TECHNOLOGY CENTER OVERVIEW

The Application of Novel Research Technologies by the Deep Pelagic Nekton Dynamics of the Gulf of Mexico (DEEPEND) Consortium

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ABSTRACT

The deep waters of the open ocean represent a major frontier in exploration and scientific understanding. However, modern technological and computational tools are making the deep ocean more accessible than ever before by facilitating increasingly sophisticated studies of deep ocean ecosystems. Here, we describe some of the cutting-edge technologies that have been employed by the Deep Pelagic Nekton Dynamics of the Gulf of Mexico (DEEPEND; www.deependconsortium.org) Consortium to study the biodiverse fauna and dynamic physical-chemical environment of the offshore Gulf of Mexico (GoM) from 0 to 1,500 m.

Keywords: deep sea, oceanography, pelagic ecosystems

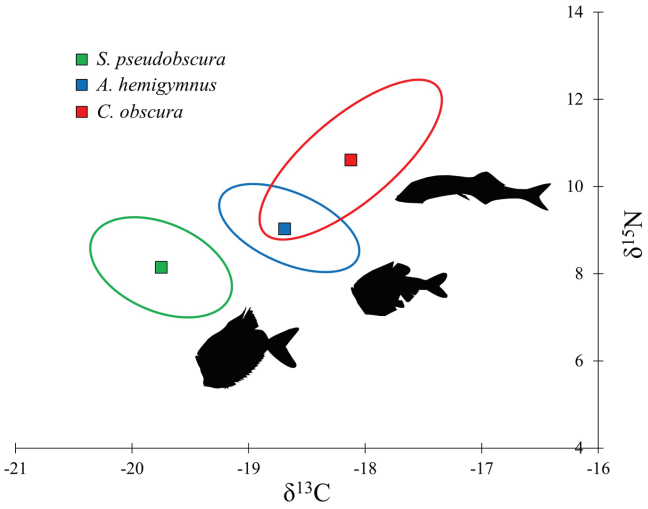
Trophic Structure: Compound-Specific Stable Isotope Analyses

Stable isotope analysis (SIA) is a popular method of delineating food web structure as it provides a view of an organism's diet over time scales relevant to tissue turnover rates rather than digestion rates. Carbon isotopes ($\delta^{13}C$) are used to determine the relative contribution of different carbon sources (primary producers) to consumers within a food web, while nitrogen isotopes ($\delta^{15}N$) are used to assign consumers to specific trophic positions. However, sampling primary producers in pelagic systems across spatially and temporally comprehensive scales is difficult and expensive.

A powerful supplementary approach to traditional SIA is the use of compound-specific SIA of amino acids (CS-SIA AA), which allows for the designation of trophic positions without the collection of primary producers. The CS-SIA AA method works

FIGURE 1

Mean bulk $\delta^{15}N$ and $\delta^{13}C$ values plotted with ellipses encompassing 95% of the $\delta^{15}N$ and $\delta^{13}C$ values for three nonvertically migrating zooplanktivorous fishes collected from different depths in the GoM. Bulk isotope data suggest that *Cyclothone obscura* (bathypelagic) occupies a higher trophic position than *Argyropelecus hemigymnus* (upper mesopelagic) and St*ernoptyx pseudobscura* (lower mesopelagic and upper bathypelagic). However, CS-SIA AA analyses suggest that the differences are caused by an enriched $\delta^{15}N$ baseline with increasing depth, rather than diet.



by incorporating data from both "source" and "trophic" amino acids. As an example, CS-SIA AA was applied to three zooplanktivorous fishes from different depths (Figure 1). Despite known similarities in their diet from stomach content analyses, bulk isotope data (particularly $\delta^{15}N$) suggested that *Cyclothone obscura* may occupy a higher trophic position than *Argyropelecus hemigymnus* and *Sternoptyx pseudobscura*. However, preliminary CS-SIA AA findings show elevated source AA values in *C. obscura*, suggesting that differences in diet are not causing the differences in bulk $\delta^{15}N$.

