State of play: the role of bacterial communities in marine eDNA decay

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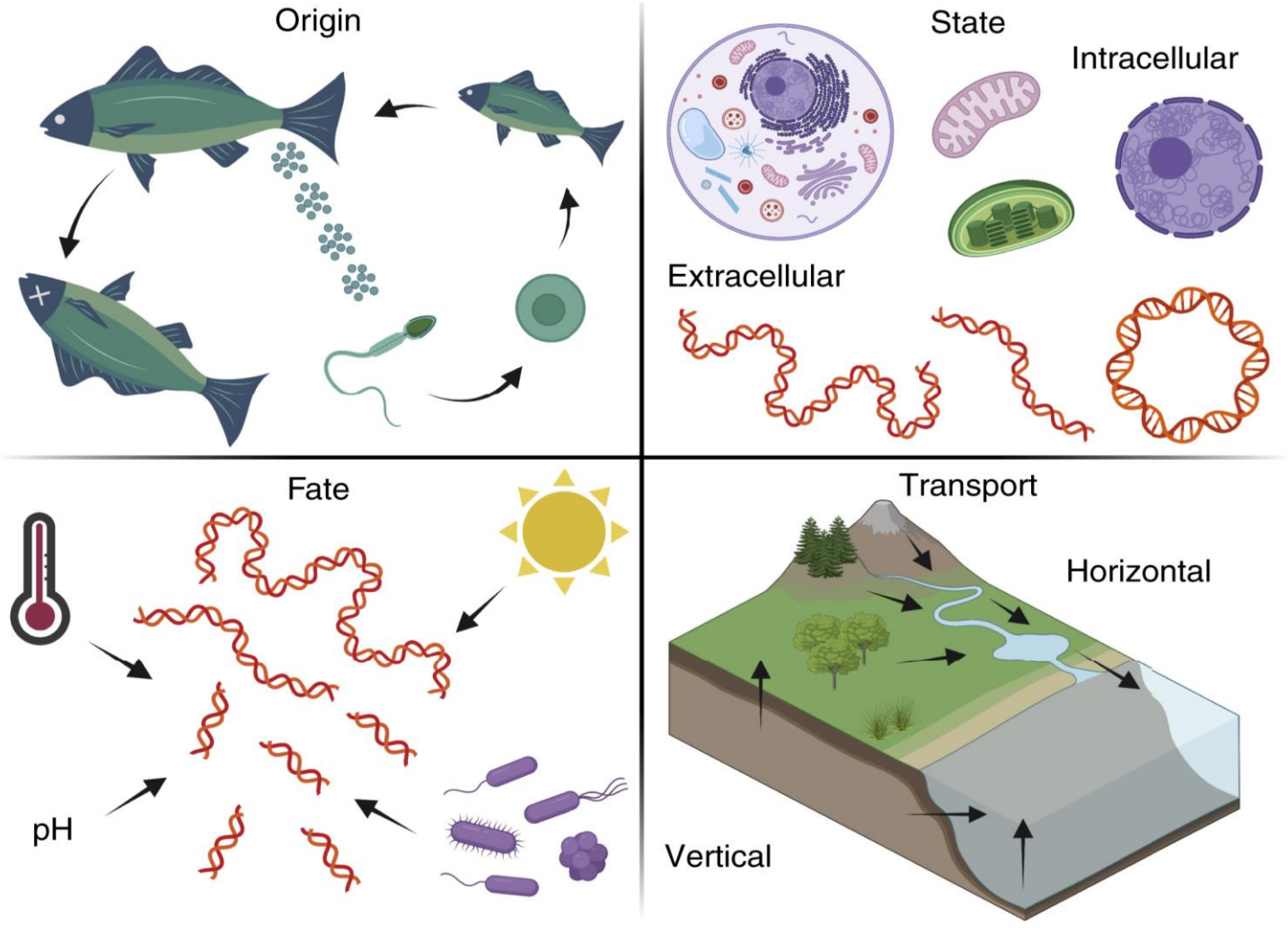
Ecology of eDNA

- Understanding the **ecology of eDNA** helps us better interpret and predict eDNA detections in the wild
- **Shedding, state, fate, and transport** each play a role individually and together
- The **role of bacteria** in decay is known to be important but mechanisms remain poorly understood



Modified from Didaskalou et al., 2022

Role of bacteria in eDNA degradation

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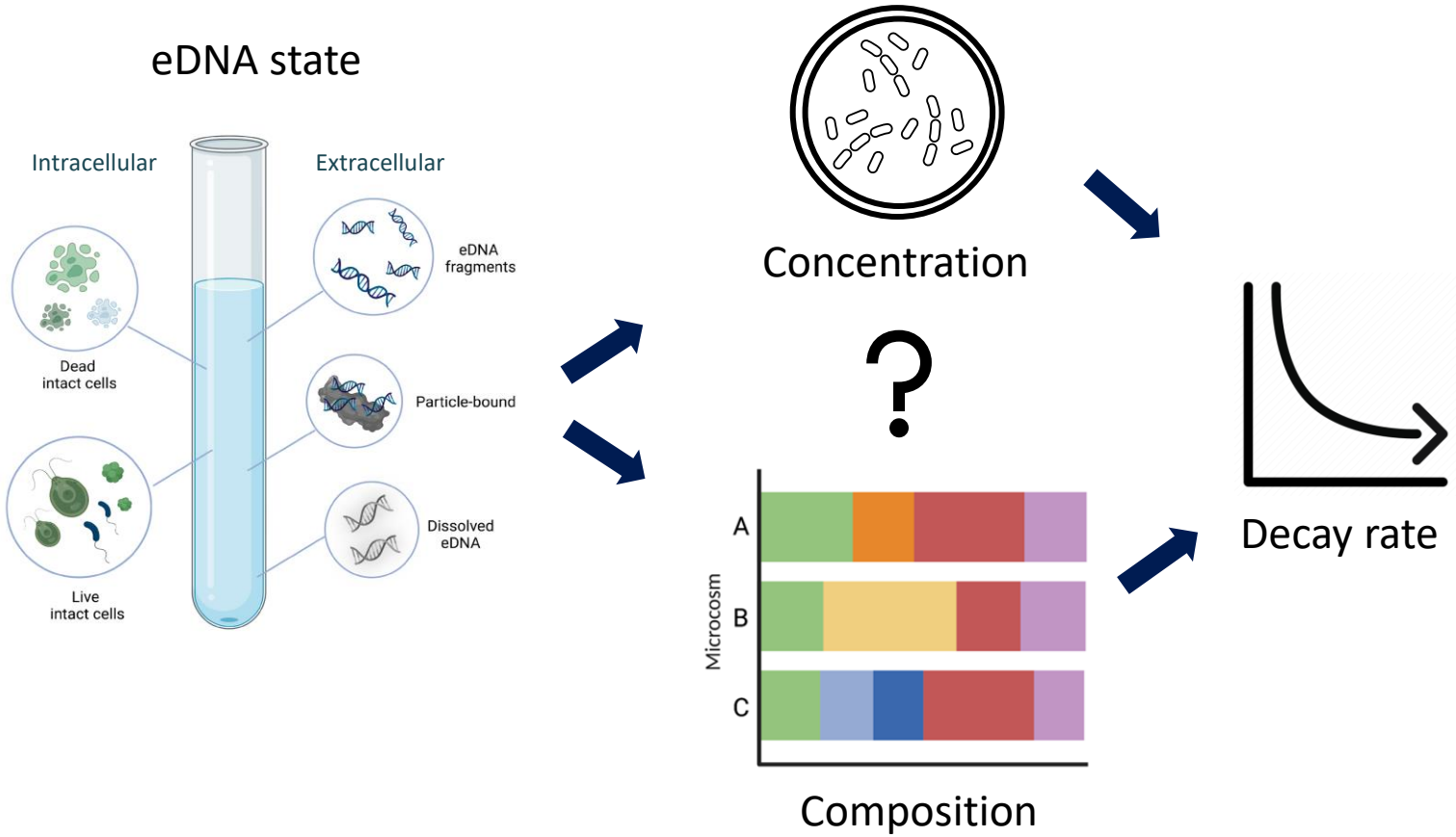

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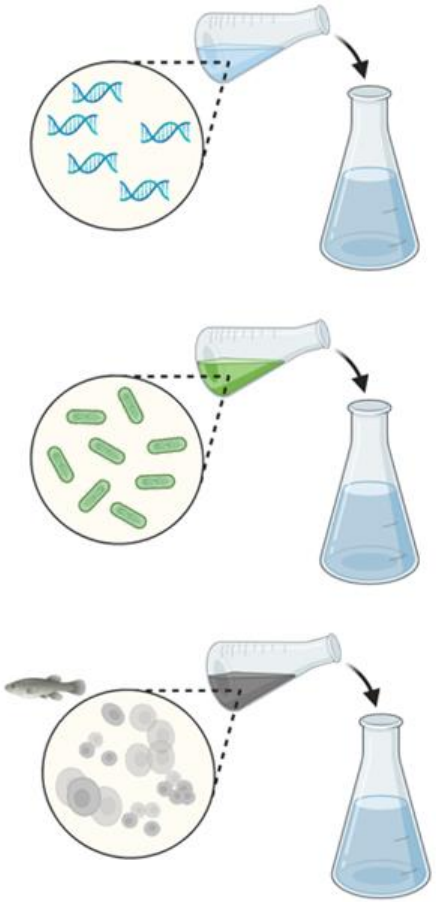
Our approach

