

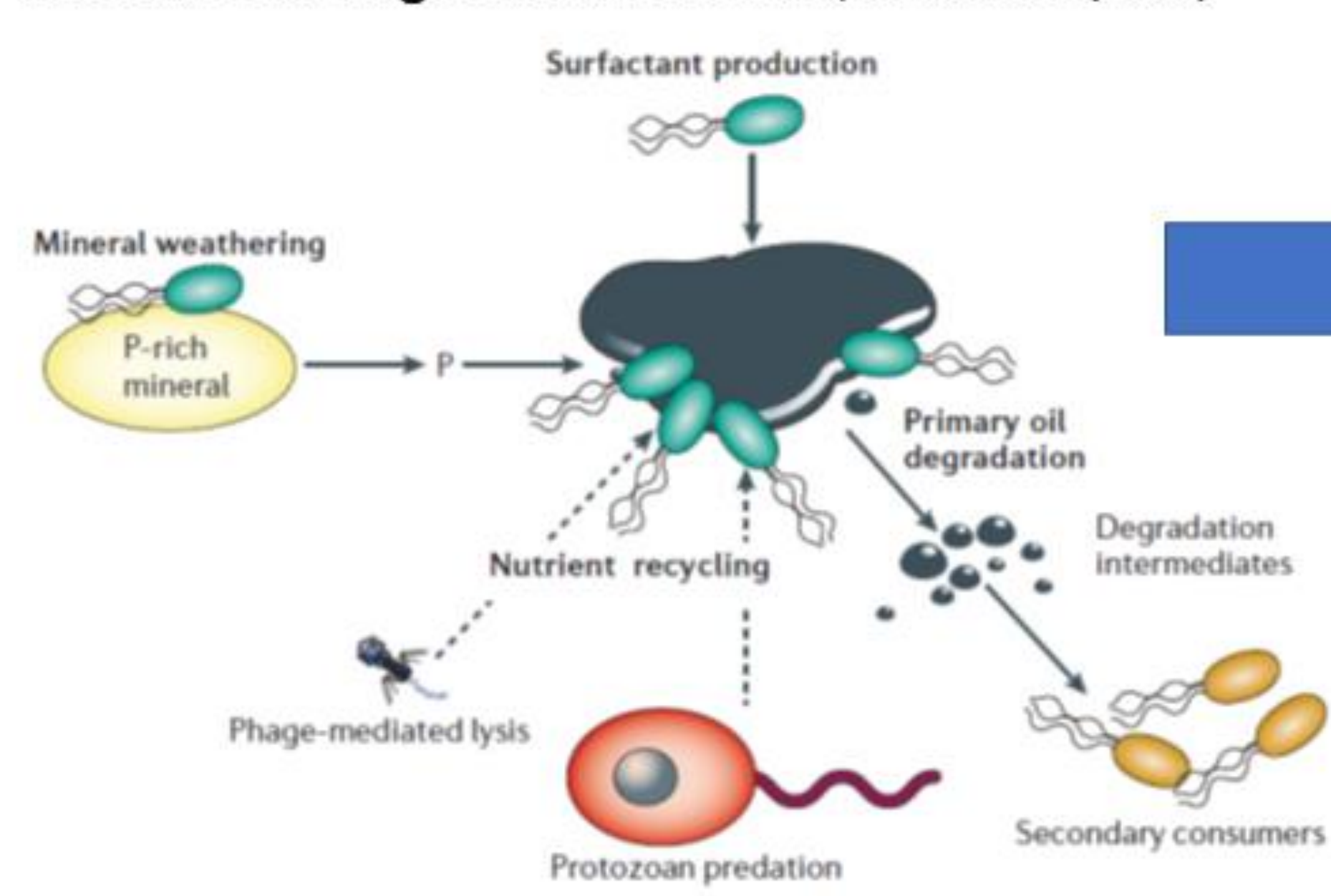
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Abstract The microbial oil-degradation network in the ocean includes direct degradation of hydrocarbons (*primary oil degradation*), as well as subsequent degradation of transformation byproducts and exopolymeric substances (EPS) through *secondary consumers*. EPS are produced by oil-degrading microbes to emulsify hydrocarbons and facilitate access to oil. Polysaccharides are the major constituent of microbial EPS in the ocean. We measured enzymatic hydrolysis of six structurally-distinct polysaccharides as indicator of microbial metabolic responses of secondary consumers to oil and dispersant additions in two laboratory incubations with either surface or deep water microbial communities from one of the most active natural oil and gas seep in the Gulf of Mexico (Green Canyon block 600). The natural assemblages were amended with crude oil (water-accommodated fraction - WAF) and chemical dispersant (Corexit 9500) in roller bottles incubated for 1 week (*Surface water*) and 6 weeks (*Deep water*). Our results demonstrate that oil and dispersant additions have the potential to change metabolic responses of *secondary consumers* with consequences for cycling of organic carbon and oil-contaminants in oil contaminated waters.

