Deep Dive! : Analysis of the Mesopelagic Prokaryotic Community in the California Current Ecosystem (2014-2021)

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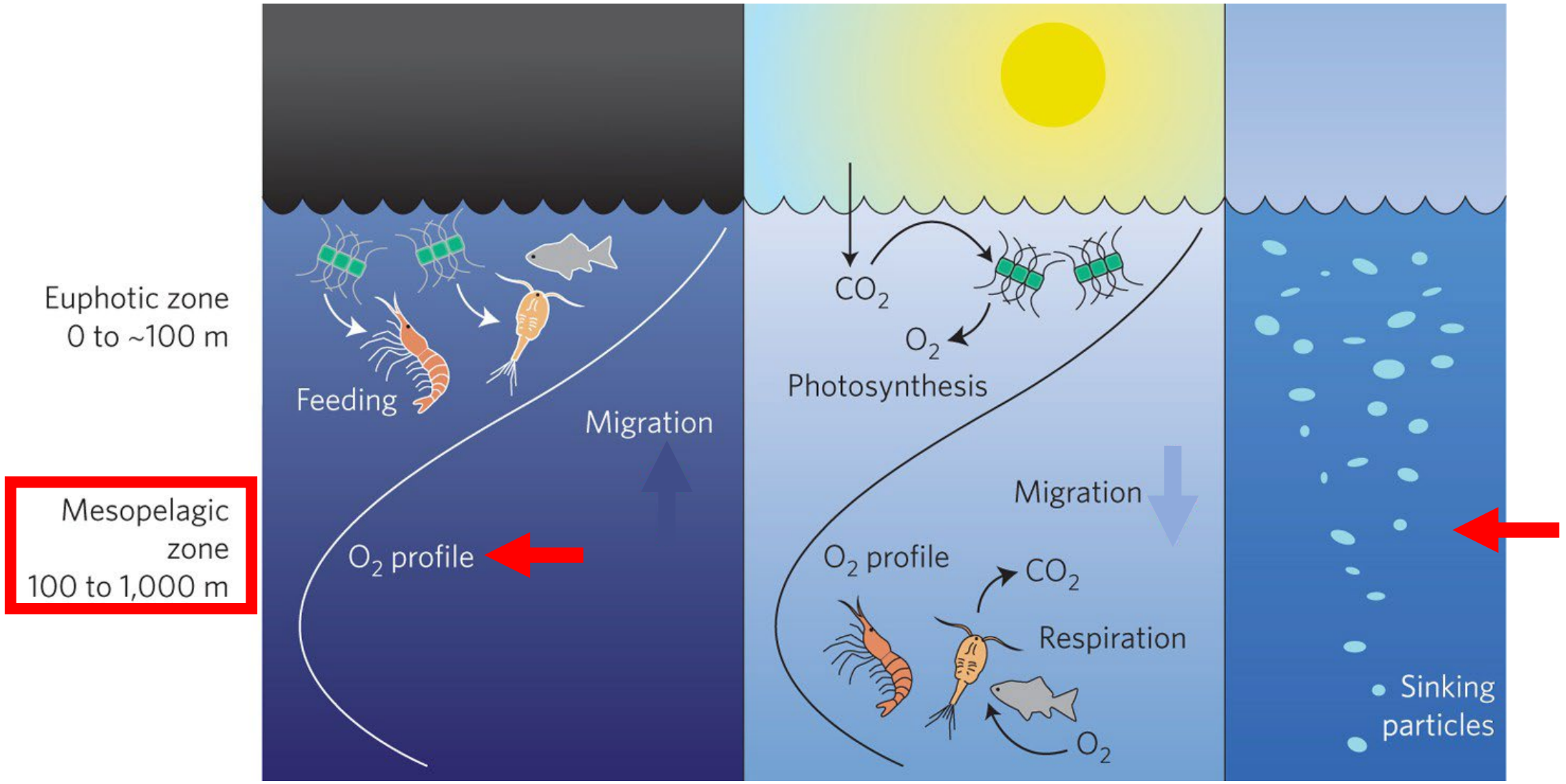
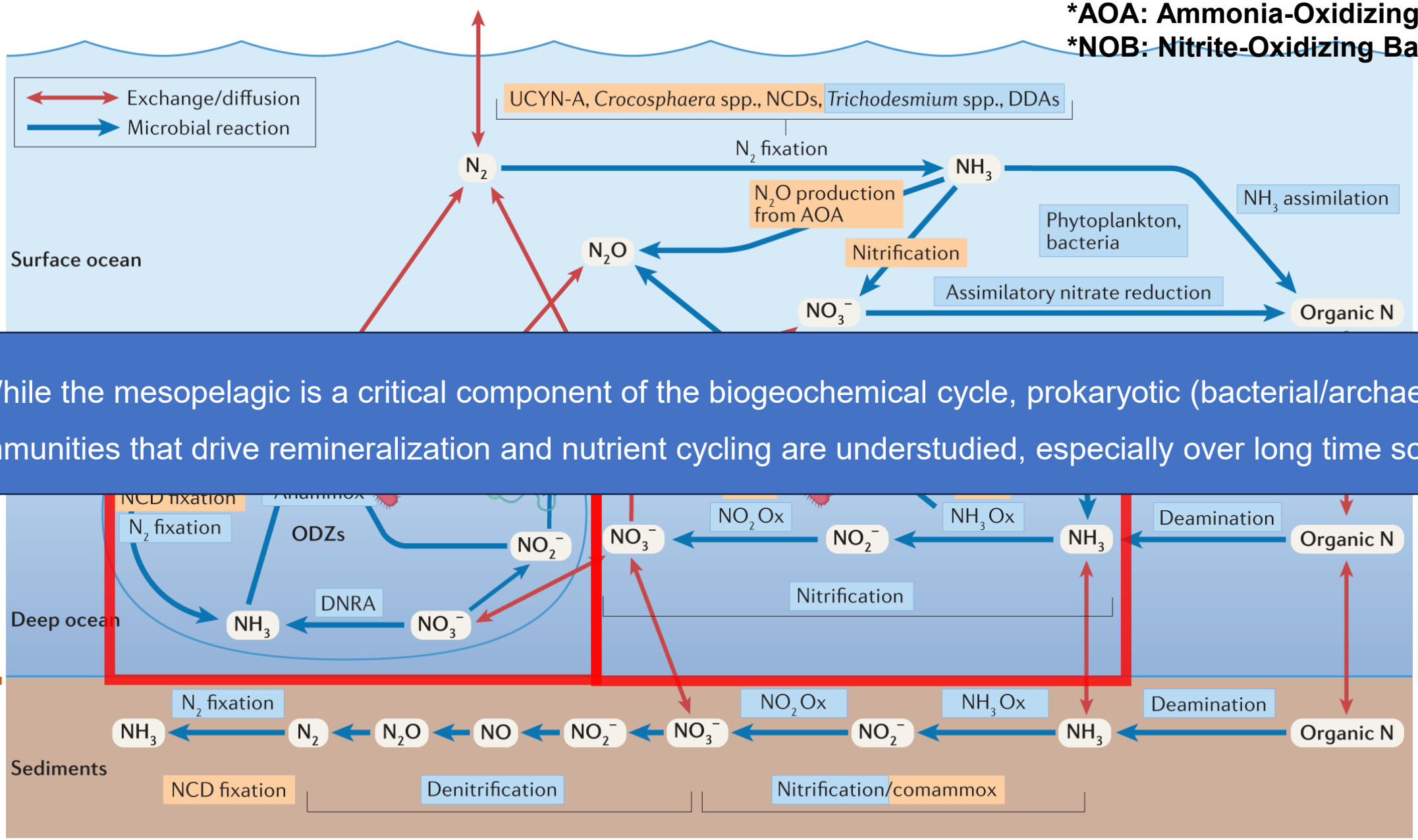
Photo credit: James Wilkinson (CalCOFI & Scripps Institution of Oceanography)

Twilight Zone (100-200 m – 1000 m)



Doney & Steinberg et al., 2013

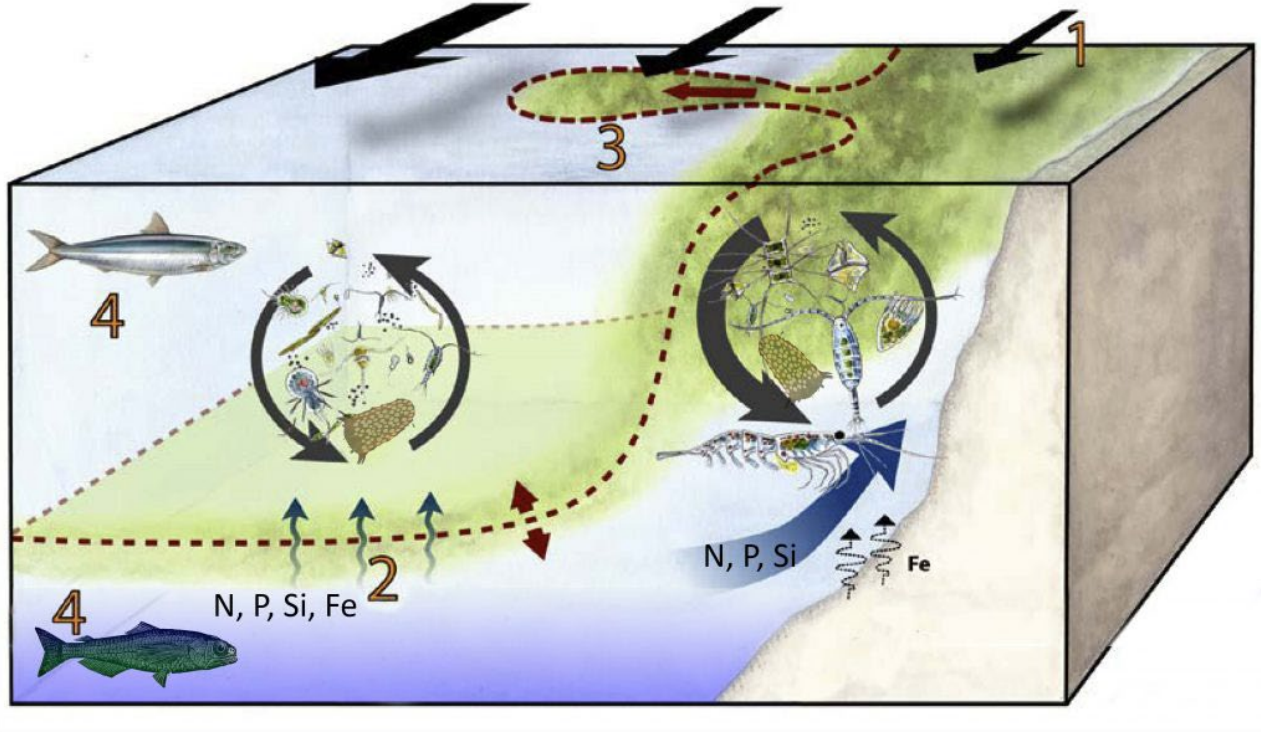
*AOA: Ammonia-Oxidizing Archaea
*NOB: Nitrite-Oxidizing Bacteria



While the mesopelagic is a critical component of the biogeochemical cycle, prokaryotic (bacterial/archaeal) communities that drive remineralization and nutrient cycling are understudied, especially over long time scales.