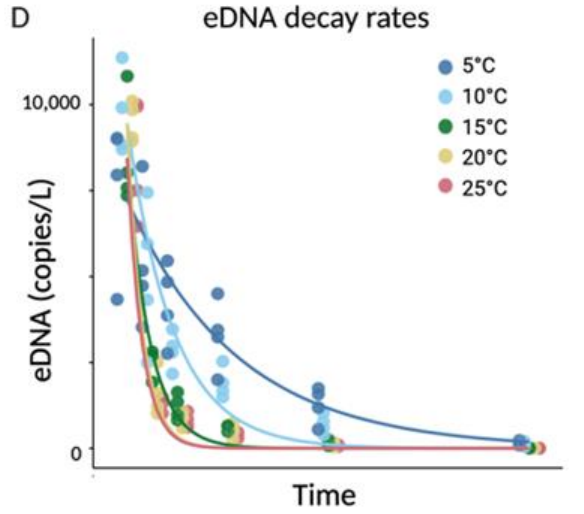
Extracellular (dissolved) DNA

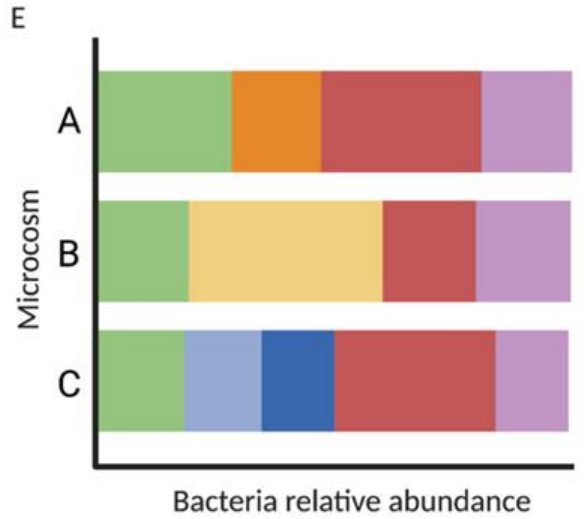
Intracellular DNA

Complex mixture



Mesocosm experiments





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Brilliant ecological insights!

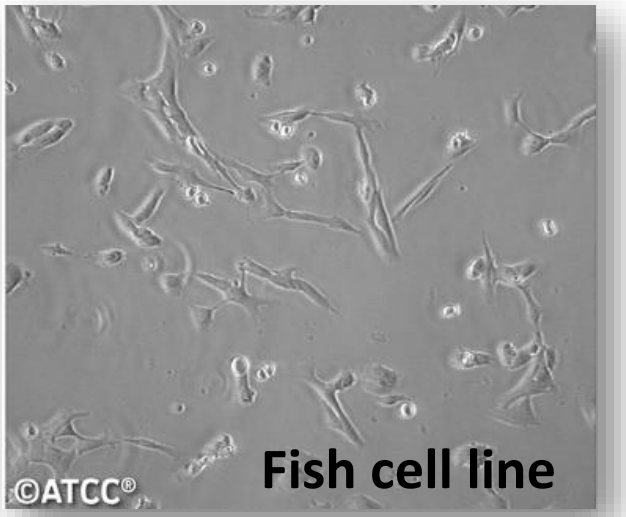
Modified from McCartin et al., 2022

Mesocosm spike-in

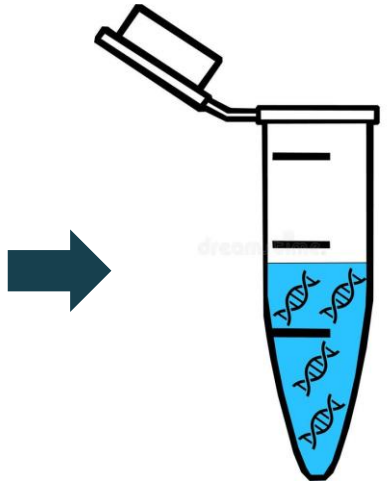
Steelhead / Rainbow trout (*Oncorhynchus mykiss*)

FISH

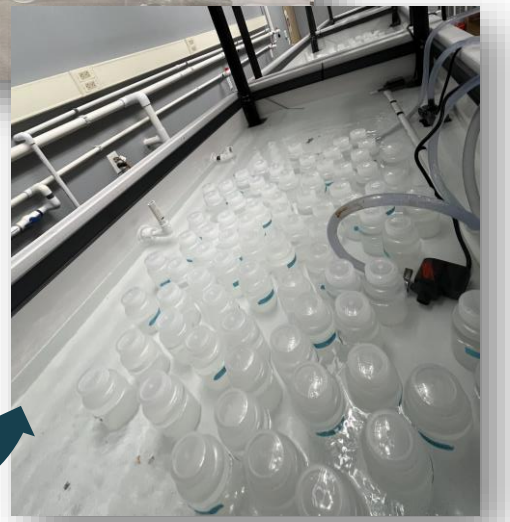
CELL



DNA



Mesocosm set-up



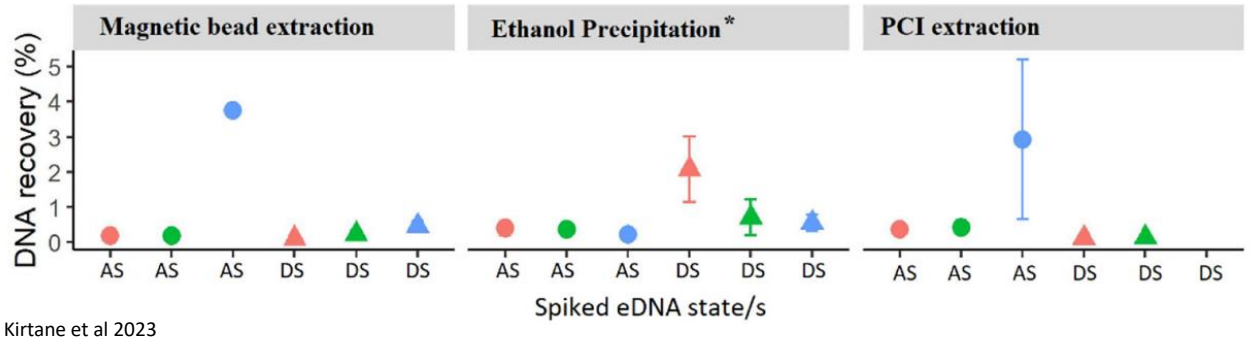
Catalina Marine Protected Area (MPA) seawater used as a medium for all mesocosms

- Three treatments (FISH, CELL, DNA)
- Five temperature (5, 10, 15, 20, 25°C)
- Eight timepoints (0, 4, 8, 12, 18, 24, 48, 72, 168 hours)
- Each treatment in triplicate
- Total = 360 samples

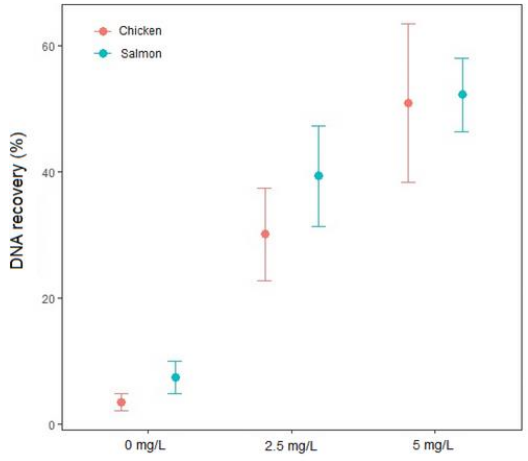
Recovering dissolved DNA

Dissolved DNA is tricky to recover

Chicken DNA target (spiked in free DNA state)