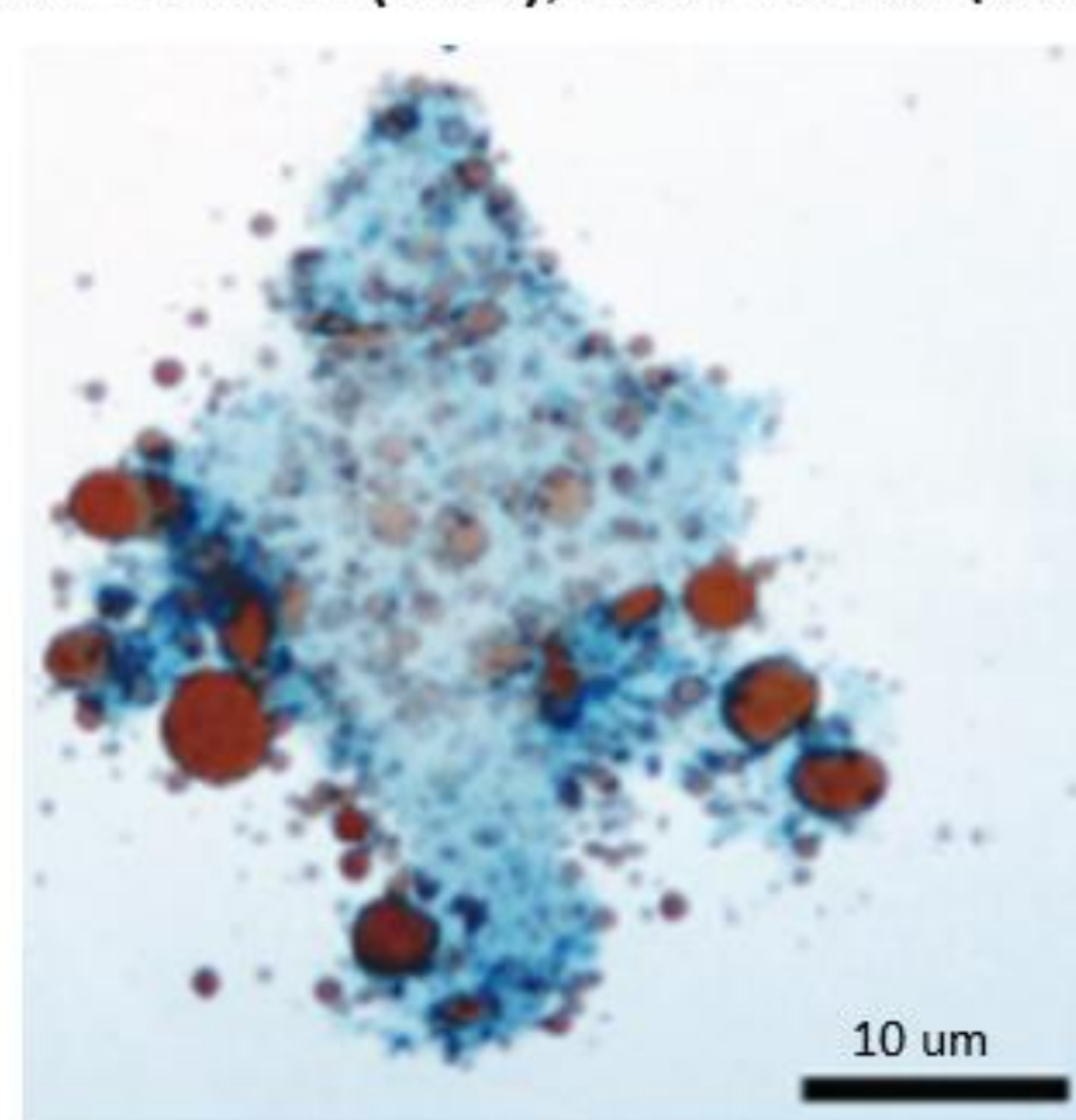
1. Background and research hypothesis

A: Polysaccharides act as emulsifier of oil and water (bio-surfactant)

