Evaluating new approaches for zooplankton monitoring and ecology studies: is eDNA a tool that is ready to advance our science?

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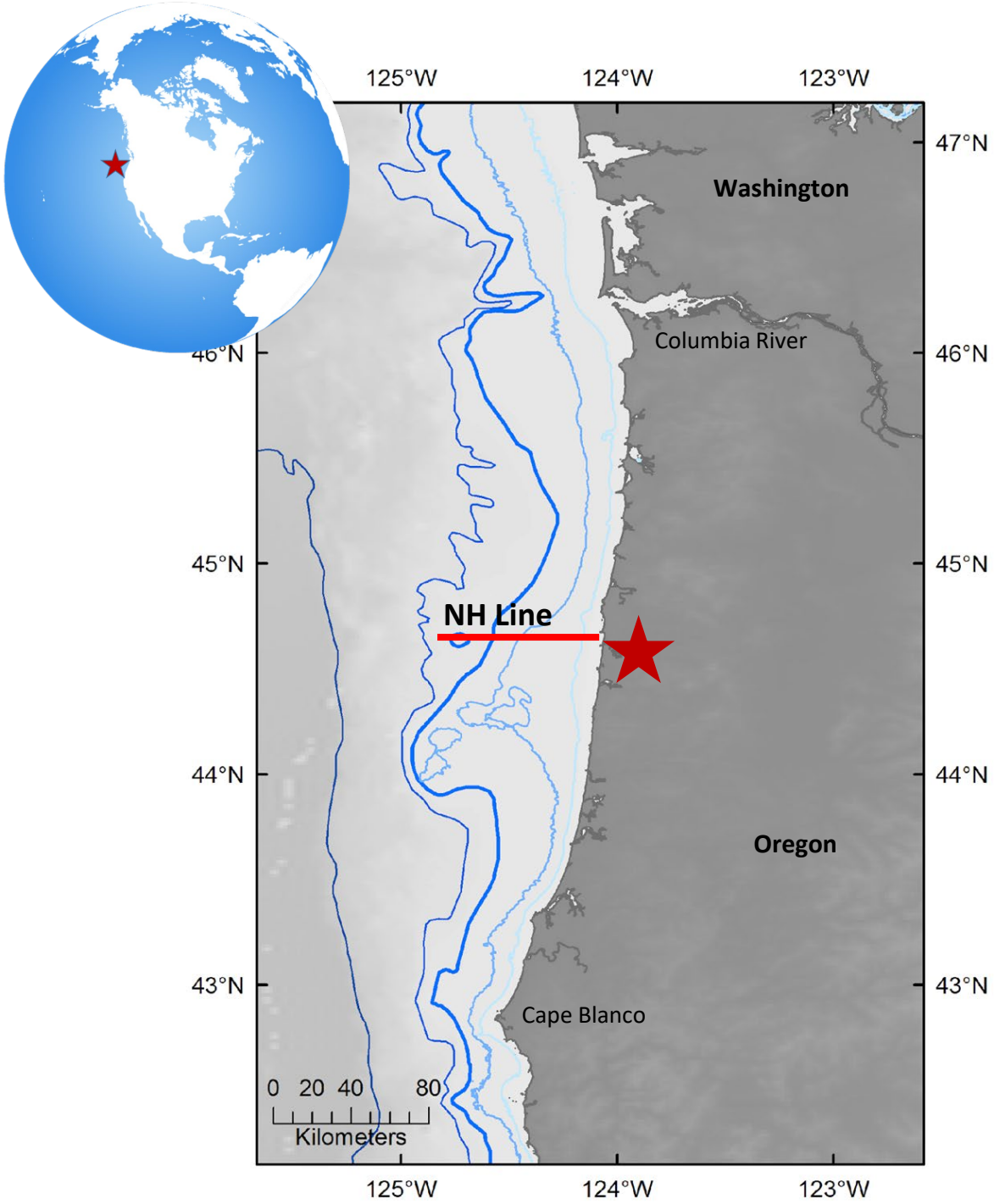


Long-term, high frequency sampling on the Newport Hydrographic Line

- Sampled every 2 weeks - monthly
  - since1996
  - 7 stations (1 – 25 nm)
  - Sentinel station NH-5 (60 m, mid-shelf)

    - Hydrography
    - Phytoplankton
    - Zooplankton
      - 0.5 m vertical net, 202-um mesh
      - 0.6 m bongo net, 335-um mesh

How could eDNA augment our monitoring program?

