Microbial extracellular enzyme activities in the mesopelagic: New insights, new questions

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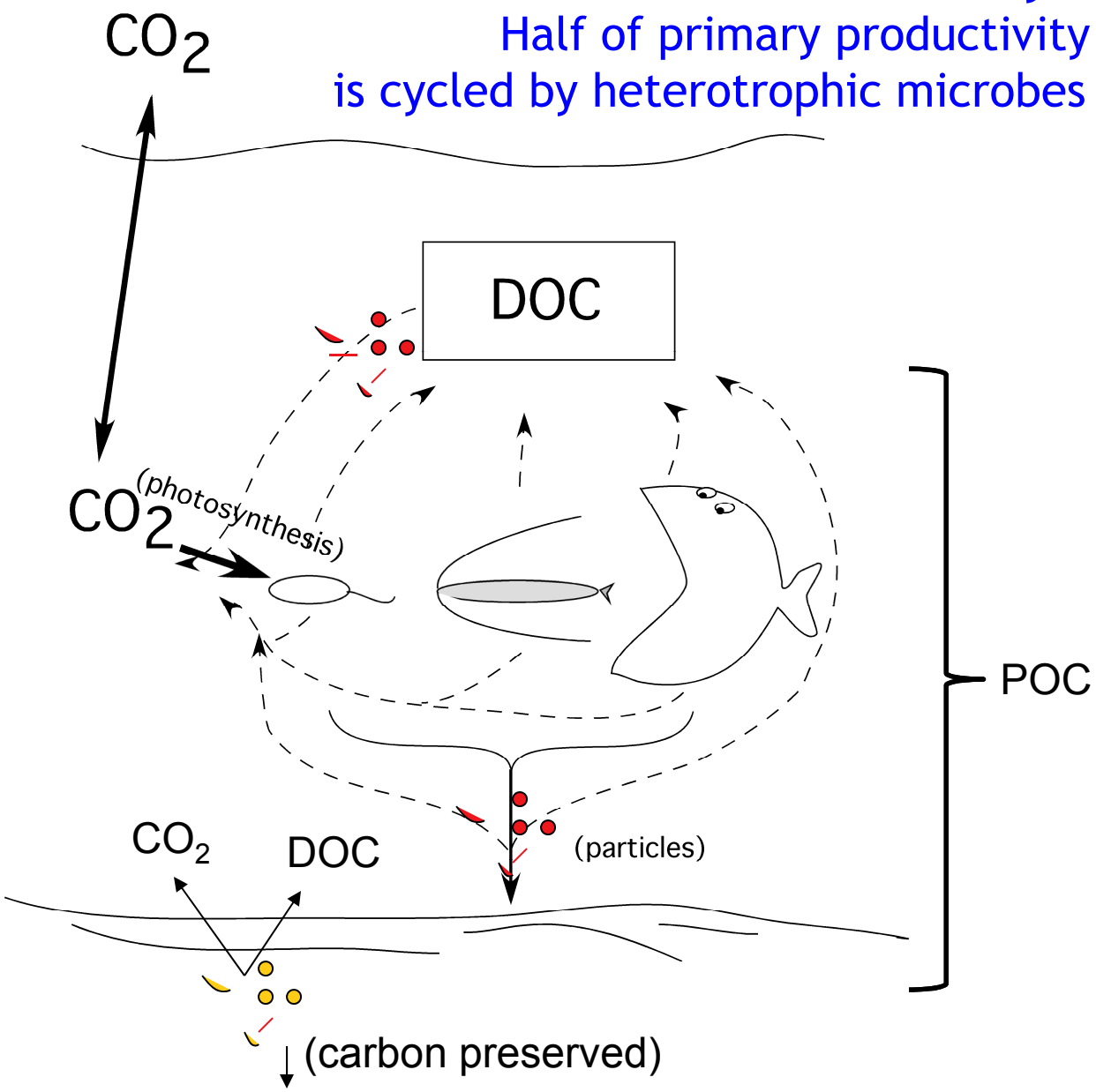
Overview

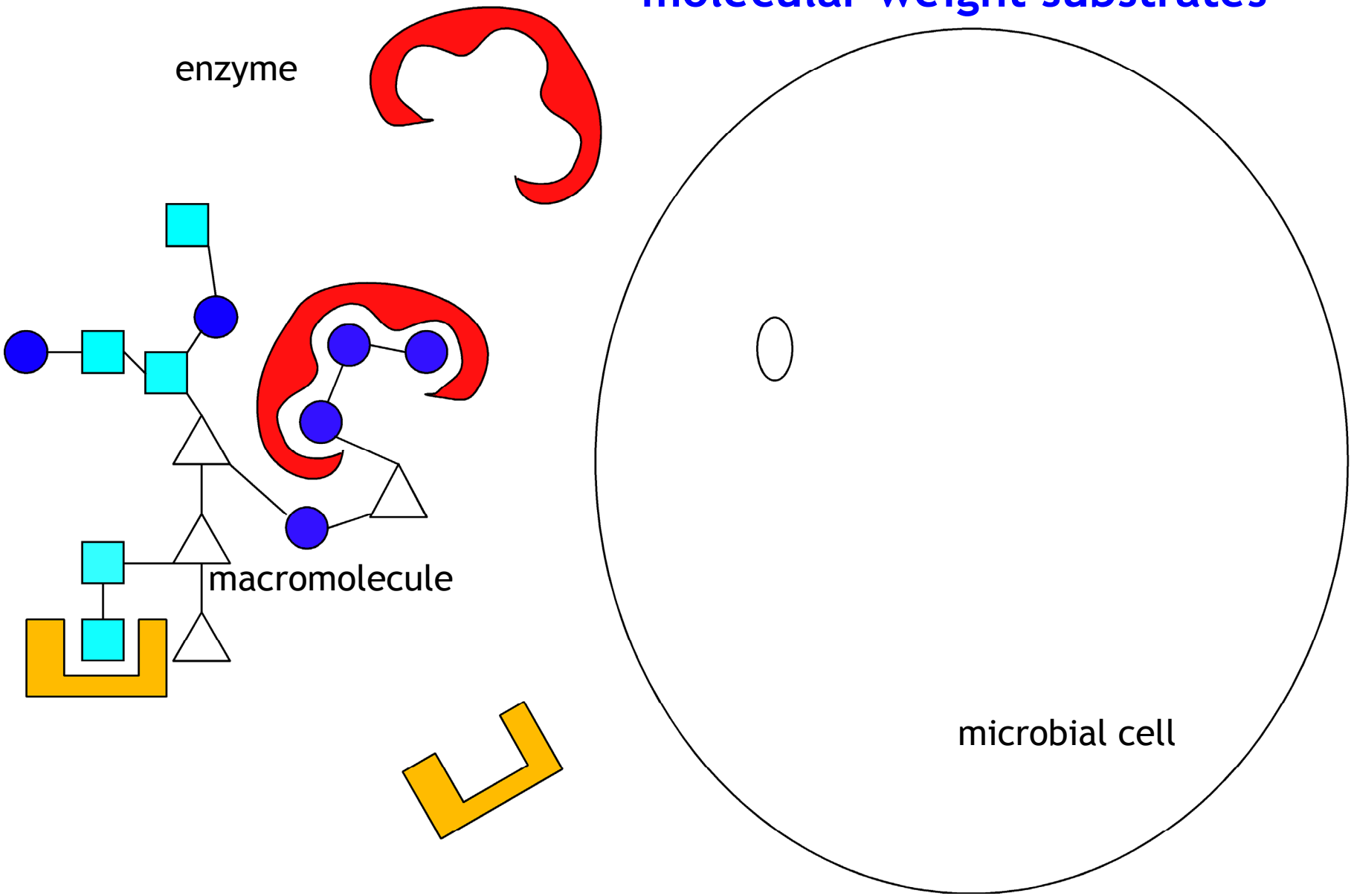
- Extracellular enzymes and the carbon cycle
  - Microbiological and genomic investigations
  - Metagenomic insights into enzyme potential
- Enzyme activities in the deep ocean
  - Water column profiles
  - Depth trends of enzymes and organisms
  - Issues of substrate structure
  - New results from the South Atlantic and Arctic
- Looking forward
  - Pressure effects
  - Particles/aggregates: community “conversations”
  - Sources and lifetimes of extracellular enzymes

Microbial activities drive much of the marine carbon cycle:

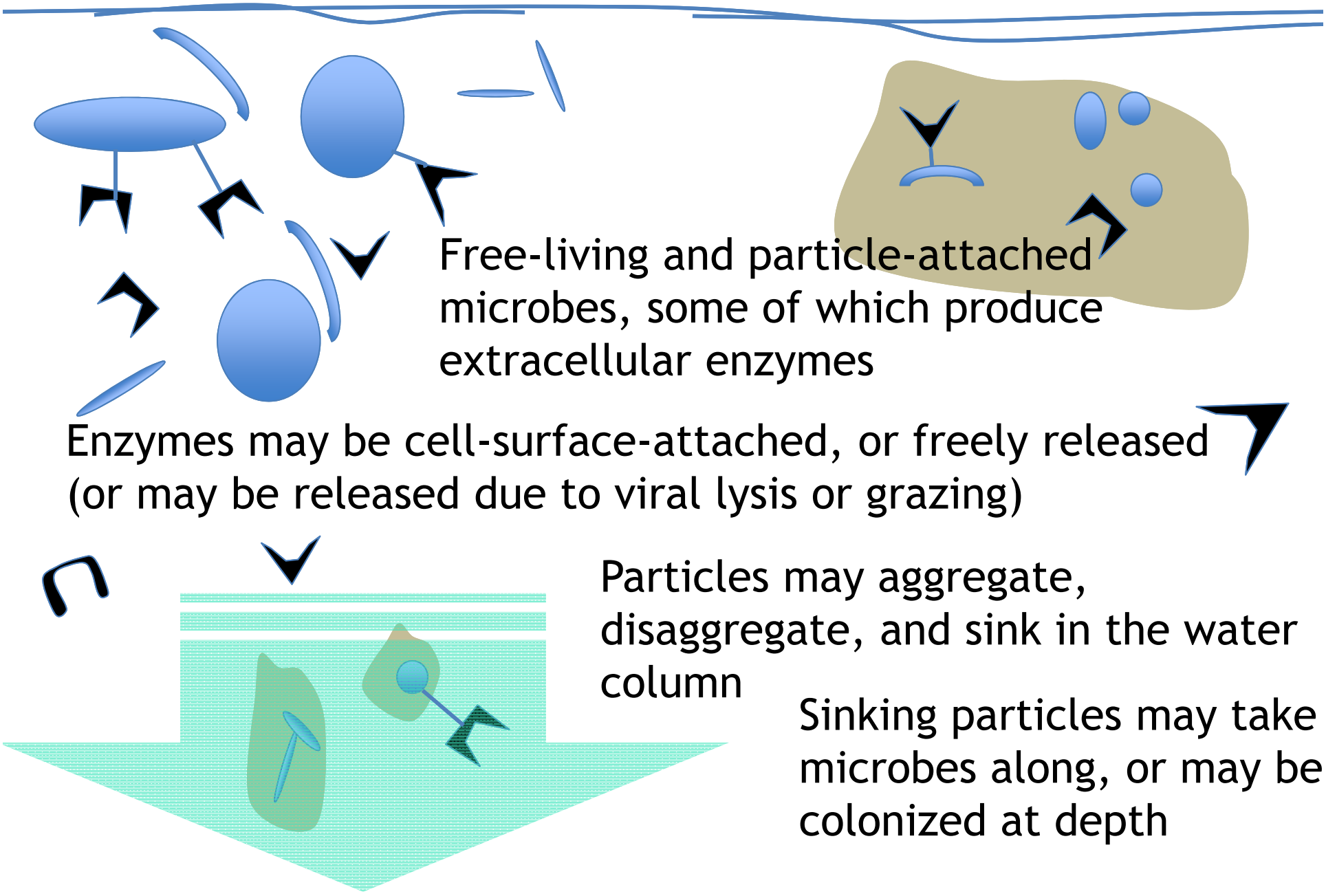
Half of primary productivity is cycled by heterotrophic microbes (Azam 1998)



Microbial extracellular enzymes initiate the remineralization of high molecular weight substrates



A plethora of extracellular enzymes in the ocean



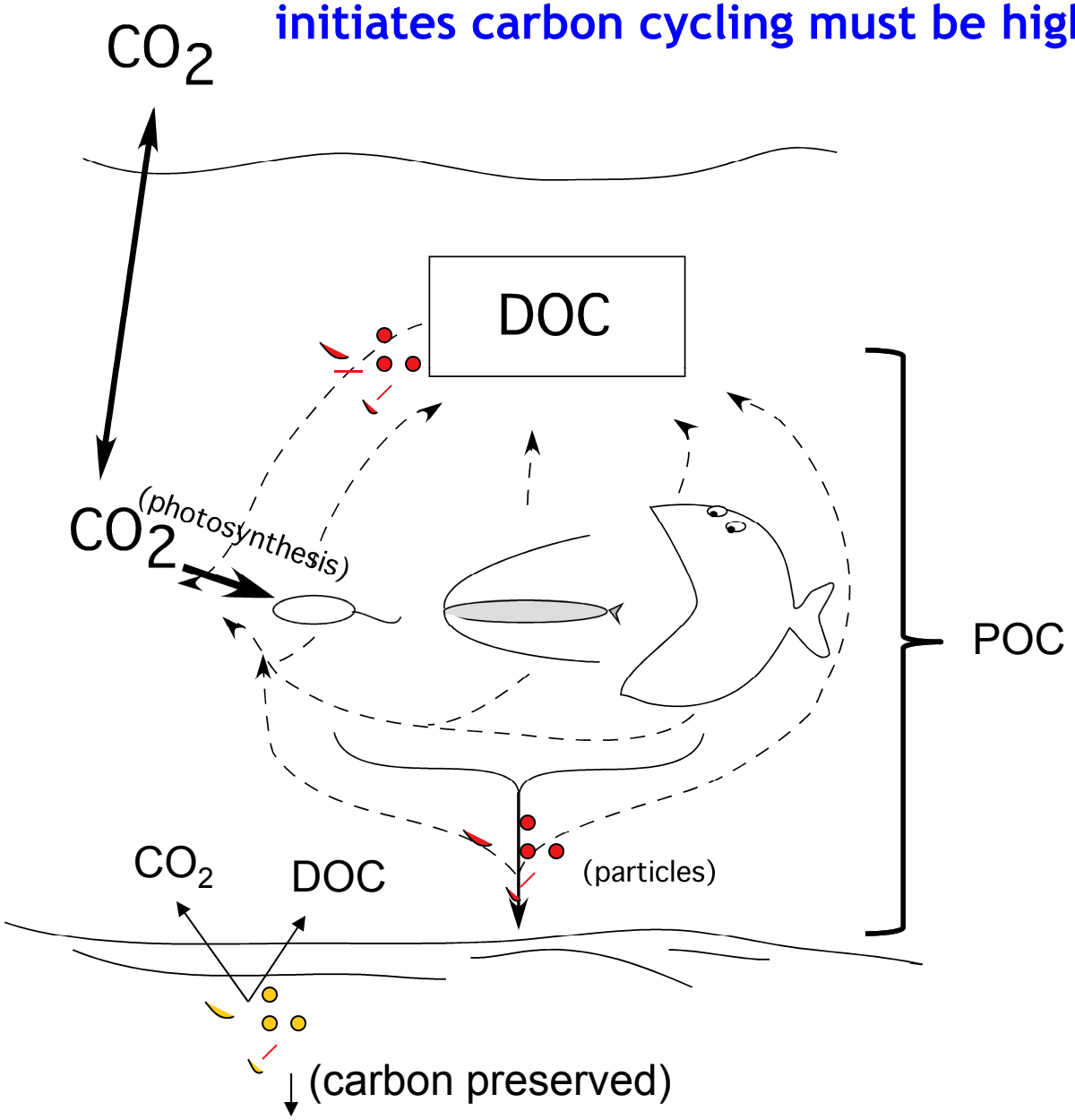
Free-living and particle-attached microbes, some of which produce extracellular enzymes

Enzymes may be cell-surface-attached, or freely released (or may be released due to viral lysis or grazing)

Particles may aggregate, disaggregate, and sink in the water column

Sinking particles may take microbes along, or may be colonized at depth

Most organic matter produced in the ocean is rapidly cycled, so **enzymatic catalysis that initiates carbon cycling must be highly efficient**



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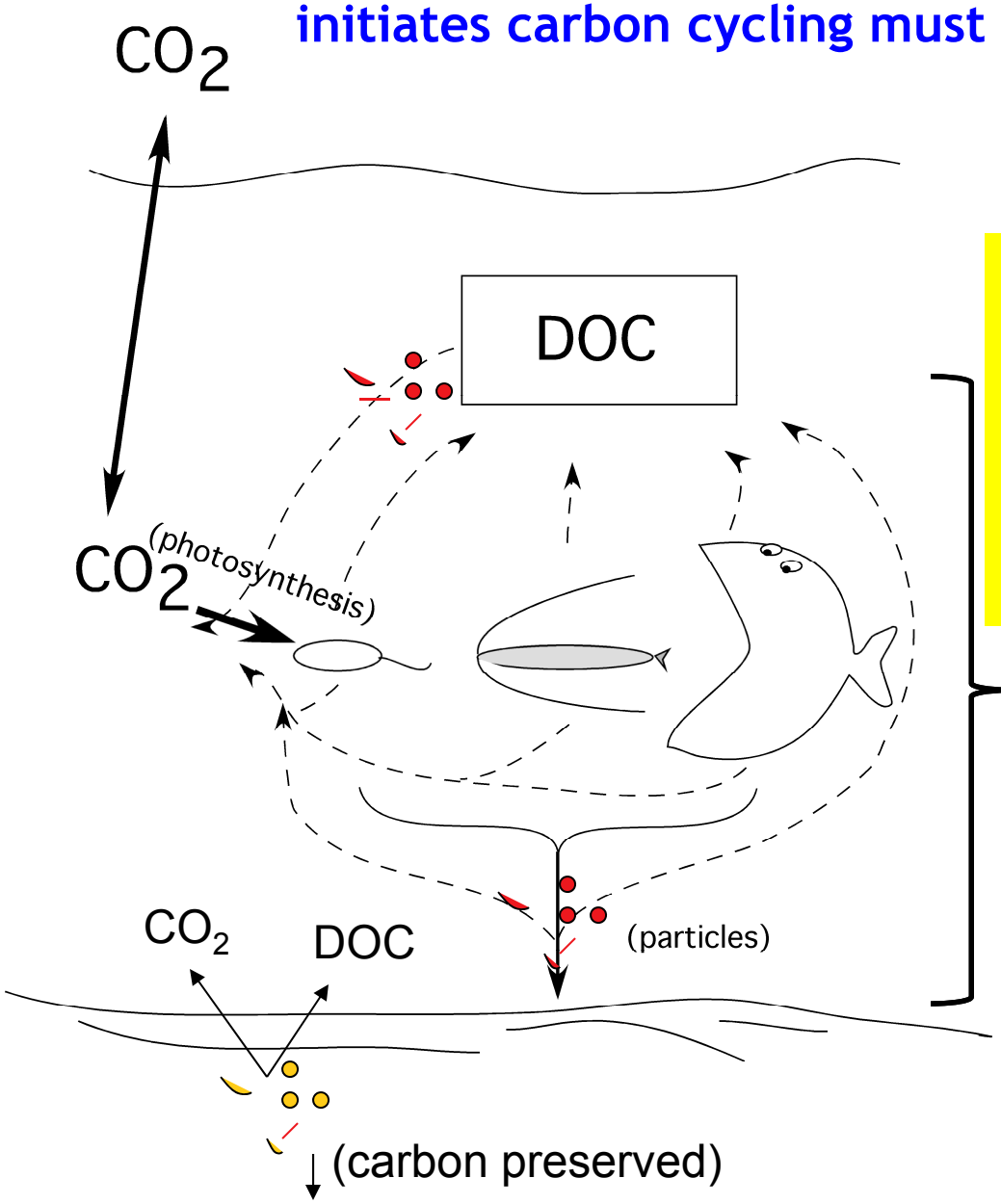
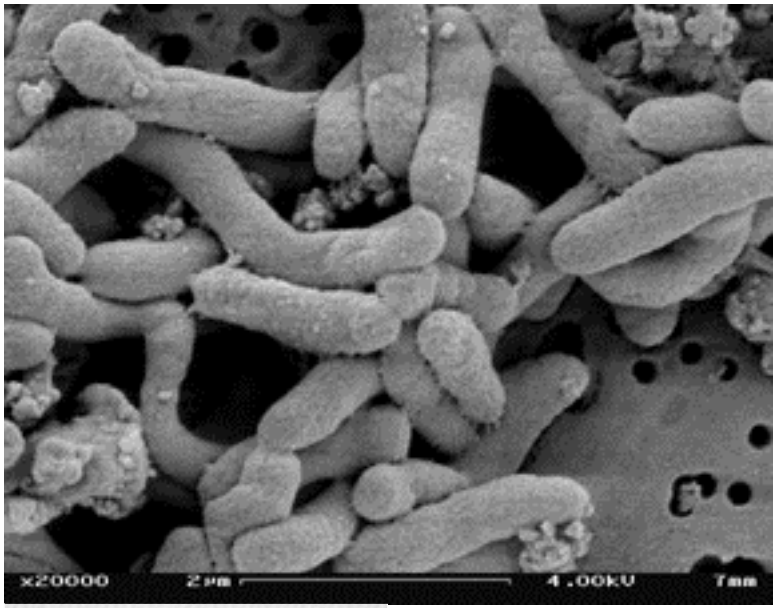
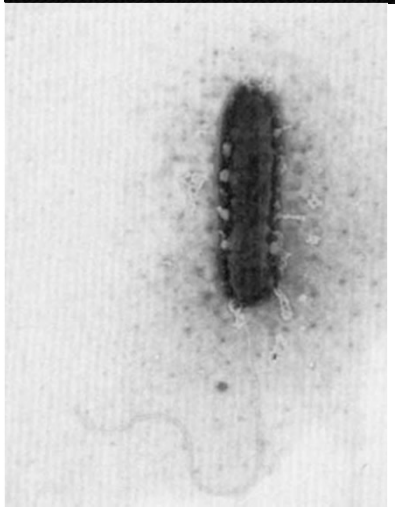