Genetic Diversity: Double-Digest Restriction Site–Associated DNA Sequencing

The level of genetic diversity found within a population is used as a proxy to measure population health. Genetic connectivity refers to the amount of genetic information shared and/or exchanged between populations. It is this exchange of genetic material across small or large spatial scales that allows for the introduction of new genes and the ability to adapt following environmental perturbations. For this reason, determining how genetic diversity is shared and exchanged within and across the GoM has huge implications for the recovery and resilience of a species and the ecosystem.

Double-digest restriction site–associated DNA sequencing (ddRADseq) is used to identify large sets of genome-spanning genetic markers to measure genetic diversity. DEEPEND utilized ddRADseq to generate unprecedented amounts of genetic data for an ecologically and functionally important array of deep-sea species (fishes, crustaceans, and cephalopods). These data allowed us to estimate levels of genetic diversity and connectivity across geographically and temporally separated samples collected from the GoM and the Atlantic. This massive sequencing effort, integrated with oceanographic and ecological data, provided an in-depth, high-resolution comparative diagnosis of the health and resilience and recovery potential of the GoM midwater (200–1,500 m) following the *Deepwater Horizon* oil spill.

Microbial Community Analyses: eDNA and High-Throughput Sequencing

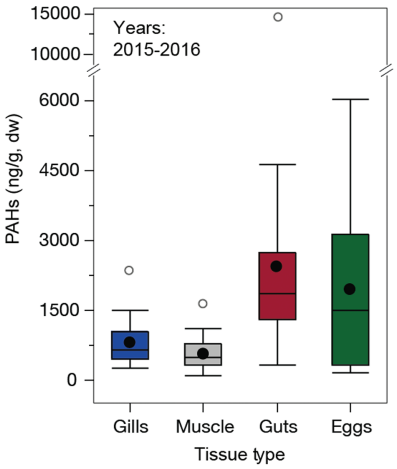
DEEPEND partner Nova Southeastern University (NSU) carried out microbial community ("microbiome") characterization and analyses of the northern GoM. To date, a total of 902 seawater samples were processed, resulting in ~88,000,000 (>60 Gb) of 16S rRNA high-quality bacterioplankton sequences. Additionally, microbiome profiles have been used to detect the movement and boundary of the Loop Current in the GoM and to ground-truth a spatial model of Loop Current dynamics. Further analysis of this system will incorporate nearly 800 collected seawater samples to build a temporal view of microbial community dynamics and assess the forces that structure these communities from the surface to bathypelagic.

Assessment of Polycyclic Aromatic Hydrocarbons in Mesopelagic Fishes

Mesopelagic fish samples were collected in 2015 and 2016 from the northern GoM to better understand mechanisms of bioaccumulation of toxic compounds including polycyclic aromatic hydrocarbons (PAHs).

FIGURE 2

PAH concentration in different tissues of mesopelagic fishes collected in 2015–2016 in the GoM. Graph shows shaded boxes as the interquartile ranges, with horizontal lines indicating median values and whiskers representing the 10th and 90th percentiles. Black and white circles denote mean and outlier values, respectively. Reprinted with permission from Romero et al. (2018). Copyright (2018) American Chemical Society.



Resultsindicated relatively high PAH concentrations in the unhatched eggs, within the range observed to cause mortality and sublethal effects in embryonic and juvenile fishes (Figure 2). Further studies are recommended for integrating PAH concentrations with population dynamics, due to the potential long-term impacts on deep pelagic communities.

Estimating Faunal Biomass and Behavioral Patterns: Acoustic Technologies

Sound-scattering layers (SSLs) are dynamic; therefore, understanding how the community structure within SSLs varies will help better characterize the important ecological role these organisms play across spatial and temporal scales. As part of the DEEPEND program, we use acoustic technologies to examine how SSL distributions respond to oceanographic conditions and focus on linking scattering responses to community level dynamics across the northern GoM (Figure 3).