Hutchins & Capone, 2022

- **Eastern Boundary Upwelling System**
- **Among the most productive coastal ecosystems in the world ocean due to seasonal upwelling**
- **Steep nearshore to offshore gradients in nutrient availability and productivity (James *et al.*, 2022)**



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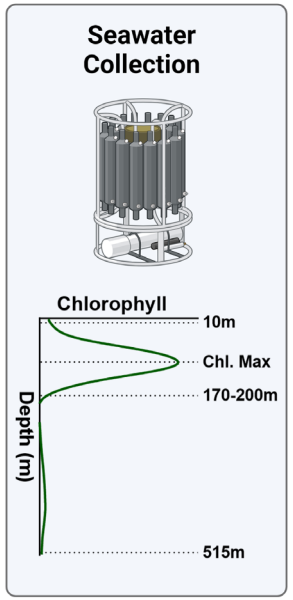
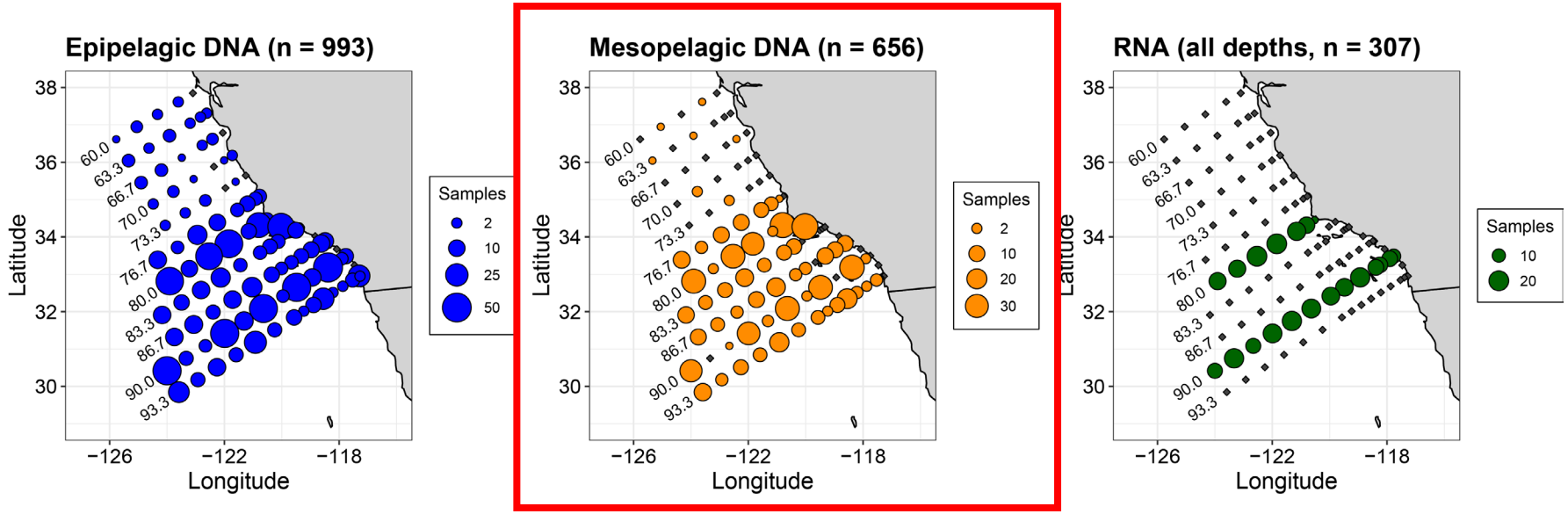
How these environmental gradients shape mesopelagic microbial community structure across space and time remains poorly understood.

Objectives

- Characterization of prokaryotic alpha and beta diversity across the upper (170 m/200m) and deeper (515 m) mesopelagic Southern CCE (2014-2021)
- Identification of key environmental variables structuring community composition at each depth layer

NOAA-CalCOFI Ocean Genomics (NCOG) Time-Series

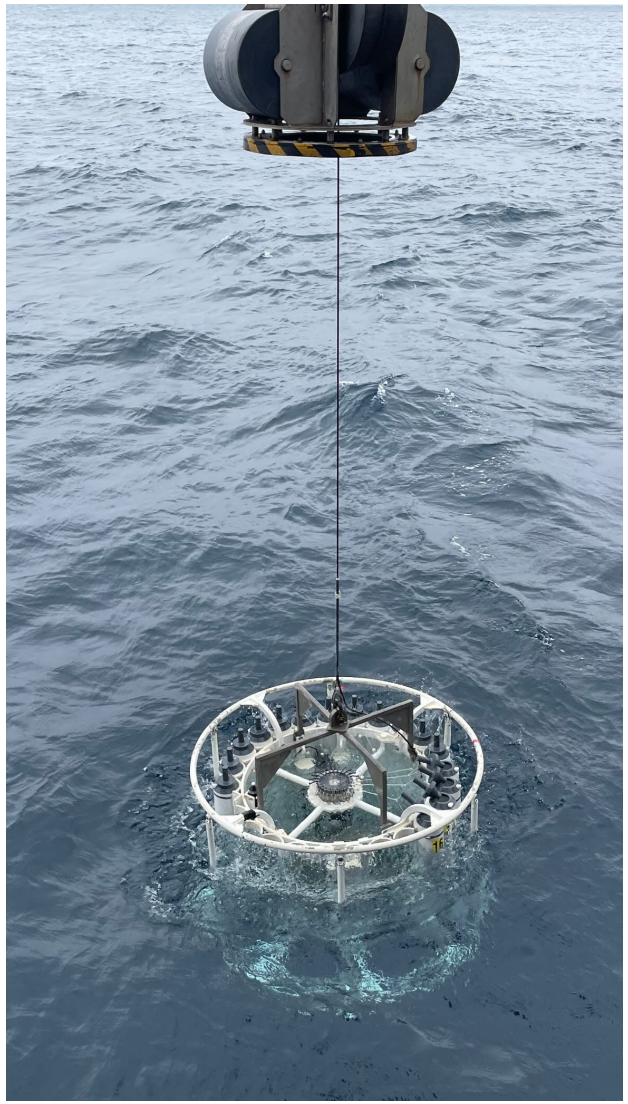




NCOG Sample Overview, 2014-2020 (Figure created by Rob Lampe)

- NCOG is a microbial time-series led by the Allen Lab (quarterly CalCOFI cruise since 2014 – present)
- On daily noon (i.e. Prodo) stations and Cardinal stations (lines 80 and 90), DNA and RNA seawater samples are collected at designated depths