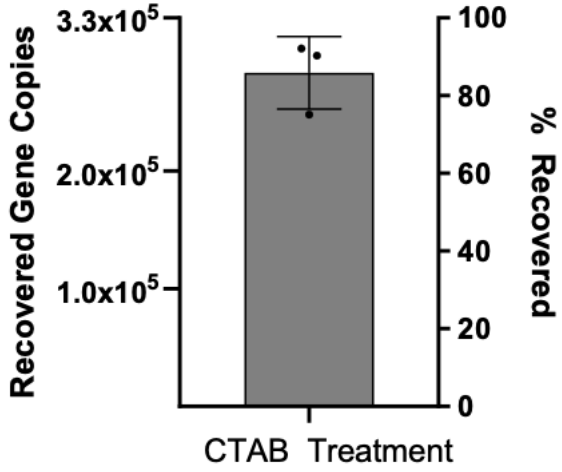


Kirtane et al 2023



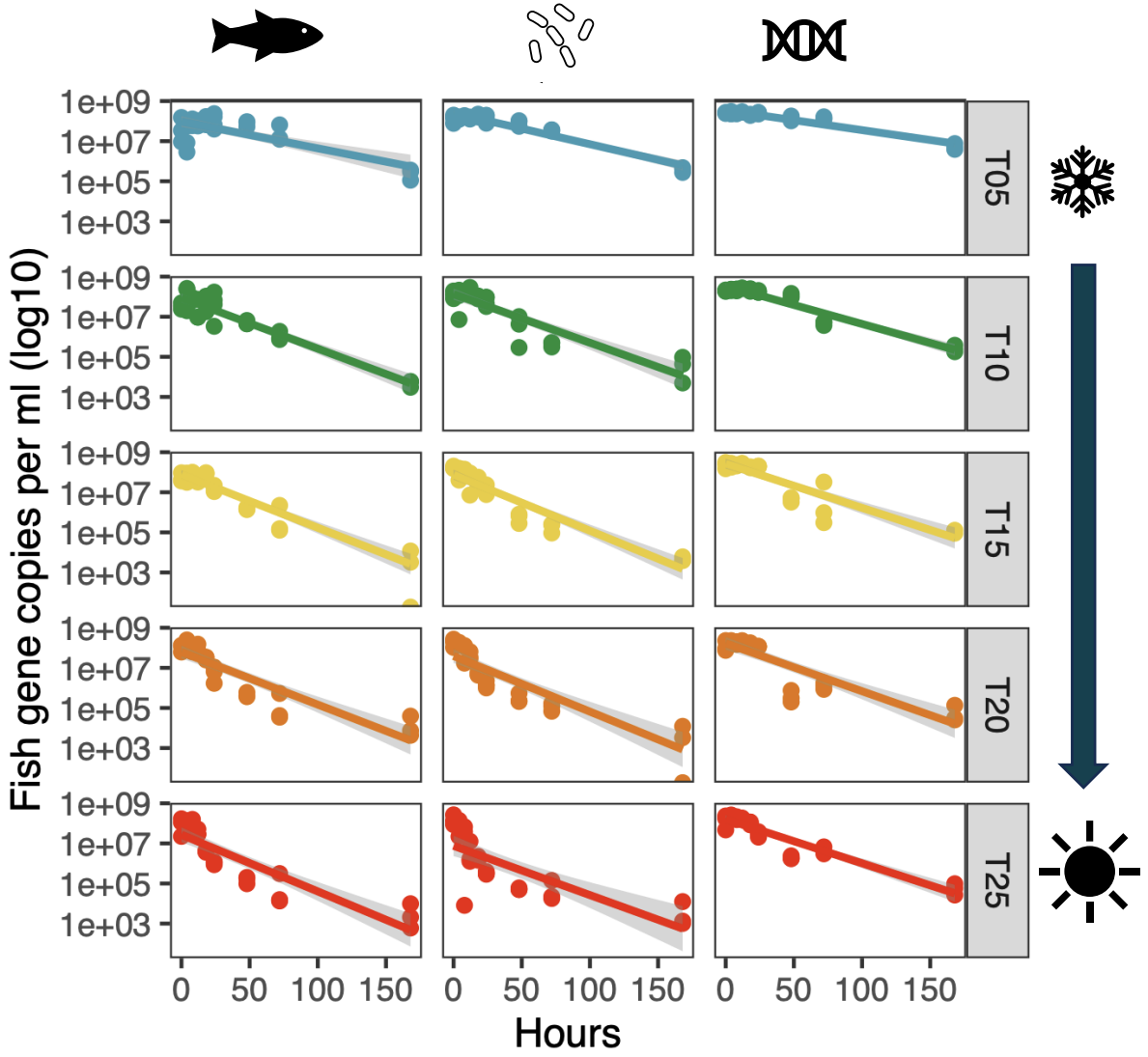
Kirtain and Deiner 2024

Our modified CTAB method recovered ~90% of dissolved DNA



- Spiked extracted DNA from *O. mykiss* cells
  - Known concentration of target gene copies
- ~80-95% recovery of dissolved DNA with CTAB
  - *O. mykiss* COI ddPCR (Brandl et al., 2015)
- Non-CTAB method recovers ~1% of DNA

eDNA decay rates vary by temperature and state



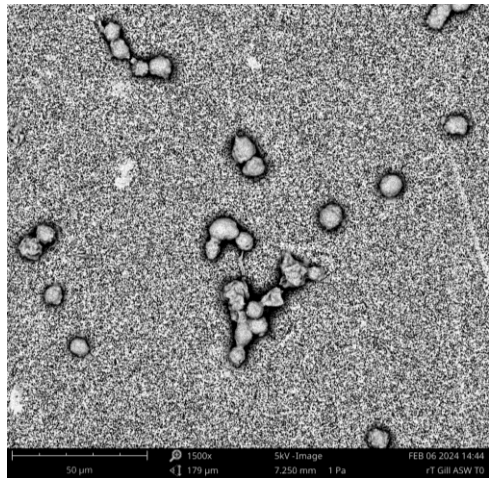
- The expected:
  - Warmer temperature = faster degradation
- The unexpected:
  - "Dissolved" DNA decayed slower than fish and cell DNA
  - At 5°C, DNA $t_{1/2}$ = 67 hours
  - At 5°C, CELL $t_{1/2}$ = 36 hours
  - At 25 °C, DNA $t_{1/2}$ = 10 hours
  - At 25 °C, CELL $t_{1/2}$ = 6 hours

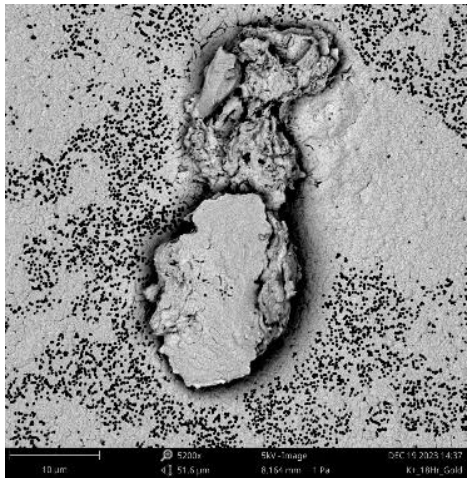
Why did the dissolved DNA degrade slowest?

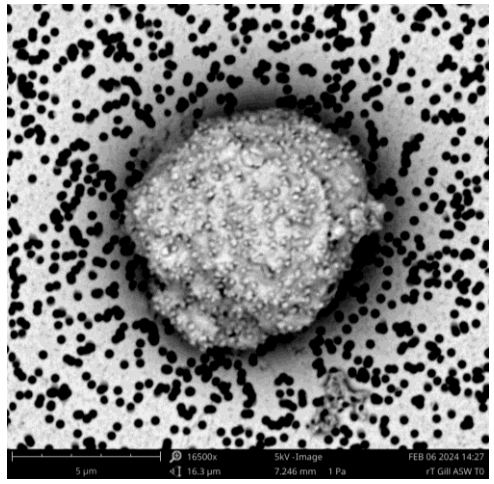
- The FISH and CELL spike-ins were introducing **more organic matter,** which bacteria can readily utilize
- In the dissolved DNA spike-in, the bacteria have **no surface to colonize**
- This may lead to decreased efficiency in DNA degradation and longer decay times

