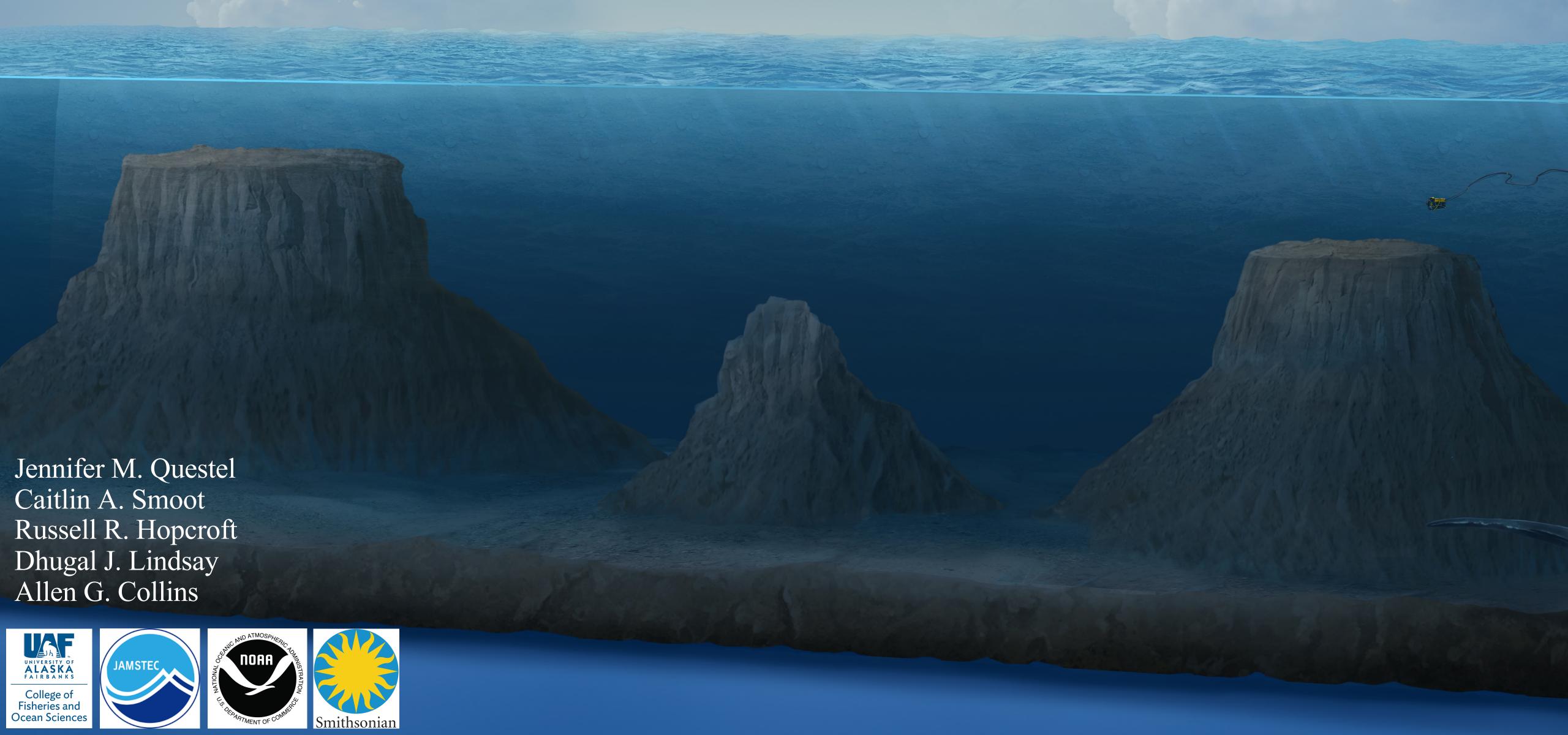
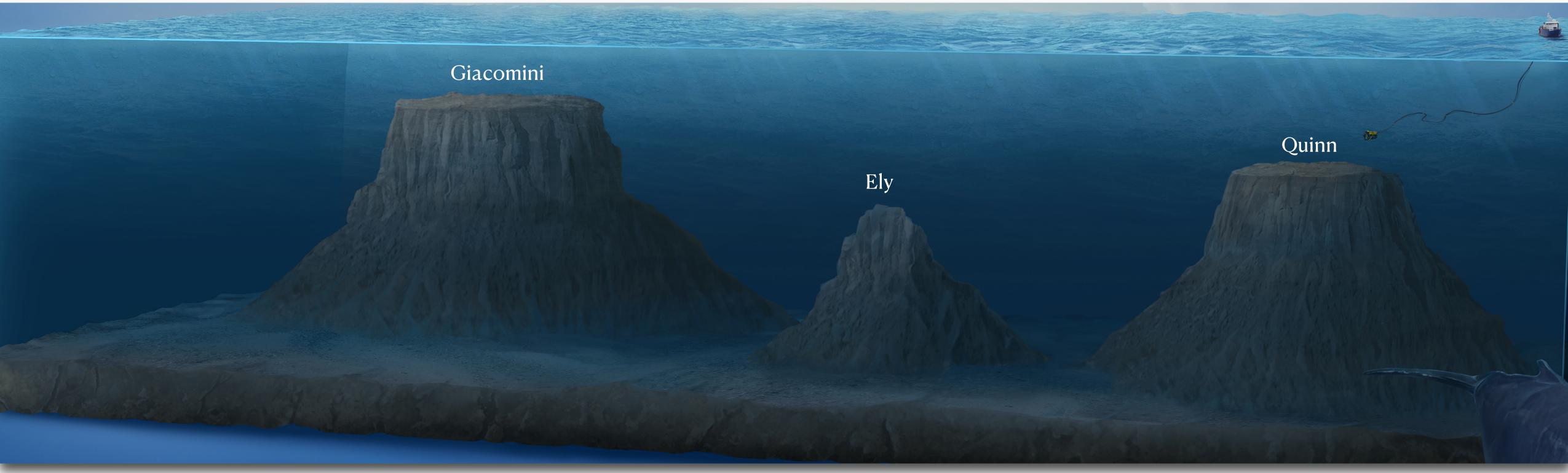
Molecular characterization of the dee-sea zooplankton community from the Gulf of Alaska Seamount Province