…but specific information about microbial enzymes and their activities focuses mostly on the surface ocean

Microbiological, genomic studies yield information about the enzymatic capabilities and genetic potential of specific organisms



(Carpenter, U.GA.)



(Akagawa-Matsushita et al. 1992)

Pseudoalteromonas atlantica
(gamma Proteobacteria)

-widely distributed in marine systems (water column; attached to surfaces)

-motile

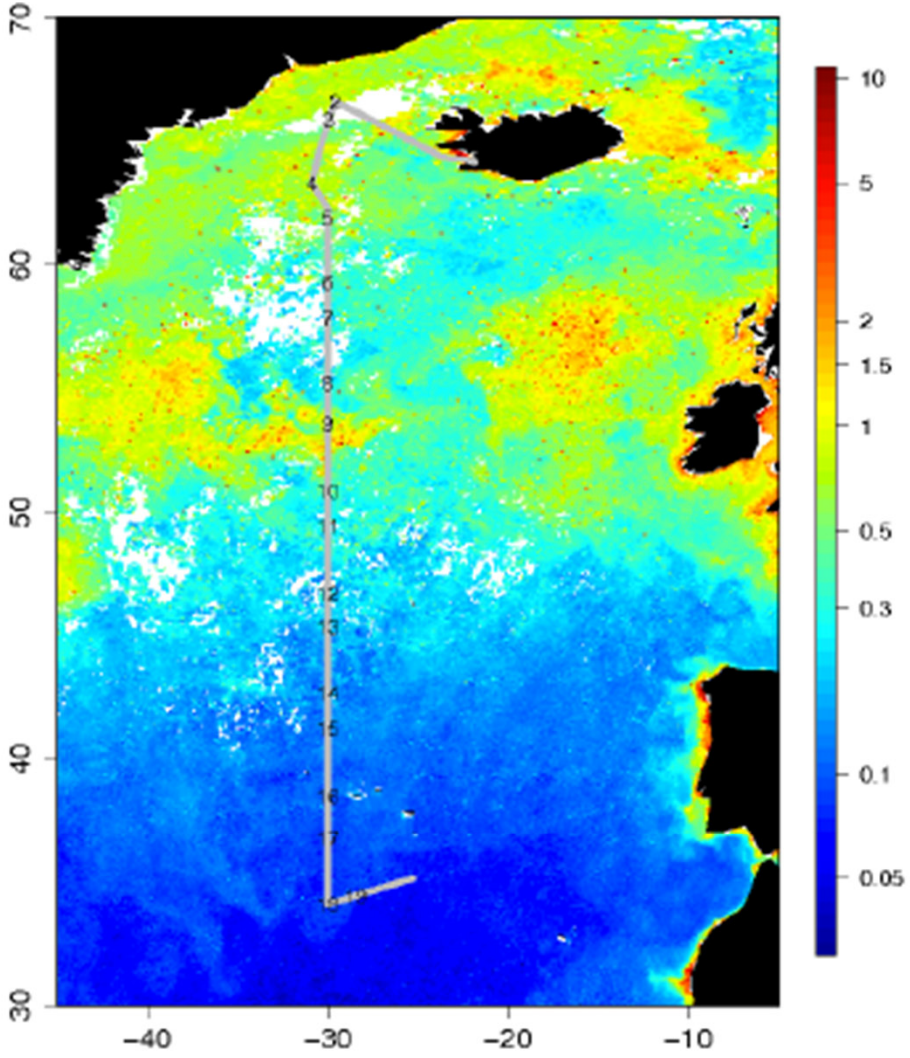
-forms biofilms

-hydrolyzes starch, agar, alginate, carageenan, and a variety of other high molecular weight substrates

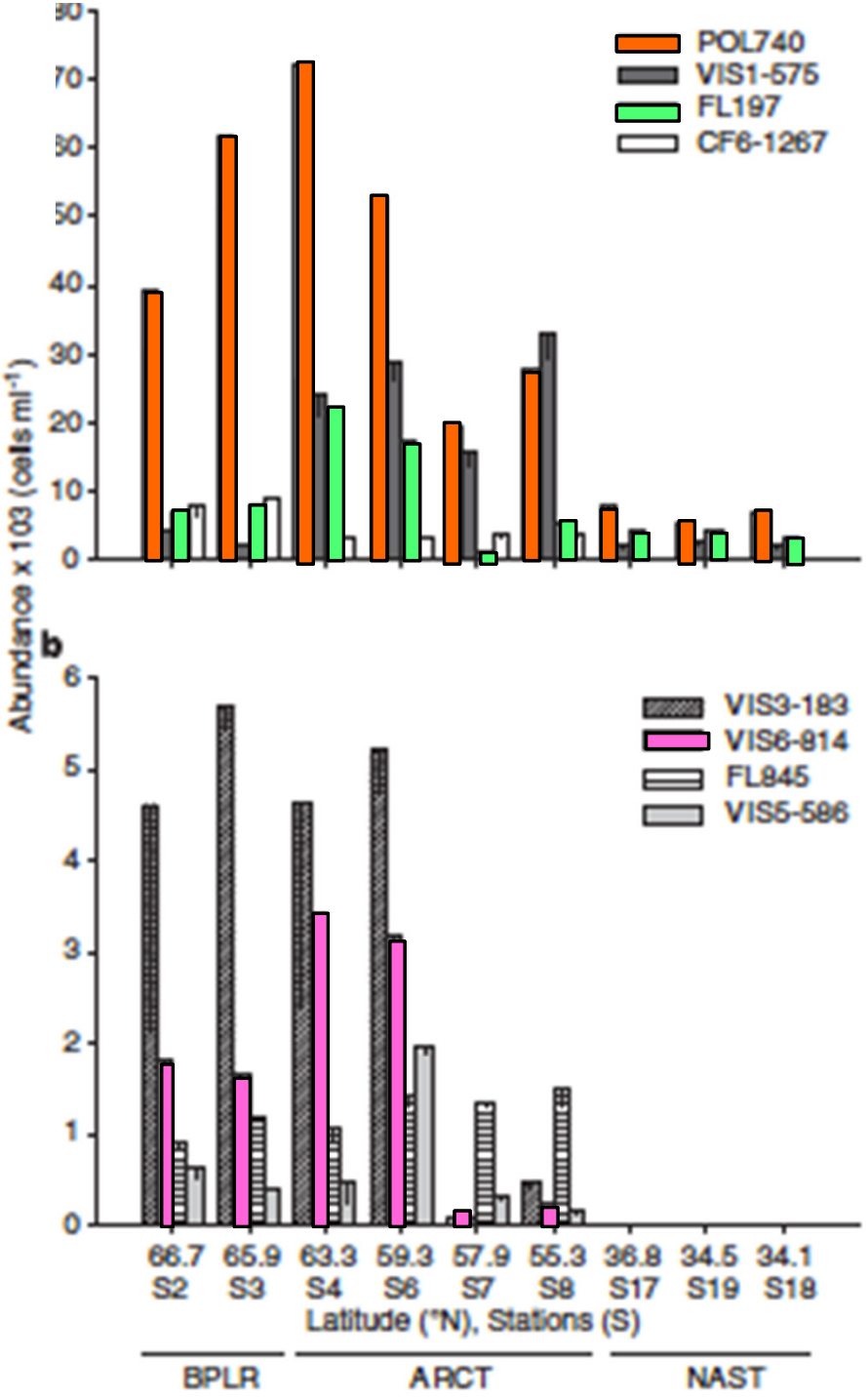
-genome completely sequenced (2006)

Spatial patterns:

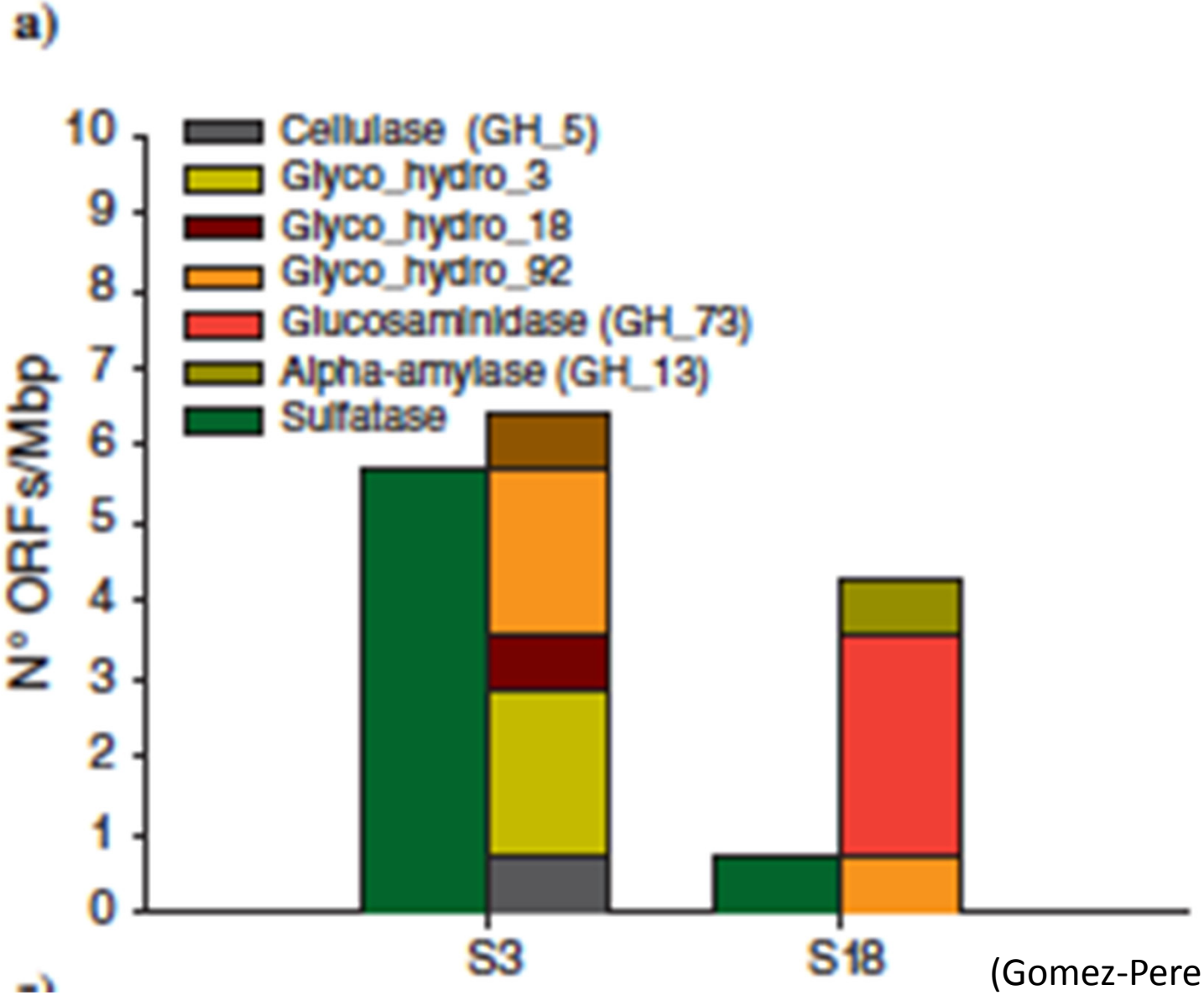
Differences in distributions of members of the *Flavobacteria*…



(Gomez-Pereira et al. 2010)



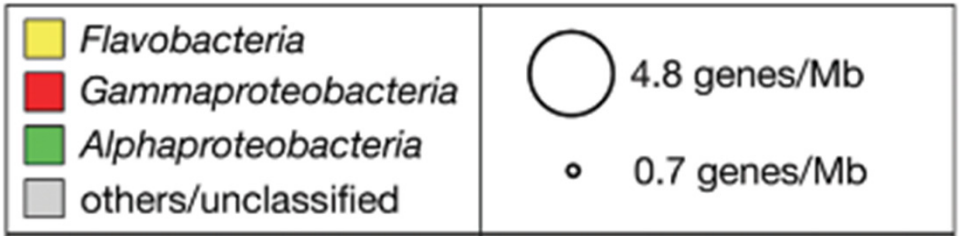
… correlate with differences in their genes for extracellular enzymes



(Gomez-Pereira et al. 2012)

Temporal patterns:

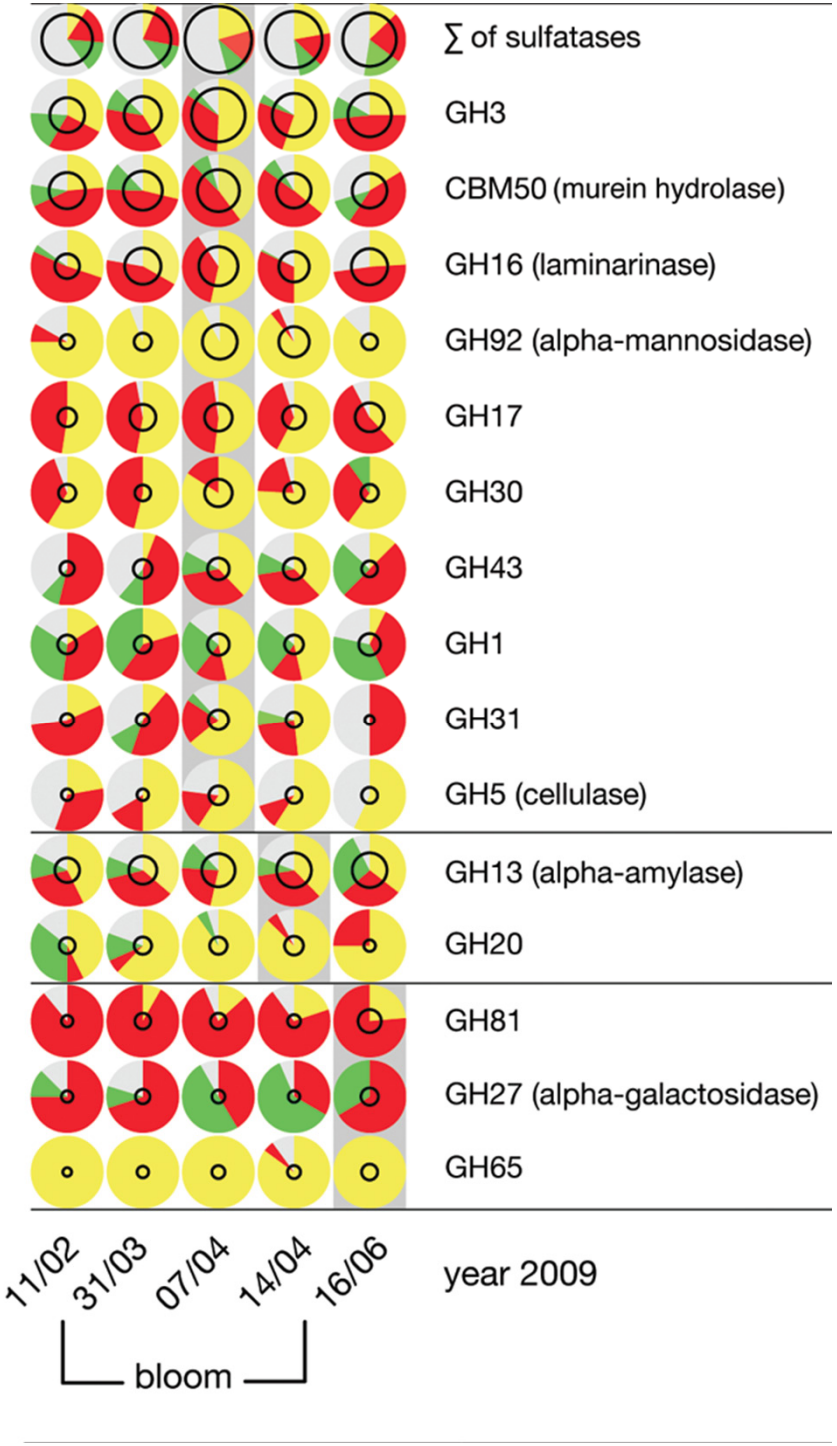
Metagenomic documentation of shifts in abundance, source of genes for polysaccharide hydrolases during a North Atlantic Bloom



-Abundance of genes corresponding to hydrolytic enzymes changed through the course of the bloom