Copepod species are used as ocean indicators to inform fishery managers on ecosystem status and to help forecast salmon returns

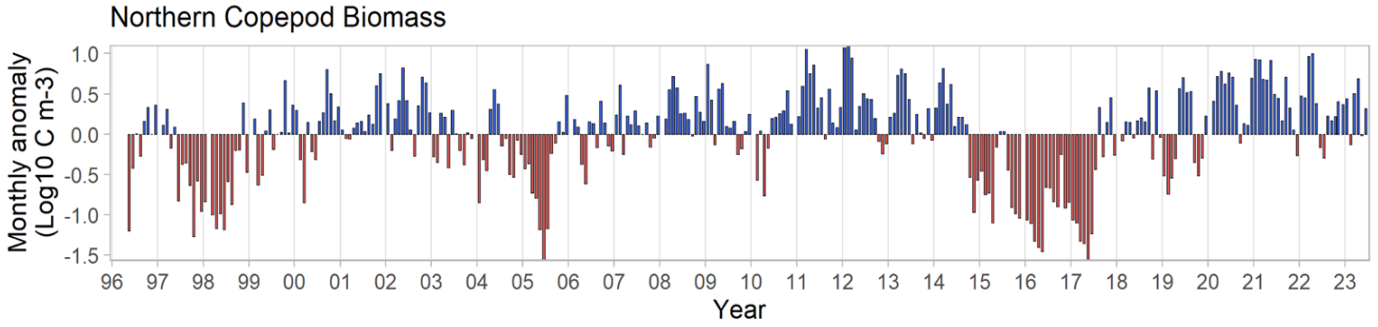
Agenda Item H.1.a
CCIEA Team Report 1 ***(Electronic Only)***
March 2024

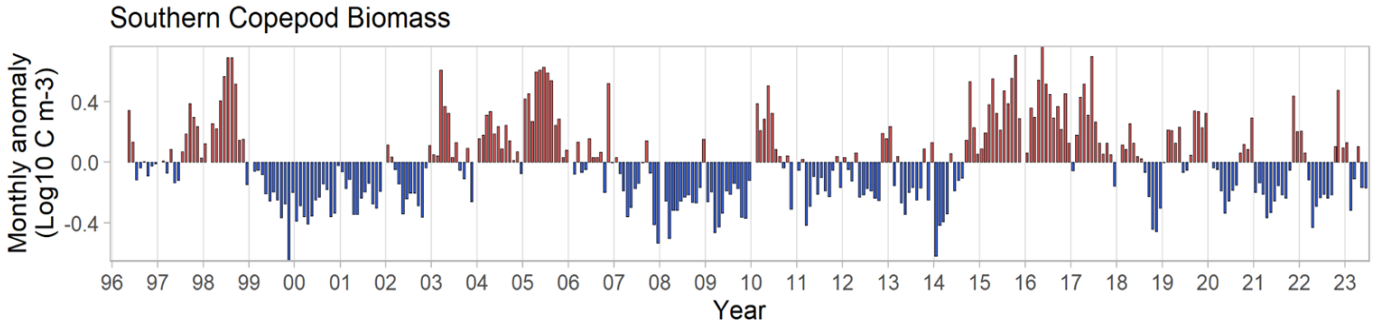
2023-2024 CALIFORNIA CURRENT ECOSYSTEM STATUS REPORT

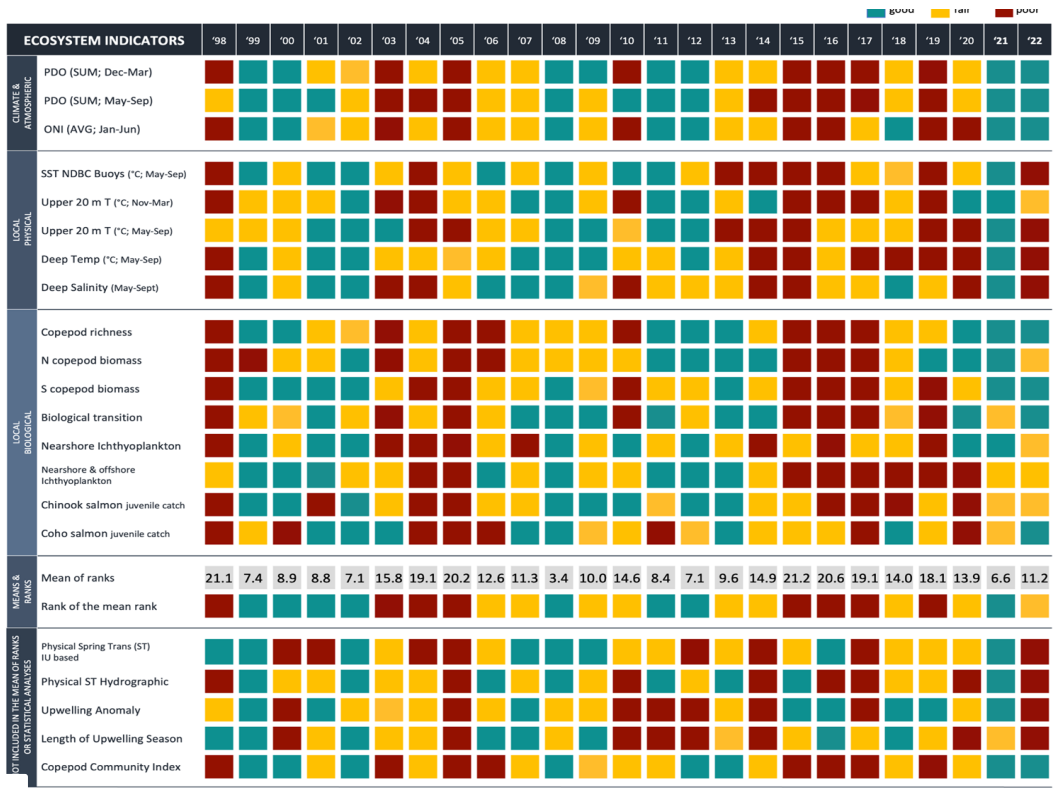
A report of the NOAA California Current Integrated Ecosystem Assessment Team (CCIEA) to the Pacific Fishery Management Council

