

O4A-1: Exploring Deep Sea Biodiversity with Environmental DNA

Annette F. Govindarajan

Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA, USA

The deep sea, including the ocean's mesopelagic zone (or "ocean twilight zone"), harbors a vast biomass that is critically important to the global carbon cycle. However, the diversity of this biomass is understudied due to the immense size of the environment and the logistical challenges of collecting samples. More information is especially needed on diel vertical migration (DVM), where typically, mesopelagic animals travel to surface waters to feed at night and return to mesopelagic depths for the day, expediting the downward movement of carbon in the process. Environmental DNA (eDNA) metabarcoding analyses have great potential to provide information on deep sea biodiversity and address hypotheses related to DVM, species distributions, and ecological interactions. Furthermore, we have found that eDNA detects animal taxa that are missed in traditional net tow analyses. However, deep sea animal eDNA signals are dilute and patchily distributed, creating challenges for eDNA research. I will discuss recent advances and results in eDNA approaches to studying deep sea biodiversity with respect to sampling, reference databases, and data interpretation. Despite potential for vertical movement of genetic material, modeled and field-collected eDNA signatures appear to reflect the depth of their source, indicating that eDNA approaches are useful for studying vertical phenomena such as DVM. Consistent with this and using newly obtained reference sequences from deep sea fishes, we found a greater diversity of mesopelagic fish in surface waters at nighttime than in the daytime. Additionally, I will show how large-volume autonomous samplers can facilitate deep sea biodiversity exploration by enabling the detection of more taxa and through their integration with diverse oceanographic platforms such as the AUV *Mesobot*, a robot designed for midwater science and exploration.

[This research is part of the Woods Hole Oceanographic Institution's Ocean Twilight Zone Project, supported by funding as a part of The Audacious Project, a collaborative endeavor housed at TED. Funding was also provided by the NOAA Ocean Exploration Cooperative Institute.]

O4A-2: Multi-Marker eDNA Metabarcoding Reveals Patterns of Multi-Trophic Interactions and Ecological Connectivity in the Mesopelagic

Nina Yang^{1,2}, Elizabeth A. Allan³, Sarah Stover¹, Benjamin Grassian^{1,4}, Joel K. Llopiz¹, Heidi M. Sosik¹, Annette F. Govindarajan¹

1. Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA, USA

2. Marine Policy Center, Woods Hole Oceanographic Institution, Woods Hole, MA, USA

3. School of Marine and Environmental Affairs, University of Washington, Seattle, Washington, USA

4. Department of Applied Ocean Physics and Engineering, Woods Hole Oceanographic Institution, Woods Hole, MA, USA

Midwater ecology and organismal interactions are difficult to study and as a result, are insufficiently accounted for in food web analyses and biogeochemical models. This hinders our ability to accurately model and forecast ecosystem function and formulate effective policies for conservation and management, especially given the growing interest in exploiting midwater resources. We used multi-marker metabarcoding of environmental DNA (eDNA) samples collected from the Northwest Atlantic Slope Water to resolve patterns of marine biodiversity from epipelagic and mesopelagic waters. Combined with statistical network analyses, we explored cross-kingdom eukaryotic community structure and taxonomic interactions with the aim of obtaining insights into food web dynamics. We found distinct, depth-specific communities comprised of unique protist, invertebrate, and fish assemblages. Ecological networks for the epipelagic, upper mesopelagic, and lower mesopelagic suggest that protists are key mediators of multi-trophic level interactions and ecological connectivity from the surface waters down to 1000 m. Additionally, network modularity and other topological features indicate greater interconnectedness in mesopelagic communities compared to the epipelagic, which may play a role in ecosystem stability and resilience. We will discuss our findings in the context of generating holistic ecological insights and the implications for deep sea ecosystems in a changing ocean to inform sustainable resource management and stewardship of the deep sea.

[This research is part of the Woods Hole Oceanographic Institution's Ocean Twilight Zone Project, supported by funding as a part of The Audacious Project, a collaborative endeavor housed at TED.]

O4A-3: Move Over Medusae: Trophic Ecology of Biomass-Dominant Gelatinous Zooplankton (Siphonophores and Pyrosomes) in the California Current Ecosystem

Elizabeth D. Hetherington¹, Alejandro Damian-Serrano², Steven H.D. Haddock³, Casey W. Dunn⁴, Hilary G. Close⁵, Shannon C. Doherty⁶, Alitzah X. Saenz¹, Phoebe J. Lam⁷, C. Anela Choy¹

1. Scripps Institution of Oceanography, University of California San Diego, La Jolla, California, USA
2. University of Oregon, Institute of Ecology and Evolution, Eugene, Oregon, USA
3. Monterey Bay Aquarium Research Institute, Moss Landing, California, USA
4. Department of Ecology and Evolutionary Biology, Yale University, New Haven, Connecticut, USA
5. Rosenstiel School of Marine, Atmospheric, and Earth Science, University of Miami, Miami, Florida, USA
6. College of Fisheries and Ocean Science, University of Alaska Fairbanks, Fairbanks, Alaska, USA
7. Ocean Sciences Department, University of California Santa Cruz, Santa Cruz, CA, USA