Relative to net sampling, acoustic technologies provide increased spatial (c. 1 m vertically, 10 m horizontally) and temporal (c. 1–5 s) resolution on the variability of the vertically migrating mesopelagic and bathypelagic communities, as well as the horizontal extent and magnitude of persistent SSLs.

To better study these communities, we deployed a Simrad wideband autonomous echosounder transceiver (WBAT) operating from 55 to 95 kHz and from 165 to 250 kHz, within the migrating SSLs during three DEEPEND survey campaigns (2016–2018). We deployed the WBAT in two different configurations: mounted to the MOCNESS frame with transducers aimed into the sampled volume of the net or attached to the conductivity, temperature, depth (CTD) rosette and suspended at depth to acquire high-resolution data within the migrating SSLs. By placing the echosounder closer to the animals at depth, we could count and measure individual animals and examine patterns in density and behavior without the need for lights or direct capture (Figure 4).

The data products that result from this technology will extend our understanding of the fine-scale structure of SSLs. These data will allow us to (1) investigate high-resolution, wideband scattering responses of the mesopelagic organisms that contribute to the SSLs; (2) examine the spatial heterogeneity of SSLs and determine the scales of patchiness; and (3) quantify *in-situ* density estimates of dominant scatterers to compare directly to acoustic data from shipboard echosounders and the MOCNESS samples.

Near-Surface Bio-optical Observations to Aid Deep Water Measurements

The northern GoM contains distinct water masses and dynamic current features, and the near-surface waters provide a pathway for flow of energy and the exchange of materials between the surface and deeper waters.

FIGURE 3

Echogram (38 kHz) illustrating diel vertical migration of the mesopelagic community in the GoM. The SSLs can be seen moving vertically during the crepuscular periods from depths near 400 m to the surface waters.

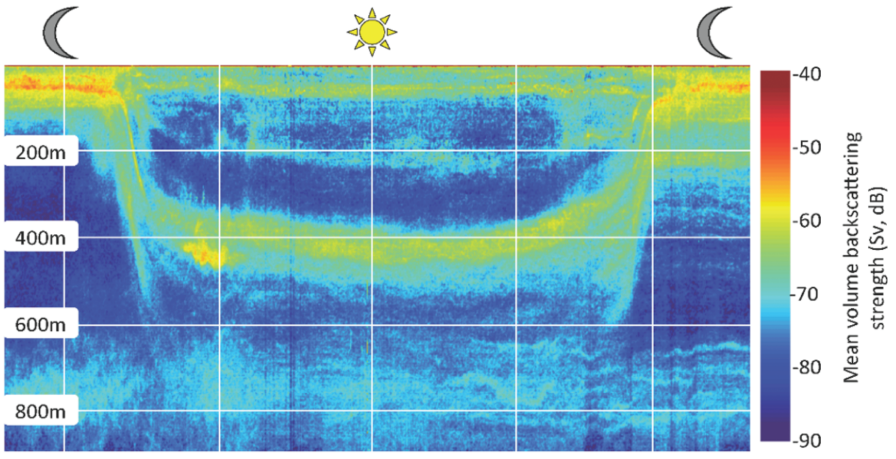
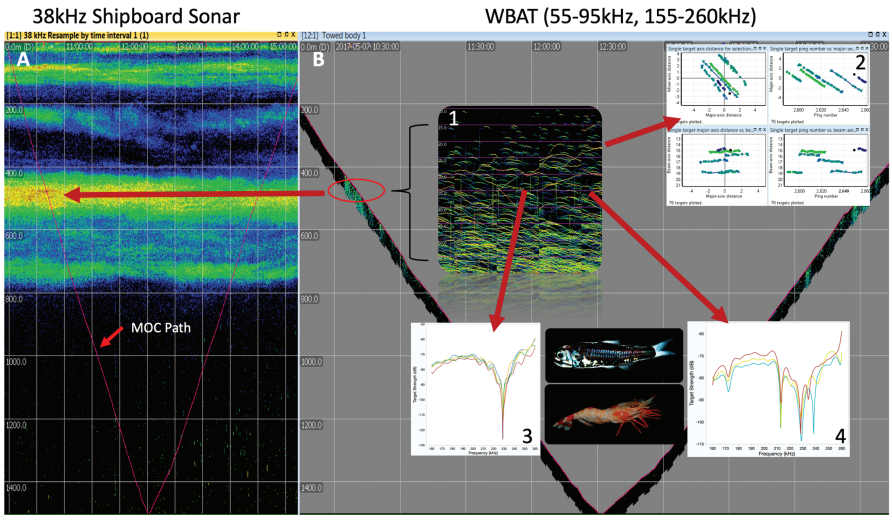