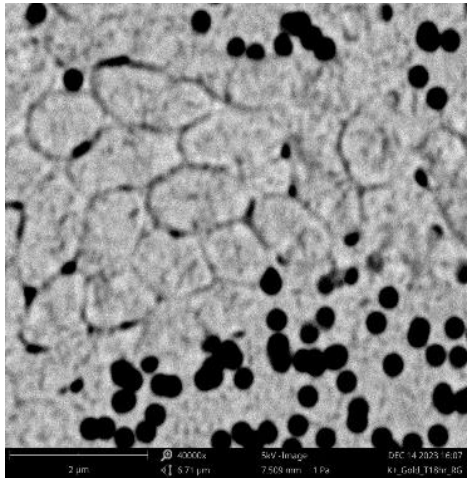
T = 0 hr

T = 24 hr



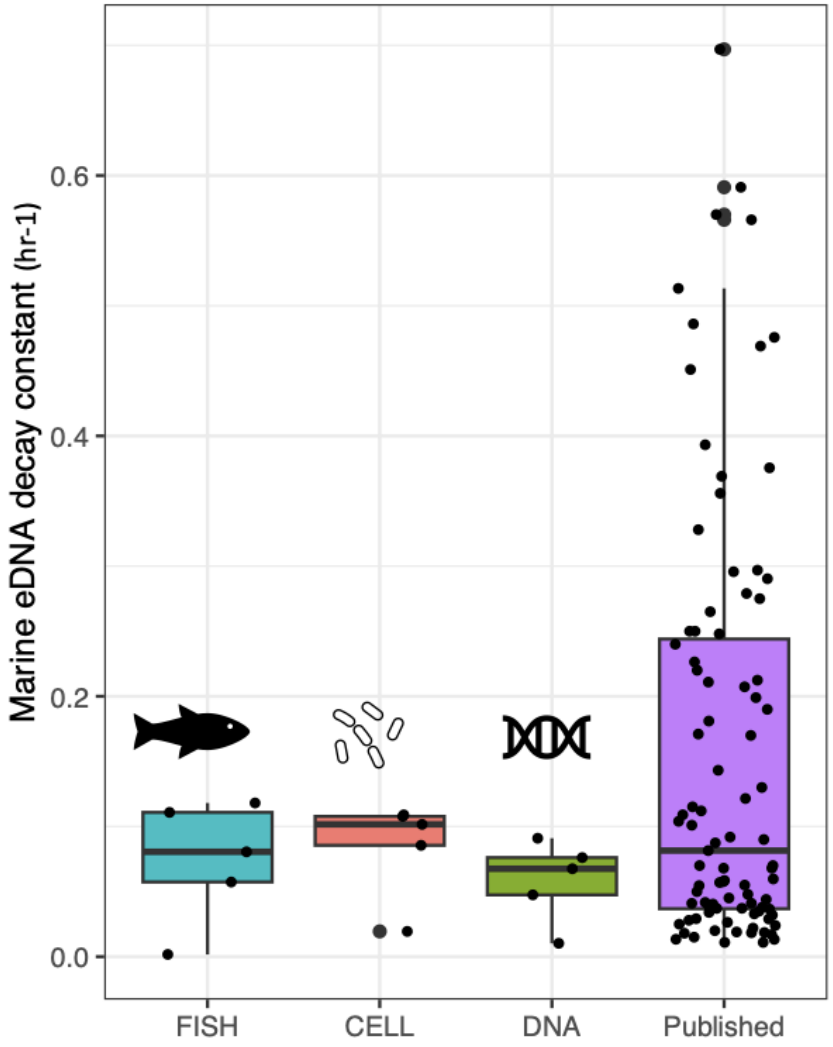






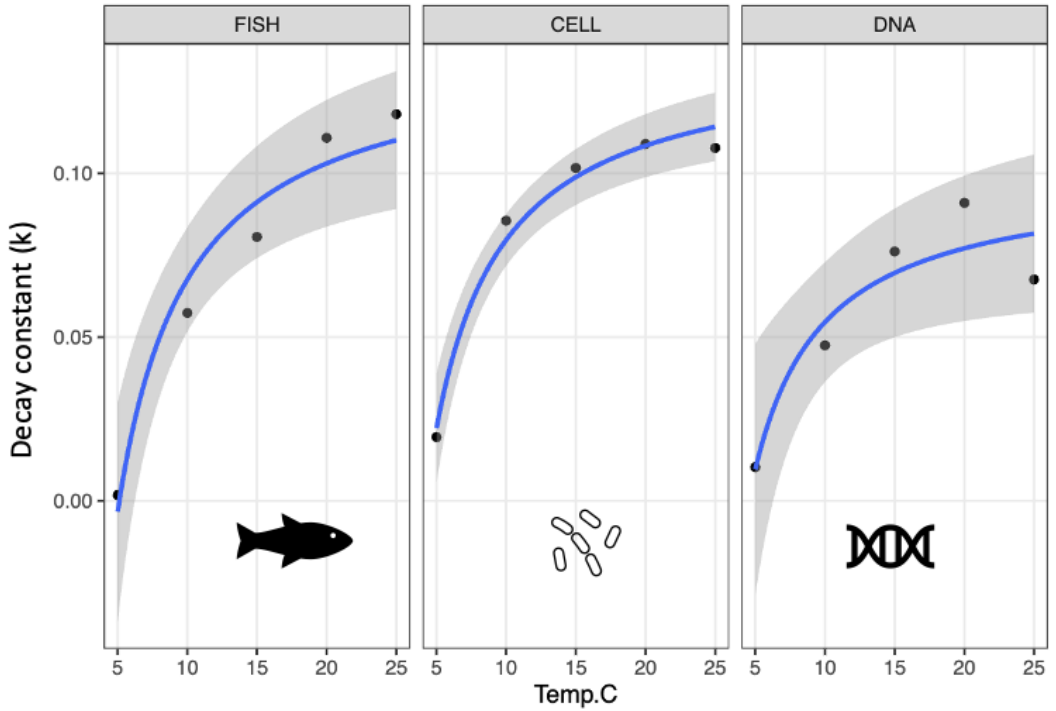
O. mykiss cell line spiked into seawater

eDNA decay rates vary by temperature and state

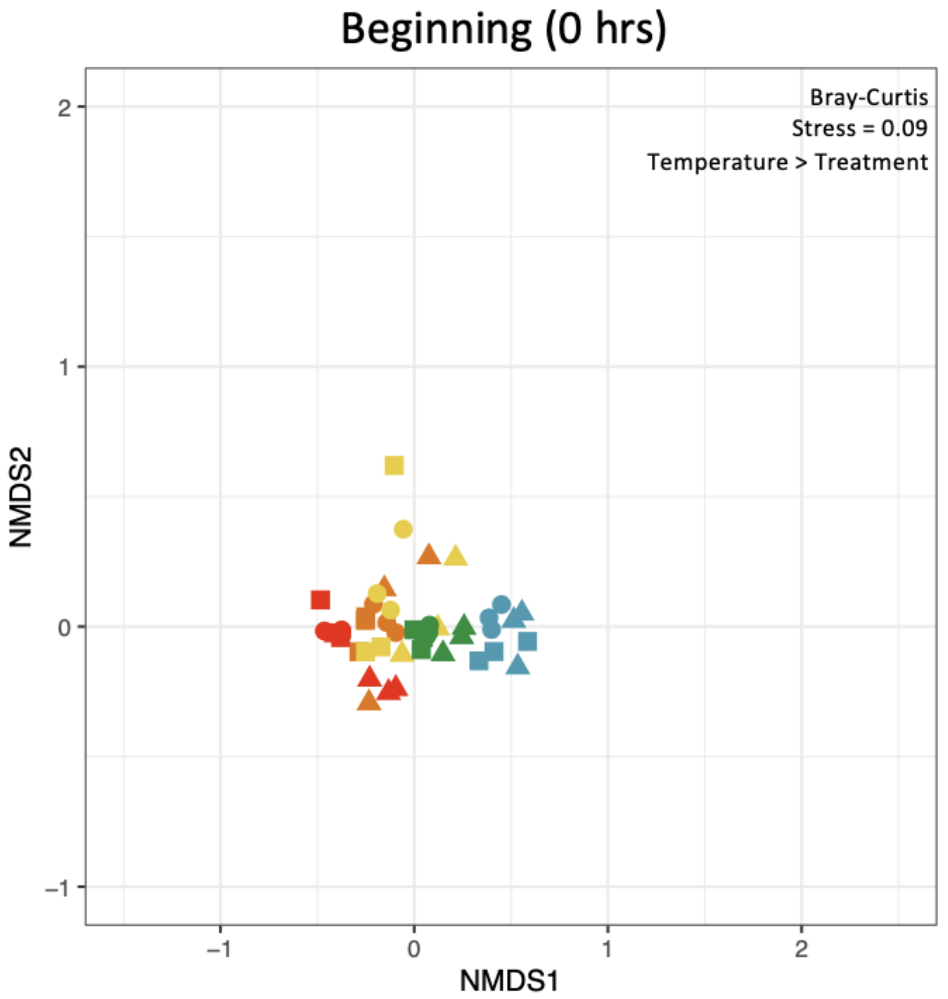


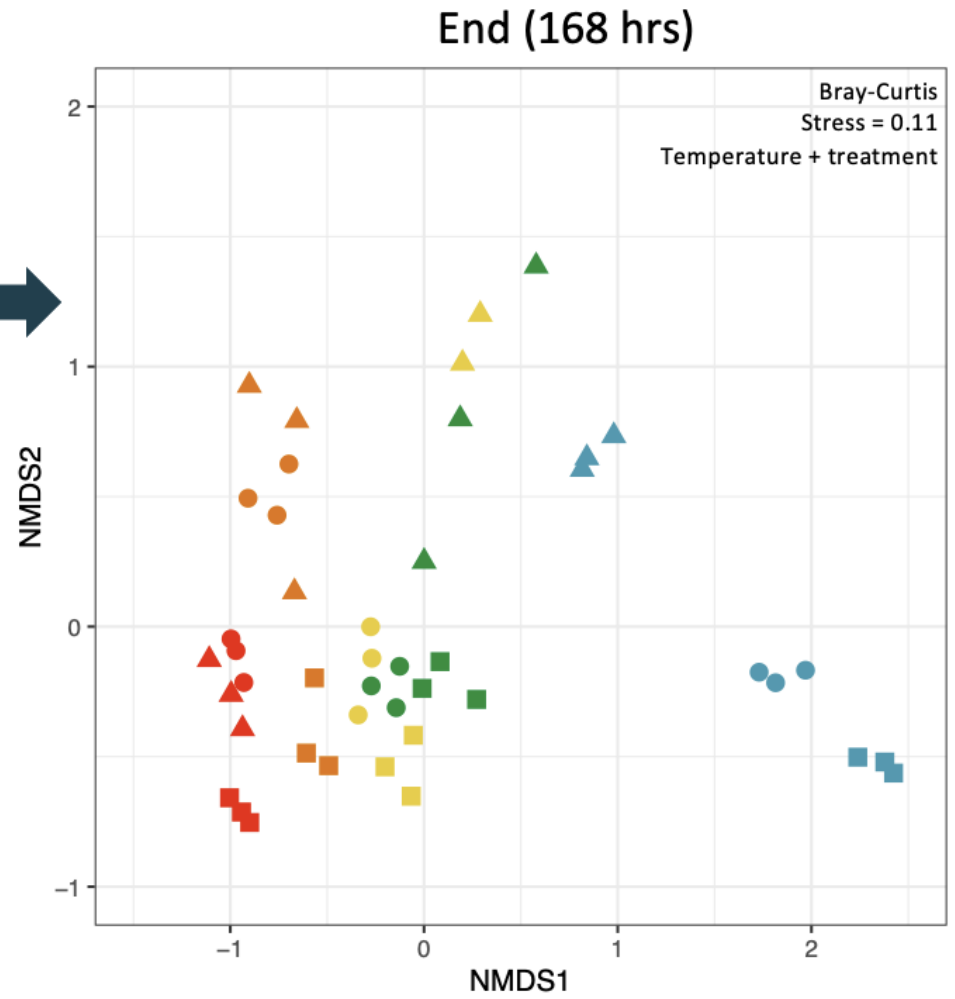
Data from Scriver et al., 2023

- All eDNA states decayed faster at higher temperatures
- "Dissolved" DNA decayed slower than fish and cell DNA