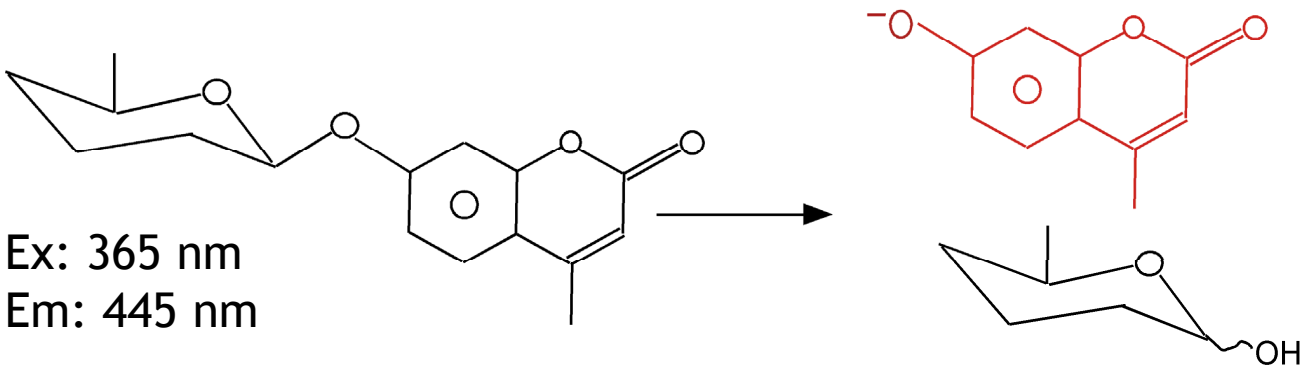
-Organisms contributing these genes also changed

(Teeling et al. 2012)



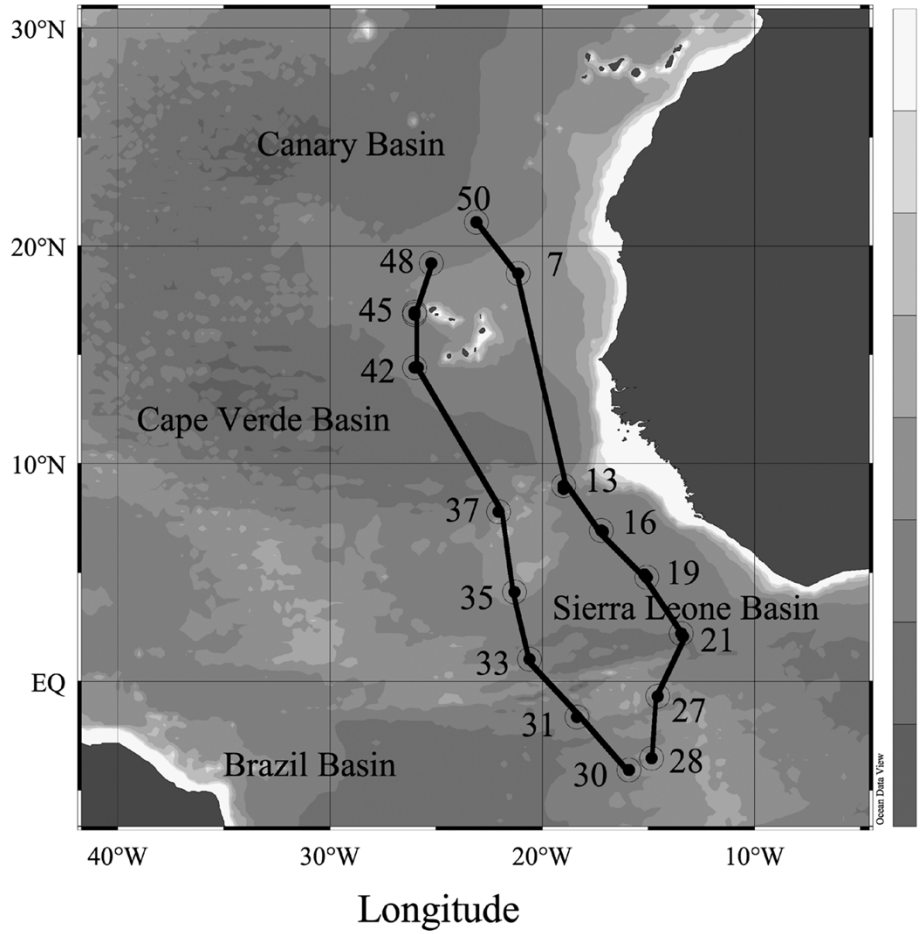
What about measurements of activity?

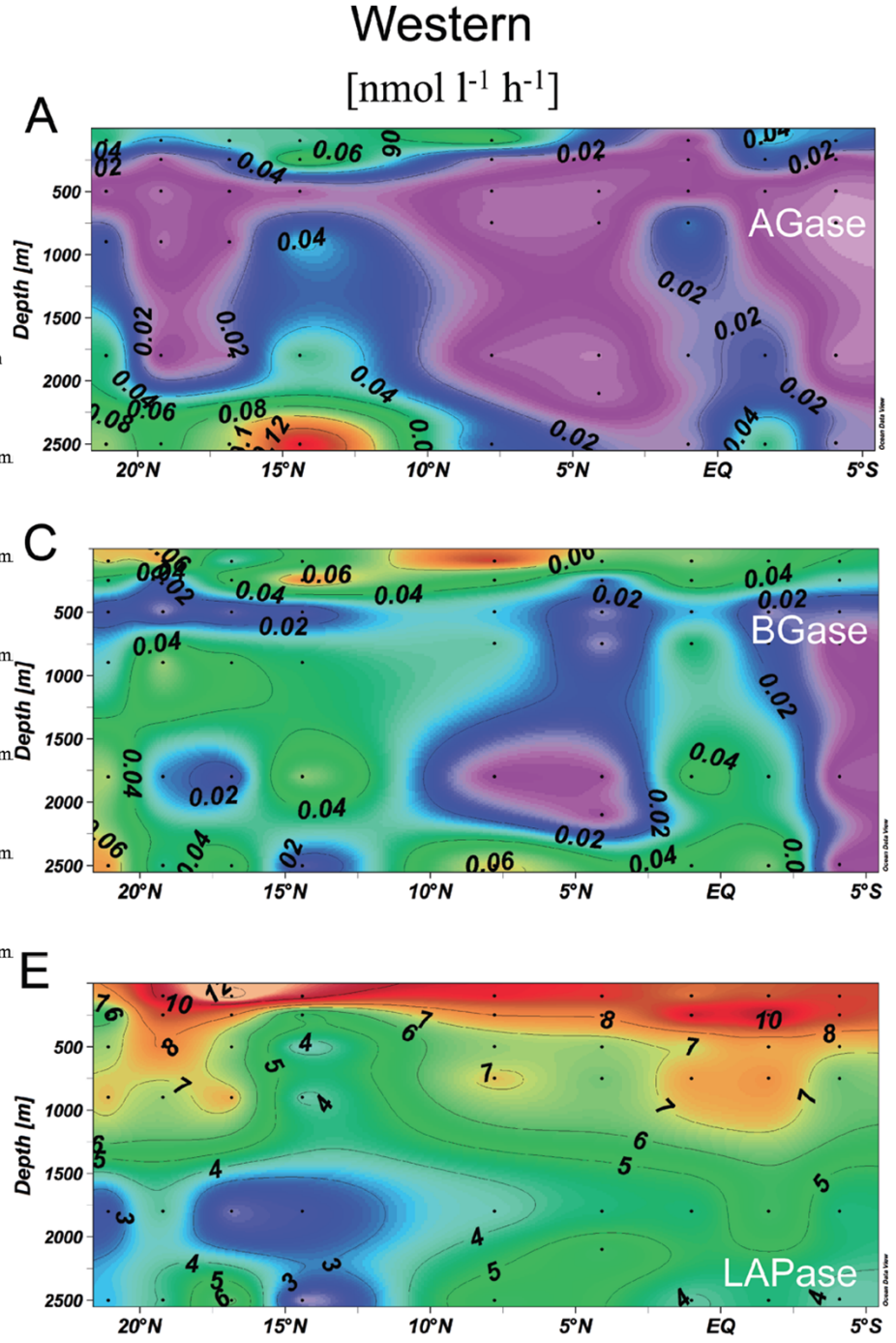
Standard methods to measure enzyme activities in field samples:



- Rapid measurements
- Widely used in environmental research
- Typically use α-/ β-glucose and leucine to represent polysaccharide and protein hydrolysis
- Comparatively few studies of meso- and bathypelagic ocean waters

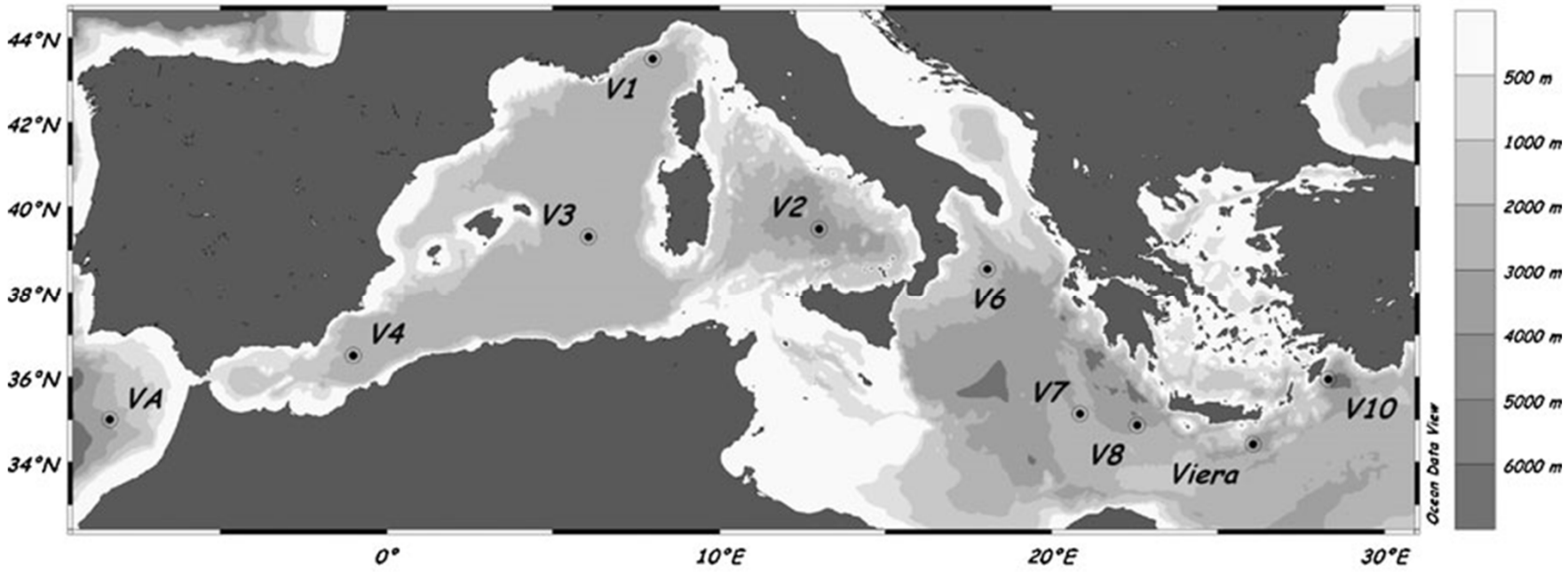
1. Variable activities in the water column: typically decrease with depth

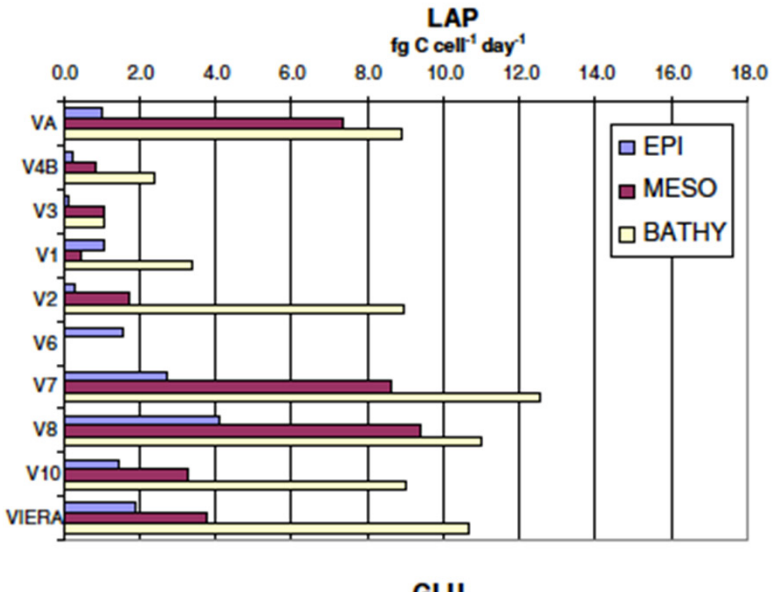


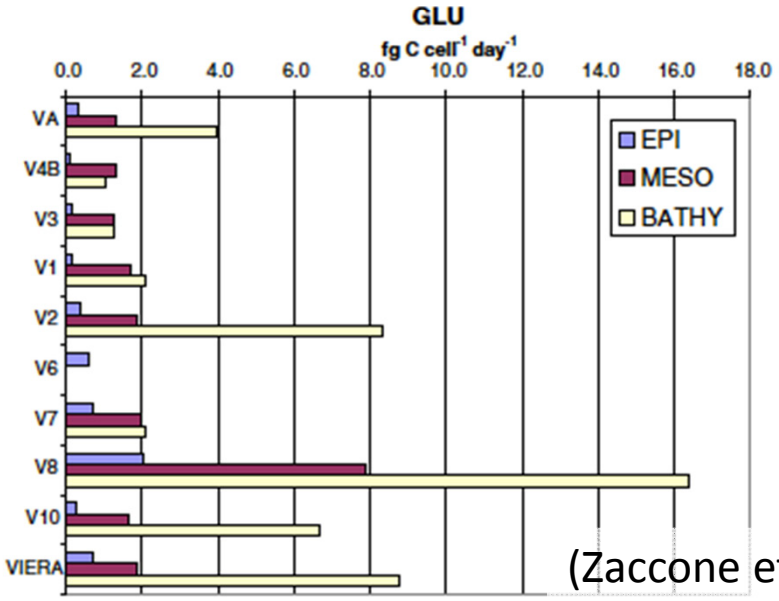


(Baltar et al. 2009)

2. Prokaryotic abundance decreases with depth in the water column, but **enzyme activities often increase on a per-cell basis**

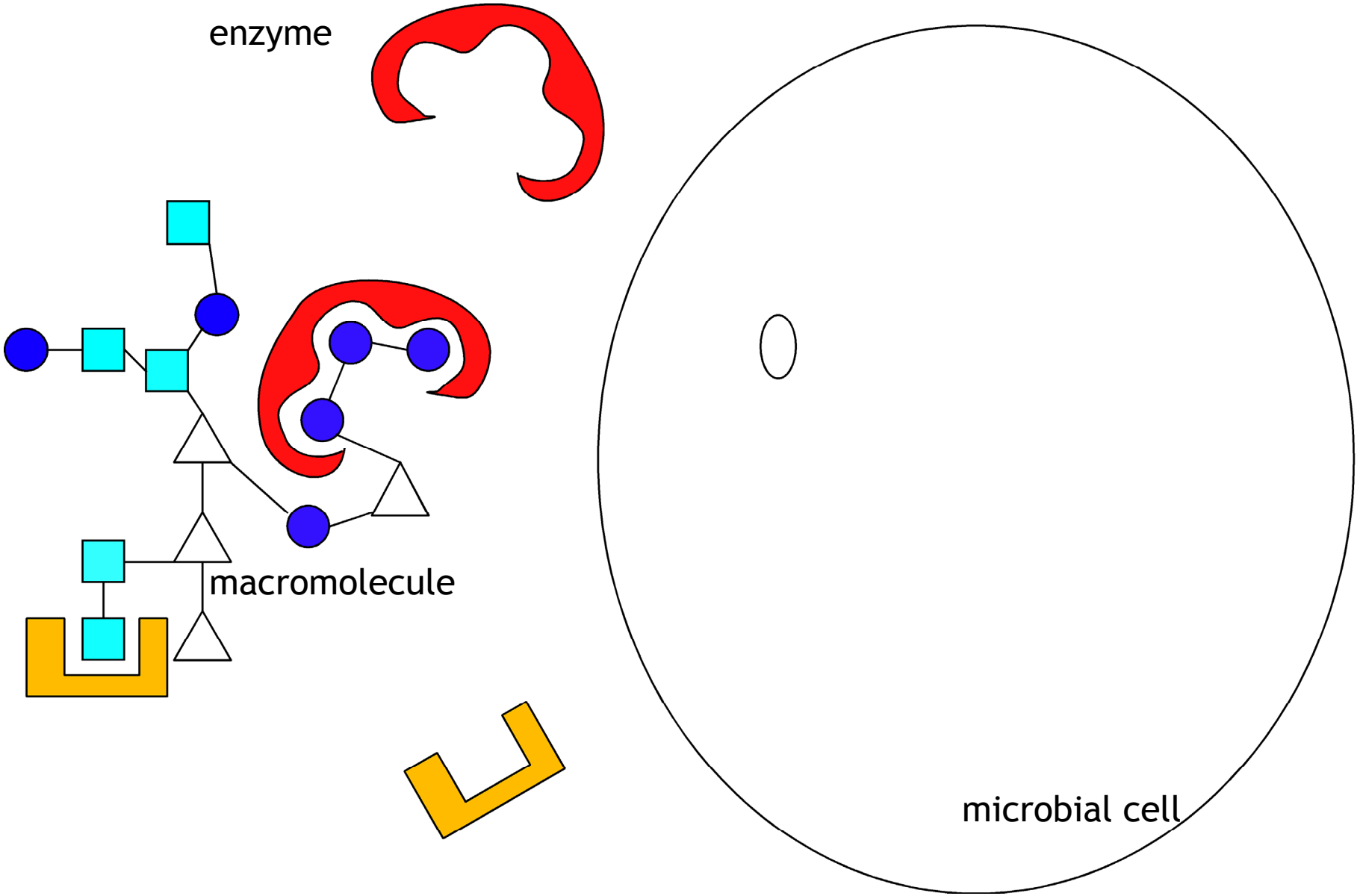




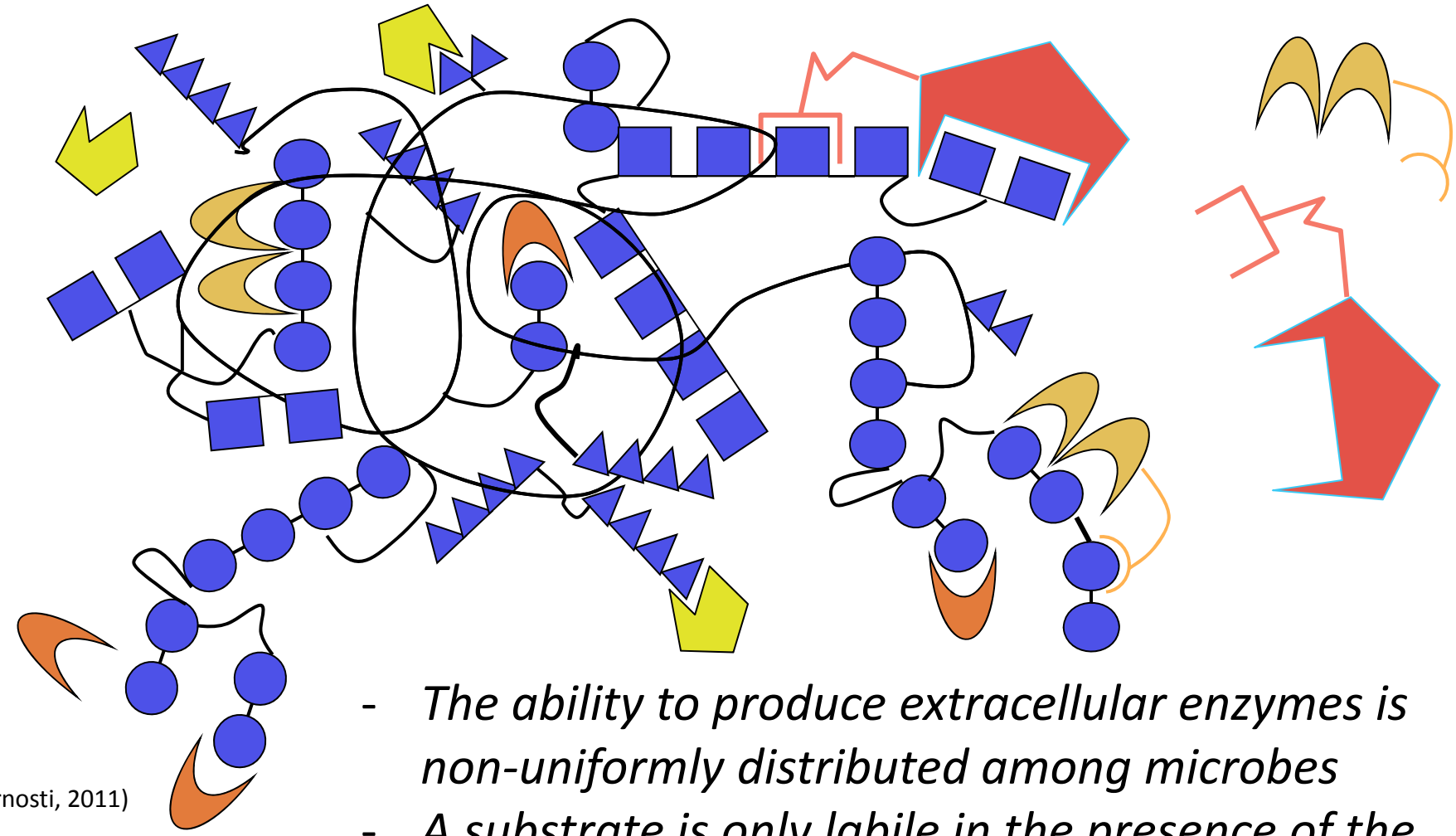


(Zaccone et al. 2012)

