

Can eDNA help us learn why anchovy abundance meteorically rose off California in 2015 and improve our ability to manage Small Pelagic Fish?

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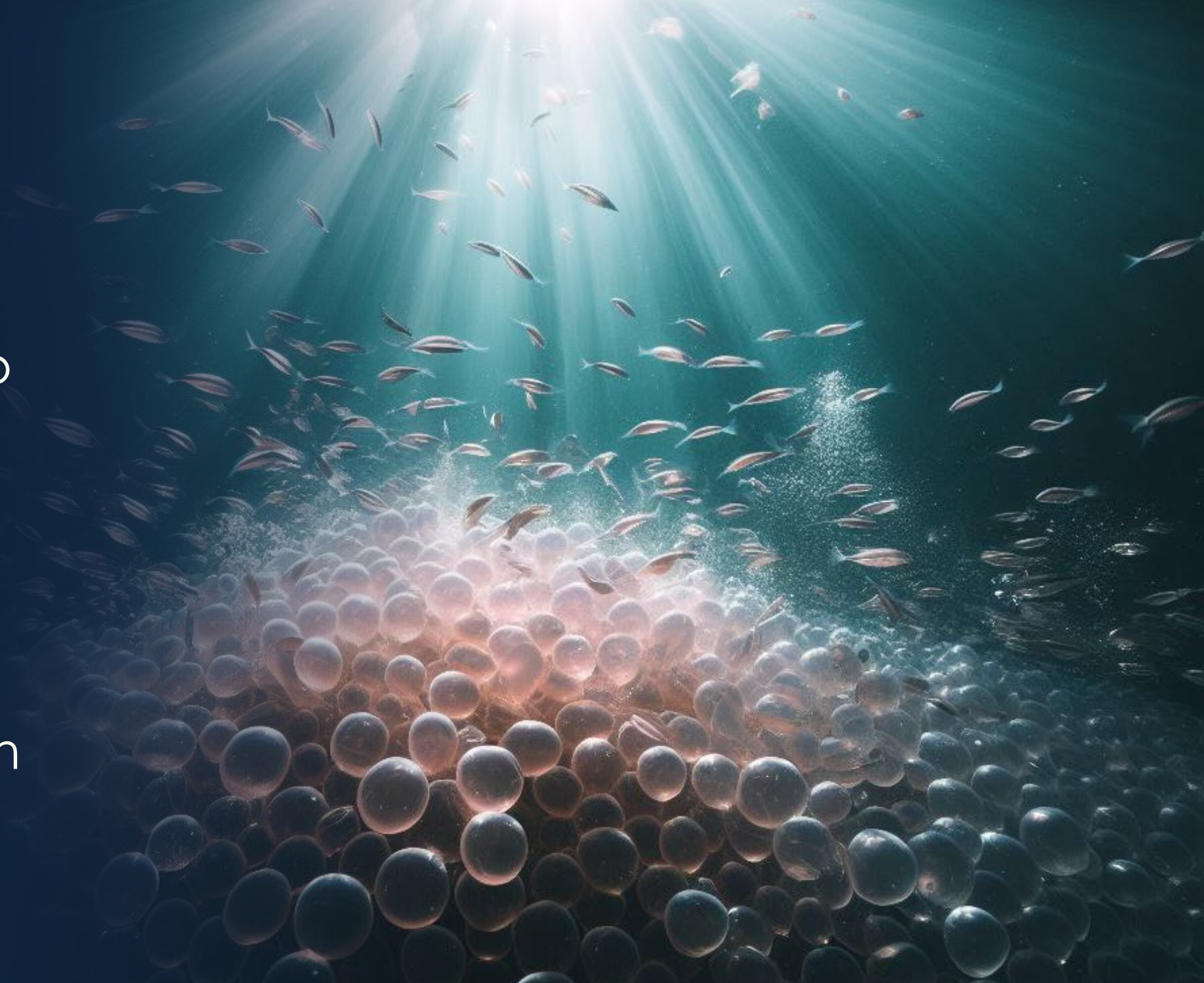
Disclaimer –this talk is about an approach that might help. We don't have a conclusive answer



The likelihood of a larval fish to see its next day is low

In a single day, up to 80% of its brothers and sisters might die

But, they are so fecund that even a small decrease in mortality can result in a lot of survivors



...If a baby fish can get through its larval phase we (humans) call them “recruits”.

At the recruitment stage natural mortality has stabilized.

Domonique Robert et al. term this the “End Point”

For example, if recruitment is high in 2026, and a small pelagic fish like an anchovy naturally lives for 3 or 4 years, then there will likely be a large number of adults in 2027 and 2028

# We're trying to understand why fish populations change in the California Current Ecosystem



One of the main small pelagic fish in the CCE are northern anchovy (*Engraulis mordax*)



Northern anchovy live for up to about 4 years, reproduce at about 1 year and reach a size of about 23 cm (9")

