolved organic matter (CDOM) absorbance coefficients at 254 nm (CDOM α_{254}) and base-extracted particulate organic carbon (BEPOM) absorbance coefficients at 254 nm (BEPOM α_{254}).

distinctive "three-peak" BEPOM fluorescence pattern occurred in the surface mixed layer where primary production was the highest (Coble, 2007). This pattern was consistent with previous observations of BEPOM in estuarine and coastal waters (Brym et al., 2014) and was likely a result of microbial-transformed planktonic material (Brym et al., 2014; Romera-Castillo et al., 2011) rather than material derived from terrestrial sources. This observation was further supported by the non-linear relationship between absorbance coefficients for CDOM and BEPOM (Fig. 5) that connects CDOM absorbance signal to planktonic sources in the BEPOM samples. Together, the absorbance and fluorescence data suggest that there was water column processing of plankton-derived material based on the presence of plankton-derived tryptophan in the BEPOM samples and the presence of microbial fluorescence in both CDOM and BEPOM. Additionally, the influence of terrestrial material from freshwater inputs was evident by humic-like fluorescence that dominated the CDOM samples.

Within the euphotic zone at Hydrographer Canyon, dissolved CH_4 was saturated (14.96 nM L^{-1}) relative to atmospheric concentrations (1.84 nM L^{-1}). Concentrations remained relatively uniform until 700 m, where a maximum of 17.87 nM L^{-1} was reached. CH_4 concentrations decreased to values below surface concentrations at bottom waters. Overall, the CH_4 concentrations did not show any major variations. Previous multibeam mapping (Skarke et al., 2014) and ROV observations at Hydrographer Canyon (Quattrini et al., 2015) suggest that this site does not contain methane seeps, and the closest known seep is approximately 7.6 km away near Shallop Canyon (Skarke et al., 2014). Uniform concentrations in the water column confirmed there was no influence of methane emissions from nearby seeps. The typical trend of high dissolved CH_4 in the surface waters reported in multiple studies (Ward et al., 1987; Conrad and Seiler, 1988; Reeburgh, 2007; Karl et al., 2008) was not seen in our results.

There were only minor variations in dissolved N_2O throughout the water column. Concentration increased with depth until reaching a maximum near the seafloor. N_2O typically displays an inverse relationship with oxygen levels, so N_2O would normally be expected to peak at the oxygen minimum (Yoshinari, 1975; Oudot et al., 2002). High N_2O concentrations in surface waters have been attributed to the biological reduction of nitrate to N_2 gas by phytoplankton (Yoshinari, 1975; Prokopenko et al., 2011). There is a lack of variation in N_2O and CH_4 concentrations throughout the water column, resulting in potentially misleading maximum and minimum concentrations that may be explained by sparse and non-discrete sampling. Thus far, there is only sufficient data on N_2O and CH_4 concentrations in surface water, and their distributions throughout the water column remain understudied (Foster et al., 2009). Future studies should coordinate the depth of the