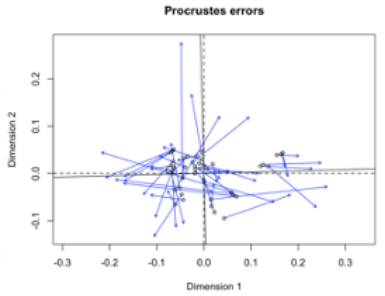
Microbial community changes over time





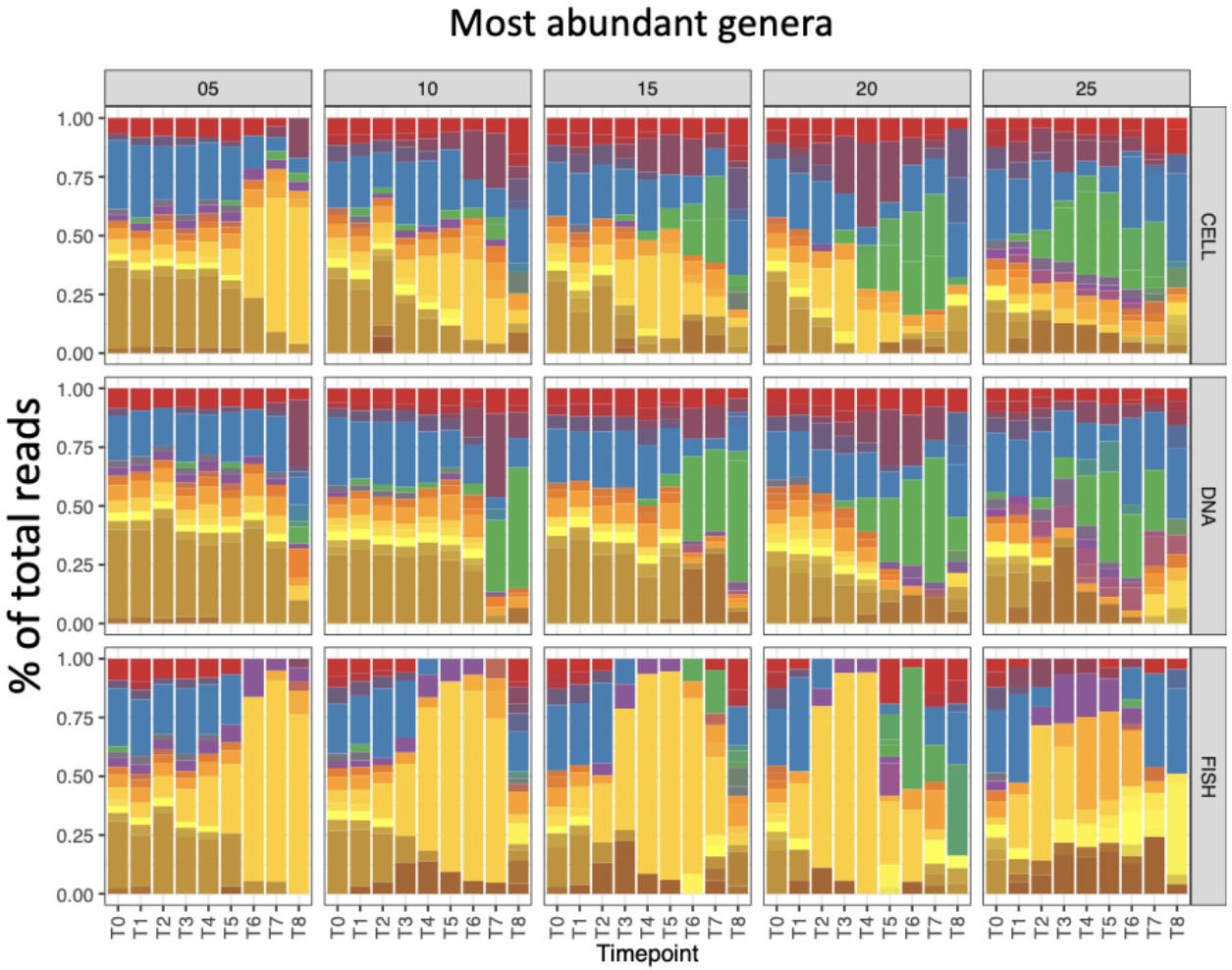
Treatment
- CELL
- DNA
- FISH

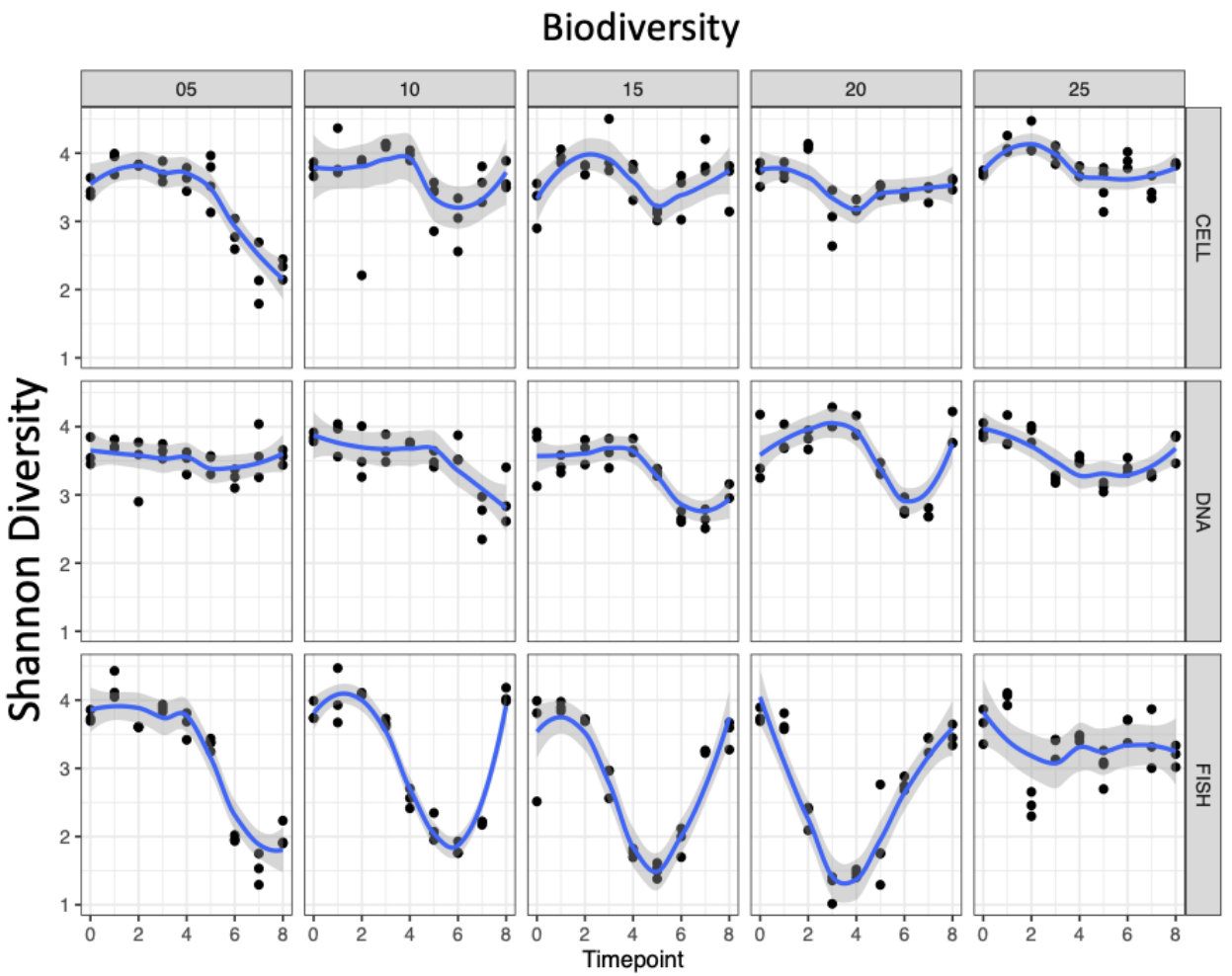
Temp. (°C)
- 05
- 10
- 15
- 20
- 25



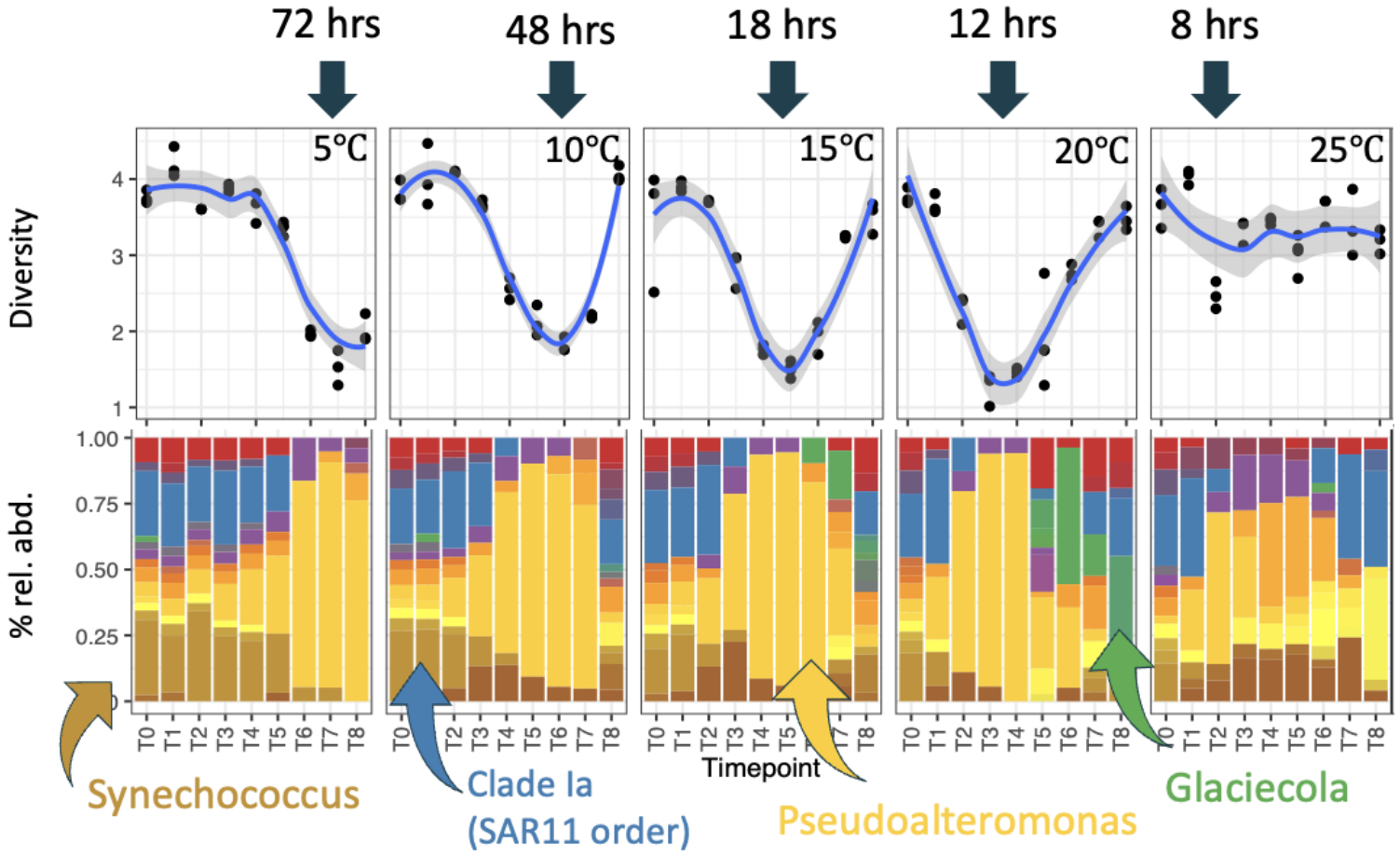
16S bacterial sequencing (515F/806R)

Microbial community changes over time