NOAA-CalCOFI Ocean Genomics (NCOG) Time-Series

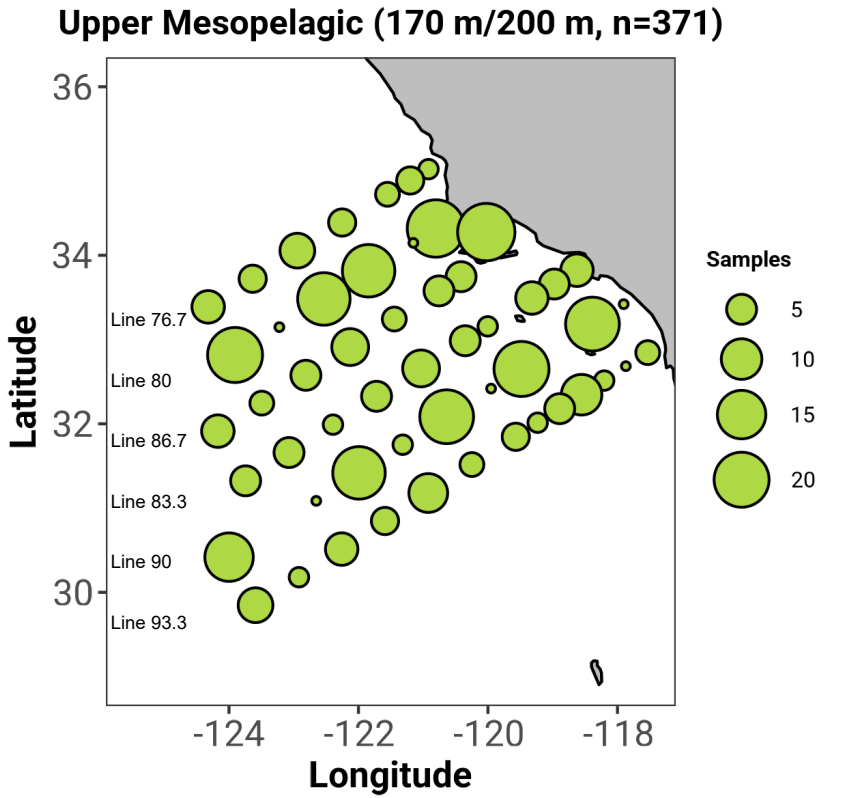


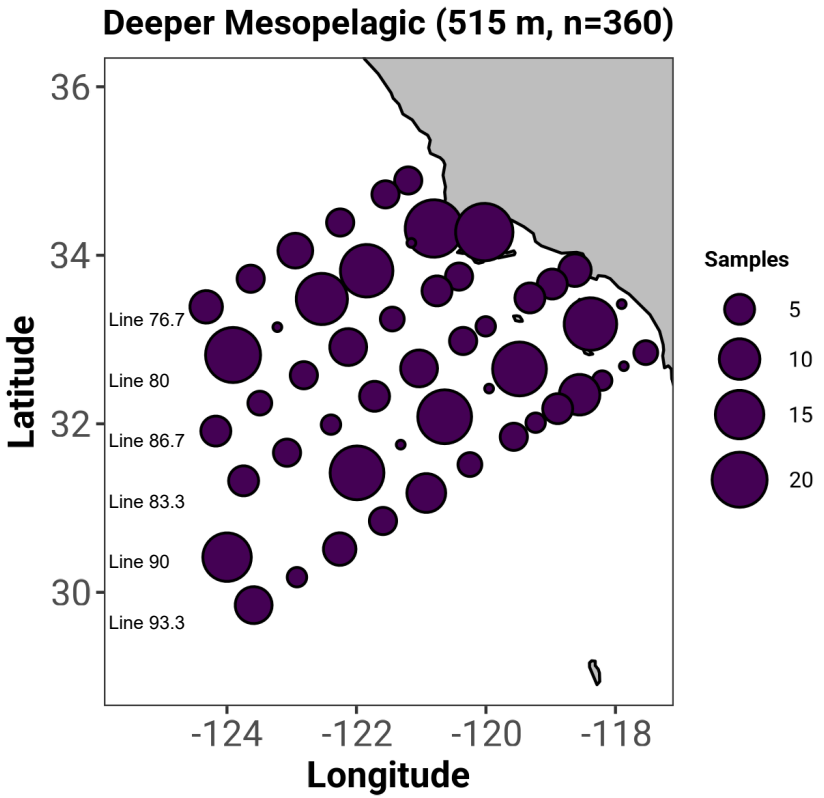


Upper Meso ('170 m')

Deeper Meso ('515 m')

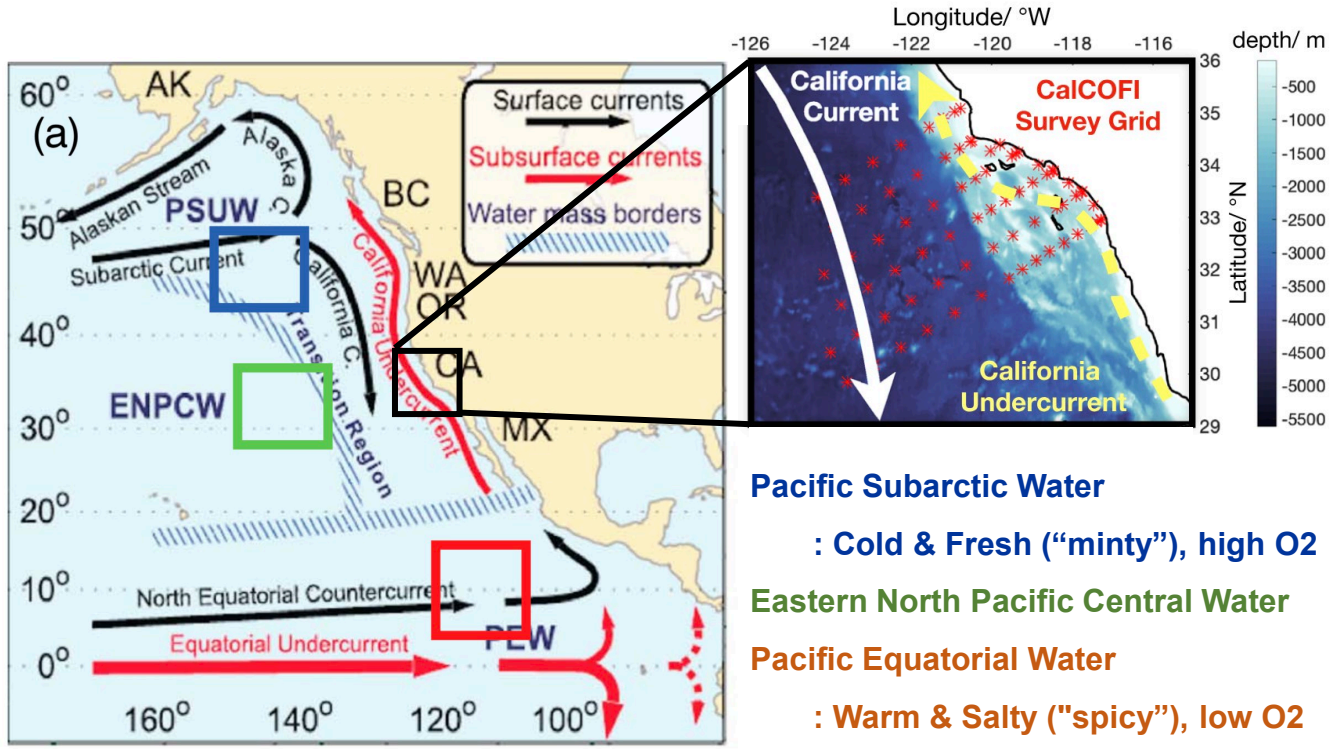






- ~6L of seawater collected from the CTD rosette from CalCOFI cruises 2014 07 – 2021 11
- DNA extracted from Sterivex™ filters from the upper (170 m/200 m) and deeper (515 m) mesopelagic
- 16S V4-V5 rRNA marker gene targeted amplicon sequencing
- Denoising (DADA2), taxonomic assignment (SILVA)

Contrasting source water influences across mesopelagic depths



Modified from Bograd et al., 2019 and Evans et al., 2020

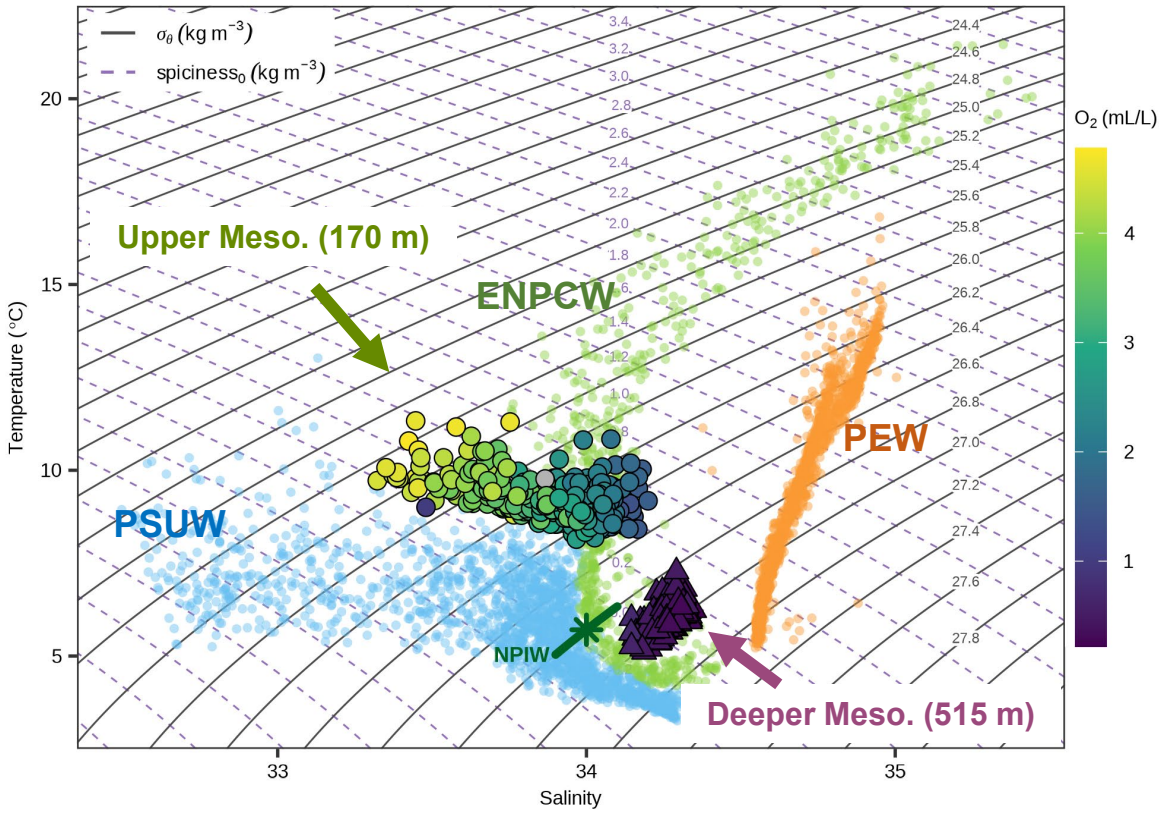
Pacific Subarctic Water

: Cold & Fresh (“minty”), high O2

Eastern North Pacific Central Water

Pacific Equatorial Water

: Warm & Salty ("spicy”), low O2



- Upper Meso (170 m): Mixing of Pacific Subarctic Water (PSUW) via the California Current core, Eastern North Pacific Central Waters, Pacific Equatorial Water (PEW) via the California Undercurrent (CUC) core
- Deeper Meso (515 m): Mixing of North Pacific Intermediate Water (NPIW) and PEW via CUC