Andrew Leising, Mary Hunsicker, Nick Tolimieri, Greg Williams, Abigail Harley

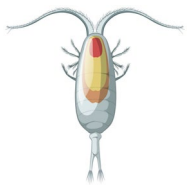
2024-01-31





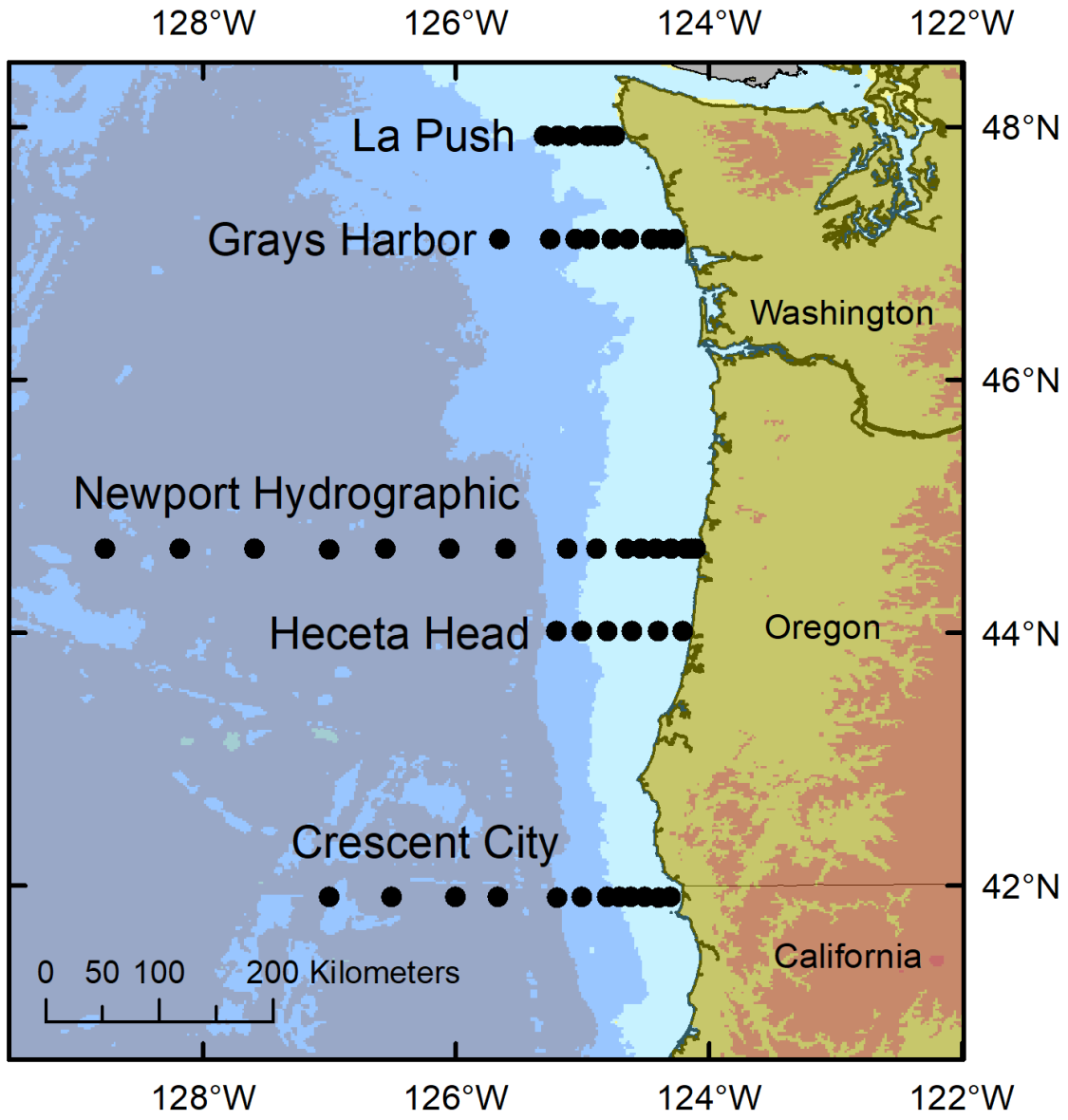


The biomass of certain copepod **species** from a **northern** (cold–water or boreal) group and a **southern** (warm–water or subtropical) group serve as ocean indicators.