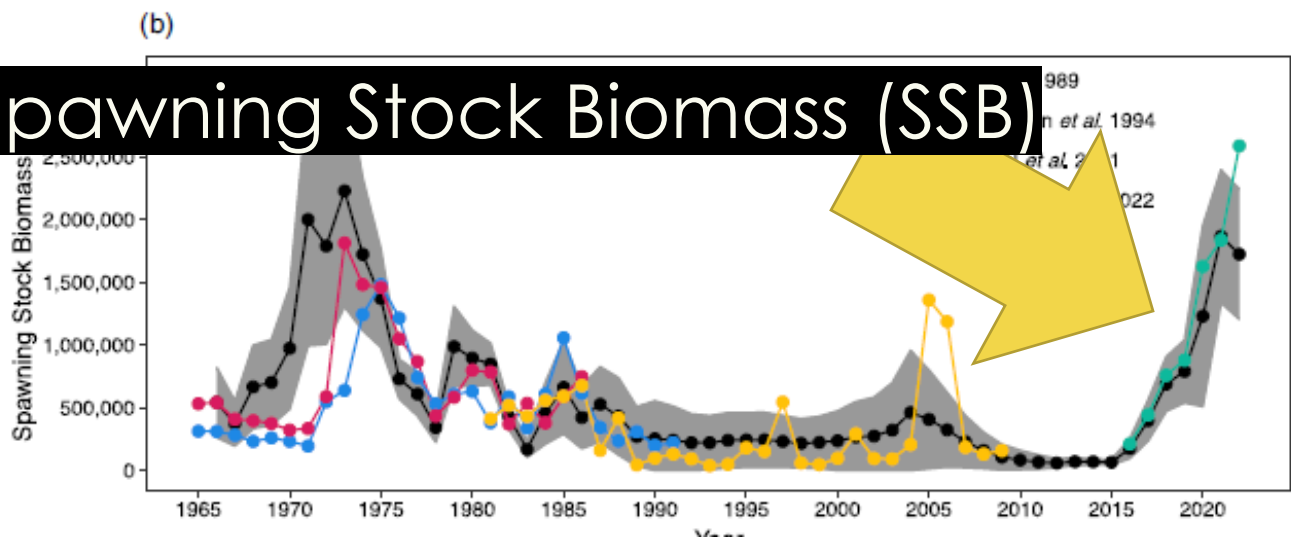
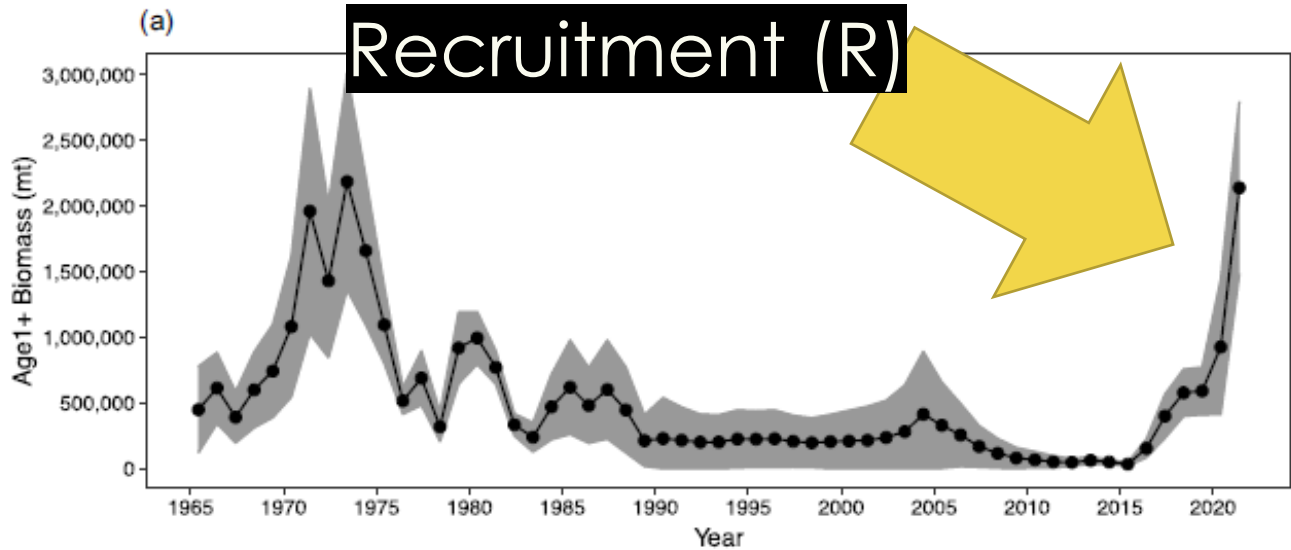
In our backyard (the California Current Ecosystem; CCE) anchovy displayed incredible population dynamics in the last decade.

In 2013, adult anchovy abundance was the lowest in recorded history

Food was scarce for marine predators



By 2019, anchovy were at a record high in the CCE  
This actually happened during my career as a fish biologist!



Again, this is real  
Why did this happen  
????

Environmental conditions have been used to predict SPF stock dynamics  
...but have mostly been insufficient



It's been pretty rough in our backyard

We thought that anchovies were supposed to do well when it is cold, but they skyrocketed in 2015 when the ocean was the warmest in observational history and these tricky bastards have flourished while the ocean has been mostly warm ever since.

The manifestation of the PDO, ONI, and NPGO off the coast of North America is a mess.

It's been hot when the PDO and ONI are low and let's not start with the NPGO.

A vibrant underwater scene of a coral reef. The water is a deep, clear blue, and sunlight filters down from the surface, creating a bright, ethereal glow. The reef is densely populated with various types of coral, including branching, brain, and table corals, in shades of brown, orange, and green. Numerous small, dark-colored fish are scattered throughout the water column, some swimming in schools and others individually. A few larger, more colorful fish, including a prominent blue tang in the foreground, are also visible. The overall atmosphere is one of a healthy, thriving marine ecosystem.

Gaining some insight  
into the world that larval  
fish actually inhabit may  
bring us closer to  
understanding the  
mechanistic drivers of  
SPF population  
dynamics

eDNA and CalCOFI might help

## What is CalCOFI?

The California Cooperative Oceanic Fisheries Investigations (CalCOFI) program is the world's longest-running fisheries survey, and we are grateful to be part of it

CalCOFI began in 1951 with the goal of understanding why sardine collapsed in our backyard in the 1940s

It evolved to monitor many aspects of the marine ecosystem – from viruses to plankton to birds to whales

# CalCOFI larval fish sampling: Coming at you since 1951

- 0.71 m diameter, 505  $\mu\text{m}$ -mesh bongo nets
- towed obliquely from a depth of 210 m
- ID larval fish species
- Time series of over 700 species of fish from 1951 to now



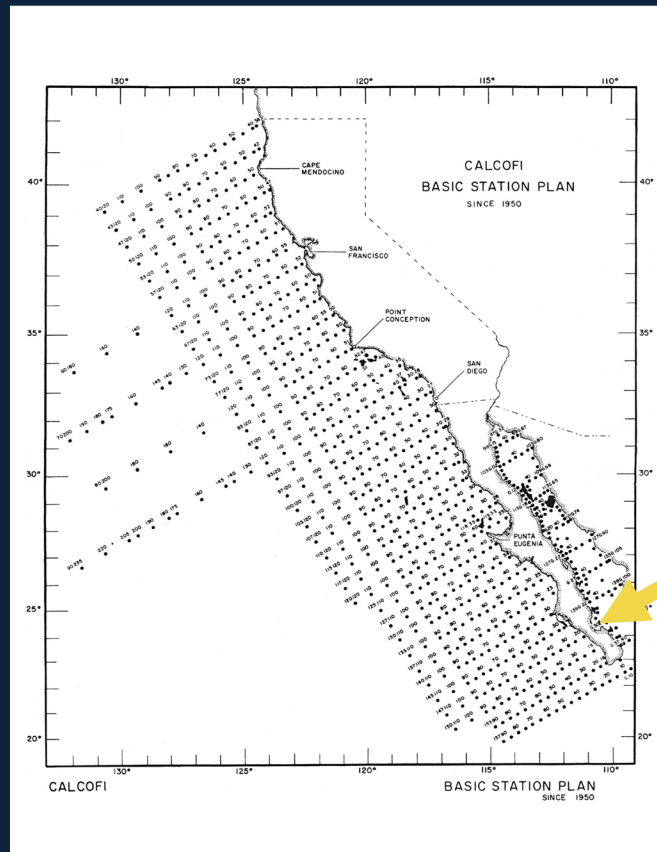
In the beginning, CalCOFI sampled from the OR/CA border to Cabo San Lucas and into the Sea of Cortez