FIGURE 4

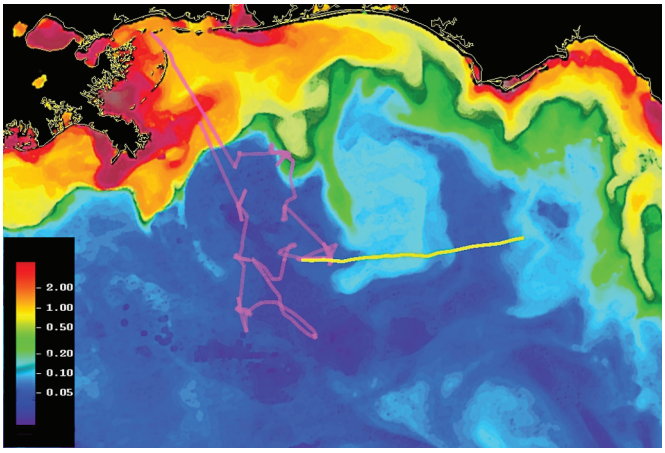
A shipboard echosounder (38 kHz; A) collected data highlighting the path of the MOCNESS and attached WBAT through the layers. Inset images (B) illustrate data output from WBAT. (1) Individual-level data resolution derived within the SSLs. (2) Results from several tracked individuals at depth providing data on behavioral responses of individuals within SSLs. (3 and 4) Wideband scattering responses of individual targets detected with WBAT within SSLs. This approach permits the investigation of a fine-scale structure within SSLs and examines taxonomic responses not interpretable from shipboard sensors. Faunal images were taken by Danté Fenolio.



Multiple images from Moderate Resolution Imaging Spectroradiometer (MODIS) instruments on the Aqua and Terra satellites (e.g., Satellite Data Products at https://optics.marine.usf.edu) were combined into relatively cloud-free composite images and transmitted to the research vessel to aid adaptive sampling of the moving waters of the GoM. An example of such a composite is shown in Figure 5, where the ship and glider tracks passed through several frontal features.

FIGURE 5

Example of an ocean color composite image sent to scientists aboard the DP05 cruise (May 2017), with ship track (purple) and glider track (traveling westward to where it met the ship; yellow). The image shows chlorophyll *a* concentration in surface waters. Note that both ship track and glider track went through several frontal features. Blue areas are clear surface waters, green and yellow areas contain more phytoplankton, and orange and red areas contain high concentrations of phytoplankton, colored dissolved organic matter (CDOM), and/or suspended sediments.



During DEEPEND cruises, an updated HOBILabs HS6 and several fluorometers were profiled from the surface to a depth of about 250 m, shortly before or after the CTD cast. The HS6 measured light backscattered at six wavelengths, while the fluorometers measured fluorescence of chlorophyll and colored dissolved organic matter. Combining scattering and fluorescence measurements with information from the instruments on the CTD rosette not only allows estimation of the vertical distribution of phytoplankton but also provides information about the characteristics of living and nonliving particles.