Northern California Current- Broadscale Surveys

- Seasonal
- 1998 – present
- Puts the NH Line in a broader context
- NOAA and UNOLS vessels
- Allows us to sample off the shelf
- Provides for greater collaborations

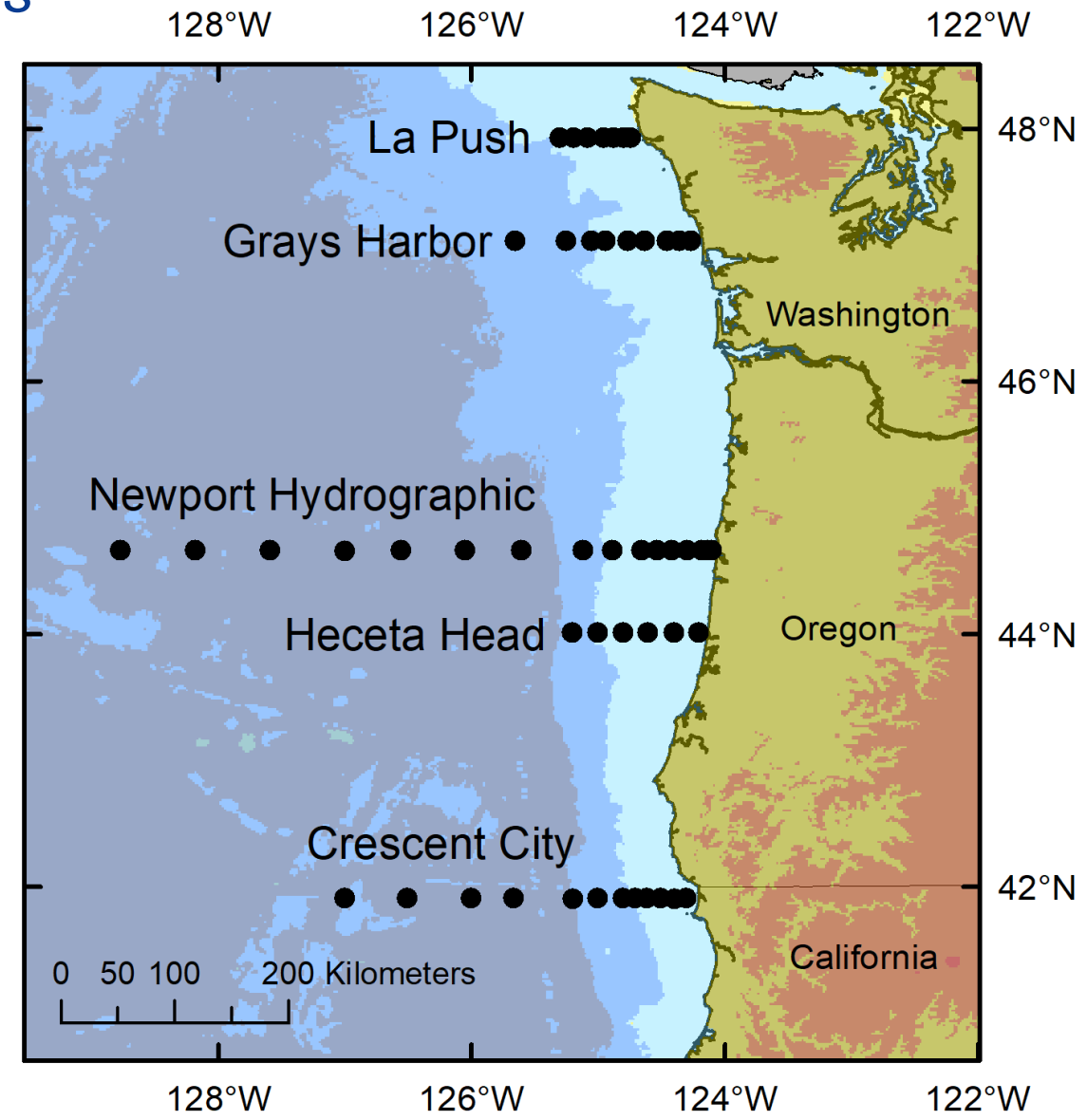


Marine Biodiversity Observation Network (MBON) in the Northern California Current: *patterns and drivers of **biodiversity** and ecosystem function from plankton to seascapes*

Lead PI: Maria Kavanaugh (Oregon State University)