Depth drives difference in alpha diversity patterns

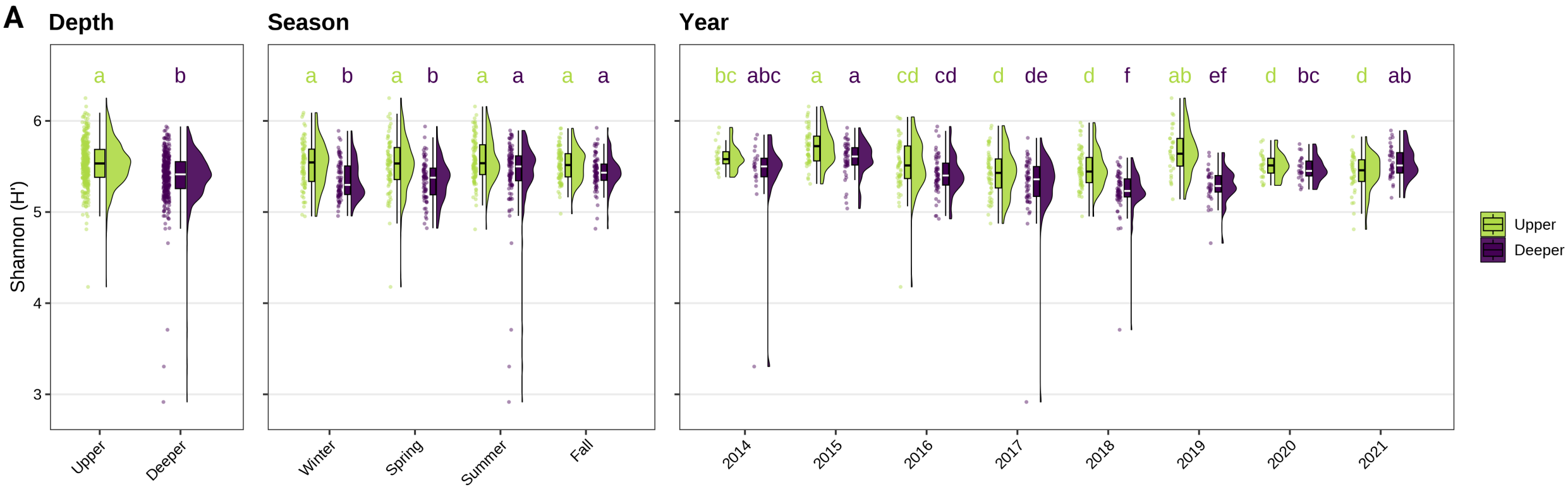


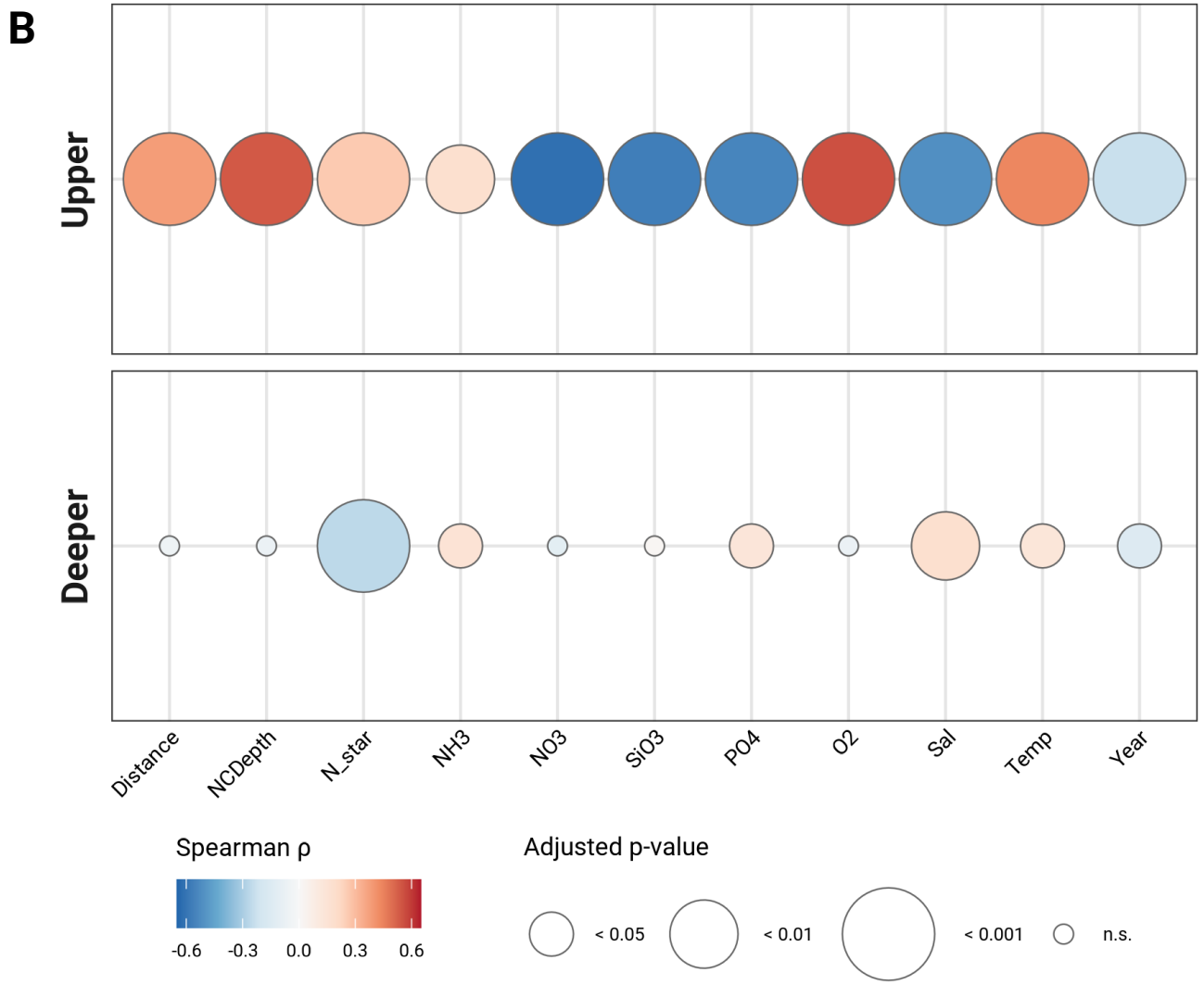
Figure (A) Shannon index (H') comparison of rarefied prokaryotic ASVs (Amplicon Sequence Variants) separated by depth class (upper and deeper mesopelagic), season, and year. Statistical differences tested with K-W tests, followed by pairwise Wilcoxon rank-sum tests and BH correction.

- Higher Shannon diversity (*evenness) at upper mesopelagic (170 m) than at deeper layers (515 m)
- No seasonal differences at the upper layers. Interannual variation at each layer

$$H' = -\sum_{i}^{S} (p_i * \ln p_i)$$

p_i : *Proportion of* ASV i
S : ASV # *(Species richness)*

Depth drives difference in alpha diversity patterns



$$H' = -\sum_{i}^{S}(p_i * \ln p_i)$$

$\boldsymbol{p_i}$: *Proportion of* ASV i

S : ASV # *(Species richness)*

Figure (B) Spearman correlation between Shannon diversity (H') and environmental variables in different depth layers with BH correction for p-values