Now we sample from San Francisco to the US/Mexico border

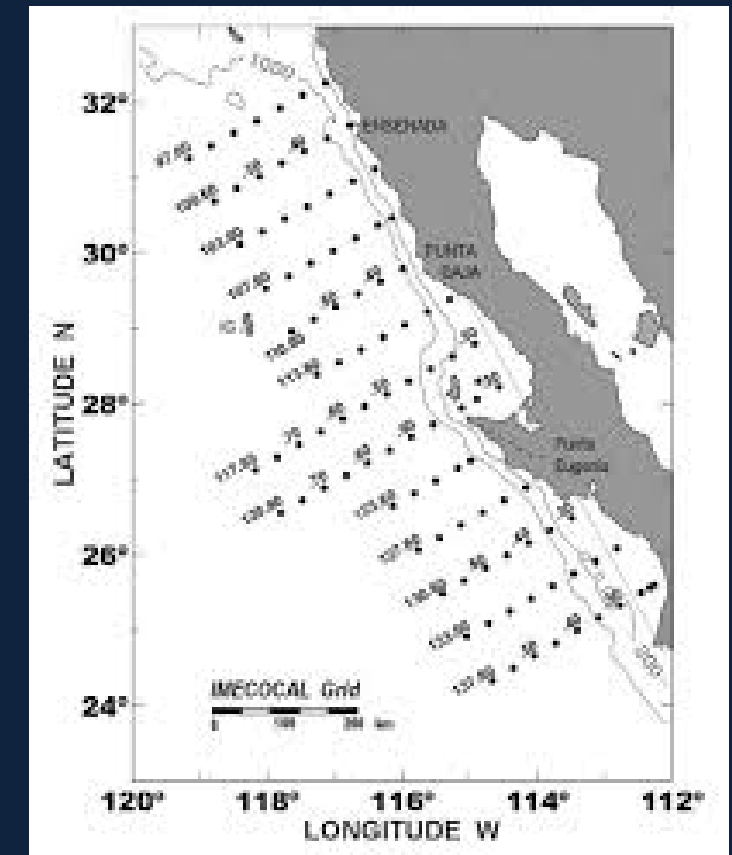
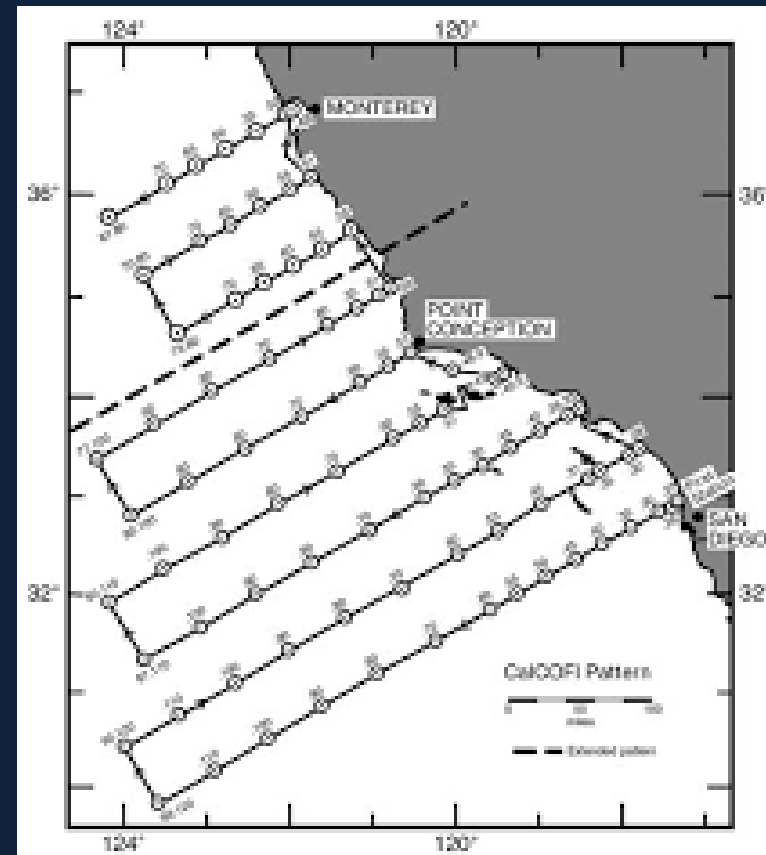
IMECOCAL sampled CalCOFI stations in the south from 1998-2016 . We miss IMECOCAL

IMECOCAL 1998-2016. We love you so much! Please come back. We're not complete without you.

CalCOFI 1950s



CalCOFI now

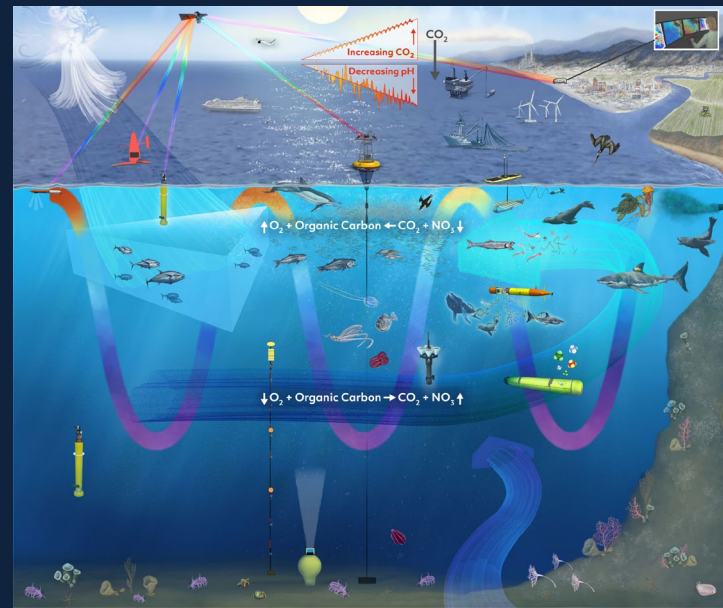


# What is eDNA?

Genetics technology has advanced miraculously in this century (just 26 years)