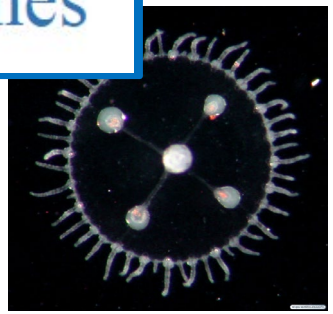
Characterize remote sensed seascapes using *in-situ* sampling:

- Phytoplankton
  - Imaging flow Cytobot (IFCB)
- Zooplankton & Icthyoplankton
  - Traditional optics, microscopy
  - In-situ Ichthyplankton Imaging System
- **Metabarcoding of eDNA** (phyto, **zooplankton**, fish biodiversity: at surface and deep chlorophyll max (using CTD flouresence)



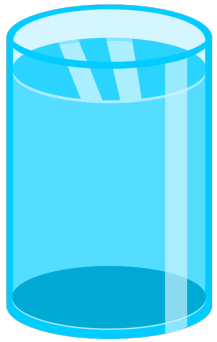
Why eDNA?
(We have a phenomenal taxonomist!)

- Session 4: Shedding new light on zooplankton: Unveiling communities, ecology, and evolution through integrated approaches



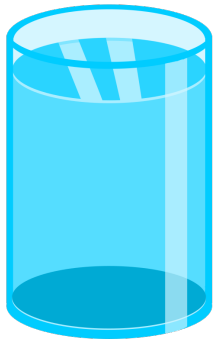
- Many soft-bodied zooplankton are destroyed in nets and thus under-represented by traditional net sampling
- Could be a better indicator for species richness, identifying rare or cryptic species hard to identify morphologically
- National Ocean Biodiversity strategy: “The implementation of the National Ocean Biodiversity Strategy should advance approaches to collect eDNA using autonomous underwater vehicles concurrently with other means to identify taxa and measure environmental change.”

eDNA Workflow:



filter & extract DNA → amplify target DNA → sequence → clean up (pipeline) → Assign to taxa using reference database

eDNA Workflow: pivotal points



filter & extract DNA

amplify target DNA

sequence

clean up (pipeline)

Assign to taxa using reference database

18S ribosomal

Cytochrome oxidase 1 (CO1) mitochondrial

CO1: More taxa identified to species?
Both markers and compare or combine?

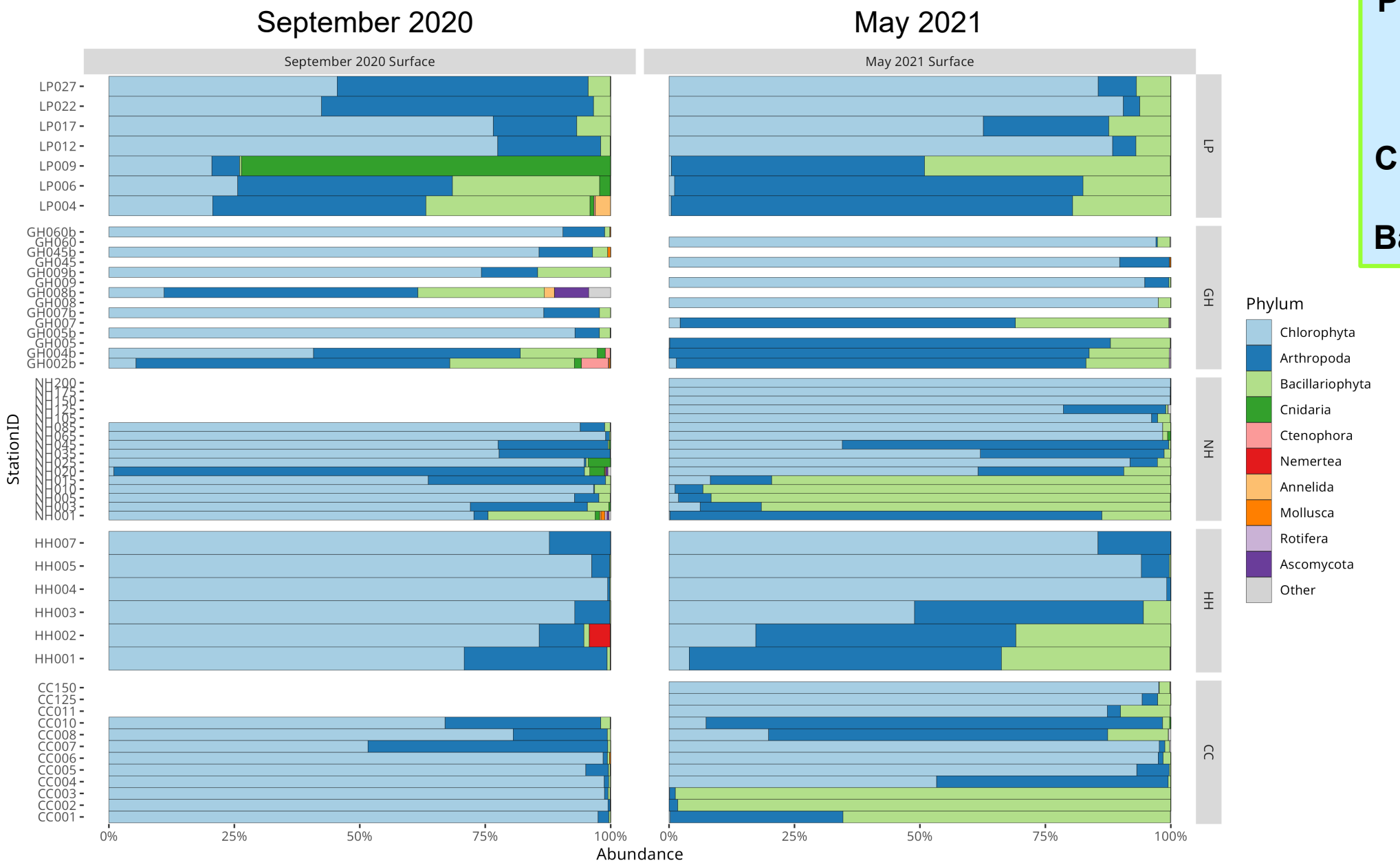
Our Journey: bioinformatic workflow with Oregon State University and advice from NOAA's Pacific Marine Environmental Lab