Depth drives difference in alpha diversity patterns

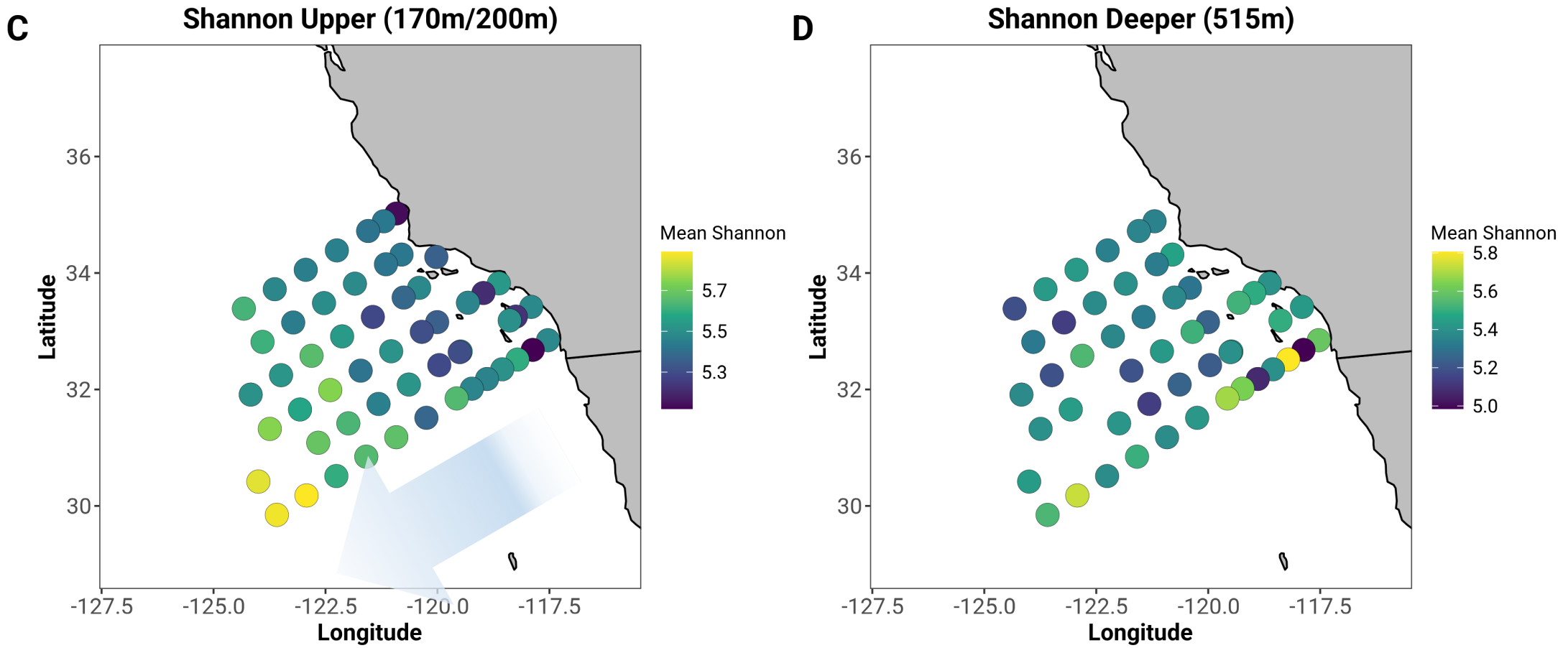


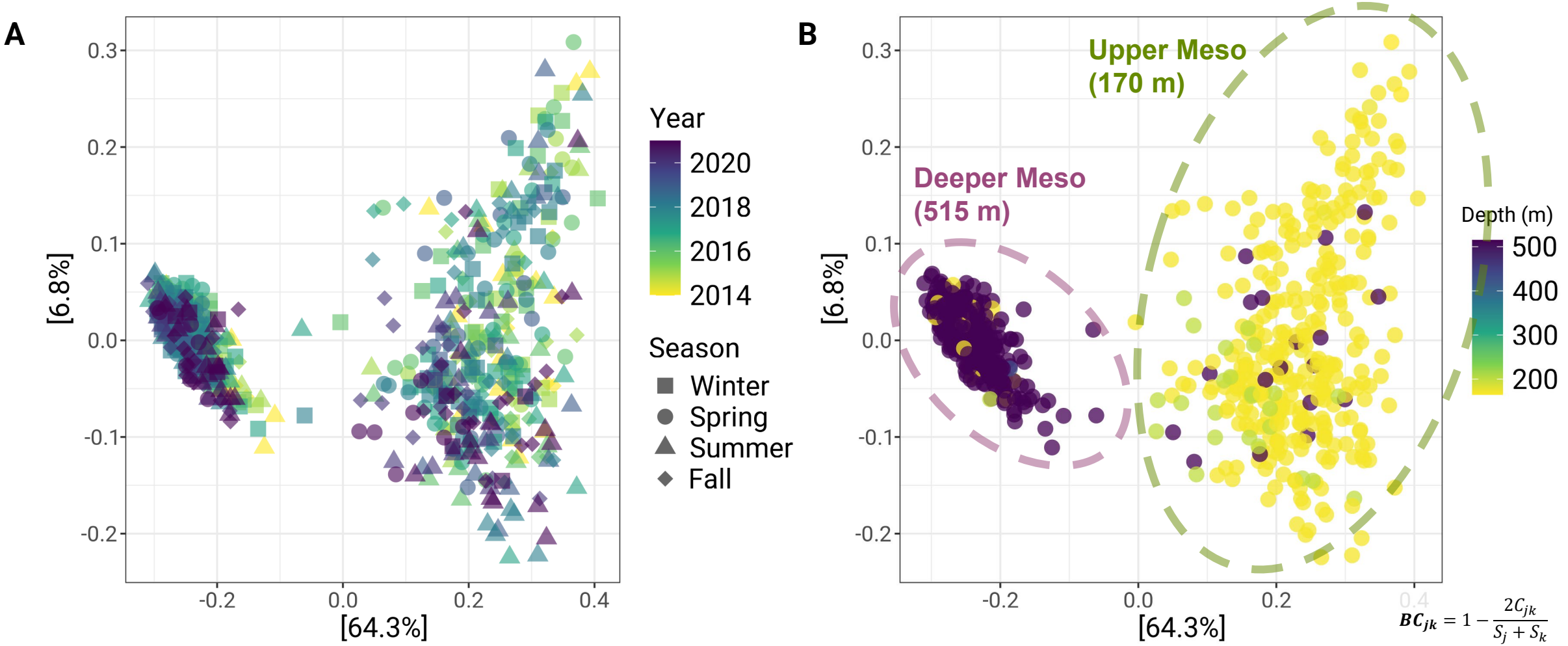
Figure (C,D) Spatial gradient of mean Shannon diversity across the CalCOFI survey grid.

- Nearshore exhibits lower alpha diversity, indicating higher dominance of certain taxa at the nearshore.

$$H' = -\sum_{i} (p_i * \ln p_i)$$

$\boldsymbol{p_i}$: *Proportion of* ASV i
S : ASV # *(Species richness)*

Depth drives mesopelagic prokaryotic community structure



Bray-Curtis (BC) distance-based Principal Coordinate Analysis (PCoA). The color schemes and shape represent **(A)** year and season and **(B)** depth, respectively.

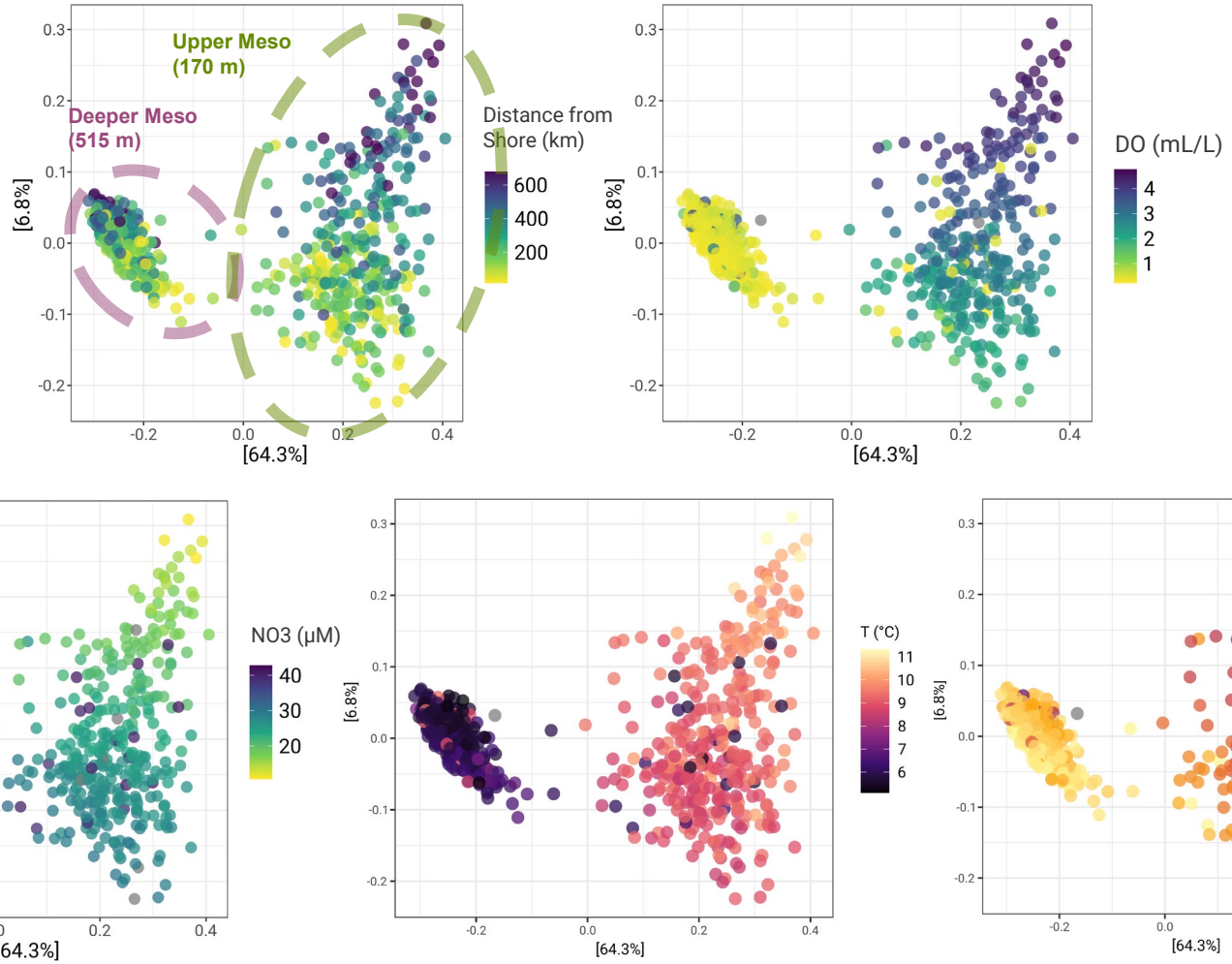
$$BC_{jk} = 1 - \frac{2C_{jk}}{S_j + S_k}$$