Nina Overgaard Therkildsen explained how we can now do low coverage whole genome sequencing to better look at fish stock structure

Environmental DNA allows us to sequence every organisms that has contributed a bit of its DNA to a parcel of water – everything from a bacteria that is collected whole to a whale that has shed a bit of snot



# CalCOFI eDNA sampling: Coming at you since 2014

- *Collect*: 0.5–2 L of seawater
- *Filter*: 0.22  $\mu\text{m}$  Sterivex-GP filter unit
- *Extract*: NucleoMag Plant Kit
- *Sequence*
  - Prokaryotes: 16S rRNA
  - Eukaryotes: 18S rRNA
- Also fish-specific eukaryotes - 12S rRNA



Can we combine ichthyoplankton and eDNA observations to better understand why anchovies blew up in the CCE in the last decade?



Can we get insight through traditional larval fish sampling and new eDNA sampling to see:

What a larval fish eats ?

And

What might be eating it?

And

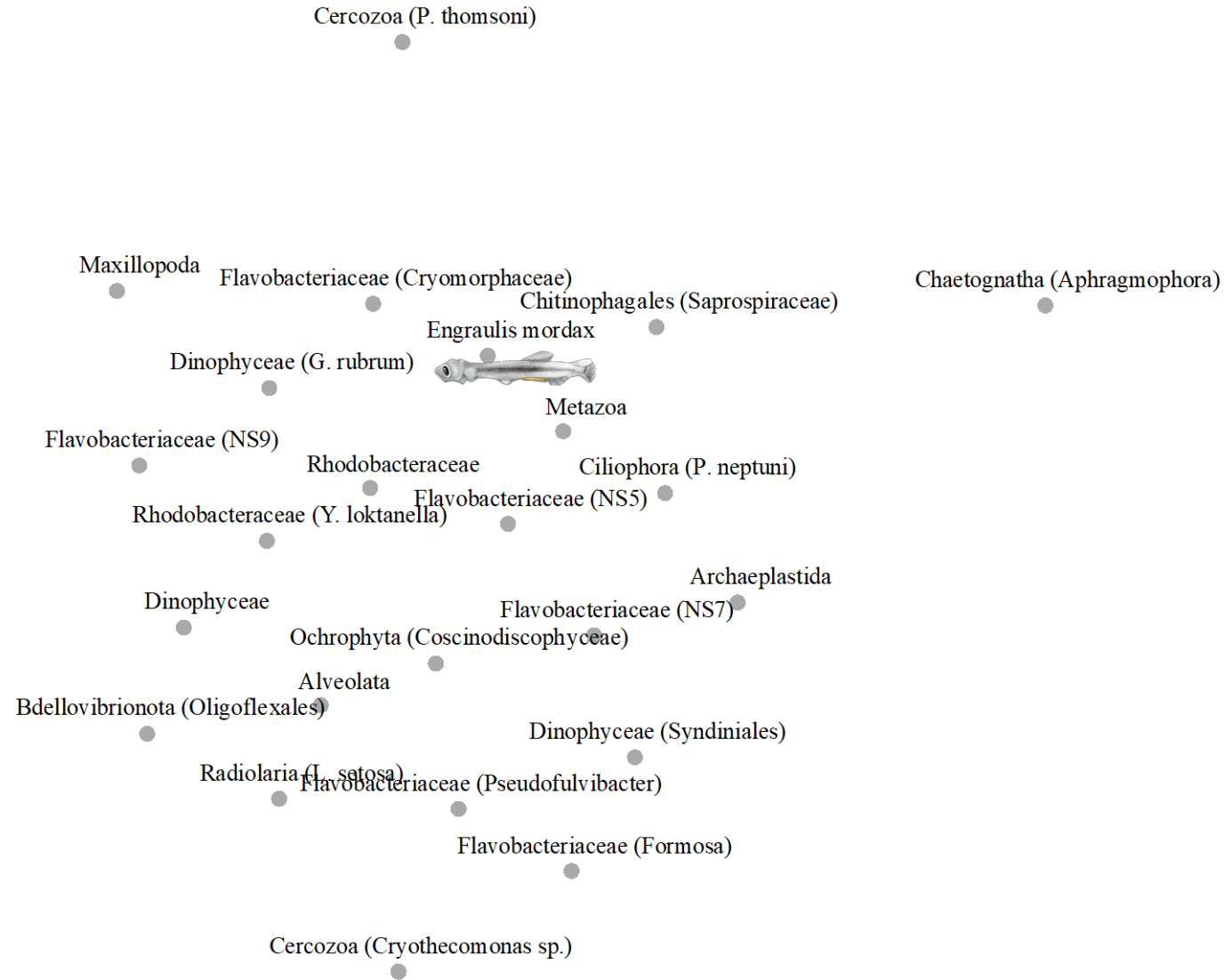
How this affects the “End Point”?