Gulf of Alaska Seamount Province • The deep sea (>1,000 m) is the largest biome yet least studied Most zooplankton data from epipelagic and nearshore regions and Ocean Station Papa Kodiak-Bowie and Cobb-Eickelberg Seamount Chain • Over 100 distinct seamounts • Lies within the Aleutian, Alaskan, and Tufts abyssal plains • Depth range: 4,000 - 5,000 m • Targeted the Giacomini and Quinn Giacomini and Quinn Bering Sea Seamounts Seamounts Eickelberg Seamount Chain $S_{eamount}Chain$ ♦ Bowie hotspot

Gulf of Alaska

NOAA OER Gulf of Alaska Seamounts Expedition (2019)





Pelagic dives to 3,000 m ROV Global Explorer

- Rotary carousel suction skid
- Detritus samplers
- UHD 4K & 3D camera systems



Depth-stratified samples to 4,500 m MOCNESS (505 μ m; left)

- 9-net system
- Net samples split for morphological and molecular analyses

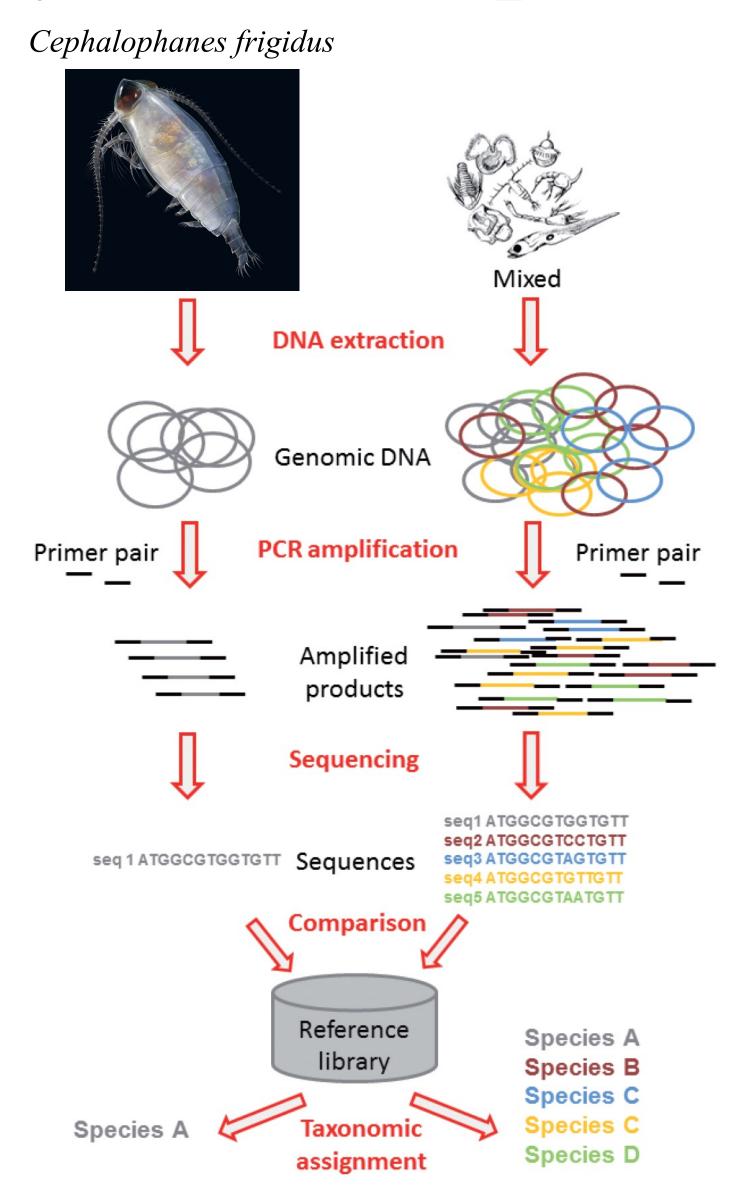
Tandem MultiNet (150 μ m; right)

- 5-net system
- back-to-back casts
- Discrete samples for morphological and molecular analyses

Molecular Analyses of Zooplankton Communities

DNA barcoding

- DNA Barcoding is a standardized approach to identification of organisms by fragments of DNA, called DNA barcodes
- Sanger sequencing technology
- Full length COI, 16S, 18S, and 28S
- DNA bracoding on organisms from all sampled stations and depths



Metabarcoding

- Taxonomic identification of organisms from complex environmental samples based on high-throughput sequencing (HTS) using DNA barcodes
- COI (~313 bp fragment)
- V4 18S rRNA (~450 bp fragment)
- Metabarcoding results from MOCNESS and MultiNet samples from GAK13.5 & GAK19

DNA barcoding of Gulf of Alaska Zooplankton

>2,000 formalin and ethanol vouchered organisms

- Each ethanol voucher has a matching formalin voucher
- At sea identifications to best taxonomic resolution
- Photographed many organisms
- Finer-scale taxonomic identifications post-cruise

DNA barcoding performed at Smithsonian NMNH

- Cnidarians and Ctenophores (2019)
 - COI, 16S, 18S, and 28S
- Arthropods and other taxa (2022)
 - COI and 18S