Issues of substrate structure



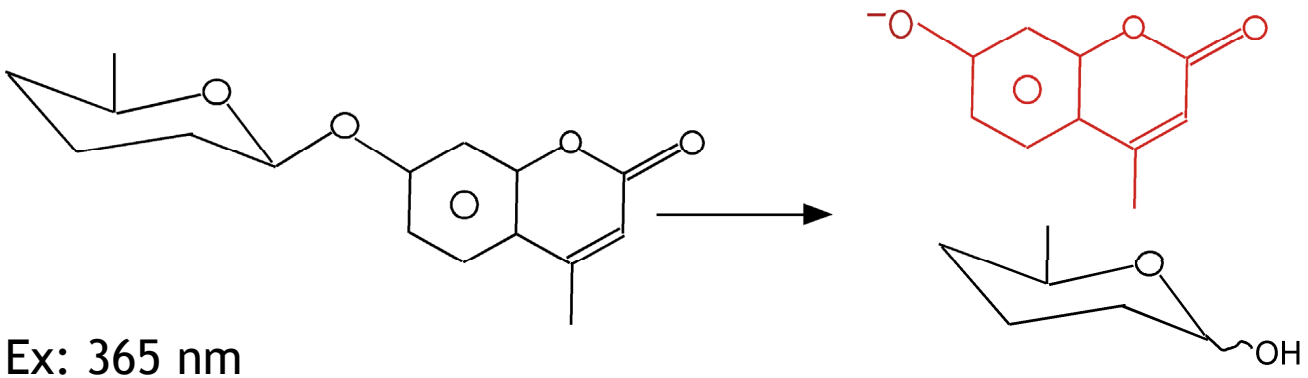
Extracellular enzymes are structurally specific



(Arnosti, 2011)

- *The ability to produce extracellular enzymes is non-uniformly distributed among microbes*
- *A substrate is only labile in the presence of the appropriate enzymes*

Standard substrates don't reflect the structural complexity of natural organic matter



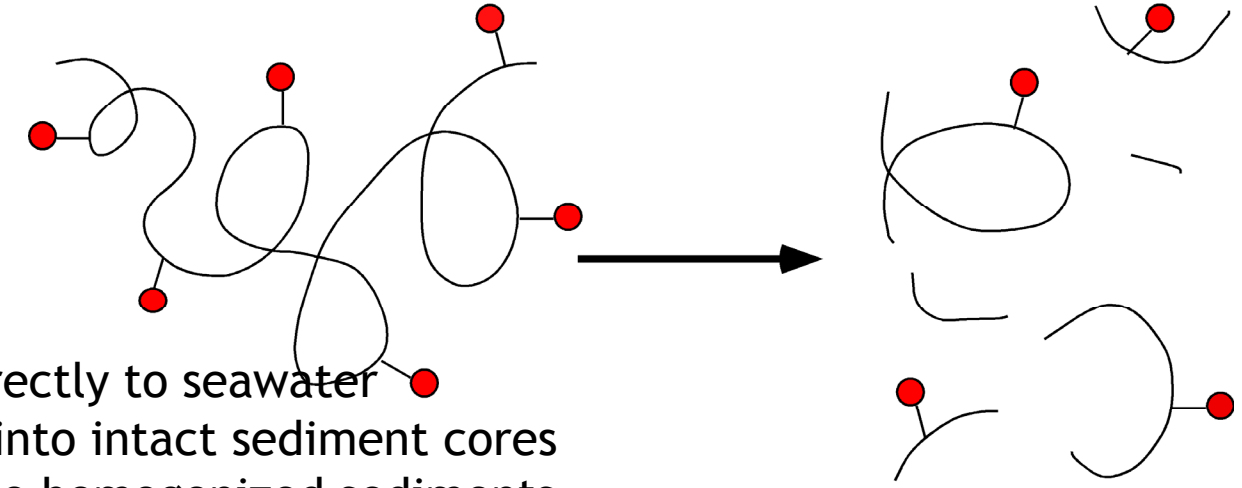
Ex: 365 nm
Em: 445 nm

-not macromolecular
(don't accommodate binding domains)
-don't measure mid-chain cleaving enzymes

-hydrolysis can occur in periplasm
(Martinez and Azam, 1993)

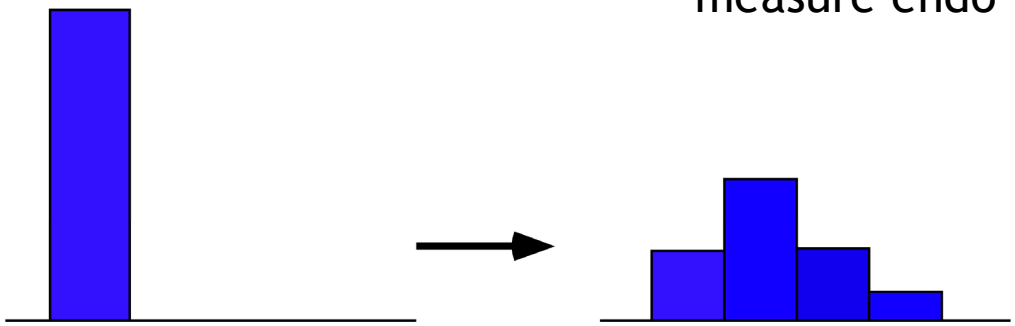
Fluorescently labeled polysaccharides

(also phytoplankton extracts, phytoplankton DOM): measure hydrolysis rates of different constituents of a major class of biochemicals



-add directly to seawater
-inject into intact sediment cores
-mix into homogenized sediments

-Ex: 490, Em: 530
-true polymers
-measure endo-acting enzymes



(Arnosti 1995; 2003)

Different organisms hydrolyzed a diverse set of polysaccharides

Three marine examples (genomes fully sequenced):

***Gramella forsetti* KT0803** *(*Bacteroidetes*)*

***Pseudoalteromonas atlantica* T6c** *(*gamma Proteobacteria*)*

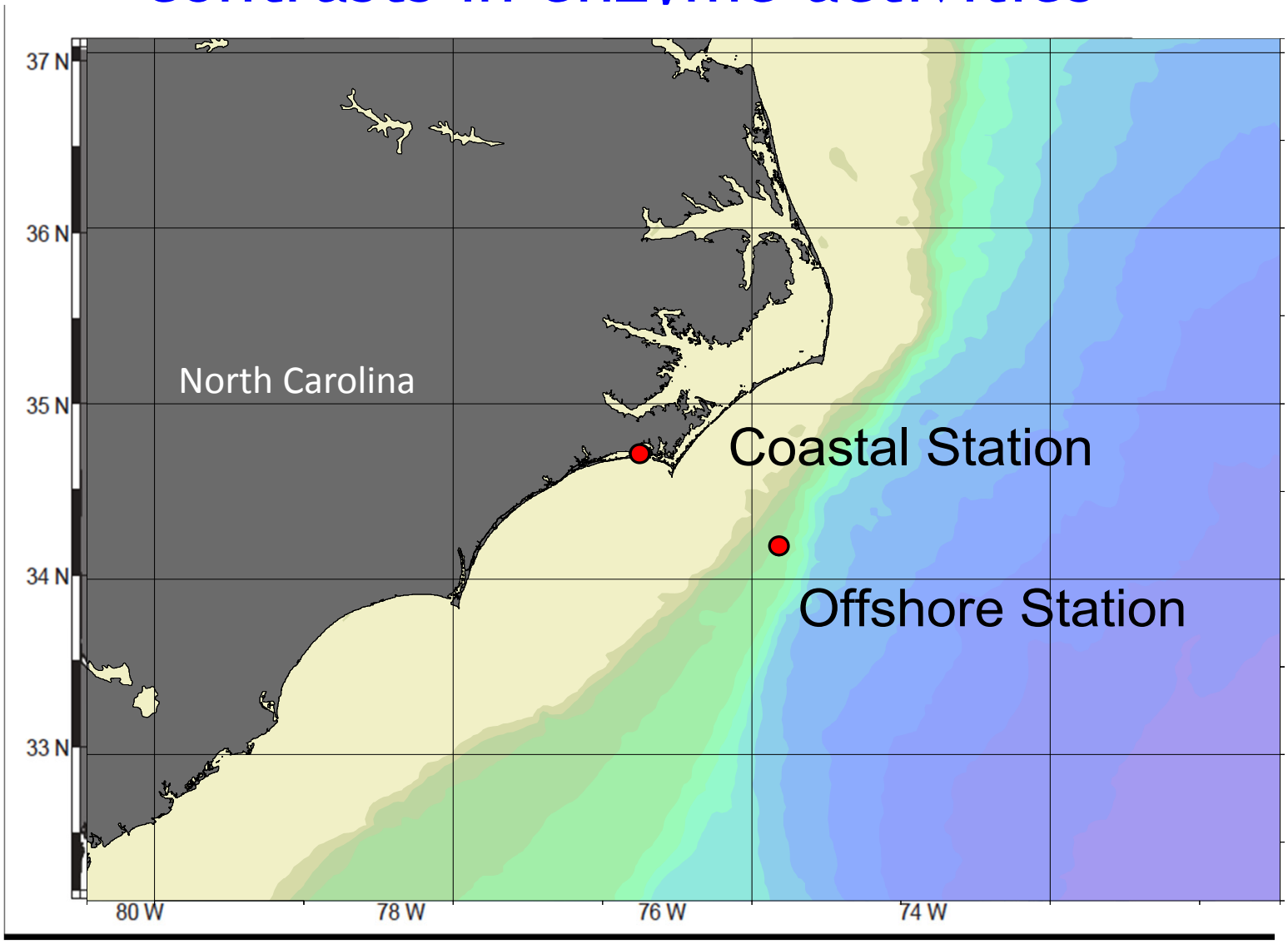
***Rhodopirellula baltica* SH1** *(*Planctomycetales*)*

pullulan	laminarin	xylan	fucoidan	arabinogalactan	chondroitin
P. atlantica		*P. atlantica*	*P. atlantica*		
	G. forsetti		*G. forsetti*	*G. forsetti*	
		R. baltica	*R. baltica*		*R. baltica*

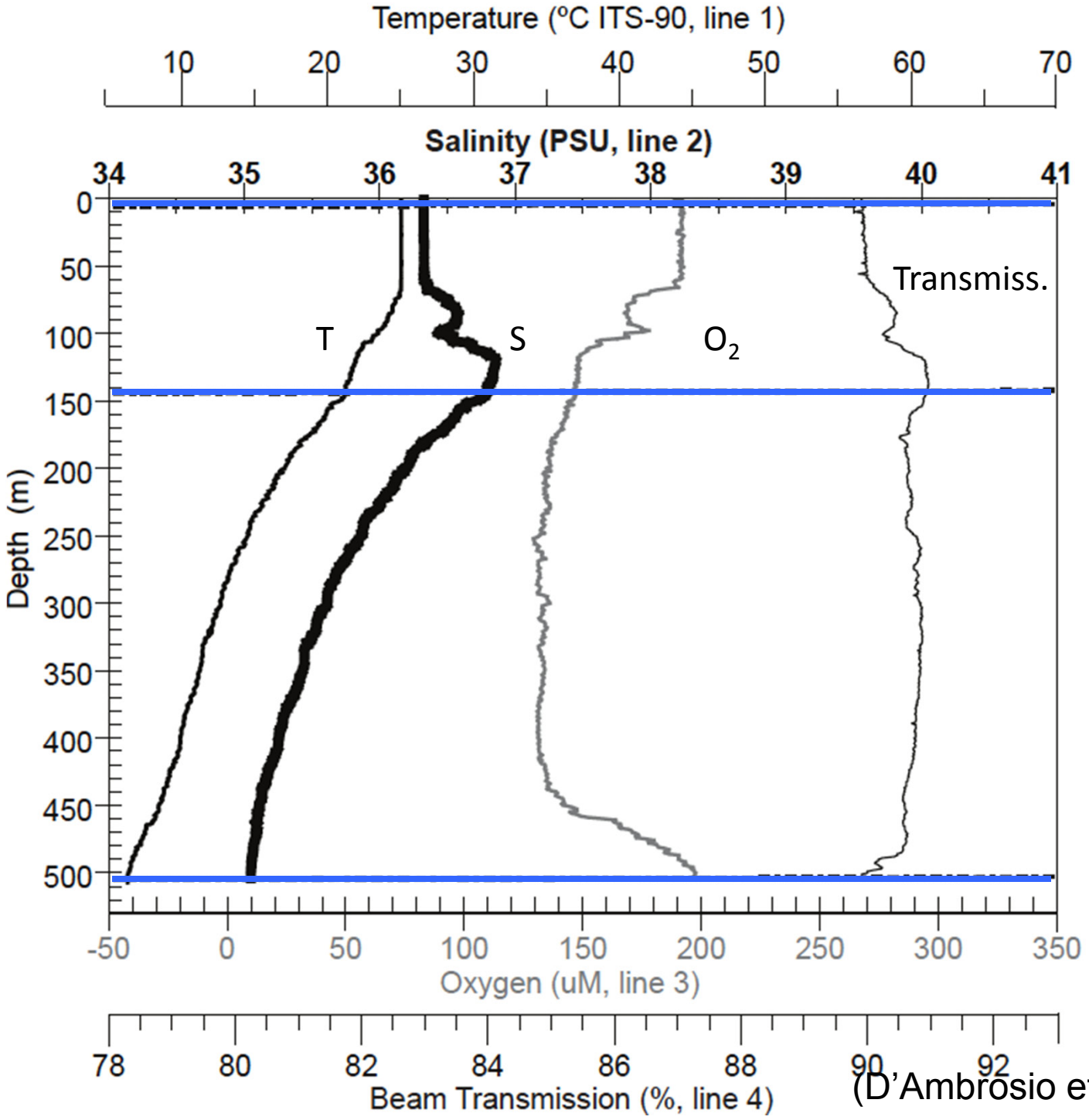
Key point: all substrates can be used by someone, but no one can use all of the substrates

The distribution of organisms in the ocean is important!

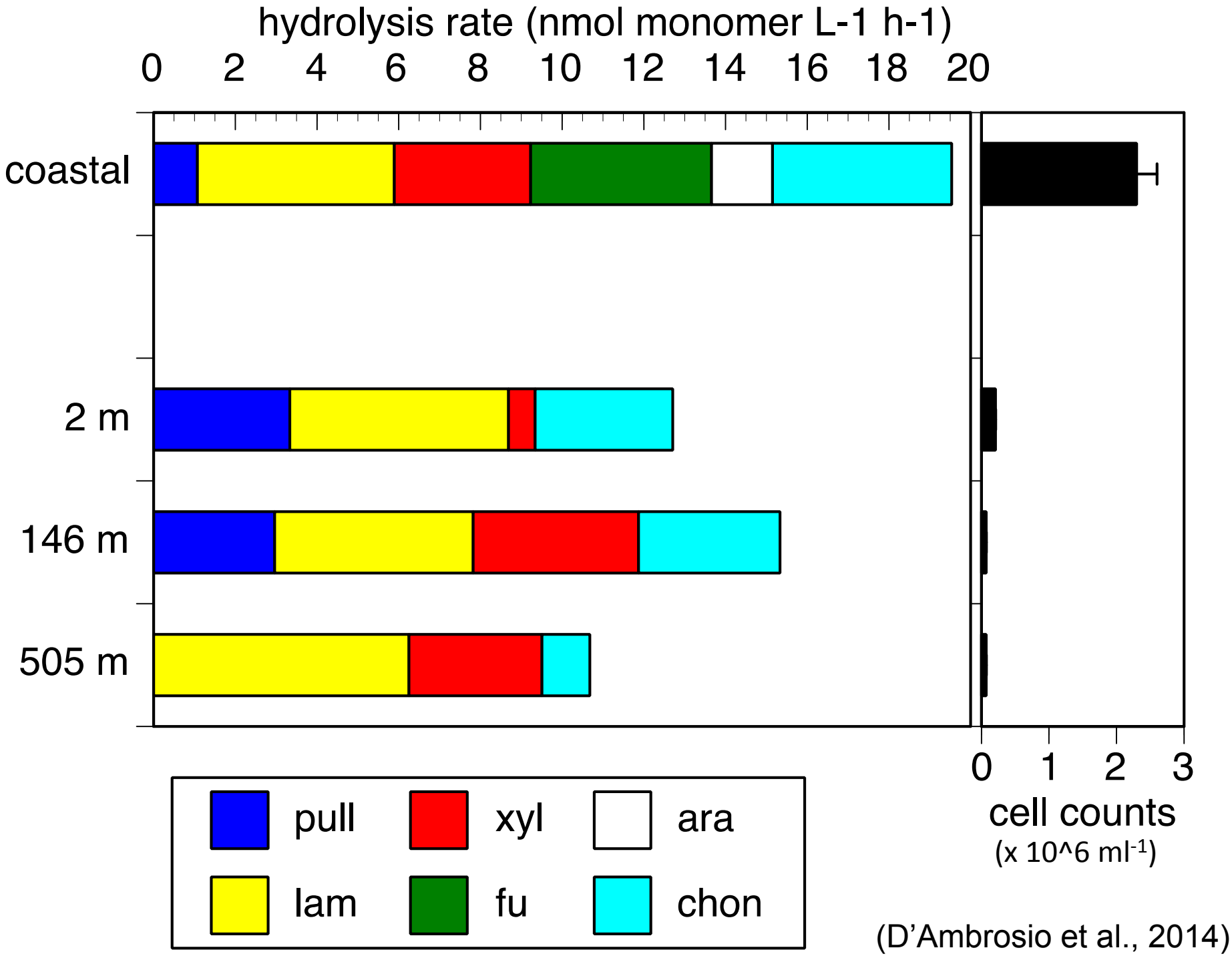
Onshore-offshore and depth-related contrasts in enzyme activities