# “anchovy water”

Alvarino 1980

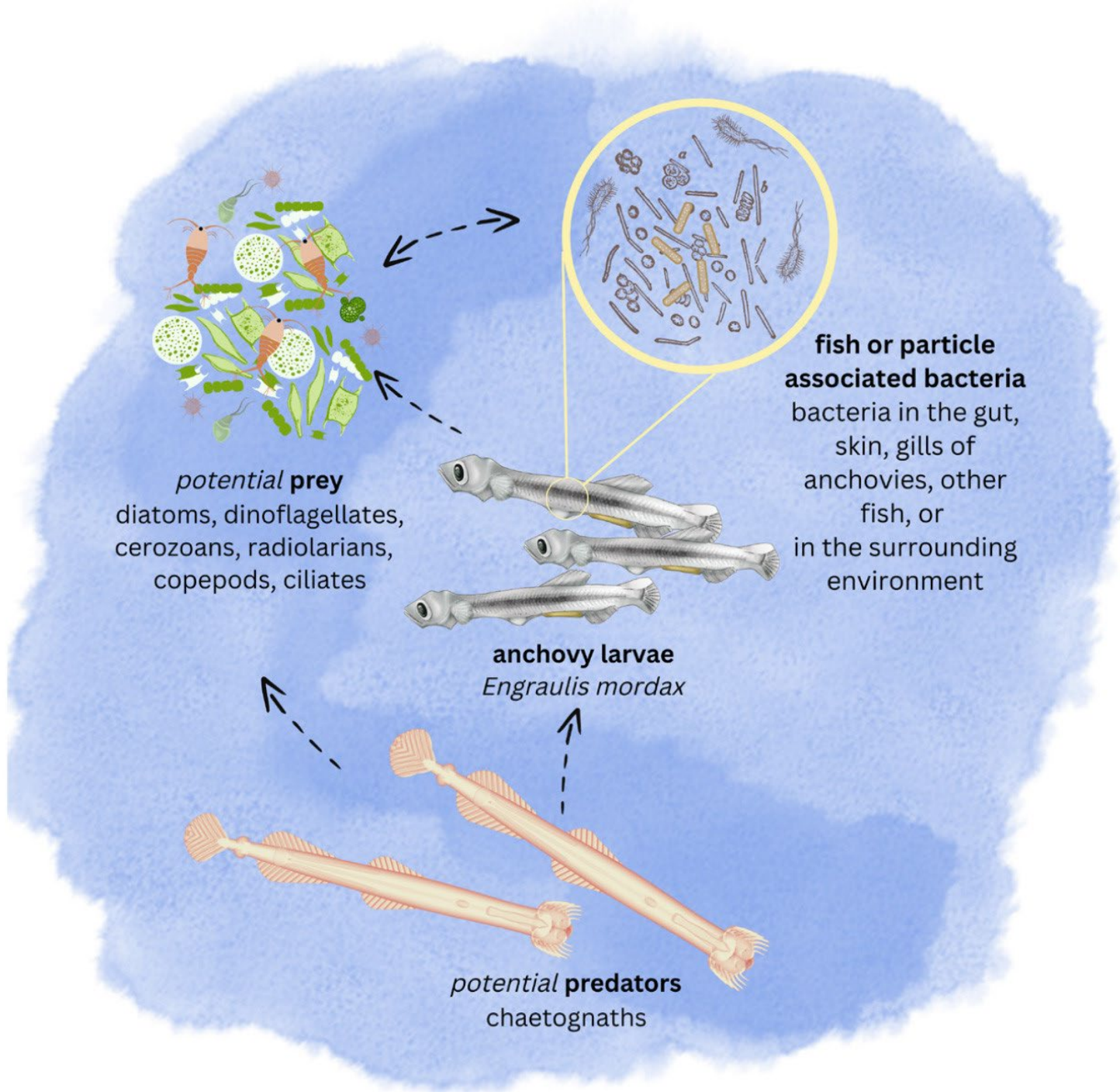
Sub-network of grouped amplicon sequence variants (ASVs) positively associated with larval anchovy provide insight into ecological habitat of larval anchovies

Satterthwaite et al. 2023



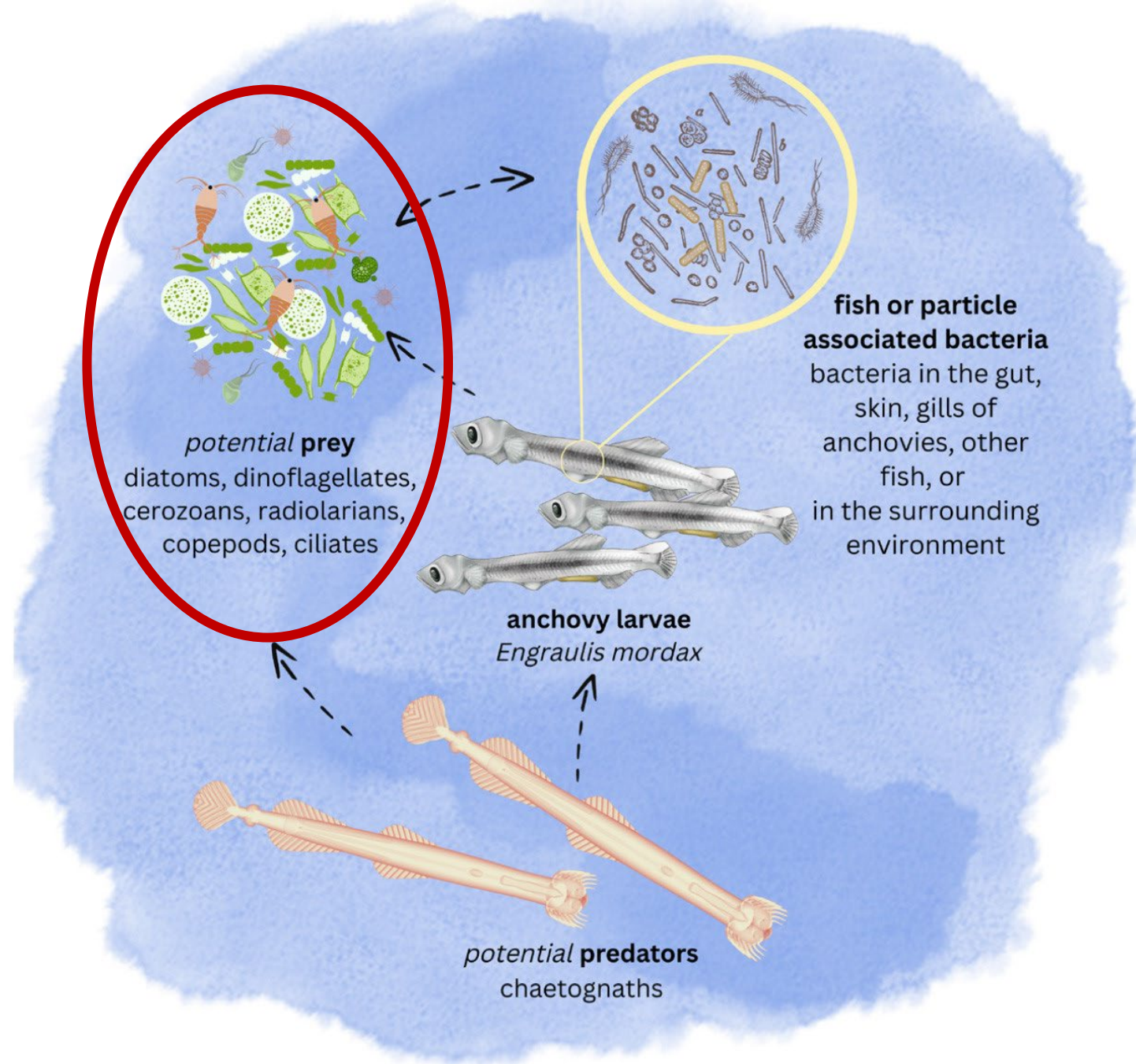
45 ASVs that clustered into 24 distinct taxa