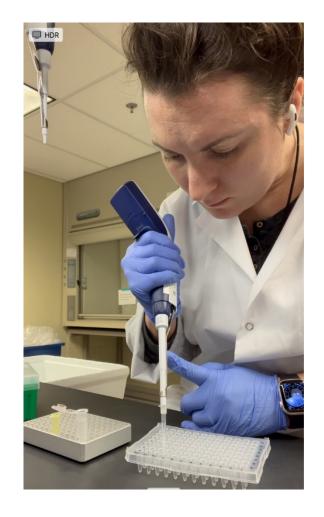




C. Smoot, D. Lindsay, and R. Hopcroft

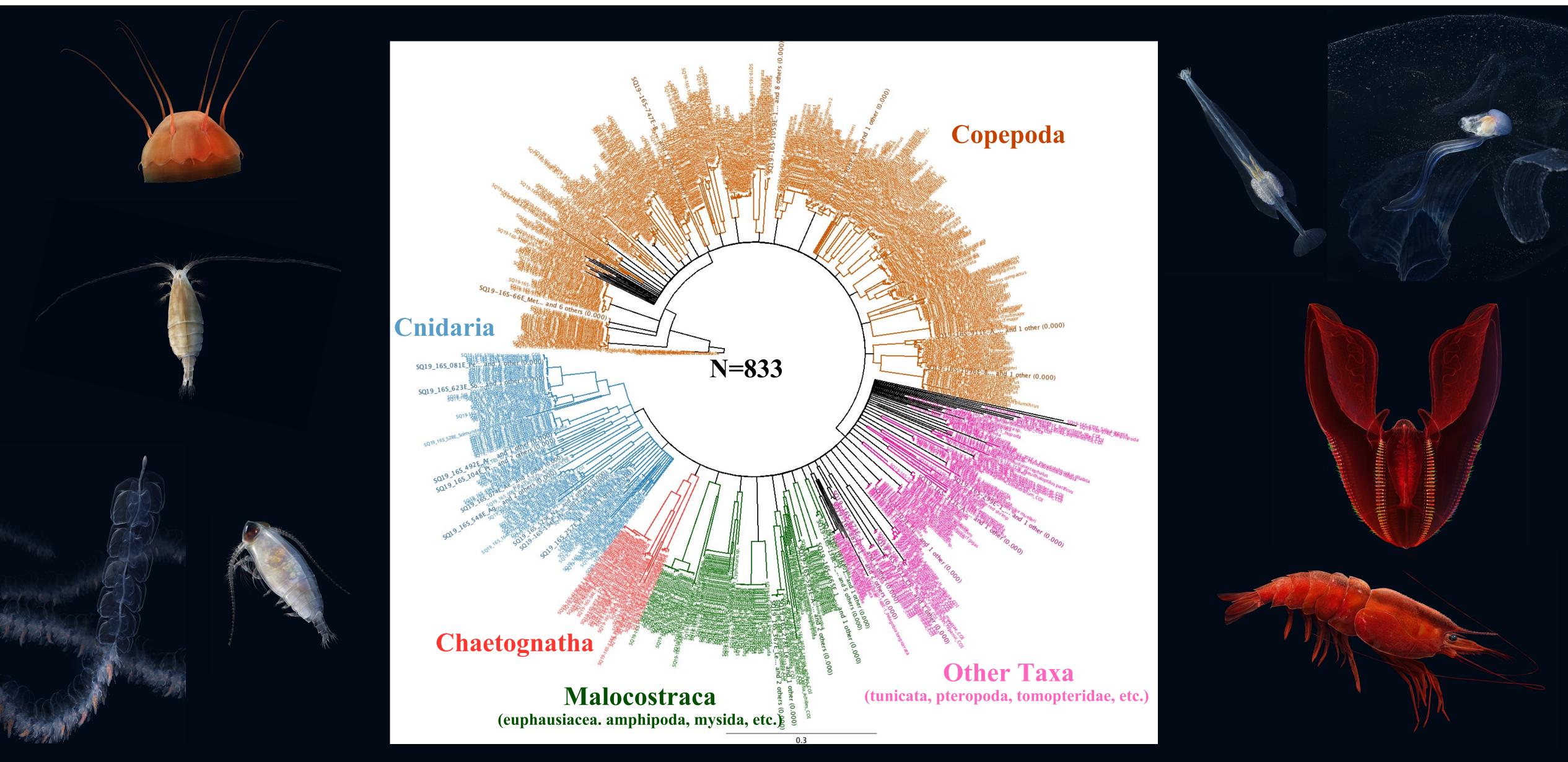


AutoGenprep 965 at Smithsonian



DNA barcoding of Gulf of Alaska Zooplankton

Neighbor-Joining tree for COI barcodes



DNA barcoding of Gulf of Alaska Zooplankton

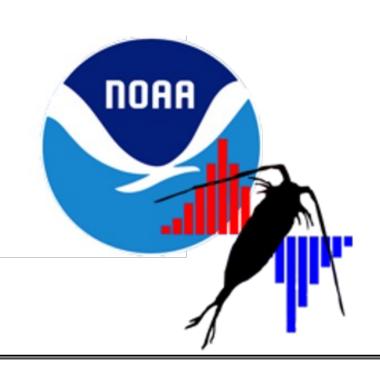
With the material identified, we have so far...