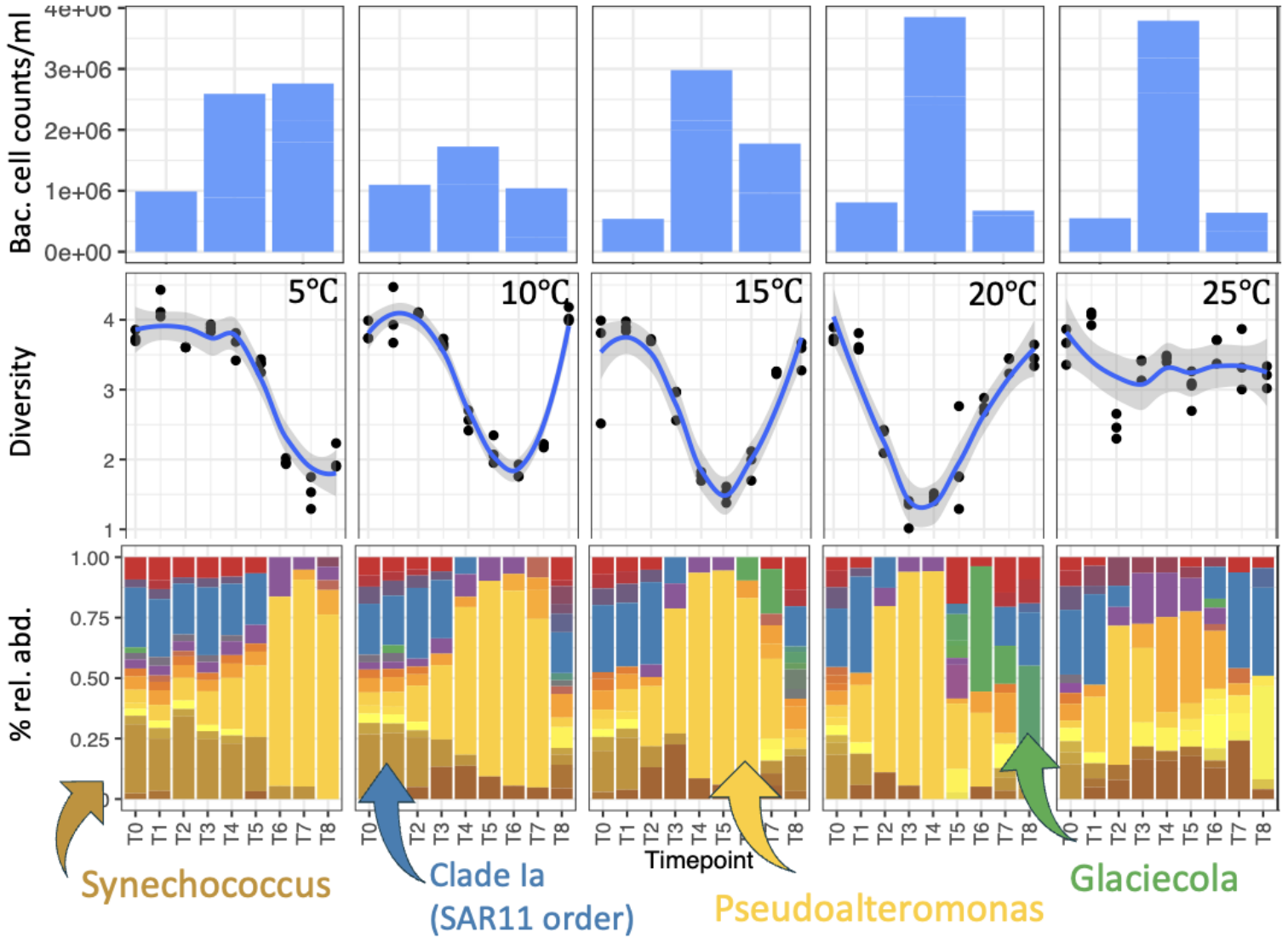


Microbial community changes over time - FISH



- Species richness drops when ***Pseudoalteromonas*** **dominates** the microbial community
- As temperature increases, the *Pseudoalteromonas* dominance occurs at **earlier timepoints**
- This **boom/bust cycle** was also apparent in bacterial cell counts