Anchovy water from eDNA analysis reveals potential prey field, predators, & microbiome associates

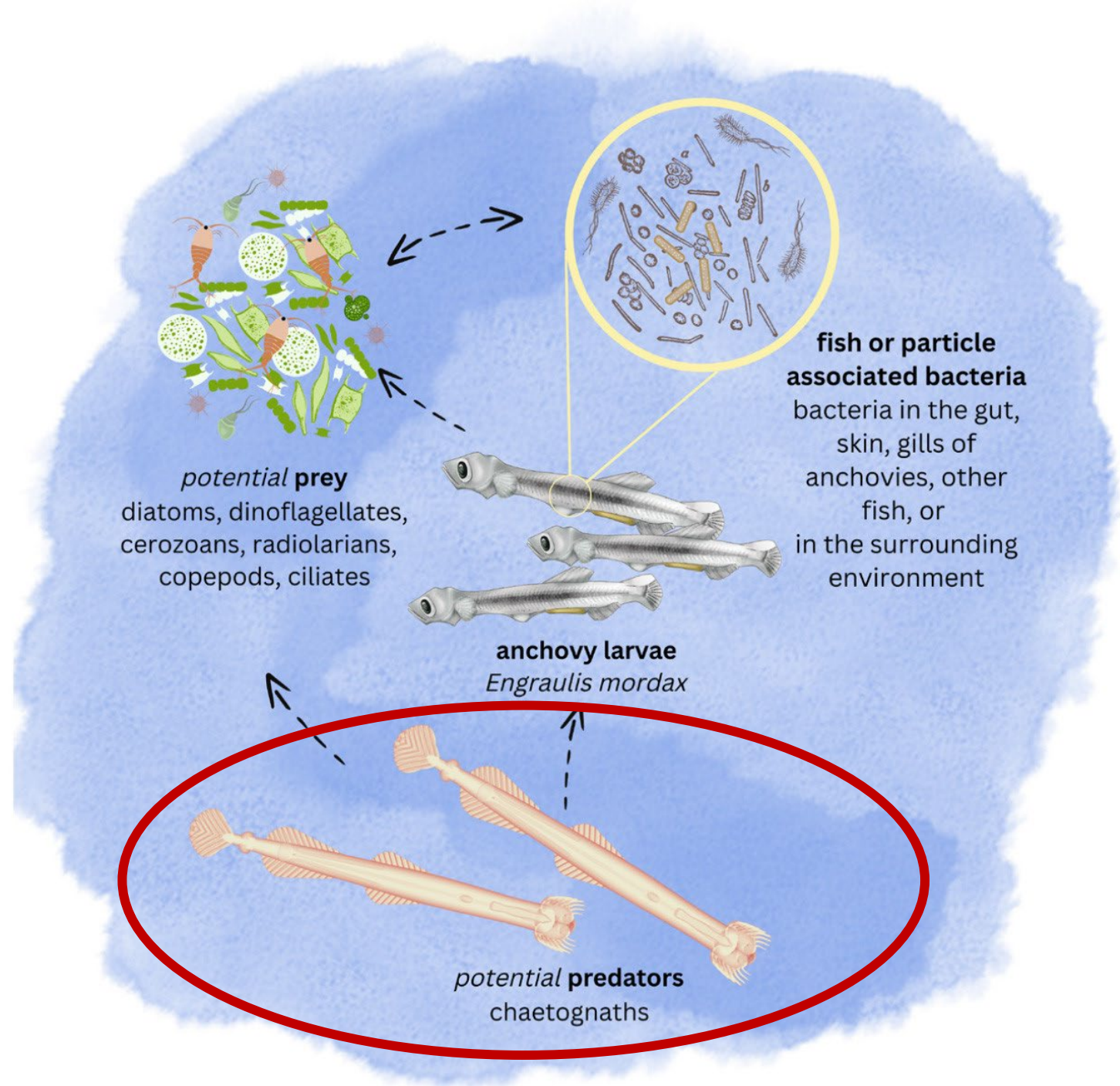


# potential prey

- ciliates (*Pelagostrobilidium neptuni*)
- cercozoans (*Cryothecomonas* sp., *Protocystis thomsoni*)
- radiolarians (*Lithomelissa setosa*)
- maxillopods (likely copepods)
- phytoplankton
  - diatoms (*Bacillariophyceae*)
  - dinoflagellates (e.g., *Syndiniales* & *Gyrodinium rubrum*)
- Most have been identified as important food sources for anchovies