- Taxonomic assignment of amplicon sequence variants (ASVs) was attempted using multiple approaches: RESCRIPt, NCBI blastn with BASTA, QIIME2 VSEARCH (MetaZooGene barcode atlas as reference), and SINTAX (pretrained CO1 model).
- Assignments to species were 700, 426, 272, or 529 ASVs (including phyto and zooplankton) according to the RESCRIPt, BASTA, VSEARCH, and SINTAX methods, respectively

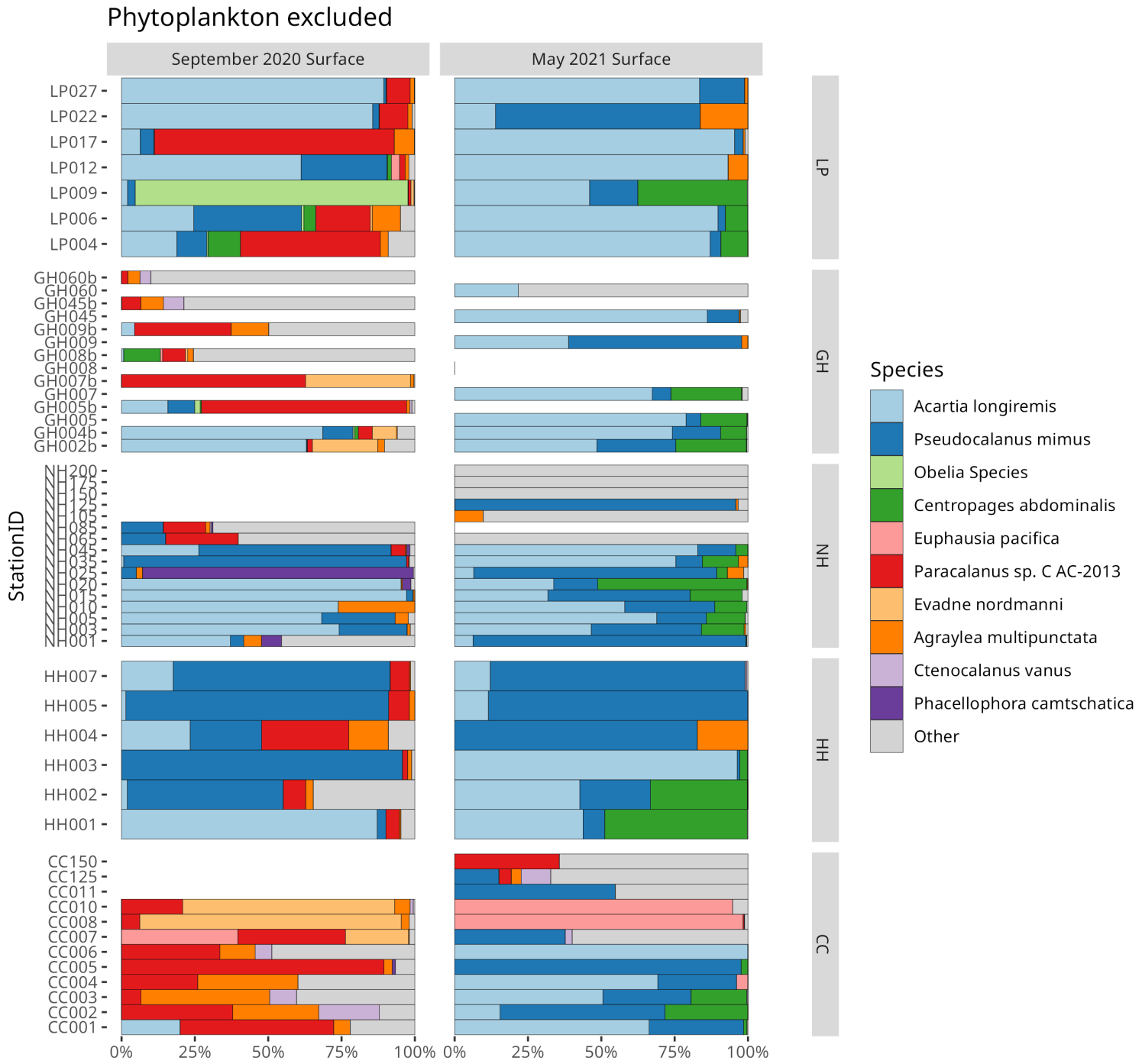
Where we just landed:

- Raw CO1 sequences were clustered into ASVs using dada2 as implemented in the qiime2 dada2 denoise-paired workflow.
- **Taxonomic classifications were made using a Naive Bayes classifier (**as implemented in the qiime2 feature-classifier fit-classifier-naive-bayes workflow**) trained on the rCRUX CO1 derep_and_clean database.**
- **844 ASVs assigned to species; 351 zooplankton species**

CO1 Results with Phytoplankton



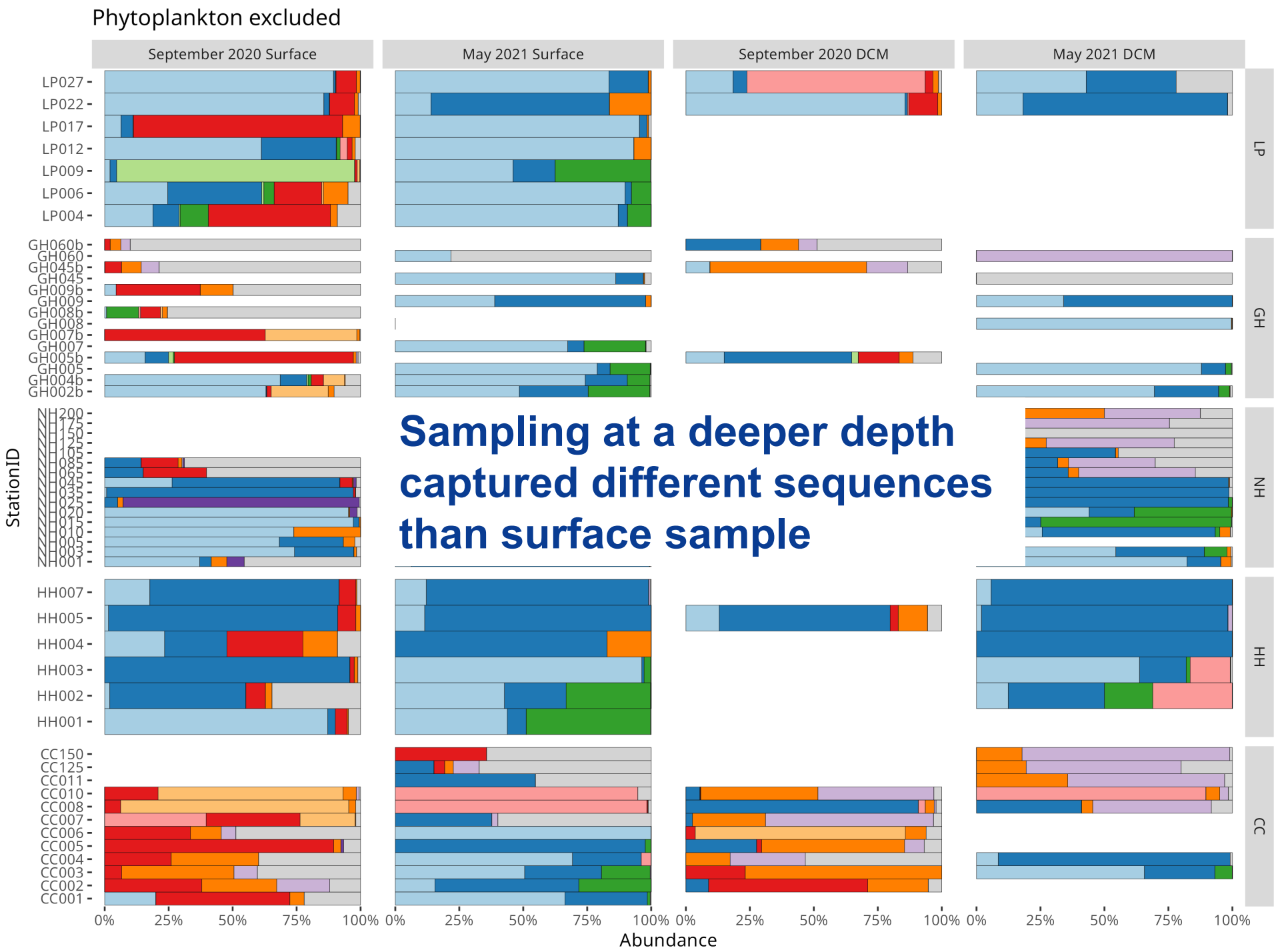
Phytoplankton dominated most of our samples: Chlorphophyta and Bacillariophyta