- Generated >3,500 sequences for 18S and COI for NE Pacific zooplankton
 - 387 new species' barcodes between 18S and COI markers alone
- Recorded multiple new observations for species in the NE Pacific (Gulf of Alaska & Ocean Station Papa)
 - (e.g., Mormonilla phasm, Batheuchaeta lamellata, Gaetanus paracurvicornis, Heterostylites submajor, Talacalanus aff. greenii)
- Collected specimens believed to be undescribed species (e.g., cnidarians, ctenophores, larvaceans)
- Obtained copepod species without taxonomic descriptions for male specimens
 - (e.g., Bathycalanus spp.)



SCOR WG157 MetaZooGene

Toward a new global view of marine zooplankton biodiversity based on DNA metabarcoding and reference DNA sequence databases.





Todd O'Brien metazoogene.org

- Stringently QA/QC database for marine metazoans
- Represents 73,373 observed species for 5 genetic markers
 - COI
 - 12S
 - 16S
 - 18S
 - 28S
- Geographic specific databases
- Downloadable files for bioinformatic pipelines

https://doi.org/10.1007/s00227-021-03887-y

REVIEW, CONCEPT, AND SYNTHESIS



Toward a global reference database of COI barcodes for marine zooplankton

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Marine fauna of the World Oceans

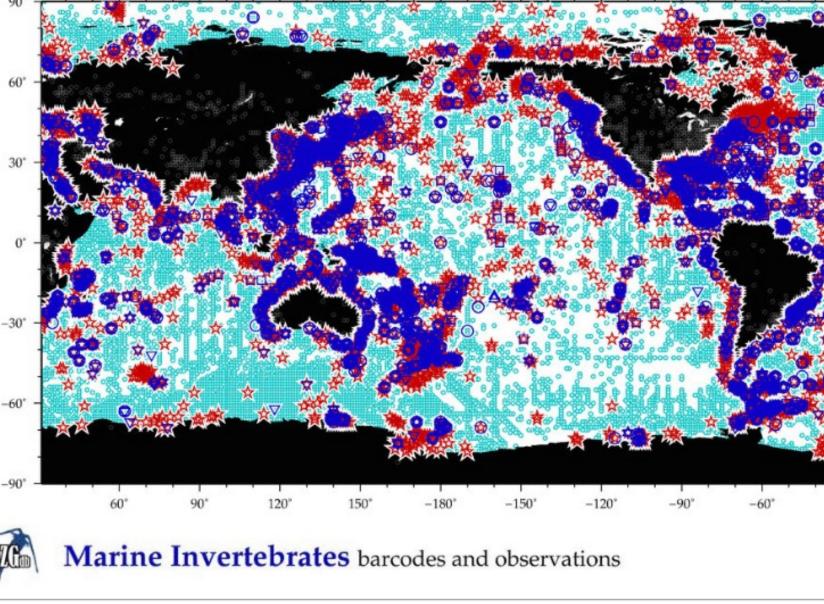
[back to MZGdb Intro Page]

Oceans/Seas/Region:

- World (all oceans)
- Arctic
- North Atlantic
 - Baltic Sea
- Mediterranean Sea
- ICES-NATL
- South Atlantic
- North Pacific
- South Pacific
 - Australia subregion
- Indian Ocean
- Southern Ocean / Antarctic

MZGdb Data Access ---> Click Here <---

All Vertebrates [13350 | 8849 | 66% | 8849 | 66%]