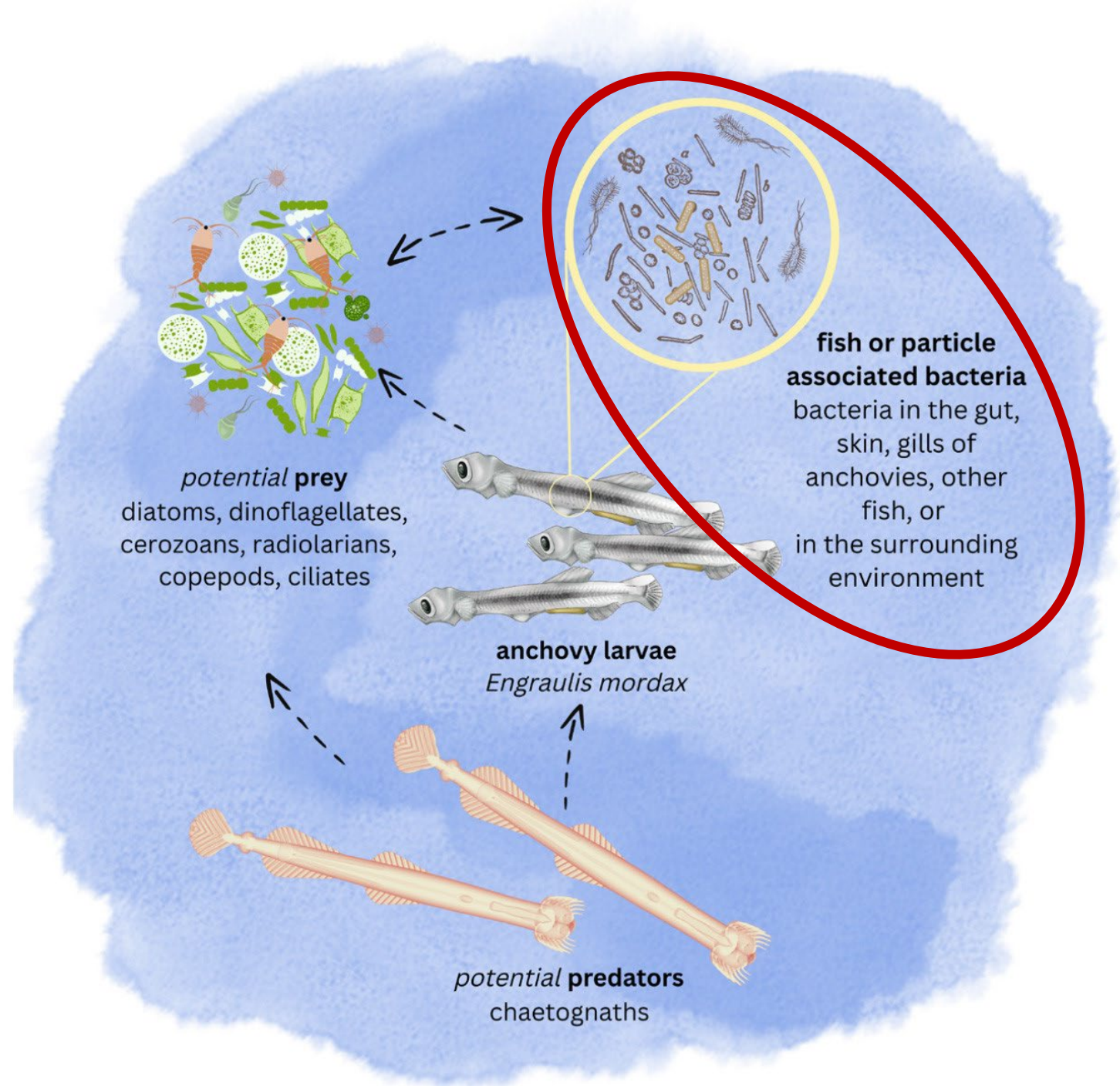


# Chaetognaths (Aphragmophora) as potential predators or competitors



# potential fish or particle associated bacteria

- Several uncultured bacterial taxa showed co-occurrence with *E. mordax* larvae
- 72% of the 16S amplicons were assigned to the order Flavobacteriales
  - Flavobacteriaceae (marine group NS5, Formosa, and Pseudofulvibacter)
  - Cryomorphaceae
  - marine groups NS7 & NS9
- Others include: Rhodobacteraceae, Saprospiraceae, Oligoflexales
- Many of these taxa have been found associated with fish gills, skin, or intestines/feces



This approach may have promise

I hope it helps. We're trying...







# Biomolecular approaches provide insight into the larval habitats of important fisheries species

- **Candidate ecological indicators**
- **Co-occurrence networks**
- **Identify species interactions**



# Next steps & future work

- Validate this study with **additional surveys concurrently sampling eDNA and fish**
- Leverage the taxonomic richness of eDNA-based monitoring products **to improve fisheries prediction models** (e.g., recruitment forecasting)
- **“Fingerprint” quality larval habitats** and provide indices of their spatial extents
- Explore the use of **network metrics in ecosystem-based fisheries management approaches**
- Conduct targeted studies to **test the connections** found through these types of ecological co-occurrence studies, such as **gut contents studies**
- Examine the **broad suite of planktonic taxa that co-occur with other important species of interest**
- Develop more **detailed reference databases** to better resolve ASV taxonomy and inform essential mechanisms.

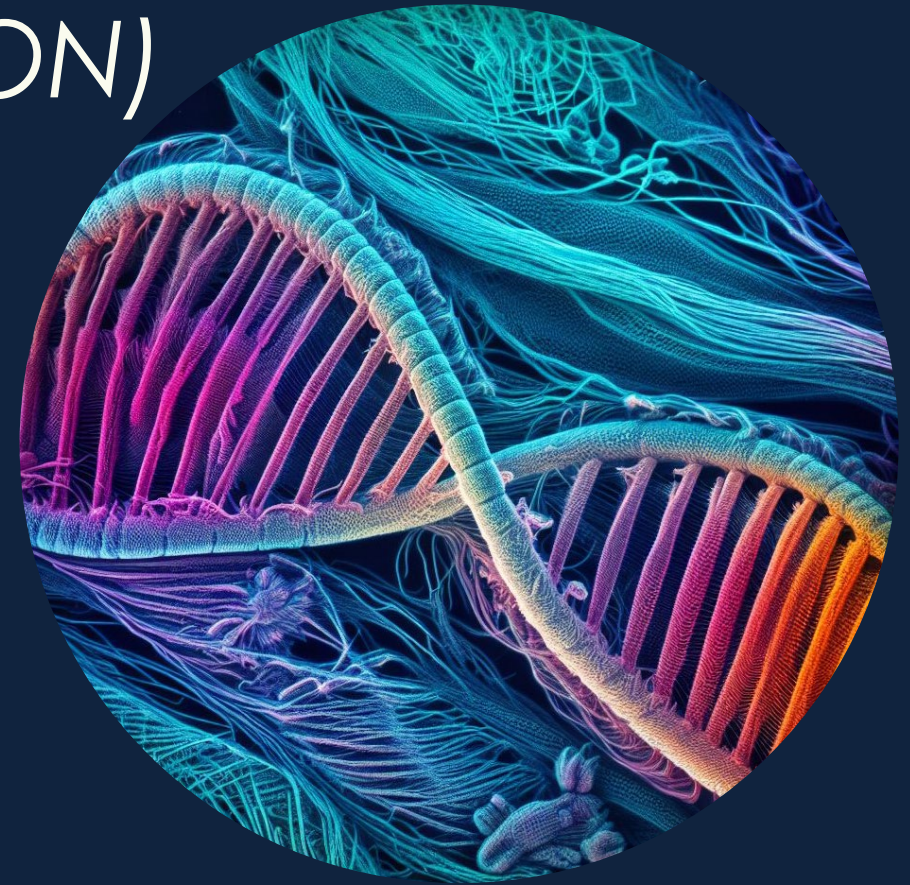


# Next steps: a sneak peek

## West Coast- Ocean Biomolecular Observing System (WC-OBON)

*Integrating & harmonizing molecular observations in support of sustainable marine management*

- Align eDNA methods in the Eastern Pacific
- Develop bioinformatic workflows & metadata standards
- Establish best practices for large scale biomonitoring



# Next steps: a sneak peek

## *Marine Mammal Remote detection via INnovative environmental DNA sampling (MMARINeDNA)*

- Understand the **transport, persistence, and distribution of eDNA** in marine environments from **marine mammals across multiple scales**
- Compare **visual, molecular, and acoustics methods** for **marine mammal detection**
- Conduct a similar study to understand **the ability of molecular plankton data to predict marine mammal abundance**



Thank you!