Microbial community changes over time - FISH



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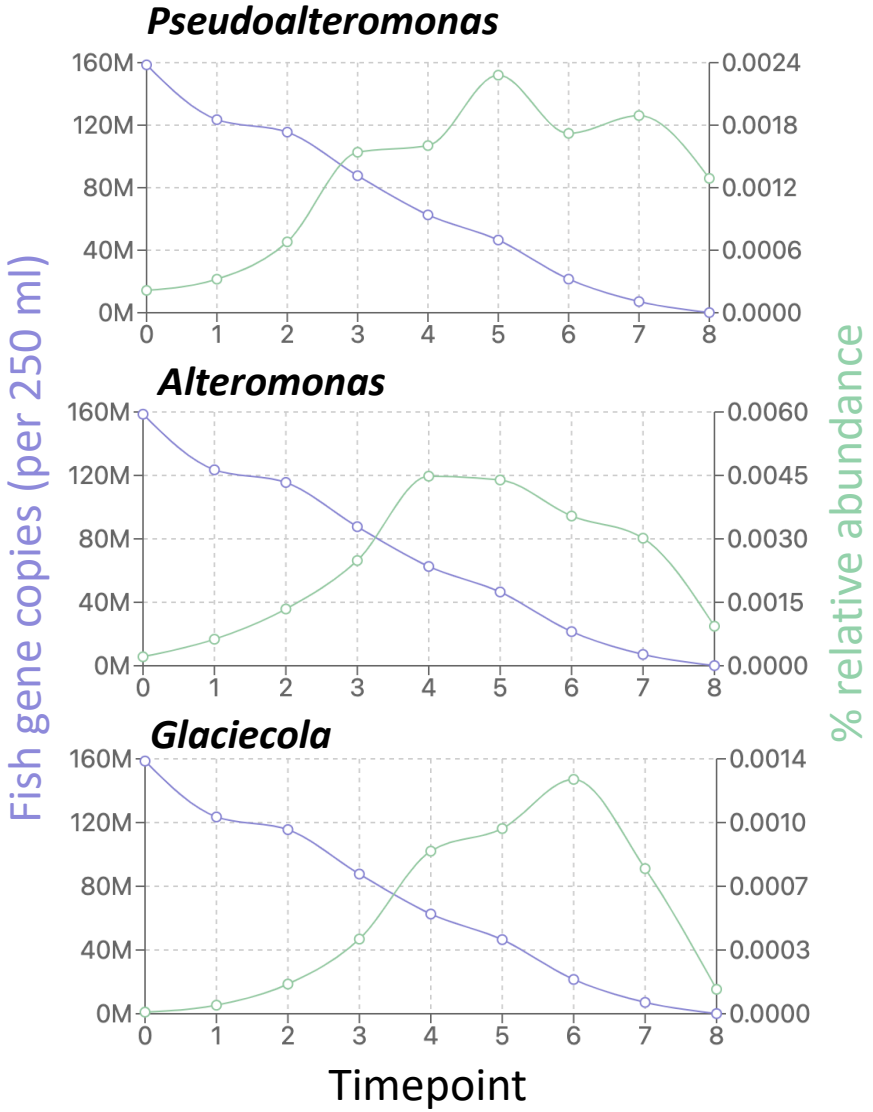
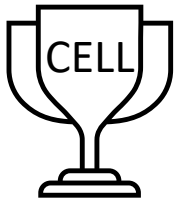
Putative marine eDNA degraders



- Known for producing DNases that can break down extracellular DNA in marine environments
- **Often found in particle-attached communities**

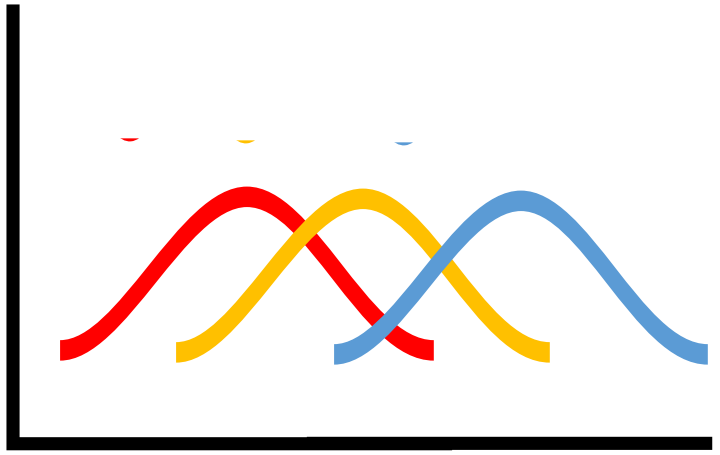


- Produces various hydrolytic enzymes and can nucleases for phosphorus acquisition
- Often found in **marine aggregates** where extracellular DNA is abundant

- Adapted to utilizing various **dissolved organic matter** in marine environments
- Can produce extracellular enzymes for substrate degradation

Conclusions

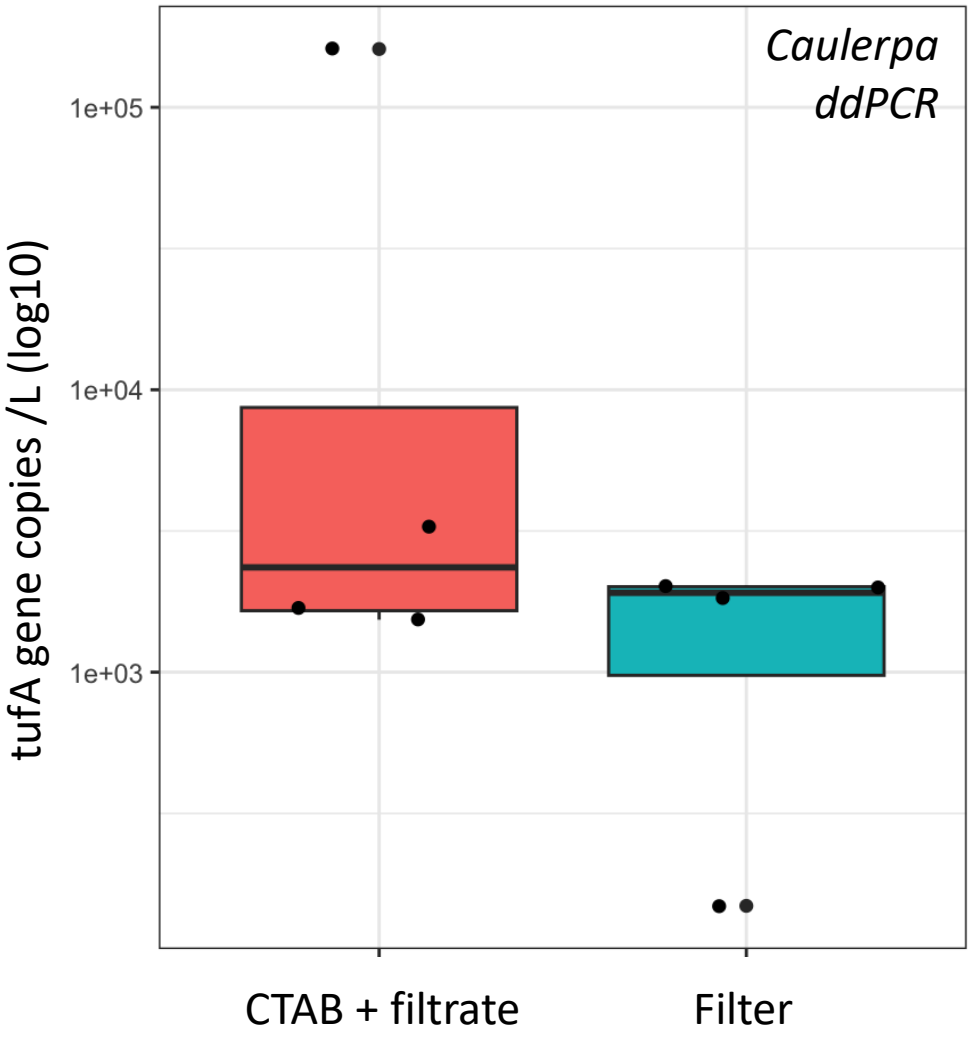
Bacteria exhibited a boom-and-bust cycle of eDNA degradation

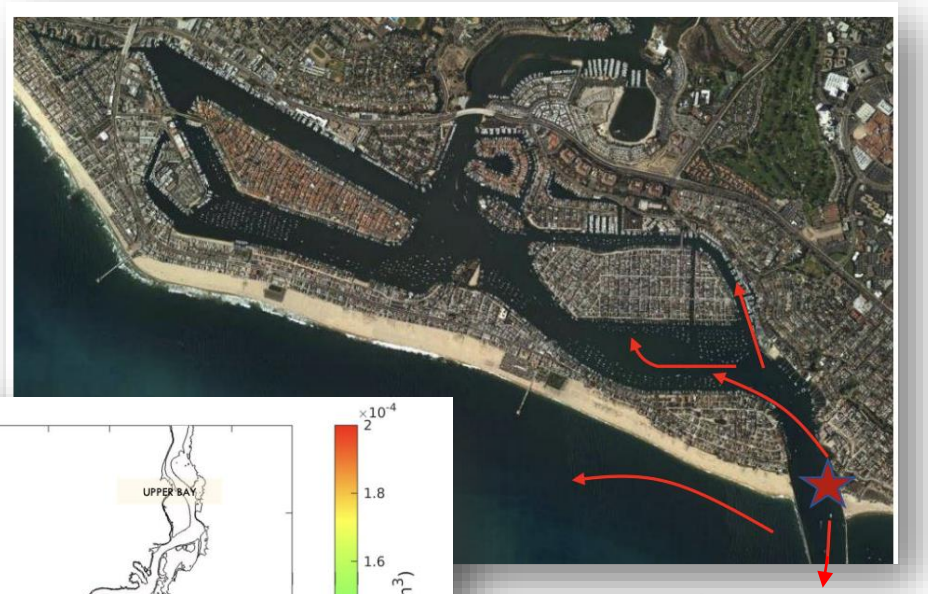


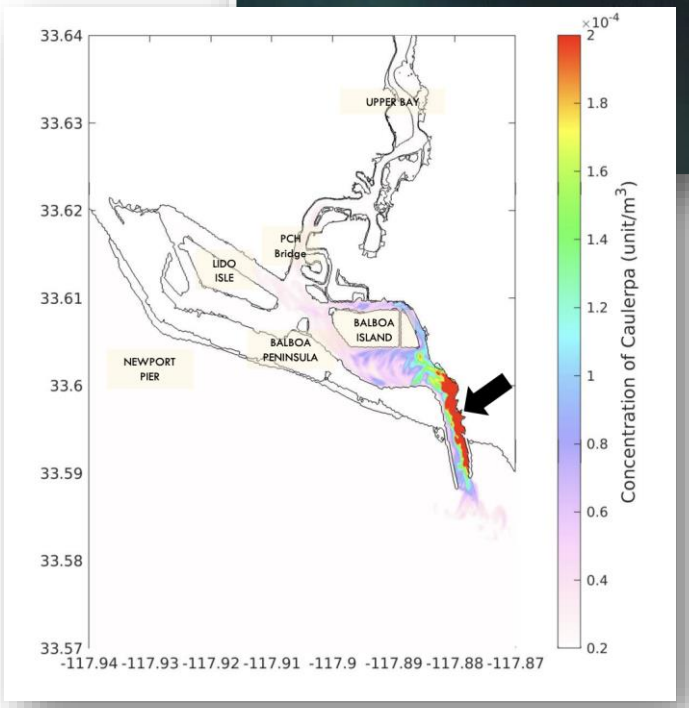
Bacterial “champion” degrader differed by eDNA state