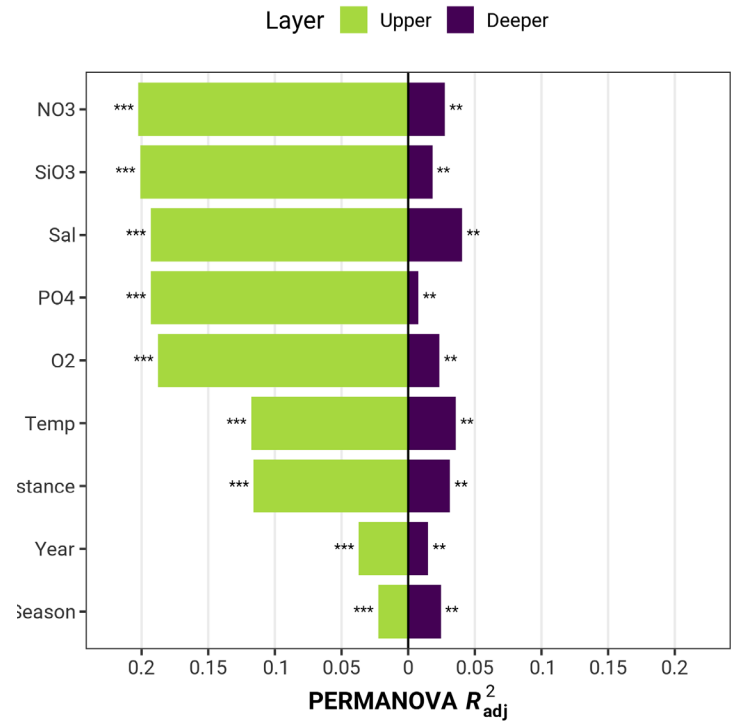
$$C_{jk} = \sum \min(x_{ij}, x_{ik})$$

S_j, S_k : total abundance in sample j, k

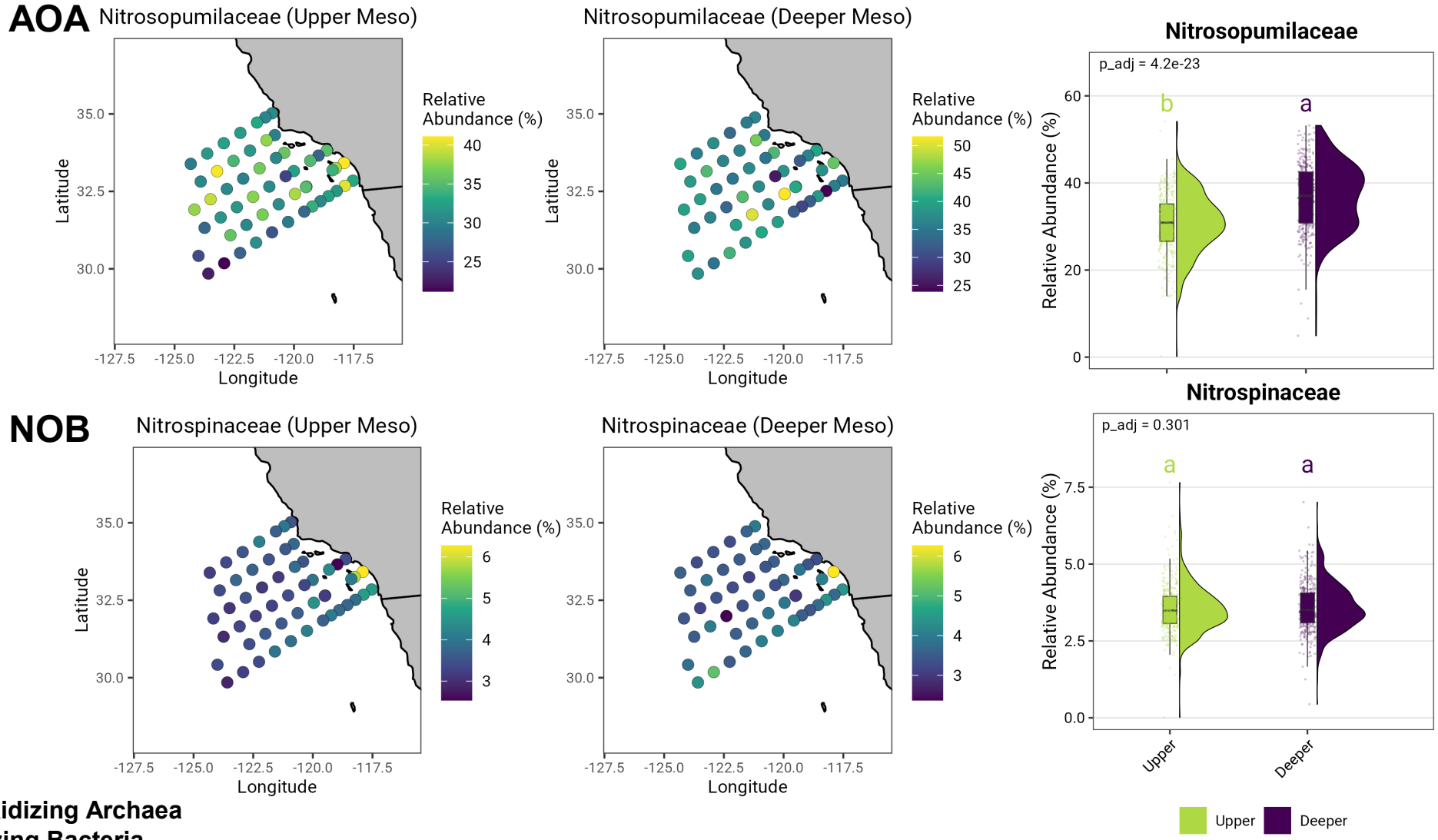
x_{ij}, x_{ik} : ASV i's abundance in sample j, k

Contrasting influence of environmental variables across depths



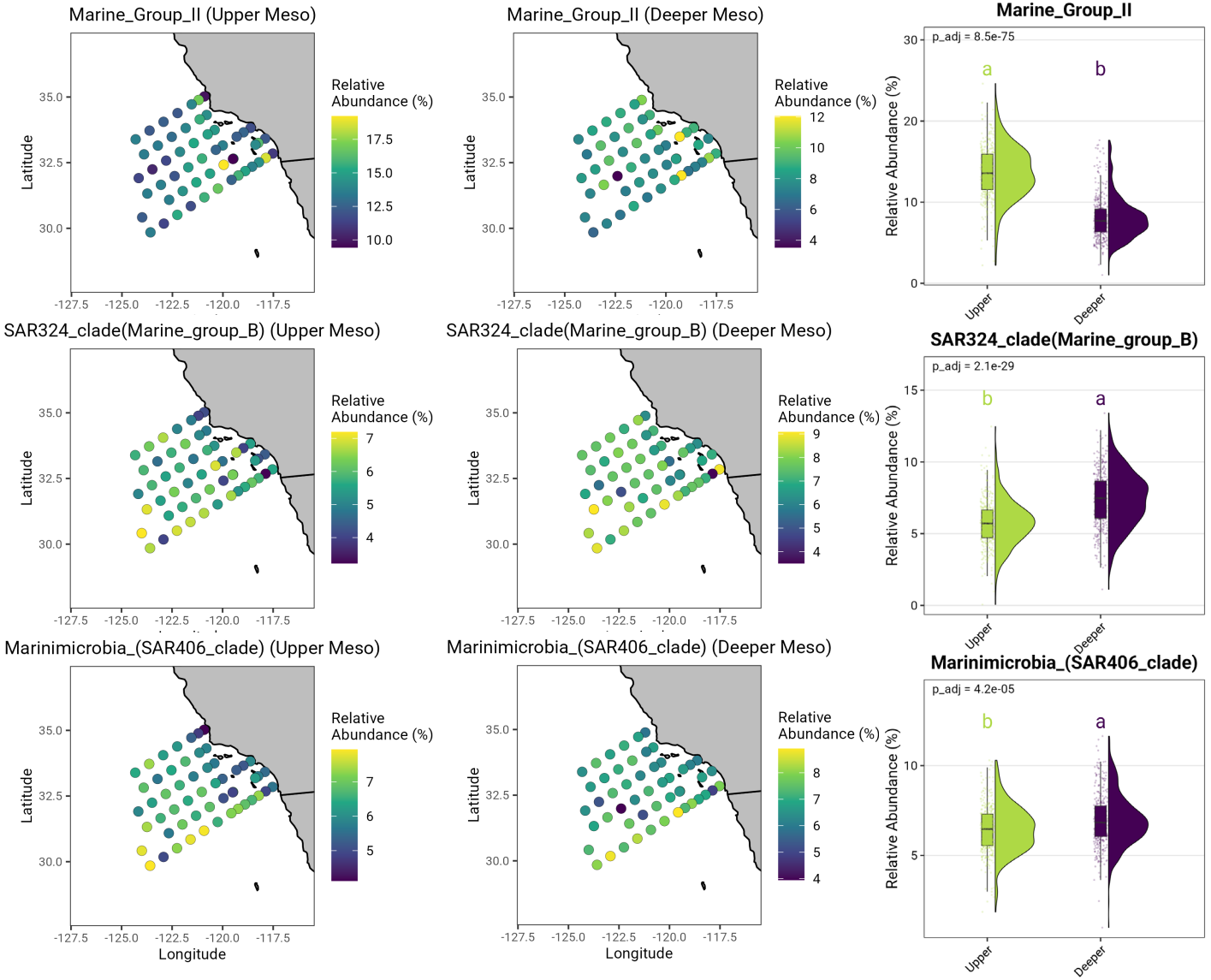


Dominance of N-cycling prokaryotes across all mesopelagic layers



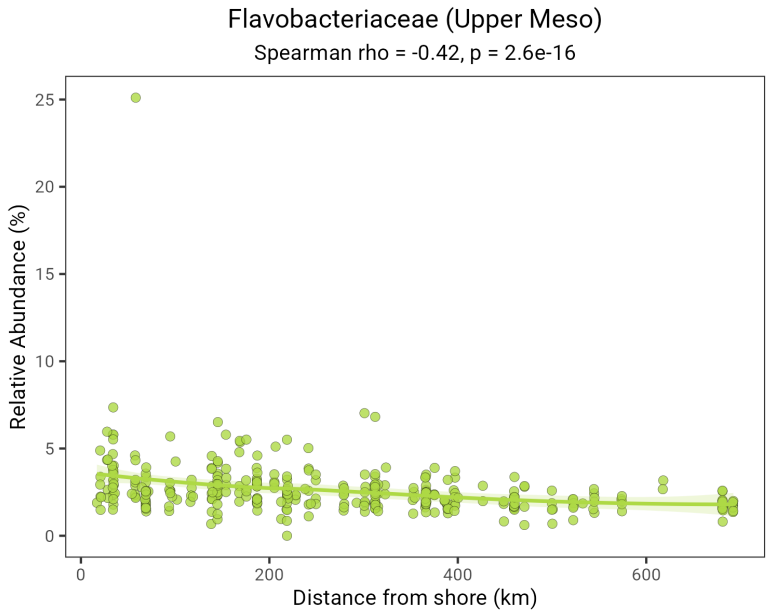
*AOA: Ammonia-Oxidizing Archaea
*NOB: Nitrite-Oxidizing Bacteria