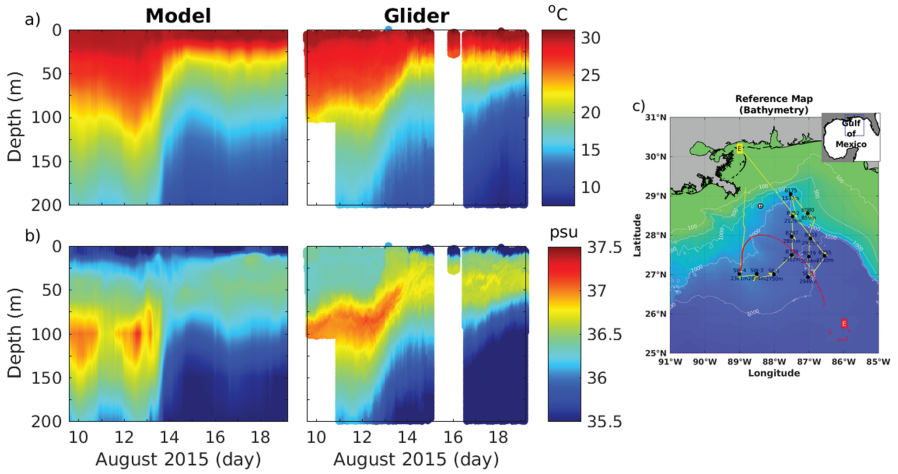
When sampling stations were occupied during the midday period, remote sensing reflectance measurements ($R_{rs}(\lambda)$, sr^{-1}) were collected from the ship. These $R_{rs}(\lambda)$ measurements assess the spectral reflectance of the sea surface, which can be used to evaluate accuracy of satellite measurements or develop bio-optical inversion algorithms relating surface water properties to above-water measurements.

Physical and Chemical Profiles of the Water Column: Slocum Electric Glider

The University of South Florida's (USF) 1,000-m Slocum electric glider was deployed in August 2015 (Figure 6c) to characterize the upper 400 m of the pelagic ocean. All gliders deployed during DEEPEND were equipped with bio-optical-physical

FIGURE 6

Qualitative matchups of (left) HYCOM simulated versus (right) glider measured (a) temperature and (b) salinity during the DEEPEND DP02 cruise (August 9–19, 2015). Reference map (c) illustrating the DP02 station locations (black dots), cruise tracks (green), and Slocum glider tracks (dotted).



sensors, including a Seabird SBE41CP CTD sensor. The glider temperature and salinity data were assimilated into the Hybrid Coordinate Ocean Model (HYCOM; Figure 6), which was used to support the DEEPEND cruises. In August 2015, for example, the glider operated to 200 m (Figure 6) and then 400 m (not shown). It transited southeasterly in an anticyclonic path at the edge of the Loop Current, and it was recovered on August 22 after 12 days of sampling (Figure 6c).

Summary

The data collected during the DEEPEND cruises have enabled various studies to understand the oceanic environments in the northern GoM including remote sensing studies of surface salinity changes (Chen & Hu, 2017); evaluation of atmospheric correction schemes for satellite measurements (Zhang et al., 2018); development of validation criteria for satellite-derived ocean properties (Barnes et al., 2019); the use of glider data to observe vertical migration of *Karenia brevis* (Hu et al., 2016); the influence of light and oceanographic structuring on acoustically observed deep scattering layer patterns (Boswell et al., 2018); the use of genome-scale ddRADseq methods to elucidate genetic diversity and connectivity patterns, and phylogenetic diversity in cephalopods (Timm et al., 2018b), crustaceans (Robalino et al., 2016; Timm & Bracken-Grissom, 2015; Timm et al., 2018a, 2018c), and fishes; use of molecular genetics to characterize microbial community structures (Easson & Lopez, 2018), which in turn can support the identification of mesoscale oceanographic features (Johnston et al., 2018); the use of PAHs to understand bioaccumulation patterns of toxic compounds in deep-pelagic fauna; and use of the HYCOM model to classify and predict physical oceanographic features in the GoM (deRada & Penta, 2018; Johnston et al., 2018). Altogether, this work represents substantial progress in understanding how pelagic ecosystems are structured and connected through the GoM across a range of spatial and temporal scales, the physical and chemical drivers that most strongly influence the fauna, and improves our understanding of how these ecosystems may be influenced by anthropogenic impacts in the future.

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