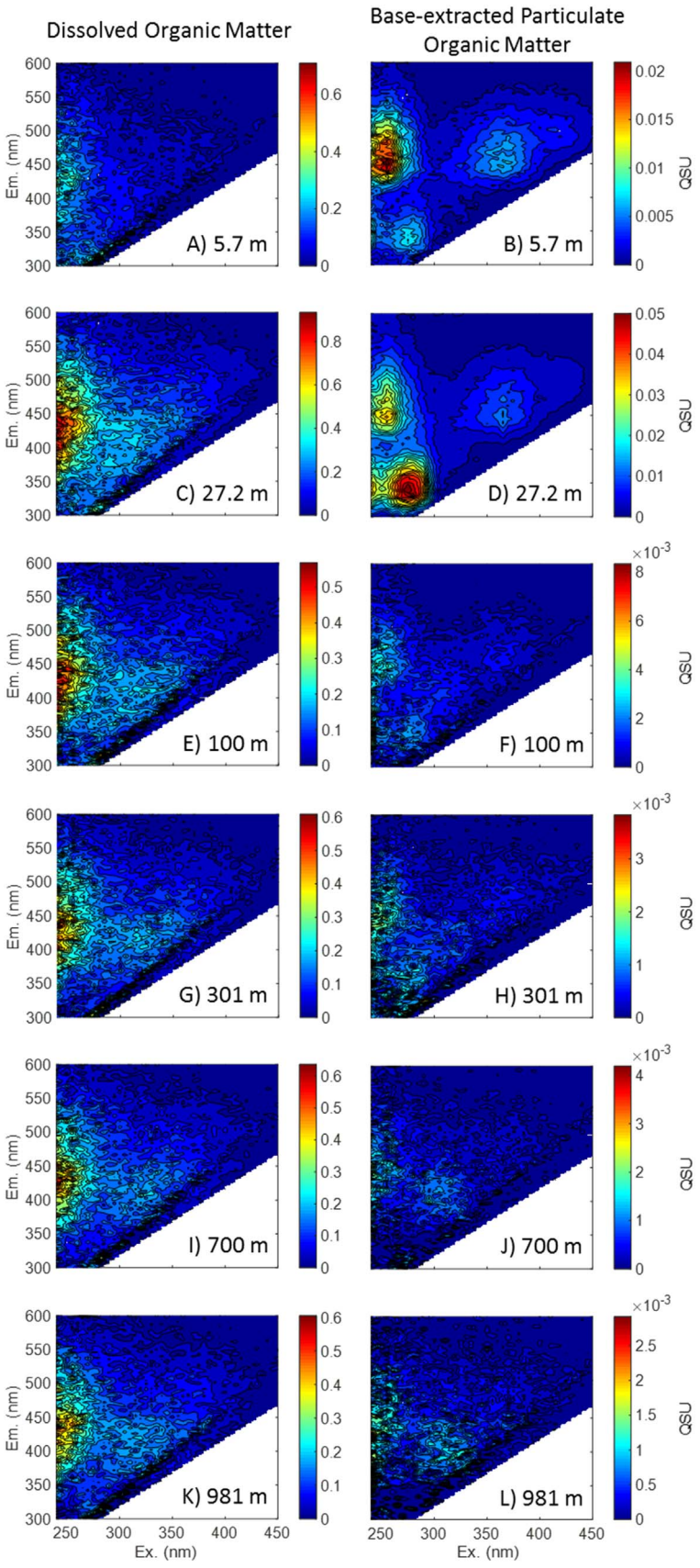

Fig. 6. Excitation-emission matrices (EEMs) of (A, C, E, G, I, K) colored dissolved organic matter (CDOM) and (B, D, F, H, J, L) base-extracted particulate organic matter (BEPOM) for six depths collected in the Hydrographer Canyon water column. EEM intensities are presented in quinine sulfate units (QSU) where 1 QSU = 1 ppb quinine sulfate. Note QSU scaling differences on the color bar for various EEMs.

organism surveys and water collection to test for correlations between biological features like zooplankton layers and water column properties.

4.3. Telepresence

Typically, participation in oceanographic expeditions is limited by available space and once at sea, communication – both from ship to shore and to the submarine – can be challenging (Marlow et al., 2017). This expedition utilized telepresence to connect shore-based researchers with the shipboard researchers physically performing scientific tasks, thereby expanding available expertise of the expedition. In-lab video and conferencing capabilities also allowed for real-time and impromptu discussion of methodologies and cross-disciplinary training of sample collection and processing. Finally, the capability to text between *Alvin* and the RV *Atlantis* will be further developed to enable real-time discussions and decisions between scientists about sample location and targeted sampling.

Recent telepresence-based expeditions on the NOAA Ship *Okeanos Explorer* have provided opportunity for midwater experts to make real-time pelagic ROV survey plans and identifications of midwater organisms from exploration command centers and personal computers (A. Netburn, pers. obs.). However, the surveys and sampling at Hydrographer Canyon were the first opportunity we know of to use telepresence to facilitate a water column manned submersible study.

5. Conclusion

The deep pelagic ocean remains one of the least explored environments on the planet. Traditional tools to study organisms in this environment are fraught with biases; net trawls often destroy fragile animals, and acoustic data lack taxonomic resolution. One of the best ways to understand any ecosystem is through direct visual observation (Fryer et al., 2002), and this is particularly challenging and rare in the deep pelagic realm. The research that has been conducted in human-occupied vehicles over the years has contributed to scientific understanding of midwater communities, providing scientists the opportunity to observe deep scattering layers (Greene et al., 1992; Barham, 1966), bioluminescence (Widder, 2010), and gelatinous and other fragile organisms (Vinogradov and Shushkina, 2002) in situ. The Johnson-Sea-Link submersible, operated by Harbor Branch Oceanographic Institute and optimized for midwater work, was, however, retired in 2011 without replacement. The Monterey Bay Aquarium Research Institute (MBARI) has been conducting midwater ROV studies since 1988 (Robison, 1993). They have pioneered methods for collecting fragile gelatinous organisms (Raskoff et al., 2003), conducting in situ respirometry, and developing other novel approaches to midwater ecology (e.g. Katija et al., 2017). MBARI has additionally conducted monthly midwater transect surveys, providing a first ever time series of organisms that are destroyed in net collections, such as siphonophores (Robison et al., 2010). However, their work has been limited primarily to the Monterey Bay off of the California coast. Expanding the capabilities of *Alvin* to include midwater work will provide unprecedented opportunity to broaden the geographic scope and depth of submersible studies of midwater organisms.

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