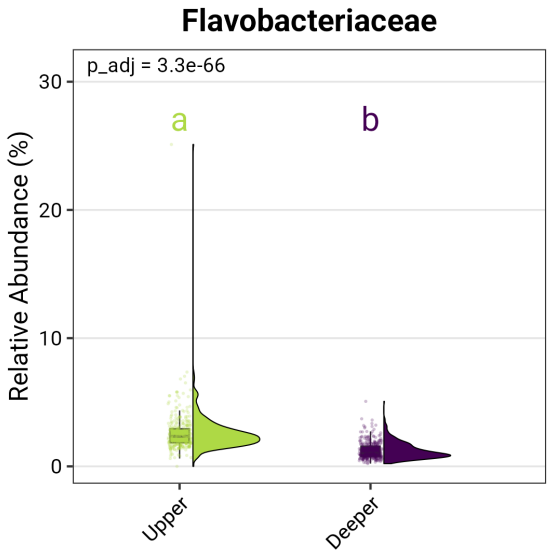
Major Prokaryotic Groups

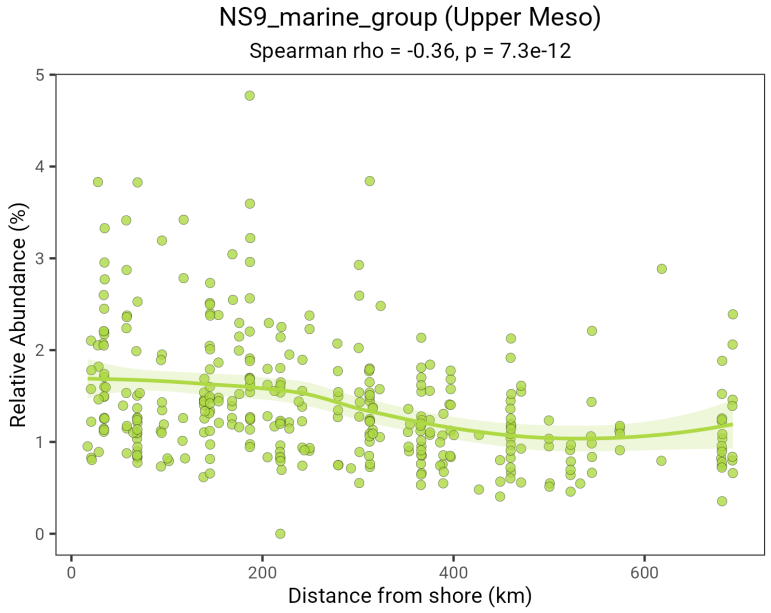


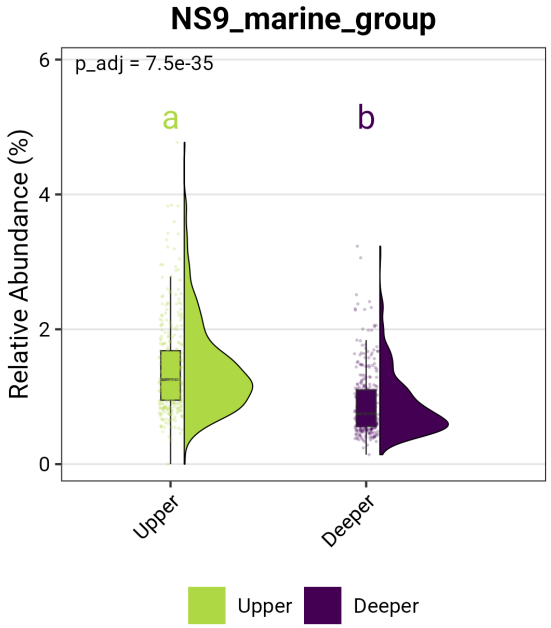
- Anerobic degradation of marine particles (Per eira et al., 2019) and dissolved organic matter (DOM) (Dai et al., 2025)
- Enriched in oxygen-deficient layers (Wright et al., 2012, Liu et al., 2024)
- Enriched in oxygen-deficient layers (Liu et al., 2 024) and denitrification and sulfur cycling proc esses (Hawley et al., 2017)

Results – Taxonomic Composition



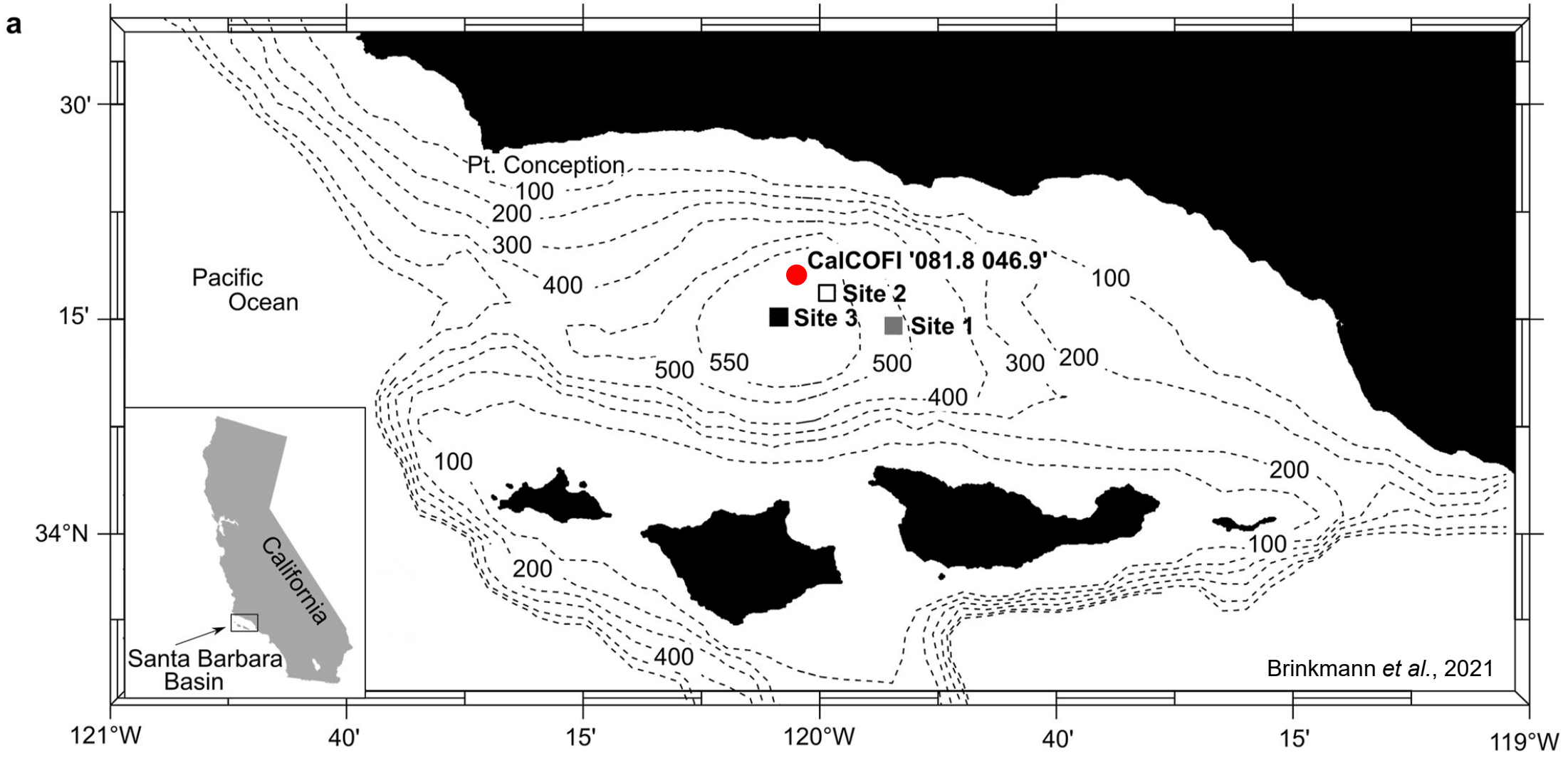






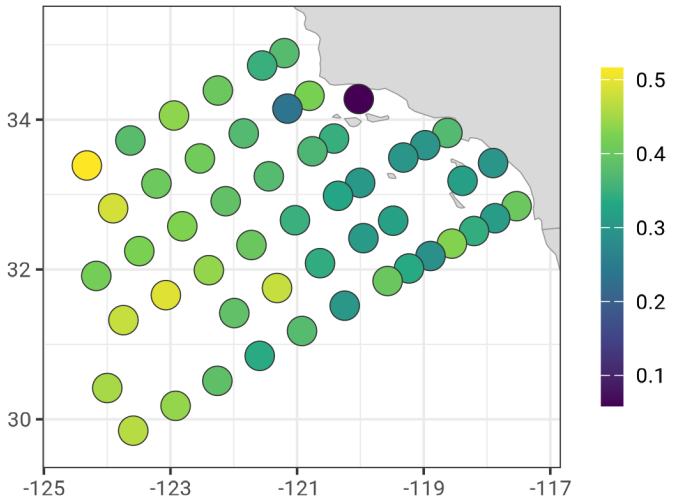
- Microbial groups in the epipelagic in the Southern CCE (James et al., 2022 and 2024) responsible for degradation and recycling of organic matter (e.g., polysaccharides) from phytoplankton are almost absent!
- And decreasing further offshore ..