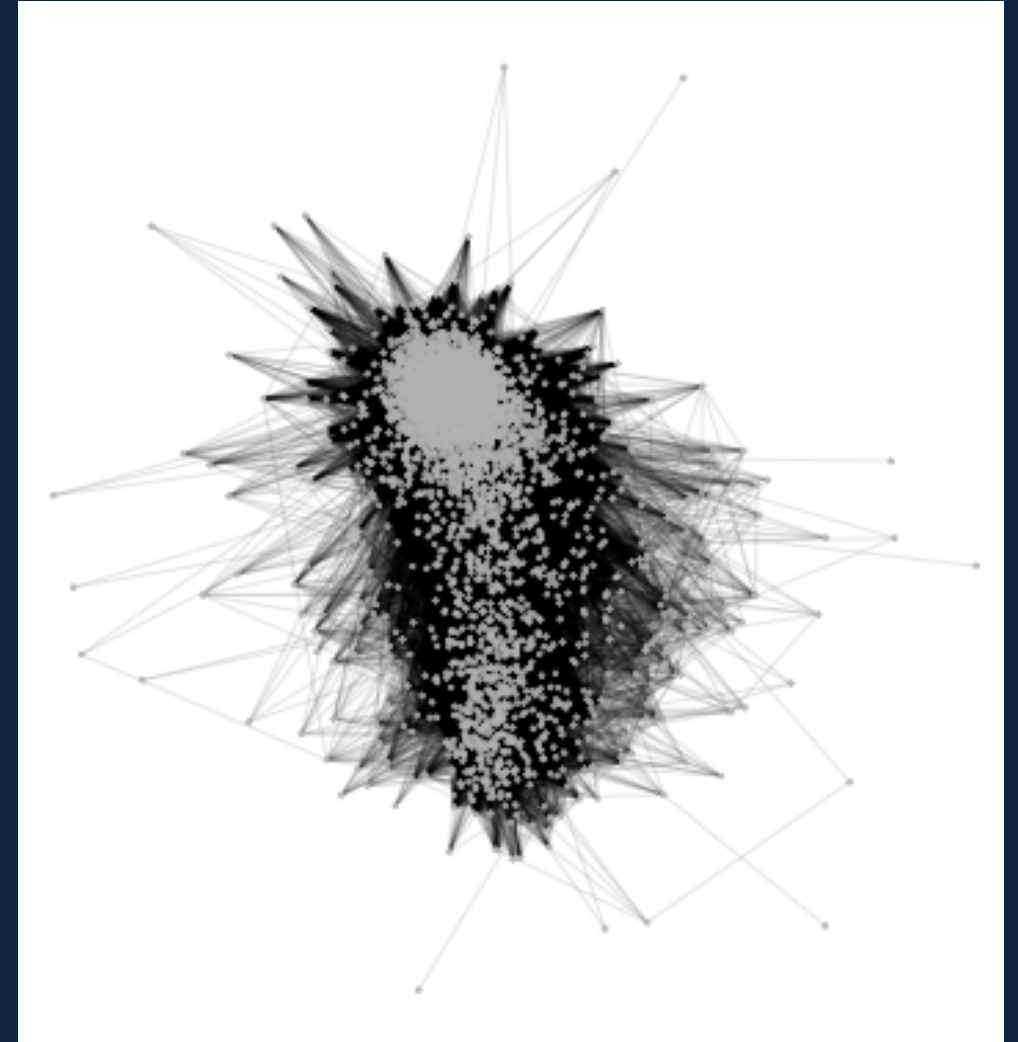
# eDNA from CalCOFI in the Southern California Current Ecosystem

- *Collected*: 0.5–2 L of seawater
- *Filtered*: 0.22 µm Sterivex-GP filter unit
- *Extracted*: DNA extracted with NucleoMag Plant Kit for DNA purification on an epMotion 5057TMX
- *Amplicon sequencing*: small subunit ribosomal RNA gene sequencing
  - V4-V5 region of the 16S rRNA gene for prokaryotes → 19,204 ASVs
  - V9 region of the 18S rRNA gene for eukaryotes → 34,454 ASVs
  - Amplified via one-step PCR using the TruFi DNA Polymerase PCR kit
    - 16S primer set –515 F (GTGYCAGCMGCCGCGGTAA) & 926 R (CCGYCAATTYMTTTRAGTT)
    - 18S primer set – 1389 F (TTGTACACACCGCCC) & 1510 R (CCTTCYGCAGGTTACCTAC)
  - Sequenced on an Illumina MiSeq lane with a 15% PhiX spike-in
- *Amplicon analysis*: analyzed with QIIME2 & denoised with DADA2
- *Taxonomic annotation*
  - 16s –SILVA
  - 18Sv9 – PR2

*For complete eDNA methods see James et al. 2022  
& posted at protocols.io*

# Ecological co-occurrence network

- Presence/absence (P/A) of ASVs from biomolecular 16S and 18S data
- Visually enumerated counts of larval fishes
- Removed taxa with little to no variance
- Explored positive & significant Pearson product moment correlations
- Taxa co-occurrence could be due to:
  - direct interactions (e.g., predation, parasitism, mutualism, or commensalism)
  - indirect relationships via associations with similar water masses or environmental characteristics.
- Co-occurrence does not prove ecological interactions – they identify hypotheses for future testing
- Mined to reveal potential ecological interactions among plankton taxa, including larvae of important fisheries species



I hope so; we are lucky to have that chance to try

Biomolecular techniques in 2026 are beyond anything I could have imagined in 2006.

And our haircuts have also improved.

Maybe eDNA can help?