(D'Ambrosio et al., 2014)



(D'Ambrosio et al., 2014)

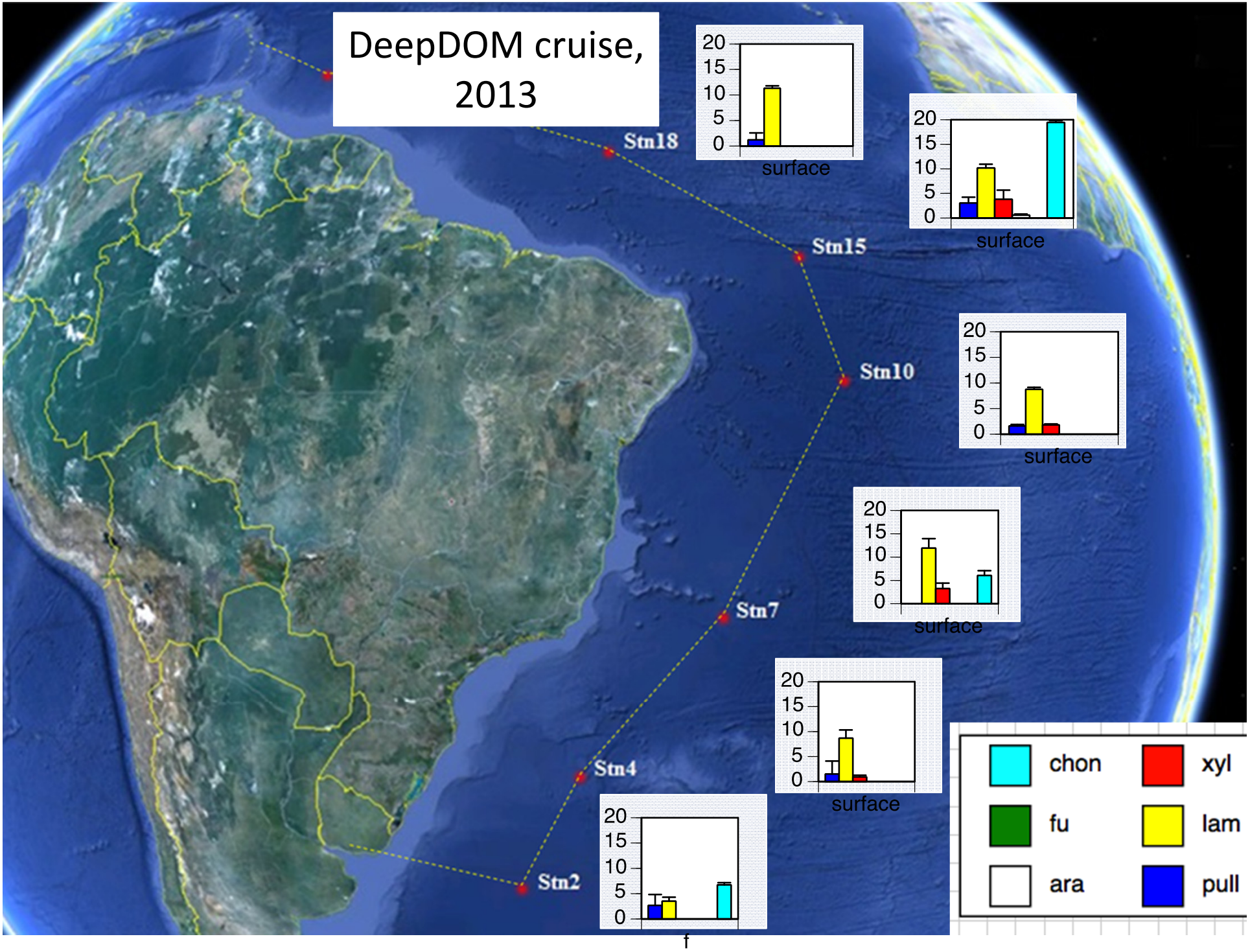

hydrolysis rate (nmol monomer L-1 h-1)
0 2 4 6 8 10 12 14 16 18 20
coastal
2 m
146 m
505 m
0 1 2 3
cell counts
(x 10^6 ml-1)
pull
xyl
ara
lam
fu
chon
(D'Ambrosio et al., 2014)

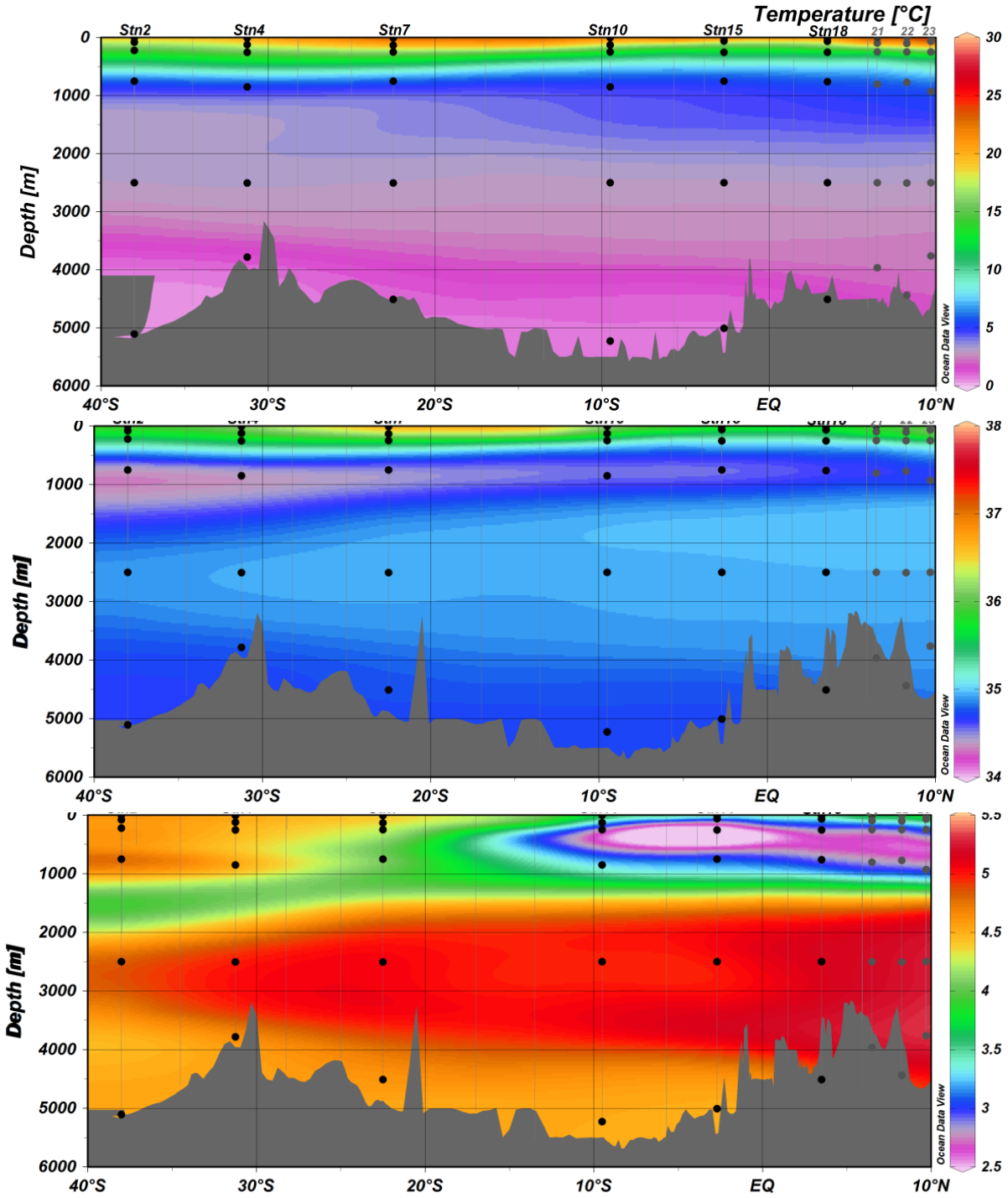
Depth- and site-related differences in enzyme activities

- All substrates hydrolyzed inshore; only a subset hydrolyzed offshore
- Changes in the spectrum of substrates hydrolyzed with depth
- Bulk parameters (temperature, cell counts) do not correlate with hydrolysis rates or patterns
- Specific enzymatic capabilities determined at fine-scale phylogenetic resolution*; also in accordance with genomic analysis of Zimmerman et al. (2013)*

Entire microbial communities, not just individual organisms, exhibit distinct substrate ‘preferences’

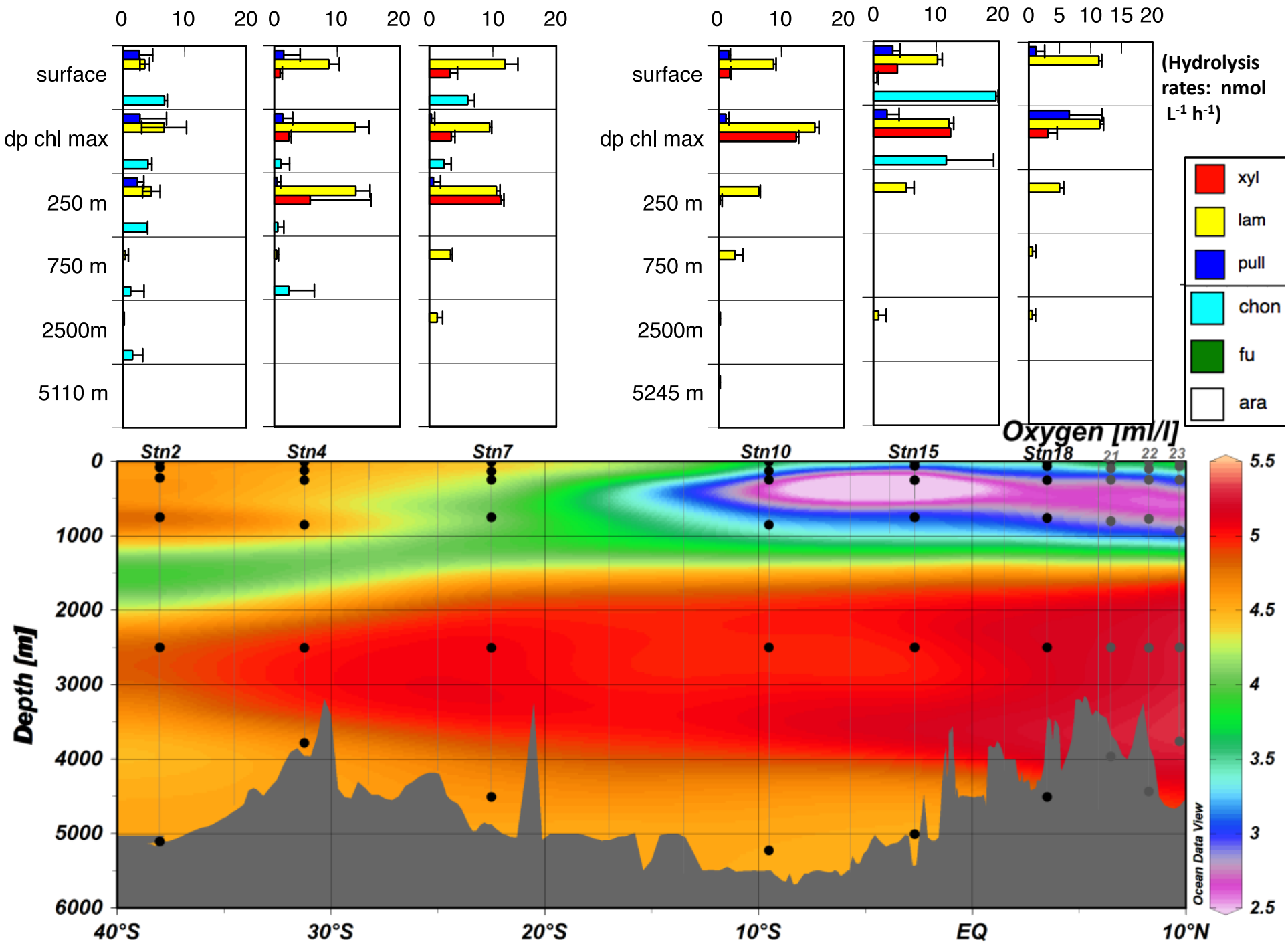
What kind of patterns are there on larger scales, and at greater depths, in the ocean?

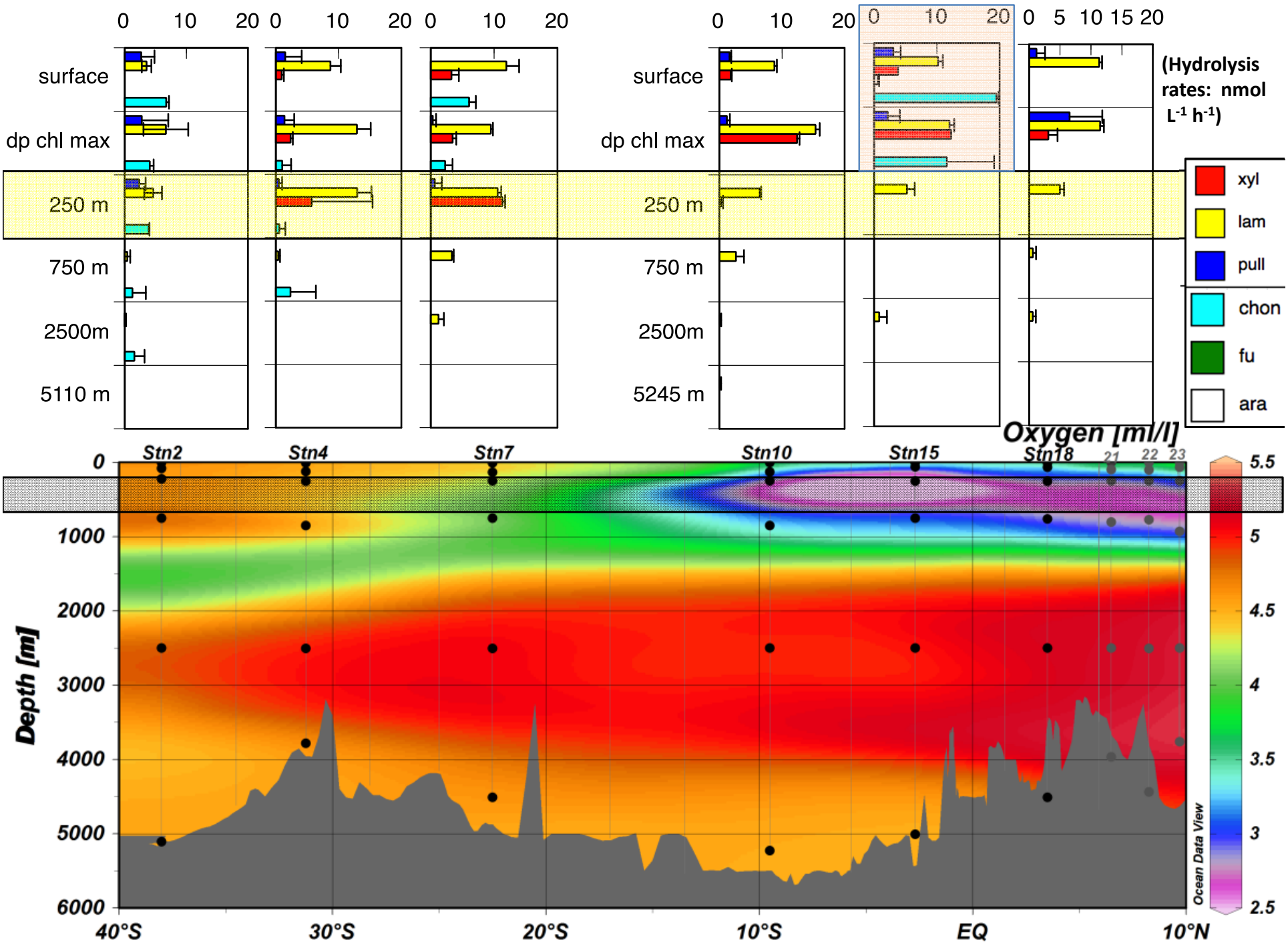

DeepDOM cruise, 2013
Stn18
Stn15
Stn10
Stn7
Stn4
Stn2
20
15
10
5
0
surface
f
chon
xyl
fu
lam
ara
pull



Sampling depths:

Surface (5m)
Deep Chl max – 70-120m
250m – upper mesopelagic
AAIW – 750-850m
NADW – 2500m
Bottom water

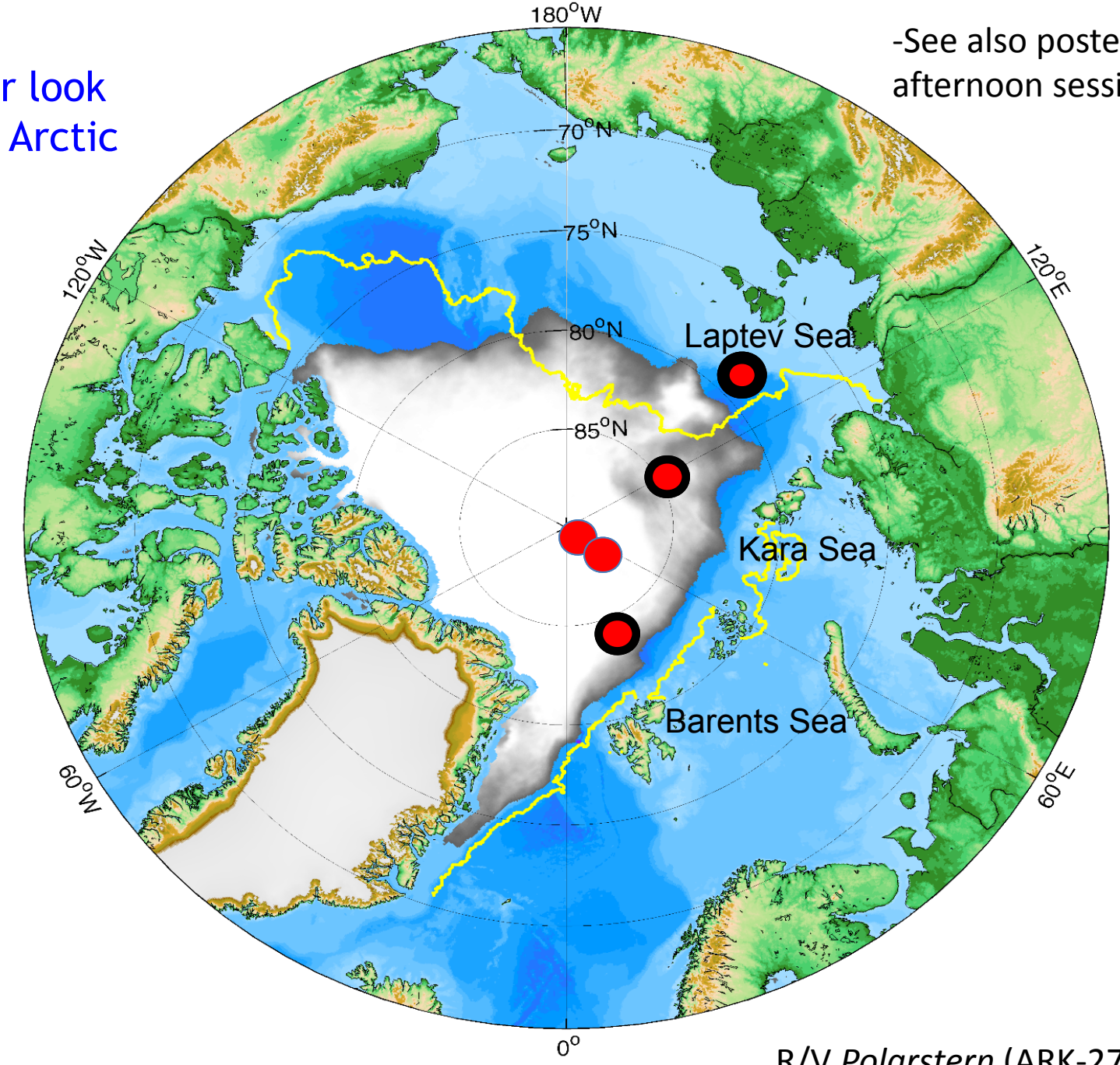

0 10 20
surface
dp chl max
250 m
750 m
2500m
5110 m
0 10 20
surface
dp chl max
250 m
750 m
2500m
5245 m
0 5 10 15 20
(Hydrolysis rates: nmol L-1 h-1)
xyl
lam
pull
chon
fu
ara
Oxygen [ml/l]
Stn2
Stn4
Stn7
Stn10
Stn15
Stn18
21
22
23
Depth [m]
0
1000
2000
3000
4000
5000
6000
40°S
30°S
20°S
10°S
EQ
10°N
5.5
5
4.5
4
3.5
3
2.5
Ocean Data View