Local hypoxia & denitrification reflected in prokaryotic communities

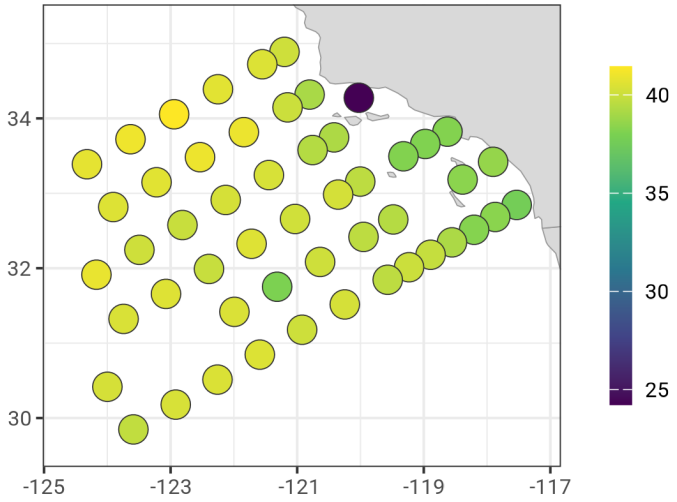


Local hypoxia & denitrification reflected in prokaryotic communities

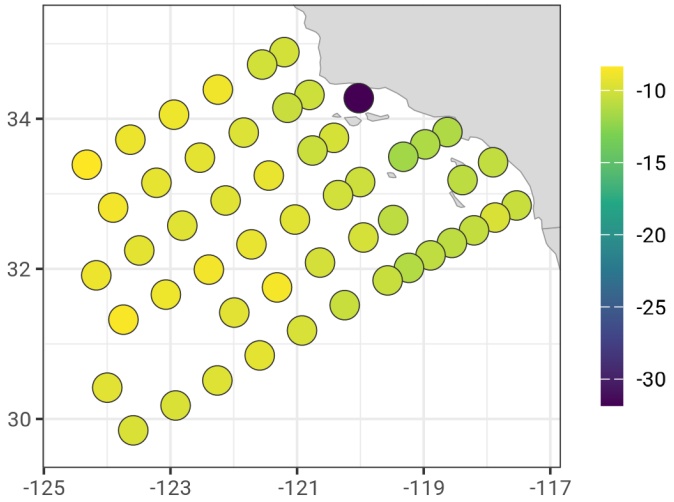
O_2 (mL L^{-1}) - 515 m



NO_3 (µM) - 515 m



N^* (µM) - 515 m

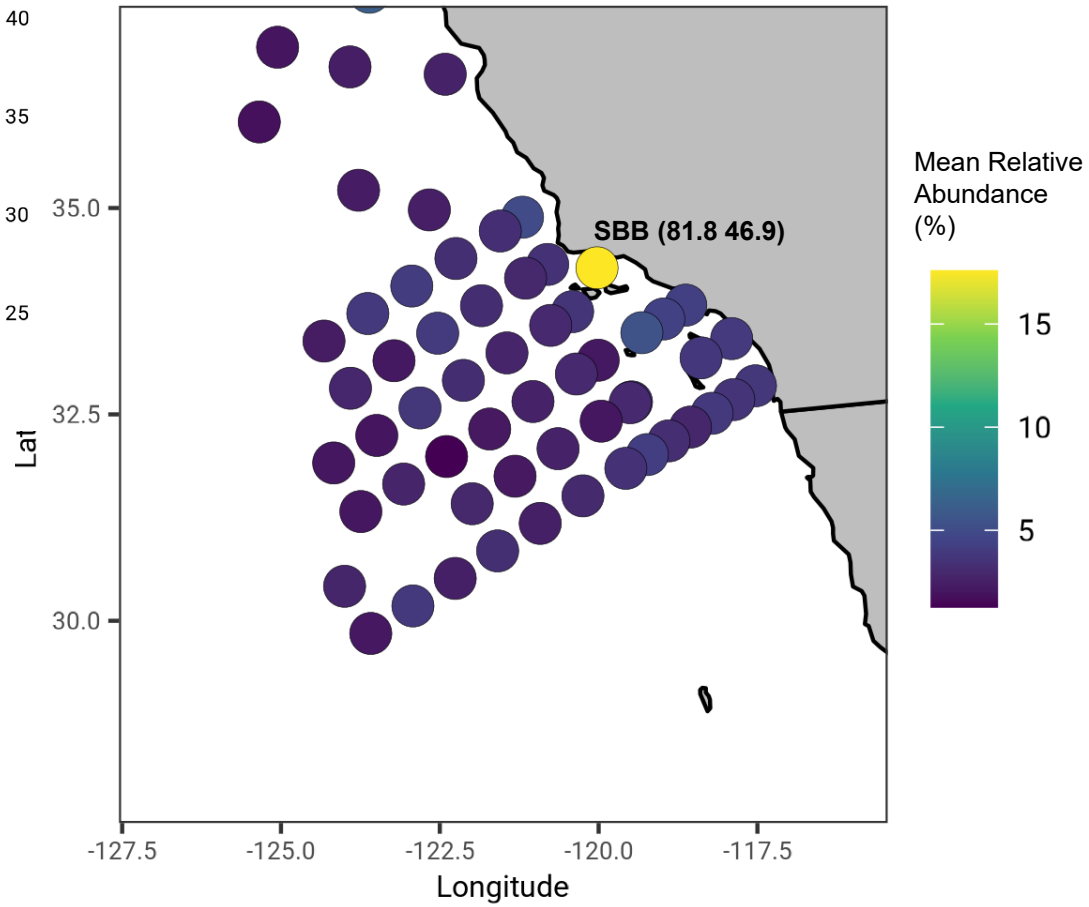


$$N^* = [NO_3] - 16 \times [PO_4] + 2.85$$

(Goericke *et al.*, 2015)

N* < 0 → denitrification

Thioglobaceae (515m)



Conclusion

- **Mesopelagic prokaryotic communities of the Southern CCE (2014-2021) exhibit distinct depth-driven structuring, with higher alpha diversity at the upper mesopelagic and clear beta diversity separation between depth layers.**
- **Water-mass properties and nutrient availability were the key variables explaining community variance, with stronger environmental structuring observed at 170m/200m than at 515m.**
- ***Nitrosopumilaceae* (Ammonia-Oxidizing Archaea, AOA) dominated across both layers (~34.1%), suggesting a central role in N cycling and remineralization throughout the mesopelagic water column.**

Acknowledgements

- Captain and crew of CalCOFI cruises (2014-2021)
- CalCOFI team at SIO & NOAA
- Steven Bograd (NOAA), Mer Pozo Buil (University of South Carolina)
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  - CCE-LTER Travel Fund

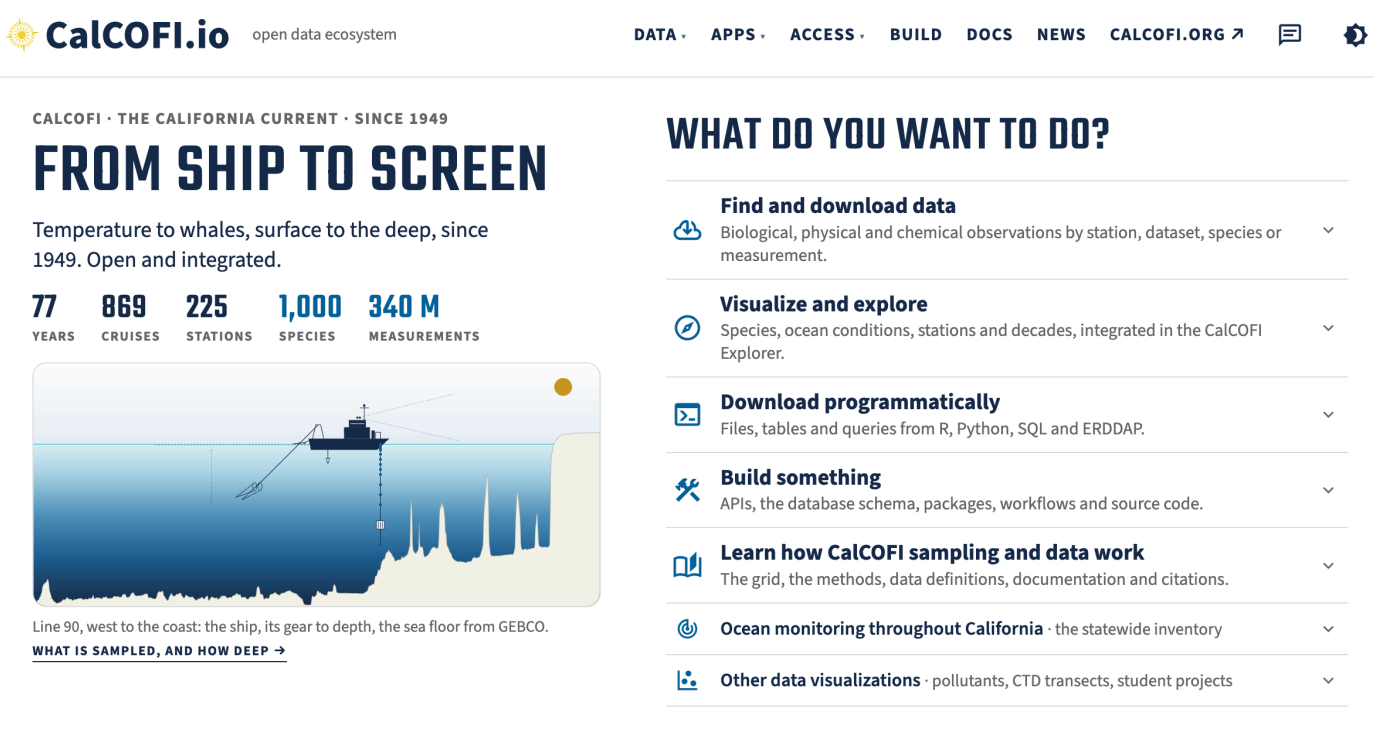


Spring 2025 CalCOFI cruise (R/V Bell M. Shimada)



Summer 2025 CalCOFI cruise (R/V Sally Ride)

Data Access & Visualization Platform calcofi.io



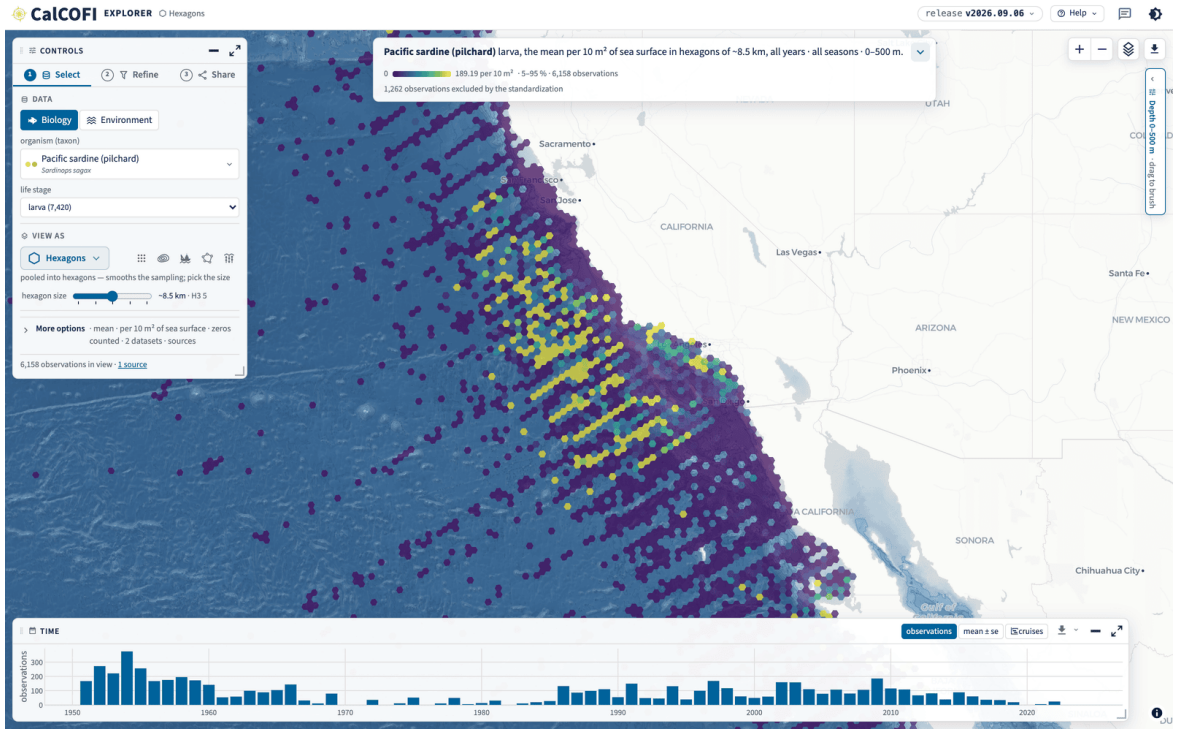


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Photo from 2026 01 CalCOFI Cruise (R/V Reuben Lasker)

Thank you!

Q&A