Crustacean Invertebrates

# of Crustacean Species	COI	12S	16S	18S	28S
21072			4074 19%	2491 11%	

Non-Crustacean Invertebrates

# of Non-Crustacean Species	COI	12S	16S	18S	28S
38951	12251		8287 21%		6531

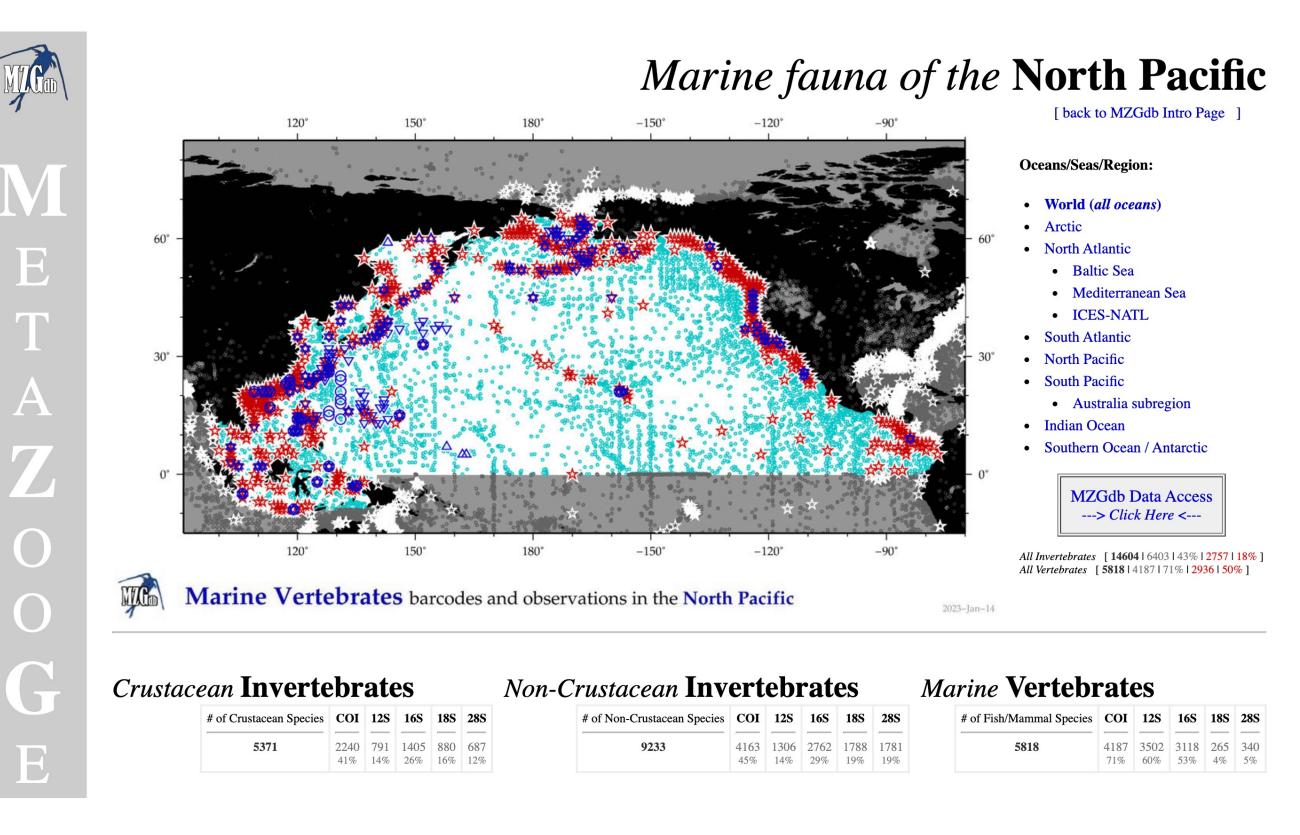
Marine Vertebrates

2023-Jan-14

# of Fish/Mammal Species	COI	12S	16S	18S	28S
13350	8849	6224	6092		597
	66%	46%	45%	4%	4%

Status of DNA Barcoding – North Pacific

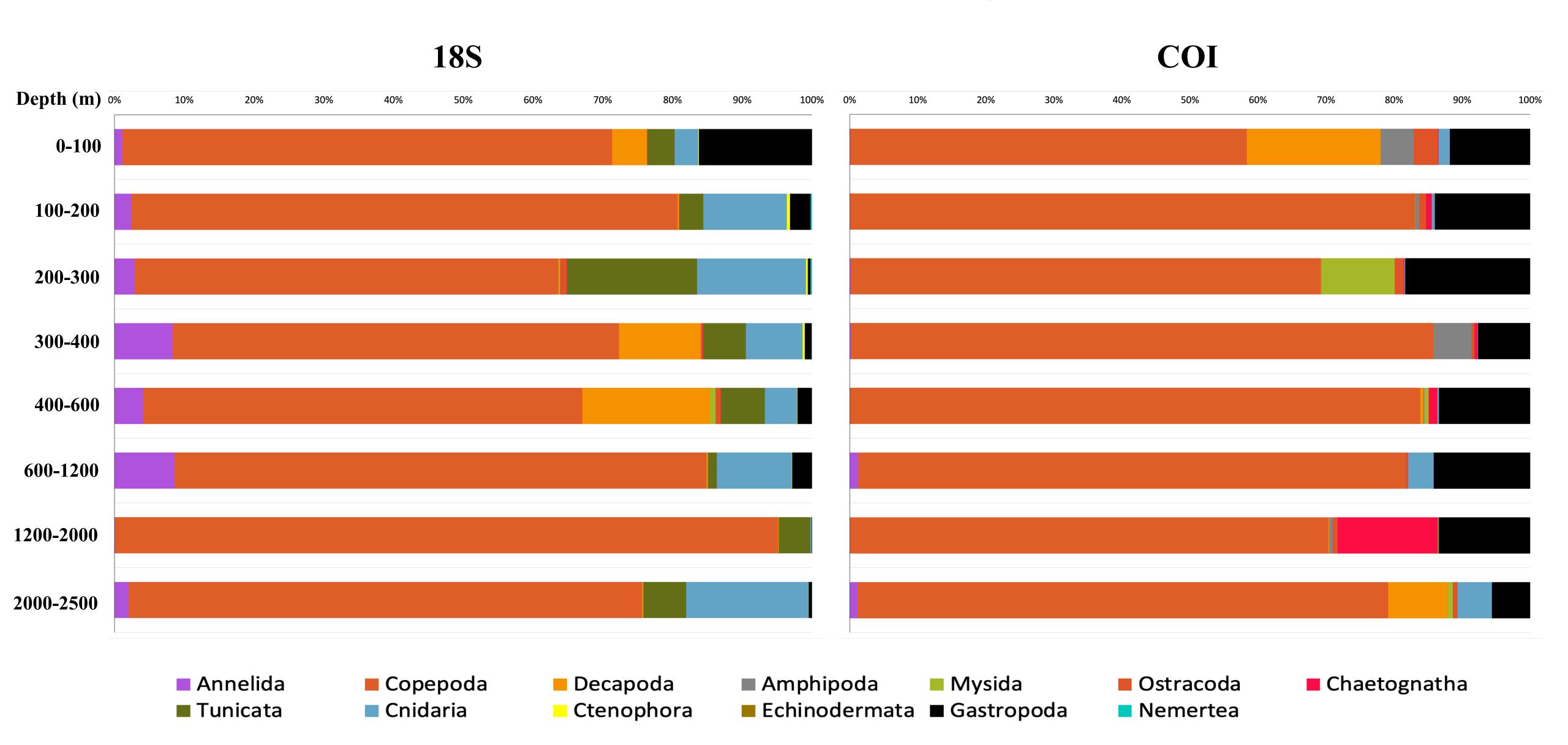
	# of species	COI	12S	16S	18S	28S
All invertebrates	14,604	,	791 (14%)	,		



Group	# of species	COI	12S	16S	18S	28S
Amphipoda	808	29%	1%	6%	13%	10%
Copepoda	908	40%	7%	14%	29%	25%
Decapoda	2,395	53%	26%	44%	14%	12%
Euphausiacea	50	84%	0%	62%	44%	18%
Isopoda	403	25%	2%	11%	6%	1%
Mysida	89	19%	2%	6%	15%	6%
Annelida	1,596	40%	3%	20%	24%	14%
Bryozoa	263	29%	3%	10%	10%	4%
Chaetognatha	39	56%	3%	8%	49%	59%
Tunicata	291	38%	2%	2%	28%	2%
Cnidaria	2,679	24%	7%	20%	16%	14%
Ctenophora	20	70%	10%	10%	65%	10%
Echinodermata	1,740	34%	9%	19%	11%	10%
Mollusca	3627	53%	24%	40%	16%	24%
Nematoda	52	0%	0%	0%	0%	0%
Nemertea	88	77%	6%	63%	34%	56%
And more						