0 10 20
0 10 20
0 10 20
0 10 20
0 10 20
0 5 10 15 20
surface
dp chl max
250 m
750 m
2500m
5110 m
5245 m
(Hydrolysis rates: nmol L-1 h-1)
xyl
lam
pull
chon
fu
ara
Oxygen [ml/l]
Stn2
Stn4
Stn7
Stn10
Stn15
Stn18
21
22
23
0
1000
2000
3000
4000
5000
6000
Depth [m]
40°S
30°S
20°S
10°S
EQ
10°N
5.5
5
4.5
4
3.5
3
2.5
Ocean Data View

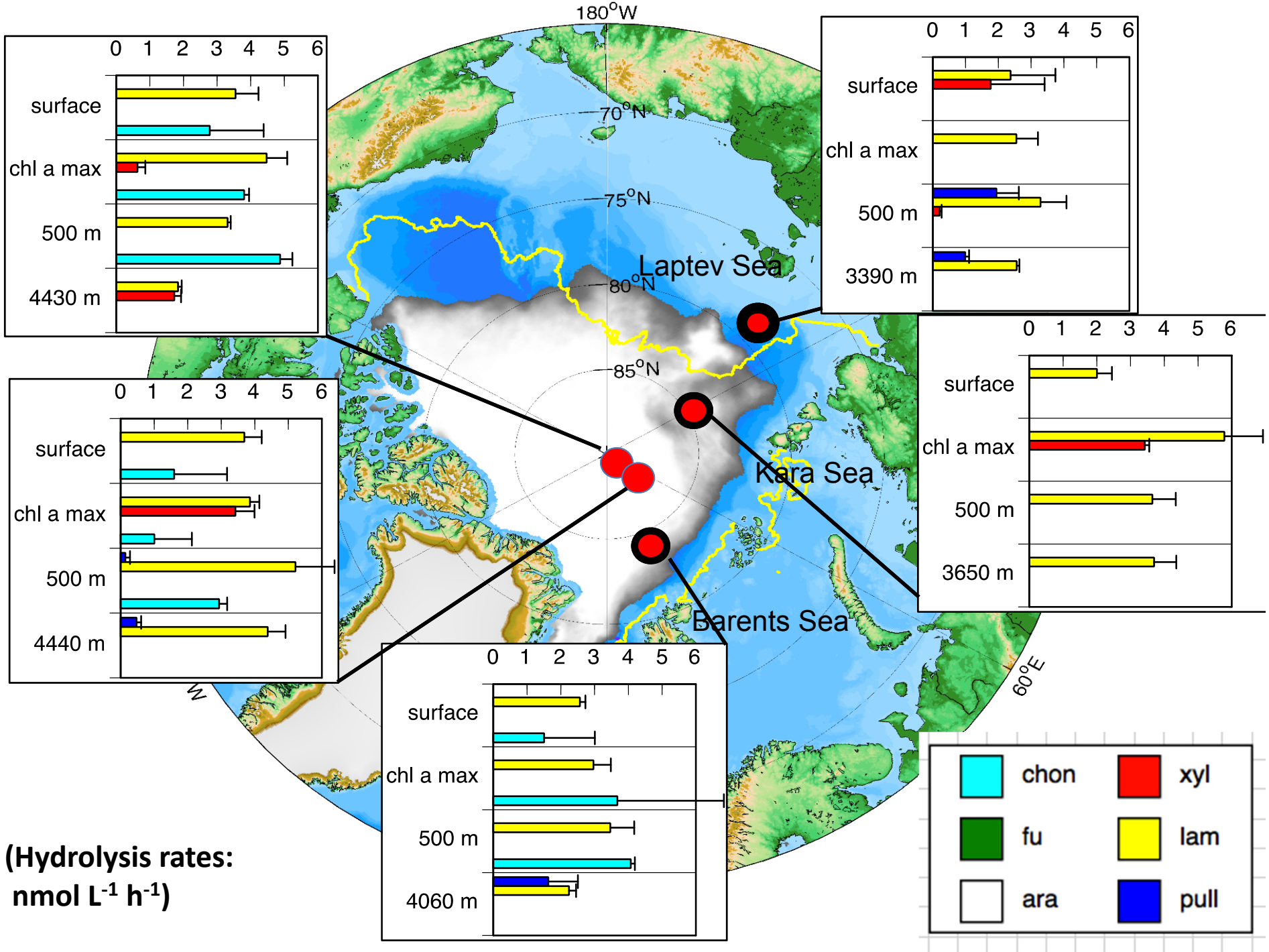
- Surface water enzymatic capabilities don't necessarily predict spectrum of activities in upper mesopelagic
- Spectrum of activities narrows in deep mesopelagic zone compared surface
- Distinct substrate-related differences in hydrolysis:
  - Some substrates (laminarin) are hydrolyzed at most stations and depths
  - Some substrates hydrolyzed rarely (arabinogalactan) or not at all (fucoidan), although they are rapidly hydrolyzed in other locations in the ocean

A deeper look into the Arctic Ocean

-See also poster during the afternoon session!



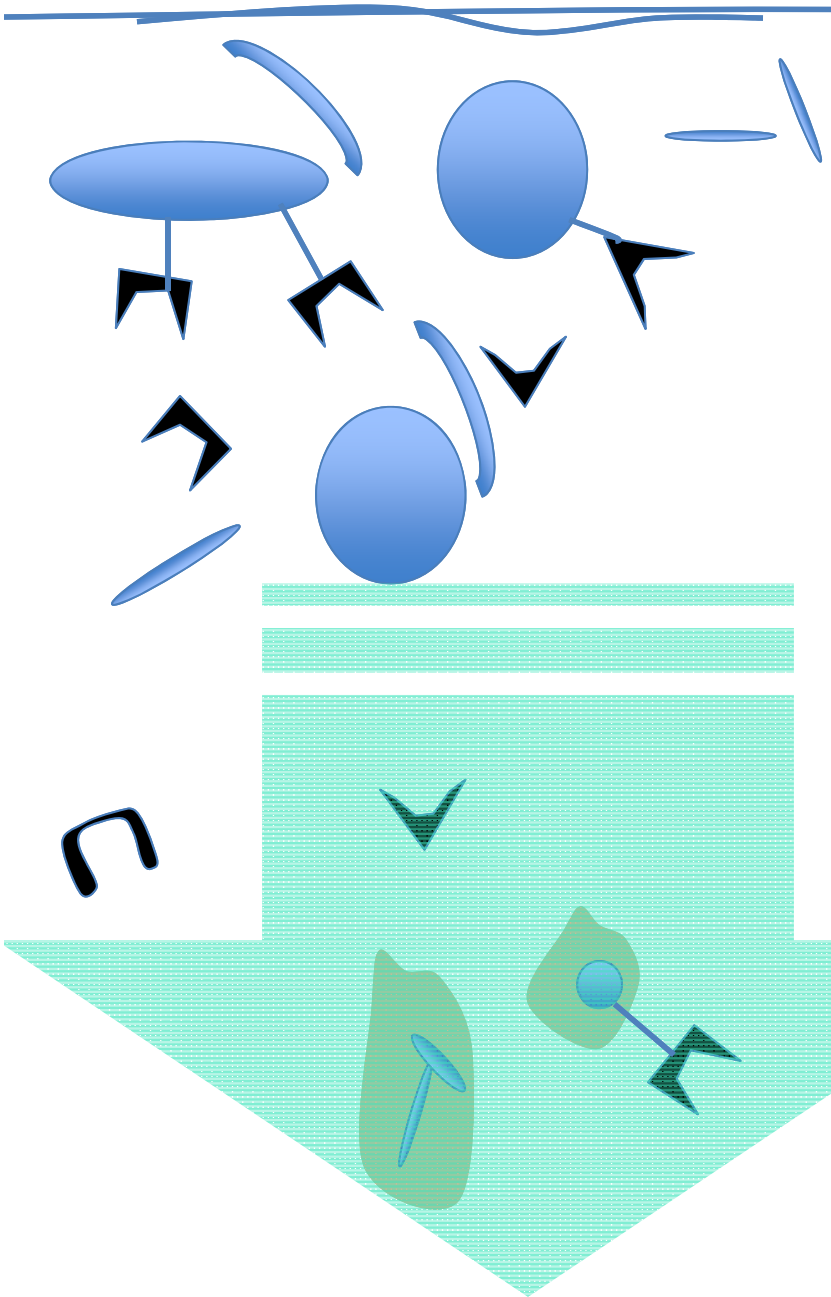
R/V *Polarstern* (ARK-27/6; fall 2012)

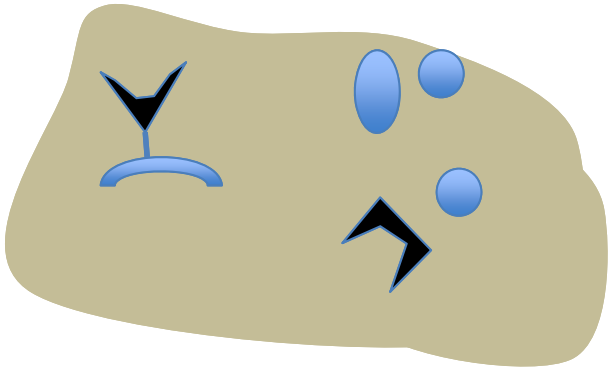


(Hydrolysis rates: nmol L^{-1} h^{-1})

- Considerable enzymatic potential, also at permanently ice covered stations, and in bottom waters at depths > 4000 m
- Spectrum of substrates hydrolyzed in surface waters is narrow
- Activity dominated by laminarin hydrolysis; also by chondroitin at the central Arctic and Barents stations

Looking forward: Some key points to consider





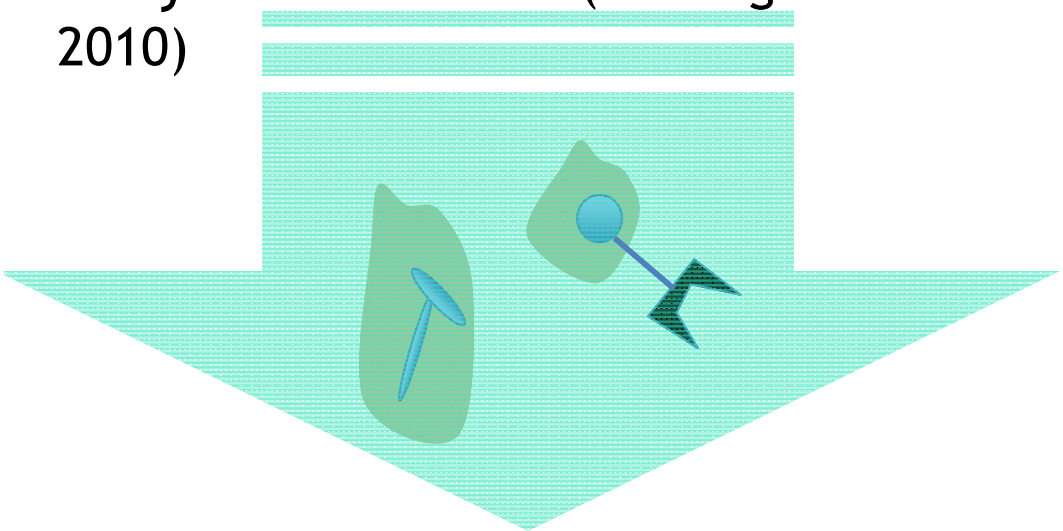
- **Pressure effects on gene expression:** some genes for extracellular enzymes transcribed only under high pressure (Vezzi et al. 2005)
- **Hydrolysis rates may be higher** under elevated pressure (Tamburini et al. 2003)

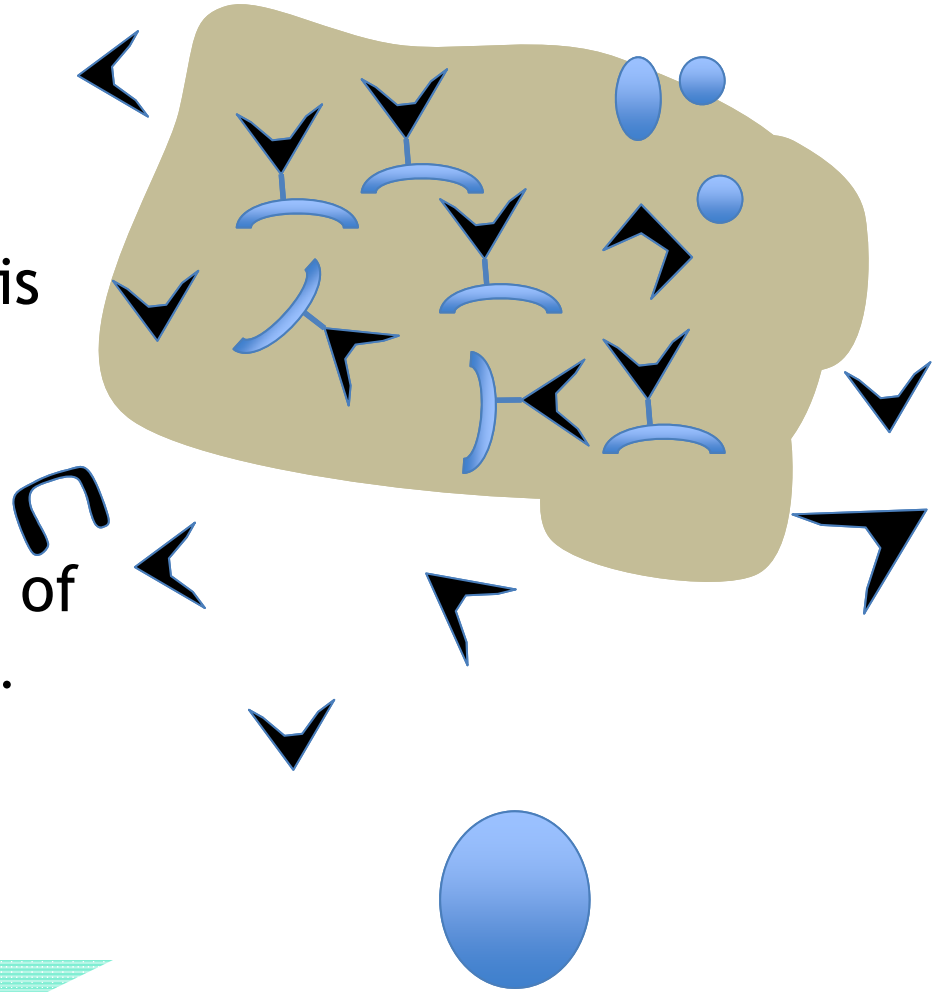
Looking forward: Some key points to consider

Effects of colonization and inter-cell communication

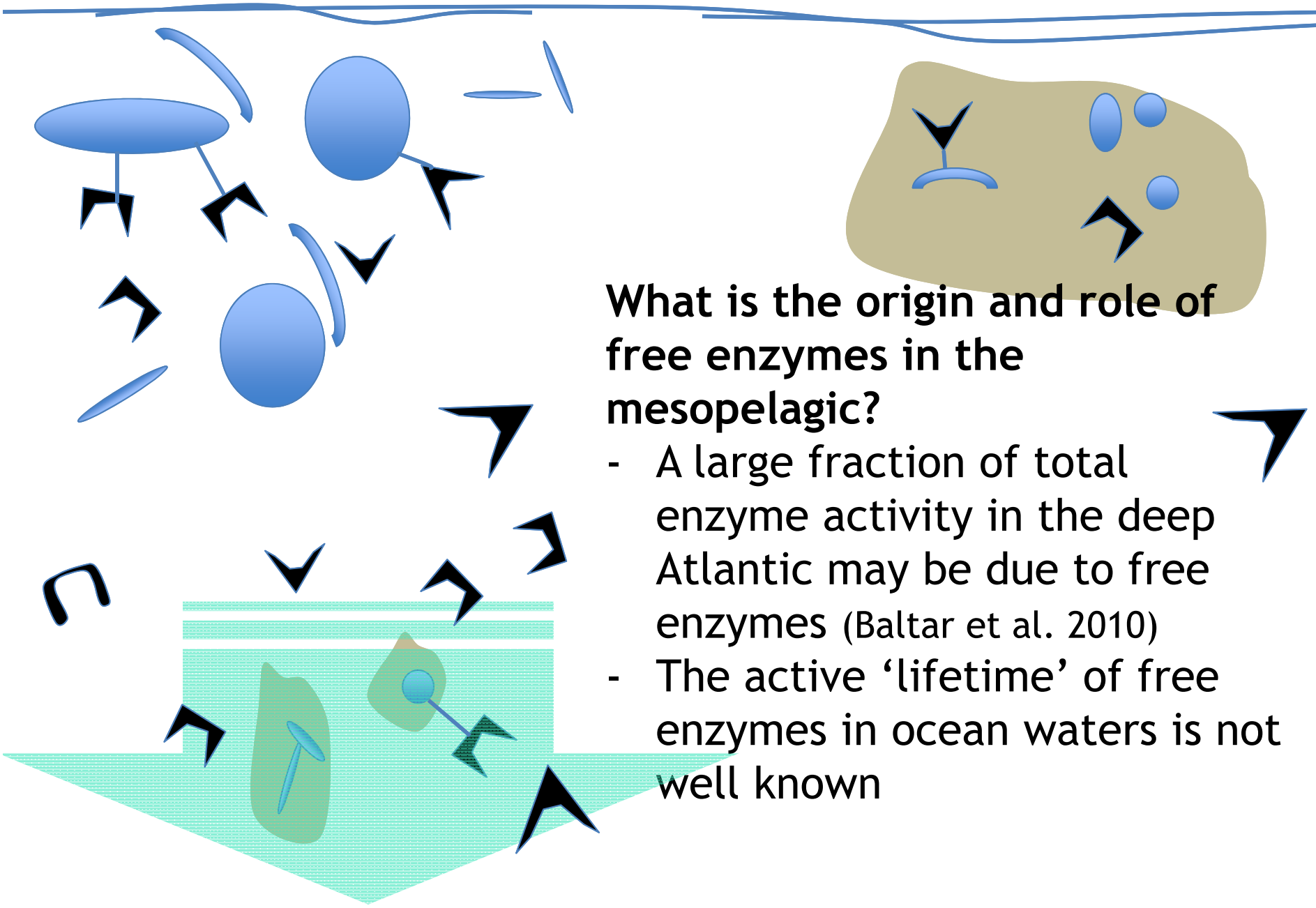
-Quorum-sensing in sinking particles can enhance hydrolysis rates (Hmolo et al. 2011)