Dissolved eDNA may persist longer than expected

Next steps: integrating decay rates into estuary models







Caulerpa

Thank you! Contact: susannat@sccwrp.org

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SCCWRP

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USC

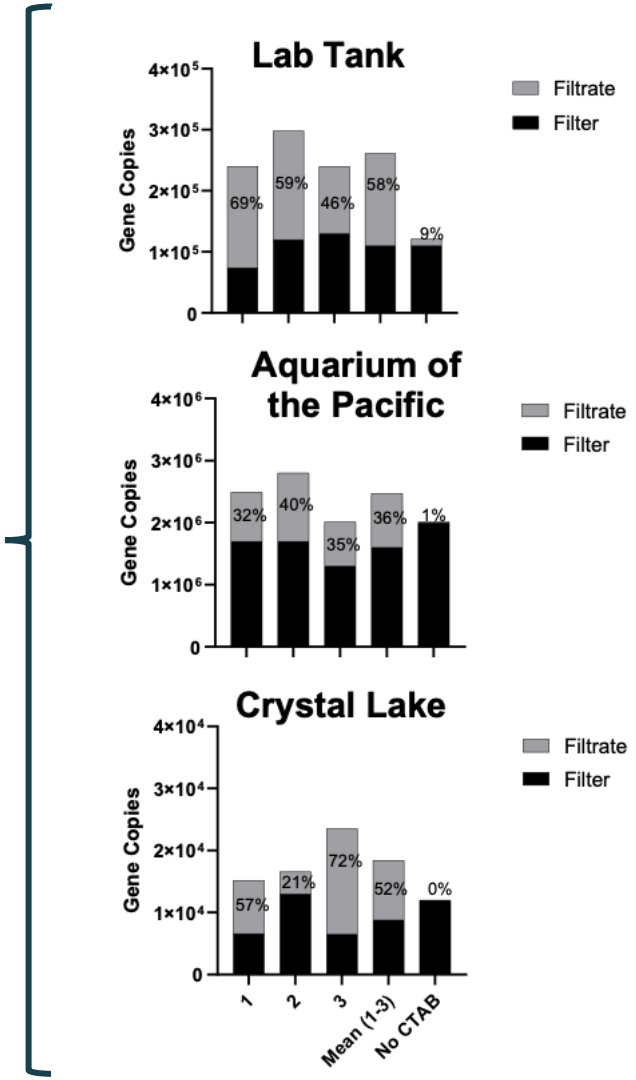
USC Wrigley Institute for Environmental Studies

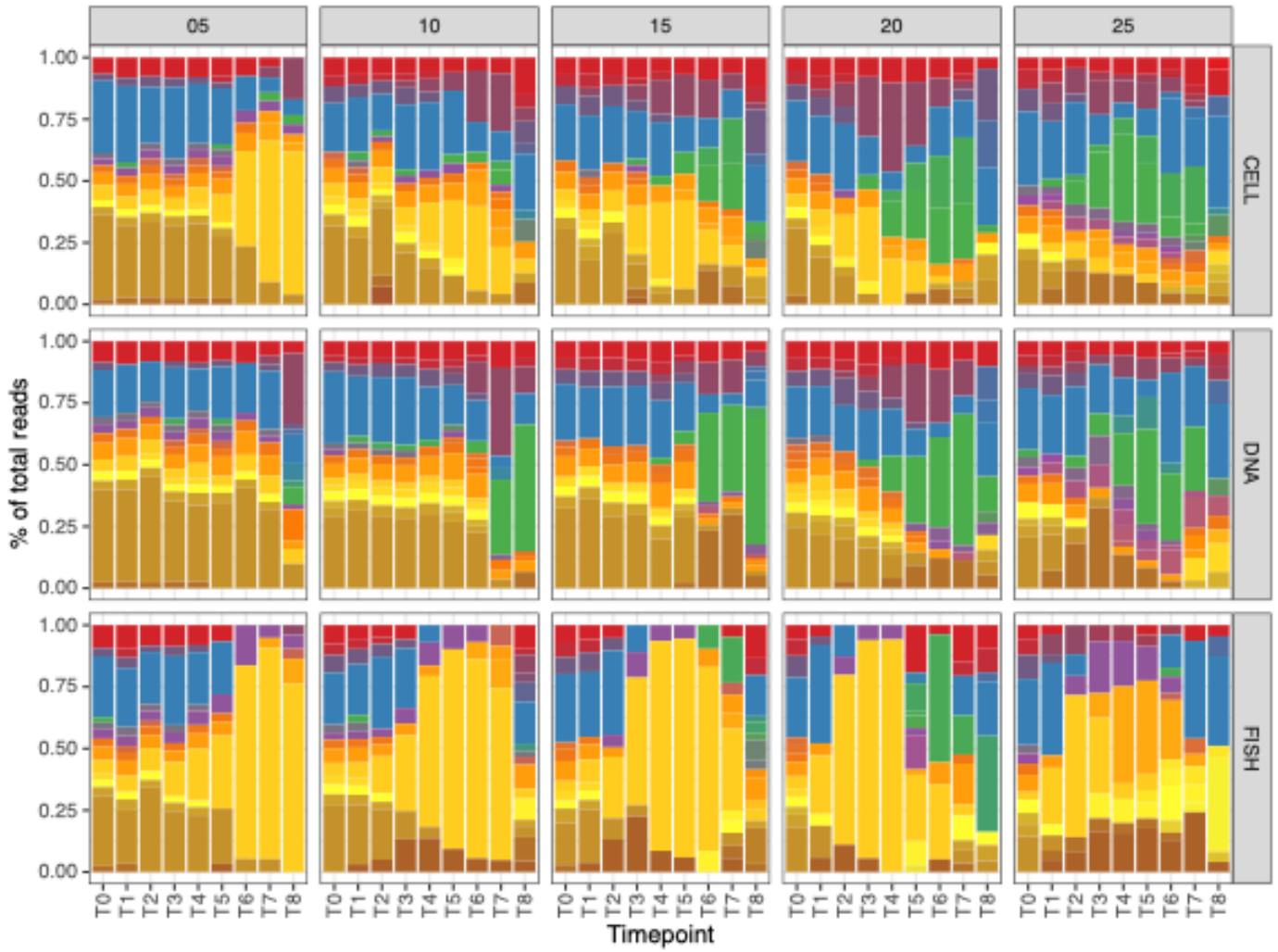
Scripps Institution of Oceanography UCSD

SCCWRP Established 1969

Next steps: what is in the dissolved DNA fraction?

~65% of of Steelhead gene copies were in the dissolved fraction

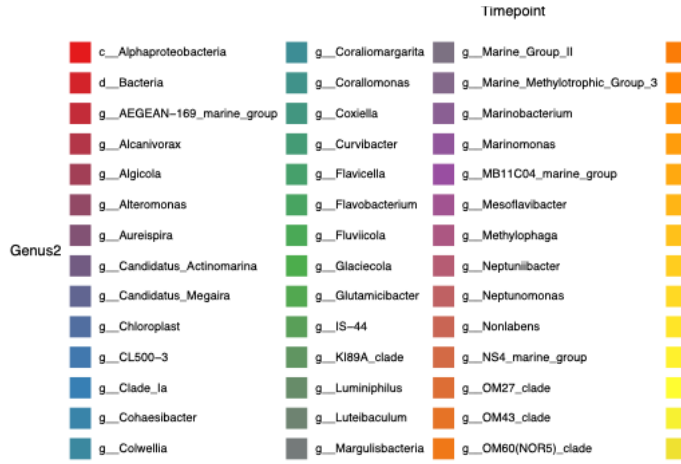




Alteromonas

Glaciecola

Pseudoalteromonas



Catalina mesocosm seawater

	Time (UTC)	Temperature (°C)	Pressure (dbar)	Salinity (PSU)	DO (ml/l)	CHLA (µg/L)	Turbidity (NTU)	Volts	NO3 µM	NO2	PO4	SIL	NH4
	4/28/24 20:27	16.2669	1.84	33.244	5.575	0.713	0.451	11.83	1.76	0.07	0.29	6.3	2.35
	Sea-Bird Scientific Water Quality Monitor (WQM) sensor												
	Wrigley dataset see here												

Sample ID	NO3	NO2	PO4	SIL	NH4	Lab Temperature		Comment
	µmol/L	µmol/L	µmol/L	µmol/L	µmol/L	Celsius		
Catalina SW	1.76	0.07	0.29	6.3	2.35	21.7		