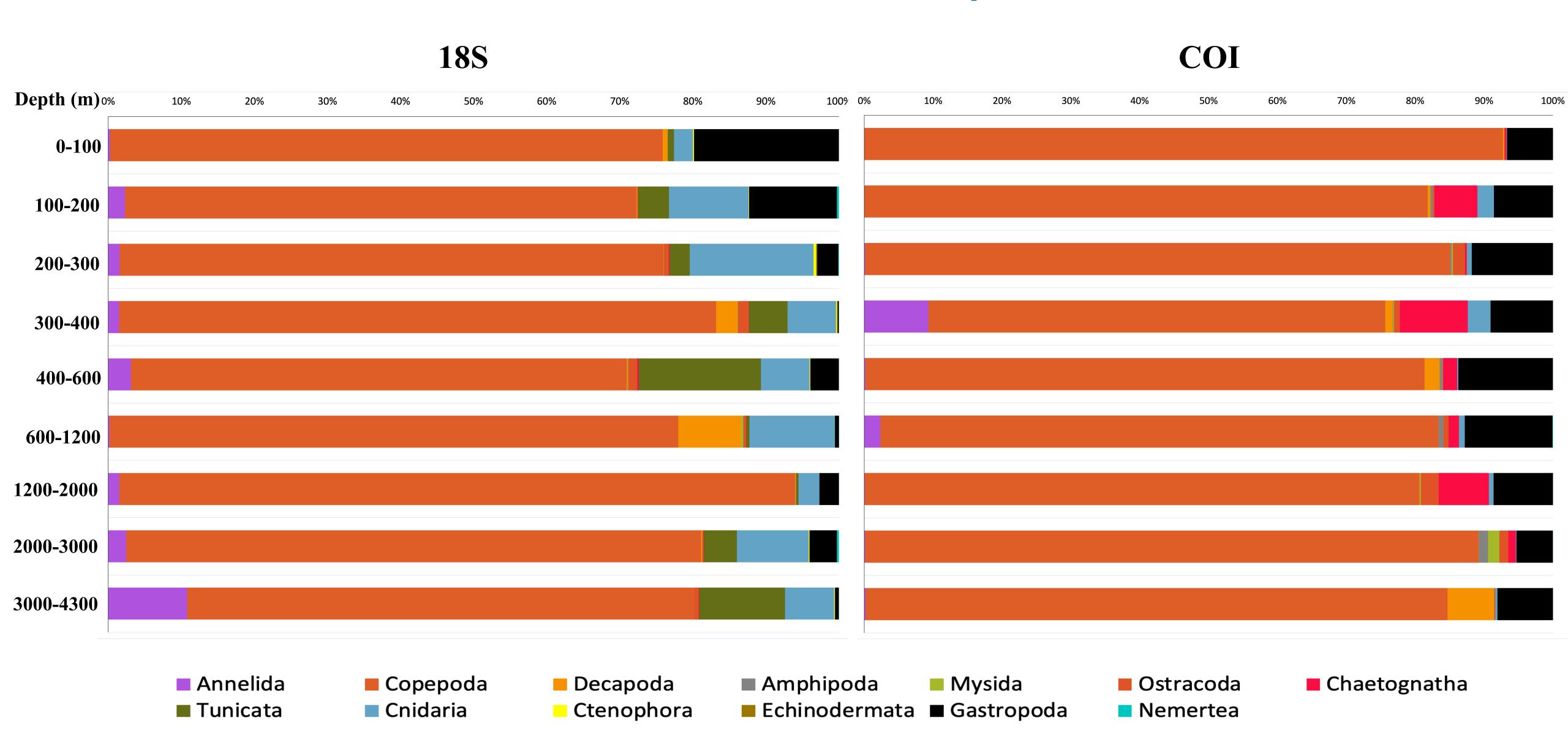
Community composition by depth

GAK13.5 MOCNESS & MultiNet - Sequence Nos.



Community composition by depth

GAK19 MOCNESS & MultiNet - Sequence Nos.



Next Steps

- Funding from NOAA OER for 2024 expedition and remaining 2019 sample analysis
- Perform integrated analysis with morphological and metabarcode data
- Ostracod identification with Martin Angel and colleagues
- Species-level analyses with COI for cryptic speciation, morphotypes, and newly described species
- Genome Skimming for barcode genes
- Keep on barcoding...
 - UN 2021 Ocean Decade Action No. 102.2
 - MetaZooGene: Metabarcoding Zooplankton Diversity



Thank you for your attention

Funding:







- Illustrations by Nicholas Bezio (SI; Bezio Studios)
- Captain and Crew R/V Sikuliaq
- Science party of the NOAA OER 2019 Seamounts expedition
- Smithsonian NMNH L.A.B
- UAF Genetics CORE lab