-Formation of aggregates can change the spectrum and rates of enzyme activities (Ziervogel et al. 2010)





Looking forward: Some key points to consider



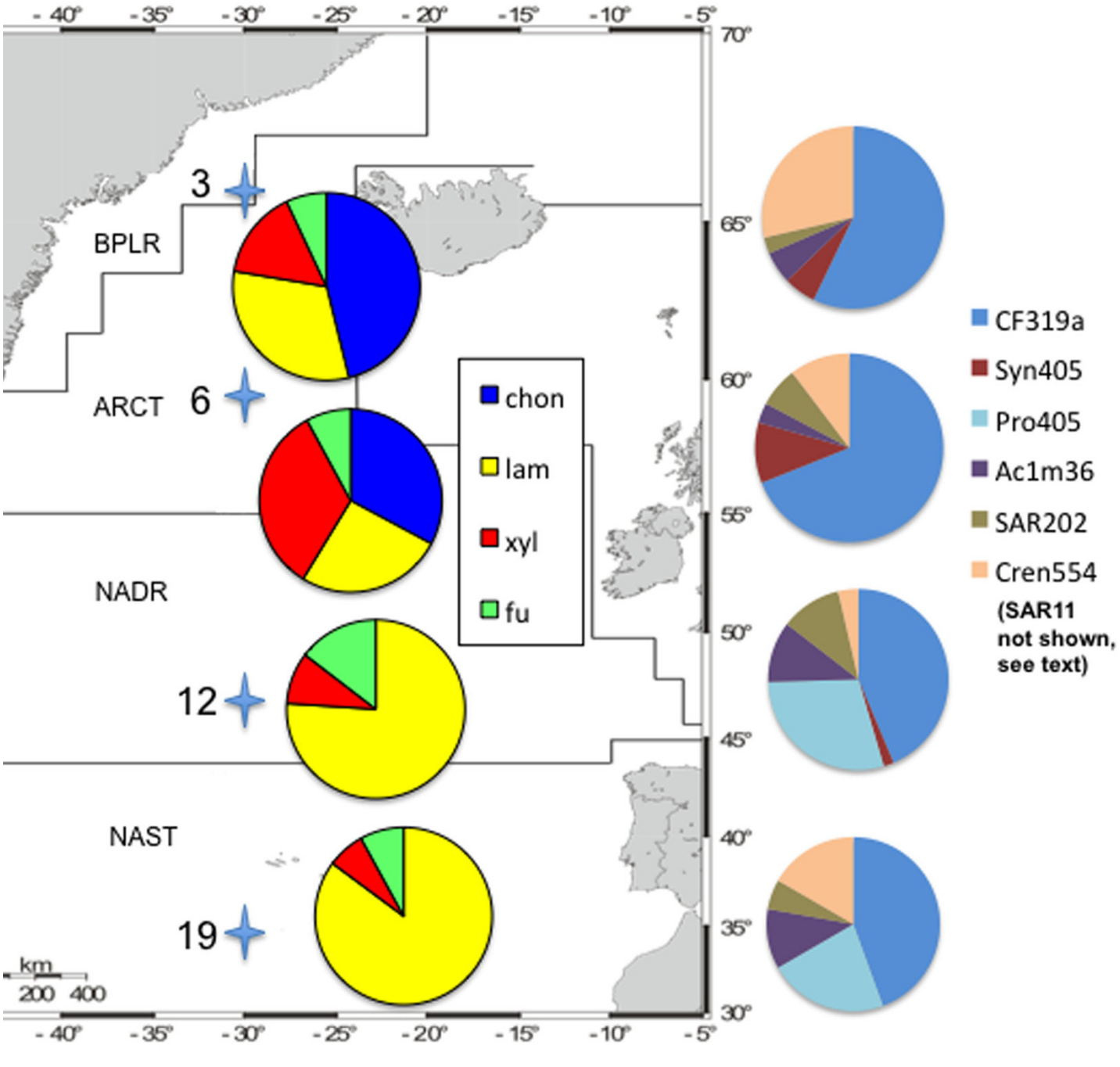
What is the origin and role of free enzymes in the mesopelagic?

- A large fraction of total enzyme activity in the deep Atlantic may be due to free enzymes (Baltar et al. 2010)
- The active 'lifetime' of free enzymes in ocean waters is not well known

Acknowledgments

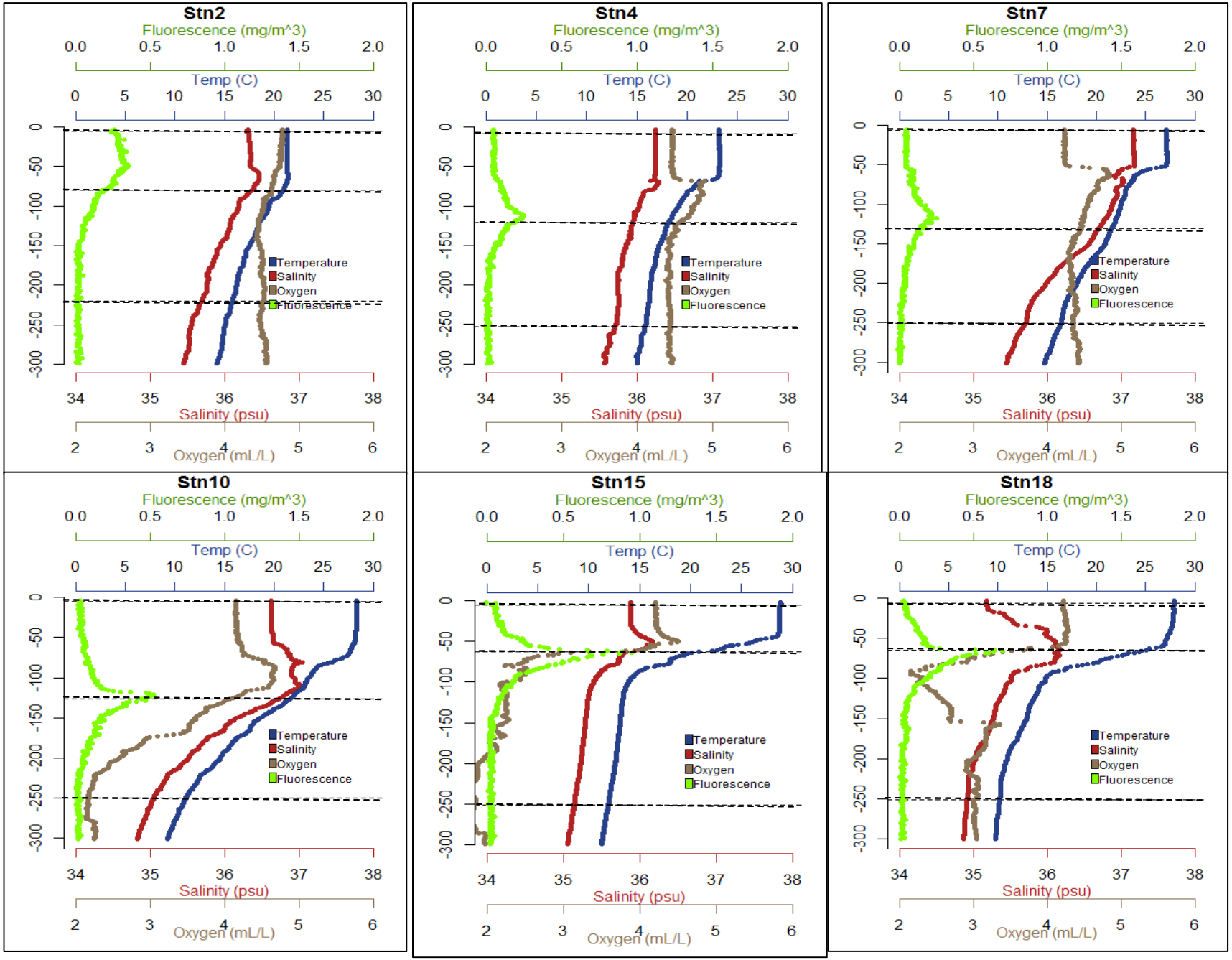
- **Adrienne Hoarfrost**, **John-Paul Balmonte**, Lindsay D'Ambrosio, Sherif Ghobrial, Kai Ziervogel, Stephanie O'Daly, Lisa Couper (UNC)
- Liz Kujawinski (WHOI; DeepDOM)
- Antje Boetius (MPI/Alfred Wegener Institute; *Polarstern* ARK 27-3)
- NSF (Chemical Oceanography; Arctic Natural Sciences)

Differences in enzyme activities of particle-associated prokaryotes



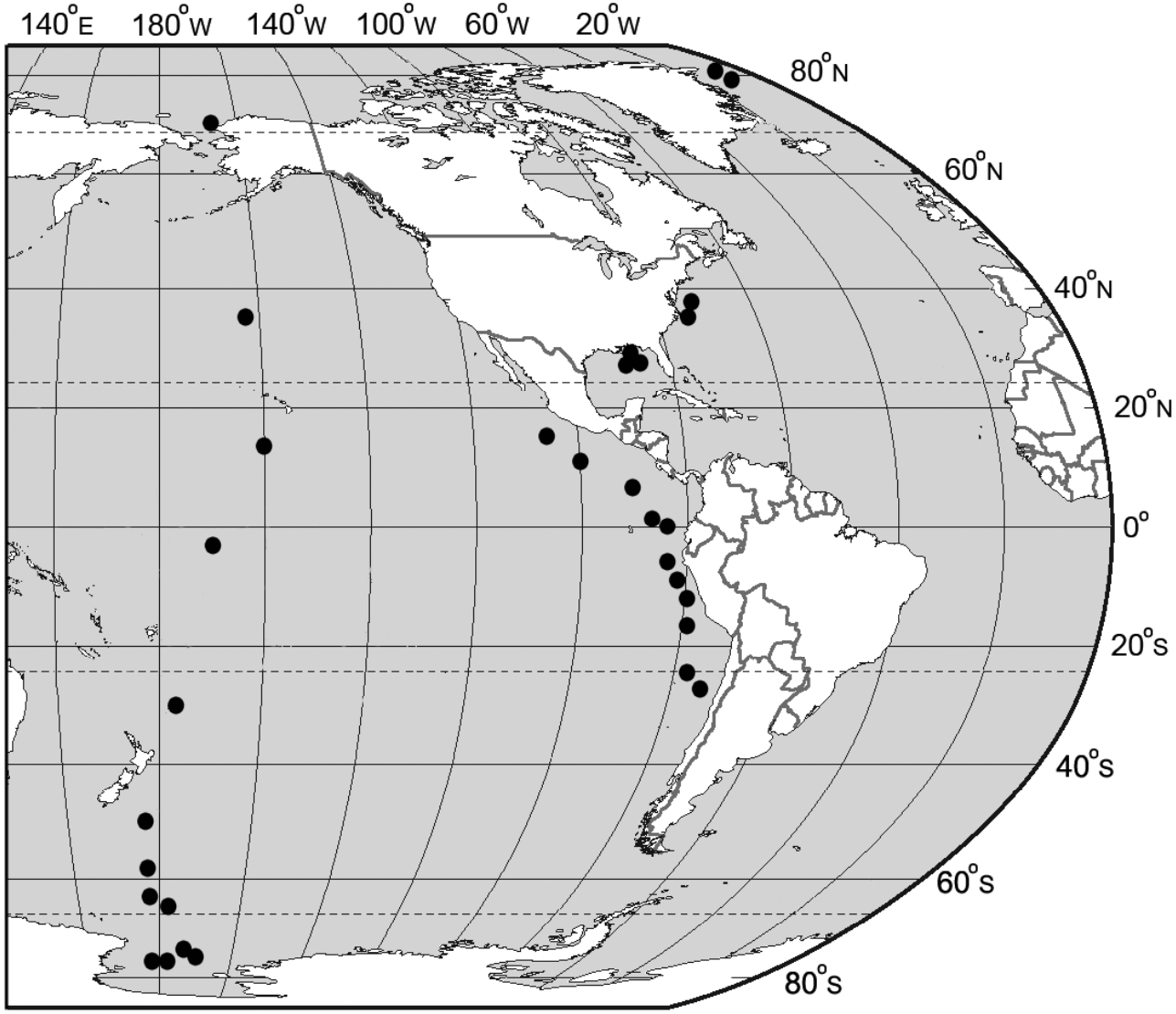
(Arnosti et al. 2012)

Surface to upper mesopelagic



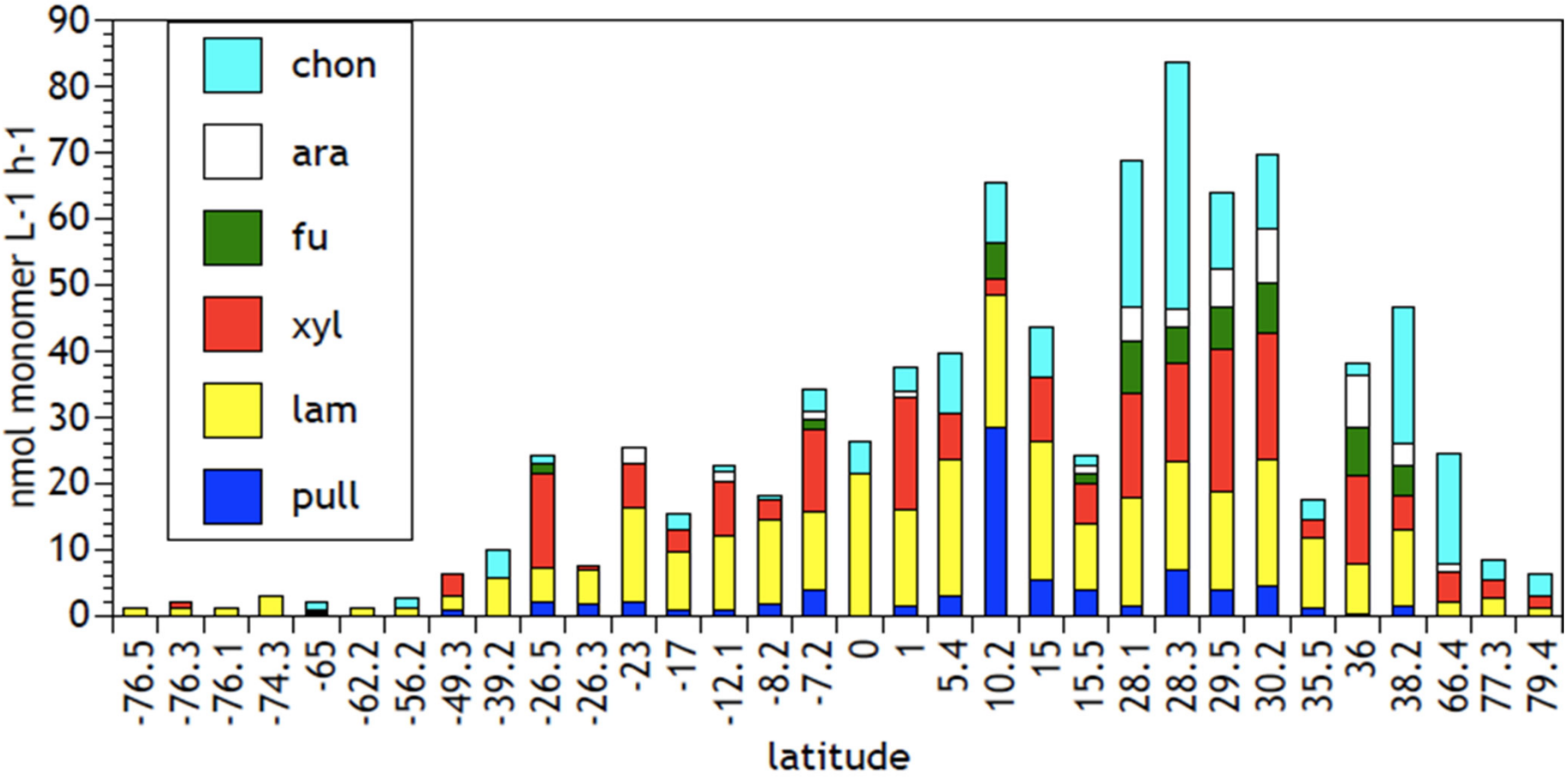
Patterns in enzyme activities on broader scales

32 sites, 76° S to 79° N



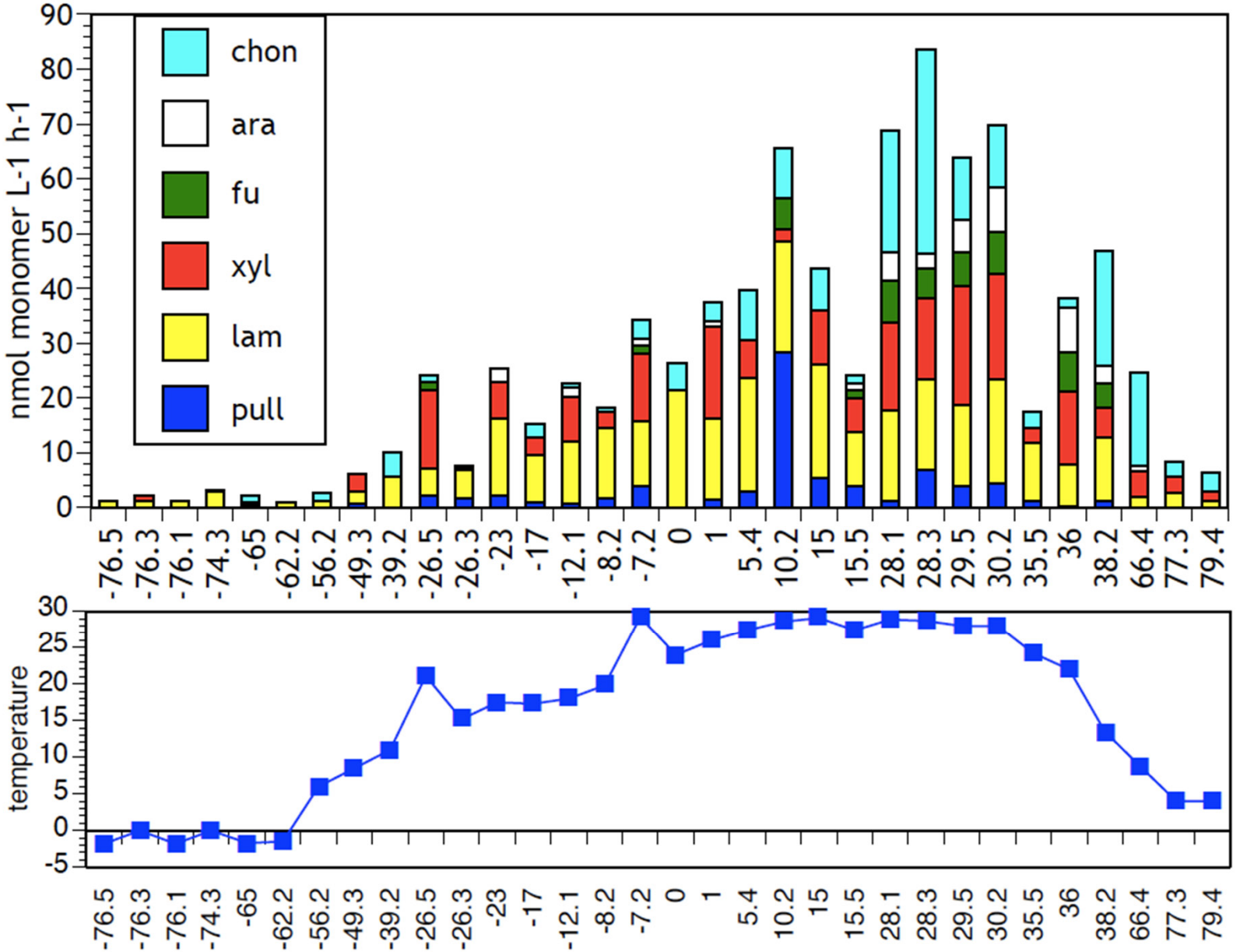
(Arnosti et al.,2011)