Gelatinous zooplankton are increasingly recognized as key components of pelagic ecosystems. We focus on two abundant and biomass-dominant gelatinous taxa, siphonophores (Hydrozoa) and pyrosomes (Tunicata). We synthesized existing literature on the trophic ecology and predator-prey interactions of siphonophores and analyzed siphonophore-prey network topology across shallow and deep habitats. Diets were distinct between siphonophore suborders and species, indicating that siphonophores occupy multiple trophic niches and prey on taxa including crustaceans, gelatinous zooplankton, and fishes. Siphonophore-prey networks were more specialized in deep-pelagic compared to upper-ocean habitats. We then sampled particulate organic matter, zooplankton, and micronekton in the California Current, concurrent with blooms of *Pyrosoma atlanticum*, and measured gelatinous zooplankton size structure and biomass across stations and depth habitats. We used stable isotope analyses of bulk tissues and amino acids to infer trophic ecology and calculate the trophic positions of siphonophores and *P. atlanticum*. Siphonophore trophic positions ranged from 2.4-4.0, which illustrates their trophic diversity. Trophic positions were consistent across depth habitats. However, the highest trophic positions were restricted to deep-pelagic physonect species, and trophic positions were strongly related to siphonophore phylogeny. Stable isotope values also indicated that deep-pelagic siphonophores indirectly rely on food webs supported by deep, small particles, whereas *P. atlanticum* relies on a shallow particle food-web baseline. *P. atlanticum* exhibited lower trophic positions compared to siphonophores and other zooplankton, suggesting that they are isotopically distinct from the primary planktonic food web pathway in the California Current. Lastly, colony size greatly influenced *P. atlanticum* vertical habitat, whereby smaller colonies were restricted to the upper 200 m, which has implications for carbon cycling. Pyrosomes and siphonophores are both colonial gelatinous zooplankton but they employ different feeding mechanisms and occupy distinct niches in pelagic food webs. These insights enhance our understanding of their vertical distribution, trophic ecology, and roles in pelagic food webs.

[This research was supported by the National Science Foundation Awards OCE-1829812 and OCE-2048210 to C.A. Choy, OCE-1829805 to S.H.D. Haddock, OCE-1829835 to C.W. Dunn.]

O4A-4: First Investigations of Dietary and Habitat Overlap in Three New Zealand Deep-Sea Squid Species

Samuel Clough¹, Amandine Sabadel^{1,2}, Kat Bolstad¹

1. *Auckland University of Technology, New Zealand*

2. *National Institute of Water & Atmospheric Research, New Zealand*

Cephalopods are vital nutrient vectors between different marine habitats and from low level consumers to apex predators. Their ecological importance will continue to rise as cephalopods fill the vacant niches left behind due to the overharvesting of their competitors. Despite their importance, 90% of cephalopod species' diets remain undescribed, and the habitat preferences of many such species are equally unknown. In this study, I aim to describe the dietary and habitat preferences of three deep-sea squids from New Zealand waters: the Angolan flying squid (*Todarodes angolensis*), the Antarctic flying squid (*Todarodes filippovae*) and the rugose hooked squid (*Onykia robsoni*). To characterize target taxon diets, morphological identification and DNA barcoding of prey tissues from specimens' gut contents was undertaken, but this method only reveals recent prey items. Longer-term comparative data can be obtained through carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope analysis of squid beaks, which are chitinous structures that are formed in layers over their lives. Therefore, by sub-sampling the beaks at locations corresponding to different life stages of the specimens and measuring their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, we can reliably quantify ontogenetic changes in habitat and assess trophic position. While there was a large degree of overlap in observed prey items from stomach content analyses between the study species, multivariate analysis reported that there was a species effect on dietary composition. This was attributed to *T. angolensis* having far more demersal and benthic prey species reported in its diet whereas *T. filippovae* and *O. robsoni* preferred pelagic prey items. All stable isotope results will be discussed during this presentation.

O4A-5: Examining the Diets of Deep-Sea Cephalopods from the Northern Gulf of Mexico Using a Metabarcoding Approach

Judkins, H¹., T. Sutton², T. Frank², M. Vecchione³

1. *University of South Florida St. Petersburg, 140 7th Ave South, St. Petersburg, FL 33701 USA*

2. *Nova Southeastern University 8000 N. Ocean Dr., Dania Beach, FL 33004 USA*

3. *NMFS National Systematics Laboratory, National Museum of Natural History, Smithsonian Institution, P.O. Box 37012, Washington, DC 20013-7012 USA*

Cephalopods are integral to the trophic dynamics of the open ocean, including the poorly known deep midwater. They are well known as prey of apex predators such as marine mammals, fishes, and birds from techniques such as visual analysis of stomach contents to identify cephalopod beaks and other ingested parts. However, there is very little information related to the specific prey of deep-sea cephalopods. Most studies report only broad categories of prey items (e.g. crustaceans, fishes). Cephalopods have a beak-like mouth with radula they use to break up food and when the resulting fragments are passed through the brain to the stomach, there are very few, if any distinguishable parts for prey species identification. The DEEPEND/RESTORE Program has conducted multiple cruises in the Gulf of Mexico where deep-sea cephalopods have been collected (2019- 2023). Midwater squid and pelagic octopod stomachs are being used for a gut-content metabarcoding project in which the identification of the prey species is possible using various DNA gene regions for specific species identification. Results from the ongoing project will be discussed to highlight the variety of prey items cephalopod species ingest in the northern Gulf of Mexico.