Removed phytoplankton and looking at relative abundance of ASVs classified to species (2020:351; 2021:443)

A number of the top 10 most abundant ASVs were species we would expect to see.

There are temporal and spatial differences

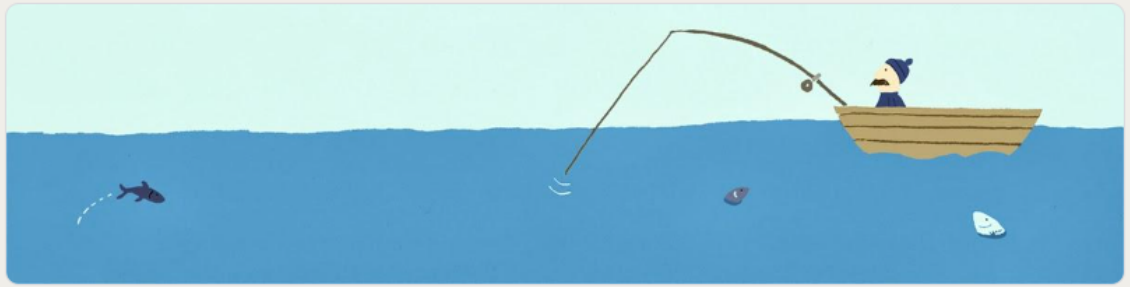

Phytoplankton excluded
September 2020 Surface
May 2021 Surface
September 2020 DCM
May 2021 DCM
LP027
LP022
LP017
LP012
LP009
LP006
LP004
GH060b
GH060
GH045b
GH045
GH009b
GH009
GH008b
GH008
GH007b
GH007
GH005b
GH005
GH004b
GH002b
NH200
NH175
NH150
NH125
NH105
NH085
NH065
NH045
NH035
NH025
NH020
NH015
NH010
NH003
NH003
NH001
HH007
HH005
HH004
HH003
HH002
HH001
CC150
CC125
CC011
CC010
CC008
CC007
CC006
CC005
CC004
CC003
CC002
CC001
LP
GH
NH
HH
CC
StationID
0%
25%
50%
75%
100%
Abundance
Sampling at a deeper depth captured different sequences than surface sample
Adding in samples from deep chlorophyll max (5-10m)
Species
Acartia longiremis
Pseudocalanus mimus
Obelia Species
Centropages abdominalis
Euphausia pacifica
Paracalanus sp. C AC-2013
Evadne nordmanni
Agraylea multipunctata
Ctenocalanus vanus
Phacellophora camtschatica
Other

Is eDNA a tool that is ready to advance our science?

Speaking for zooplankton in the Northern California Current, **NO**.

- Need for more complete and common reference database!
  - Current results are missing many main taxa (database issue, DNA or sequencing issue):
    - At least one species of euphausiids, amphipods, chaetognaths, pteropods
    - Is taxonomic classification of ASVs as complete as possible?
- Need for standardization of workflow, maybe not?
- eDNA can complement net sampling and morphological data but how do we incorporate data from multiple tools? What is the question?
- Not enough experienced bioinformaticians (in house)
  - Job Announcement: CIMAR PIFSC Bioinformatician recruitment open. “Together with other new hires at NOAA Science Centers across the nation, be a part of the newly established NMFS ‘Omics Network designed to collaboratively develop powerful, reproducible, and streamlined end-to-end operational workflows that automate the analysis of eDNA metabarcoding data.”

Steps forward:



Assembling a List of Monitored Marine Species in the Northeast Pacific to Inform DNA Reference Barcoding Efforts

The ask:

A list of species targeted/observed by your biological monitoring program in the Northeast Pacific.

Zachary.Gold@noaa.gov

W1: Reference sequence databases for global zooplankton biodiversity: Optimization, applications and user guidelines
Discussion sessions and breakout groups will focus on the selection of genes and gene regions, sequencing technologies and platforms, bioinformatics pipelines, and inter-comparison and inter-calibration of the results for local-to-global characterization of marine zooplankton diversity.

Thank you!

My co-authors, funding agencies and collaborators on the Northern California Current MBON project

And special thanks to Zach Gold (NOAA-PMEL), Kathleen Pitz (MBARI)