Hydrolysis rate vs. latitude

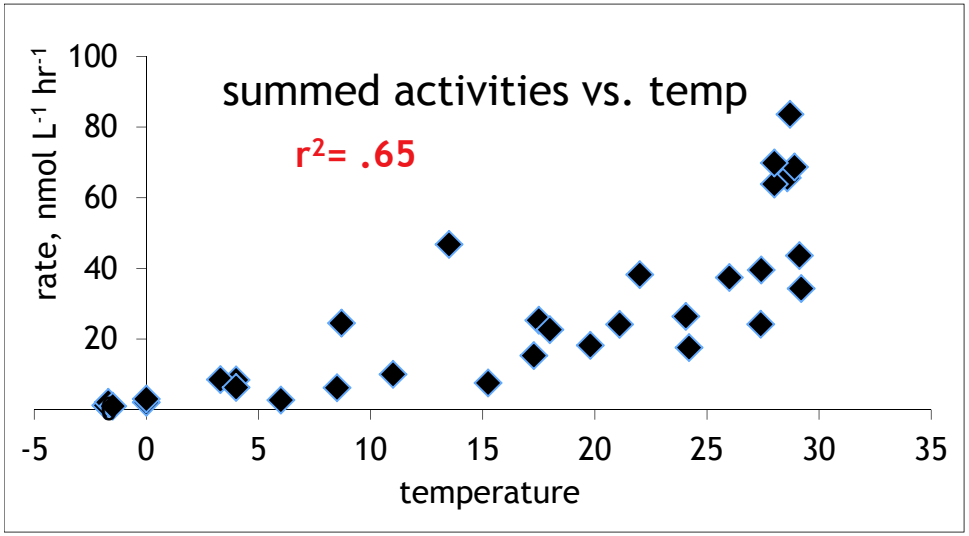


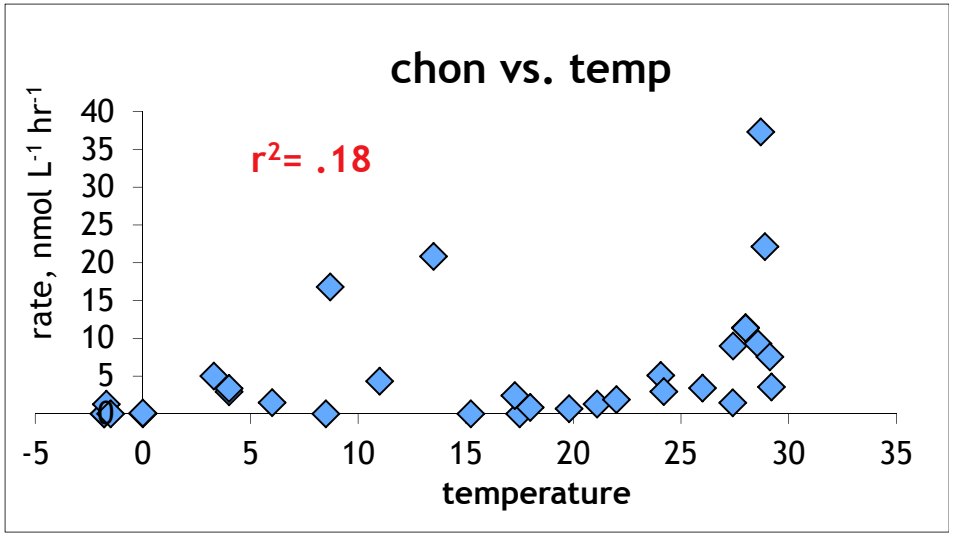
(Arnosti et al., 2011)

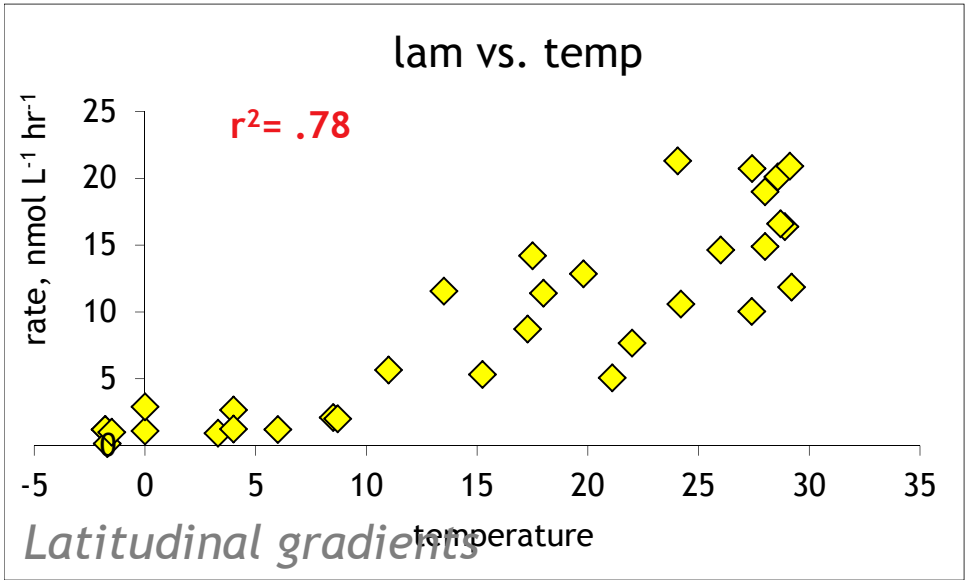
A correlation between hydrolysis rates and temperature

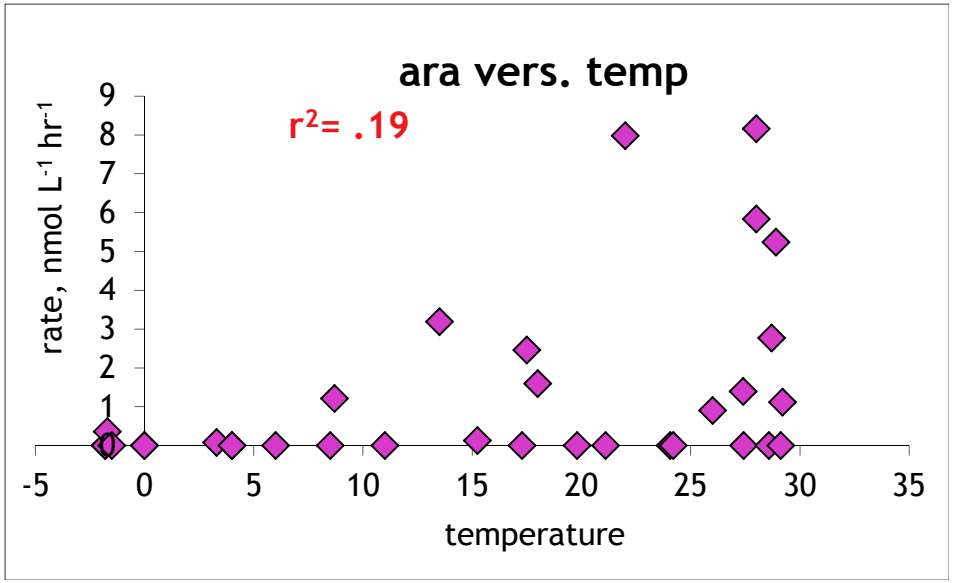


Statistical correlations support a relationship between laminarin hydrolysis and temperature









Latitudinal gradients

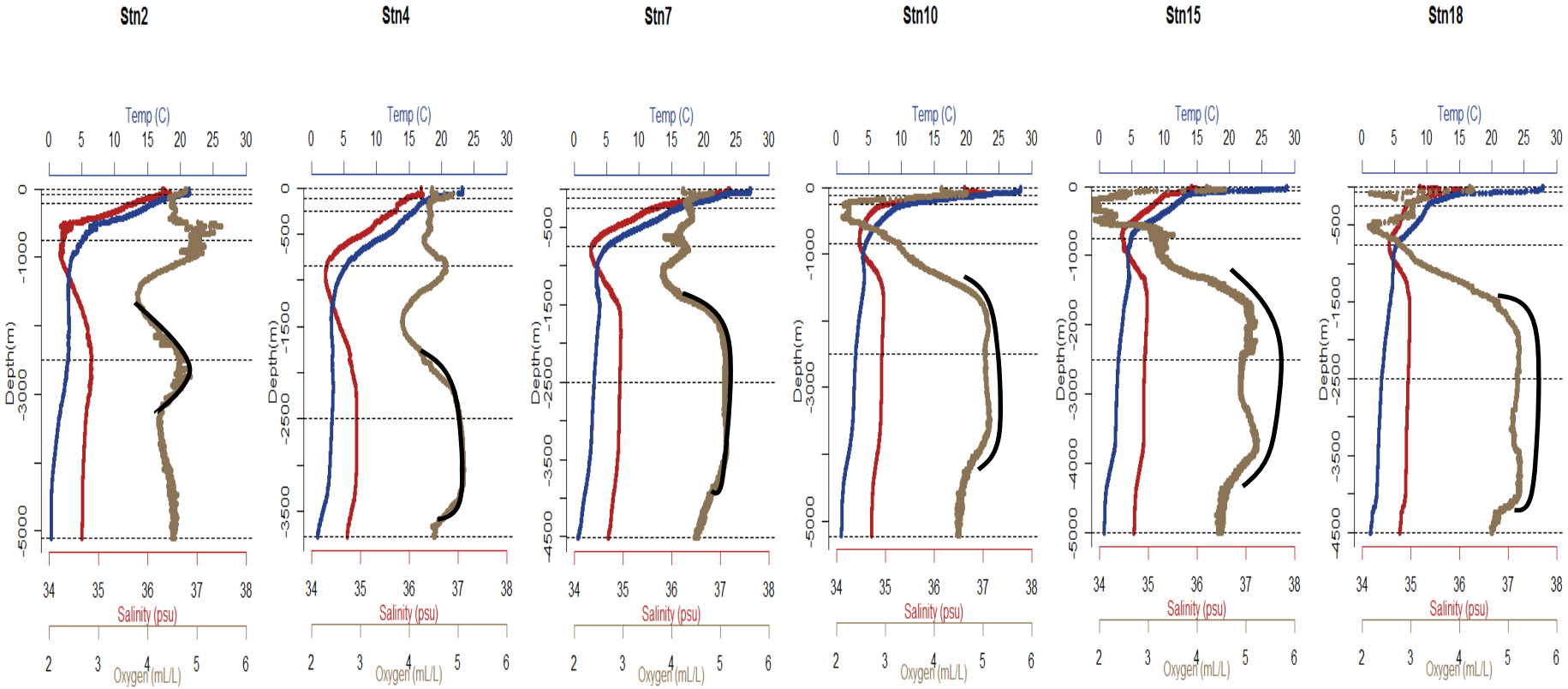
Possible limitations in gene expression: *Pseudoalteromonas atlantica* fucosidase

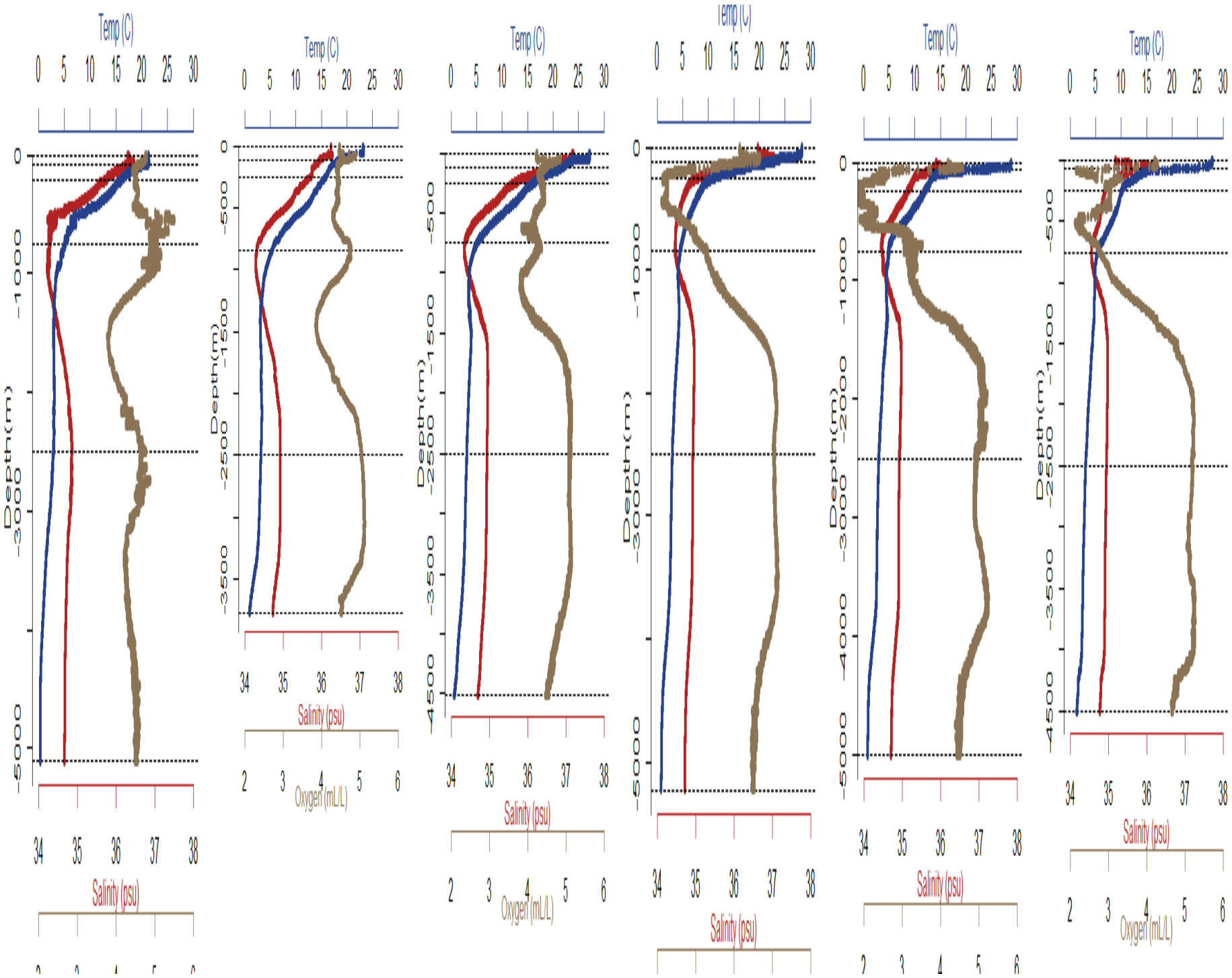
>> 32 sample sites are represented in this data set

Genes more wide-spread than activity?

Depth profiles

South → North

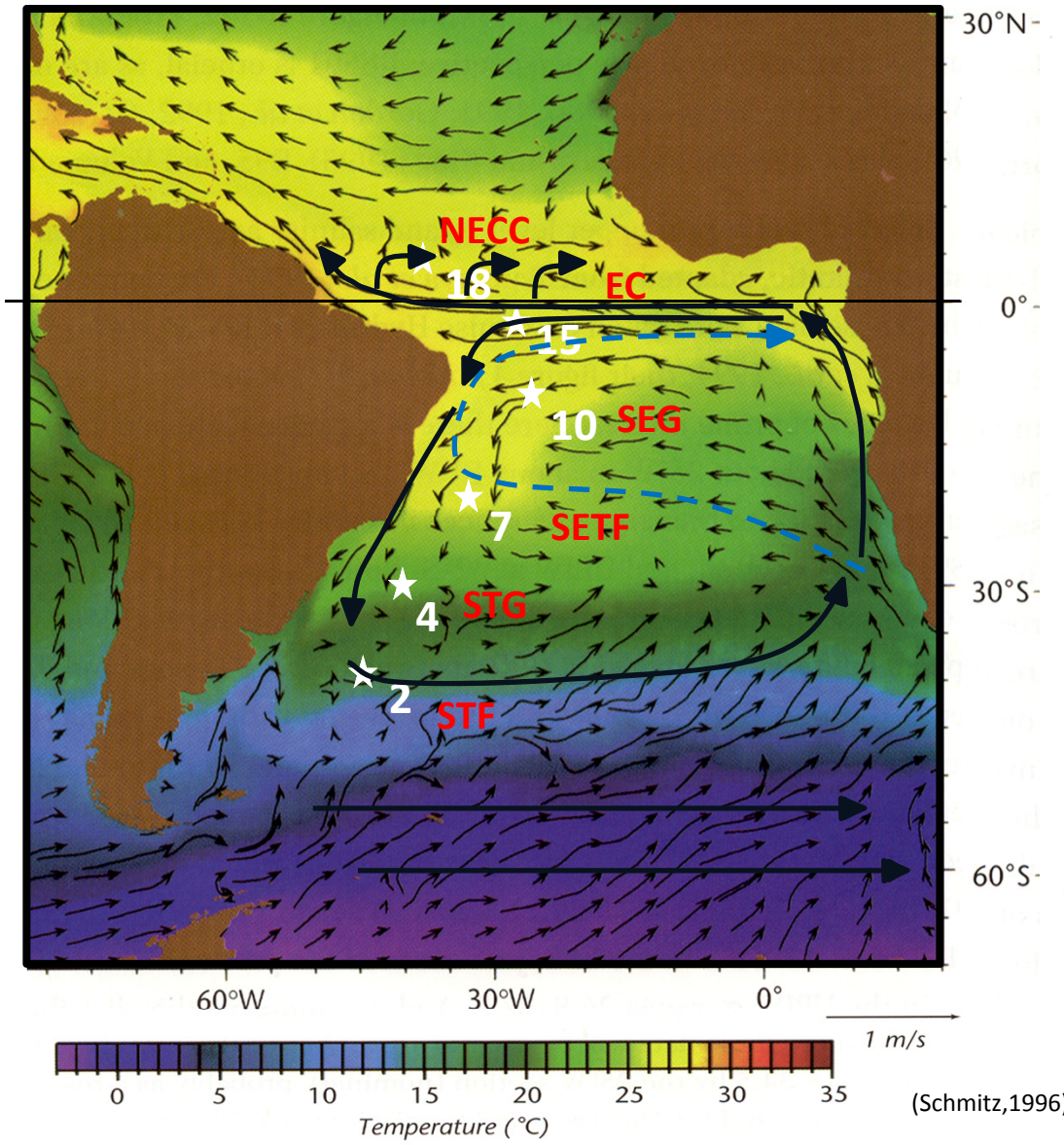



Temp (C)
0 5 10 15 20 25 30
Depth(m)
0
-500
-1000
-1500
-2000
-2500
-3000
-3500
-4000
-4500
-5000
34 35 36 37 38
Salinity (psu)
2 3 4 5 6
Oxygen (mL/L)

SST and Surface Circulation

Fronts vs. gyres vs. ECs

- Stn2: subtropical front
- Stn4: subtropical gyre
- Stn7: subequatorial-subtropical front
- Stn10: subequatorial gyre.
- Stn15: EC
- Stn18: NECC



(Schmitz,1996)

Depth profiles