

Discovering food web interactions in the ocean twilight zone: metabarcoding analysis of the diet of mesopelagic fishes



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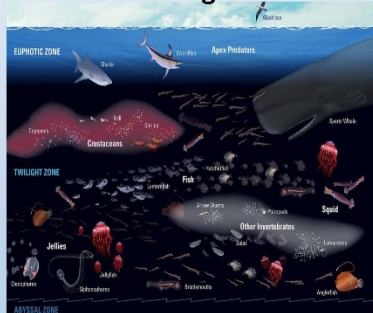


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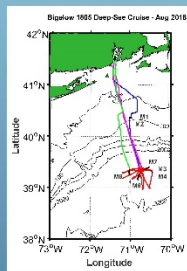
Ocean Twilight Zone



Abstract

- Ocean Twilight Zone or mesopelagic (200m – 1,000m depth) holds the largest biomass of organisms
- Mesopelagic fishes have largest biomass of any ocean domain
- Target species chosen to reflect diversity of behavior (DVM) and diet
- DNA extracted from gut contents, metabarcoding analysis using V9 hypervariable region of 18S rRNA
- Results analyzed using custom scripts and reference databases to classify sequences and operational taxonomic units (OTUs)
- Metabarcoding results compared to morphological / microscopic analysis of gut contents

Cruise Track HB-1805



Cruise track with transit legs to and from port (blue and green); transit legs for crew drop-off (purple); gear deployments; and sampling locations (Red)

Methods

- **DNA Extraction:** Complete gut content DNA was extracted using phenol chloroform isoamyl (25:24:1) and cleaned using the Power DNA Clean-Up Kit (MO Bio Laboratories)
- **PCR Amplification:** Protocol and primers for V9 region of 18S rRNA (Amaral-Zetter et al. 2009)
- **High-Throughput Sequencing:** Illumina MiSeq
- **Bioinformatics:** Analysis directed with Mothur (Ver. 1.40.2) on UCHC Xanadu cluster using the SILVA 132 reference database

Metabarcoding and Morphological ID of Prey Results

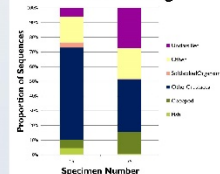
Hygophum hygomii

- Hygom's Lanternfish
- Max size 70mm
- Circumglobal
- Complete DVM
- Known prey: zooplankton

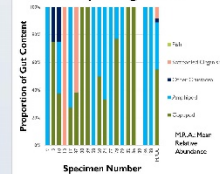


Photo: Paul Gruber (WHOI)

Metabarcoding



Morphological ID



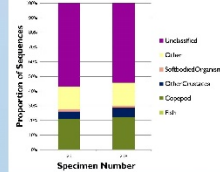
Agyropelecus aculeatus

- Silver hatchetfish
- Max age 2 years
- Circumglobal
- Complete DVM
- Known prey: zooplankton

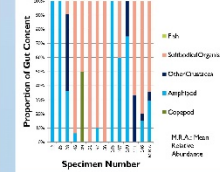


Photo: Paul Gruber (WHOI)

Metabarcoding



Morphological ID



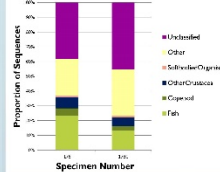
Nemichthys scolopaeus

- Sender snipe eel
- Common but patchy
- DVM mixed reports
- Known prey: zooplankton

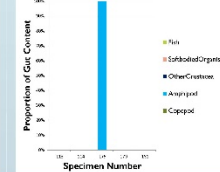


Photo: Paul Gruber (WHOI)

Metabarcoding



Morphological ID



Conclusions & Future Directions

- *Hygophum hygomii* gut sequences had similar amounts of prey such as soft bodied organisms and copepods which is comparable to morphological identifications
- Similar prey are seen in all species guts (Eucarida and copepods), but in different proportions; lack of amphipods in metabarcoding results due to lack of representative sequences in reference database.
- Soft bodied organisms seen in morphological identifications were classified further by metabarcoding
- Technical metabarcoding replicates provide evidence of reliable and reproducible results
- Future plans to use V4 analysis to determine sequences in "unclassified" area and to also use COI analysis for species level classification

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References

- Amaral-Zetter, L.A., McIlmont, E.A., Ducklow, H.W., and S.M. Huse (2009) A Method for Studying Protistan Diversity Using Massively Parallel Sequencing of V9 Hypervariable Regions of Small Subunit Ribosomal RNA. *Genes*, PLoS One 4: e6372
- Irigoien, X., Kleyer, T.A., Russel, A., Martinez, U., Boyra, G., Acuna, J.L., Boile, A., Echovarría, F., González-Gordillo, J.L., Hernández-León, S., Agustí, S., Alanes, D.L., Duarte, C.M., and Kasarvoti, S. (2014) Large mesopelagic fishes biomass and trophic efficiency in the open ocean. *Nature Communications* 5:3271

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Abstract

The ocean twilight zone or mesopelagic (200m – 1,000m depth) holds the largest biomass of organisms in the ocean and supports the largest diurnal migration on the planet. The food web dynamics of these deep ocean regions are central to the functioning of the ecosystem, determining responses to climate change, and resilience to commercial fishing. Mesopelagic fishes were collected using mid-water trawls from the Slope Water of the Northwest Atlantic Ocean during summer 2018 and spring 2019. Fishes were removed from samples immediately and frozen in liquid nitrogen. Selected fish specimens were partially thawed for species identification and dissection of gut contents. Specimens were paired by species and size for comparative analysis of gut contents by either morphological (microscopic) taxonomic identification or metabarcoding analysis. Extracted DNA was sequenced for the V4 hypervariable region of the 18S rRNA gene using an Illumina MiSeq sequencer. Results were analyzed using custom scripts and reference databases to classify sequences and operational taxonomic units (OTUs) into major groups of organisms. Additional analysis with more variable markers (including COI) is planned to identify species of the major groups for direct comparison with morphological taxonomic results. This study will contribute to new understanding of the role of mesopelagic fishes in the pelagic food web and the ecosystem dynamics of the ocean twilight zone.

Keywords:

mesopelagic fishes, food webs, metabarcoding, ocean twilight zone

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