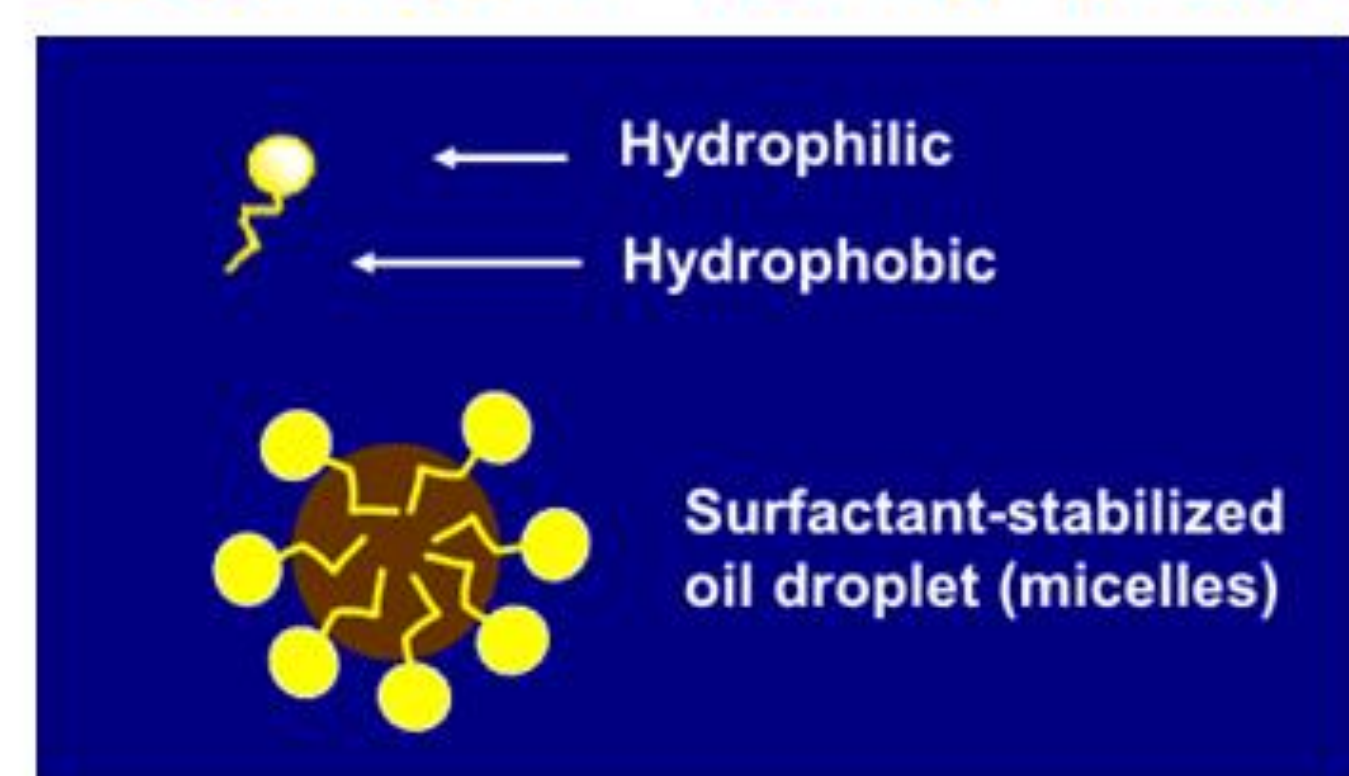
Microbial oil-degradation network, Head et al. (2006)



Halomonas with oil (red) and polysaccharides (blue), Gutierrez et al. (2013)



B: Surfactant - surface tension



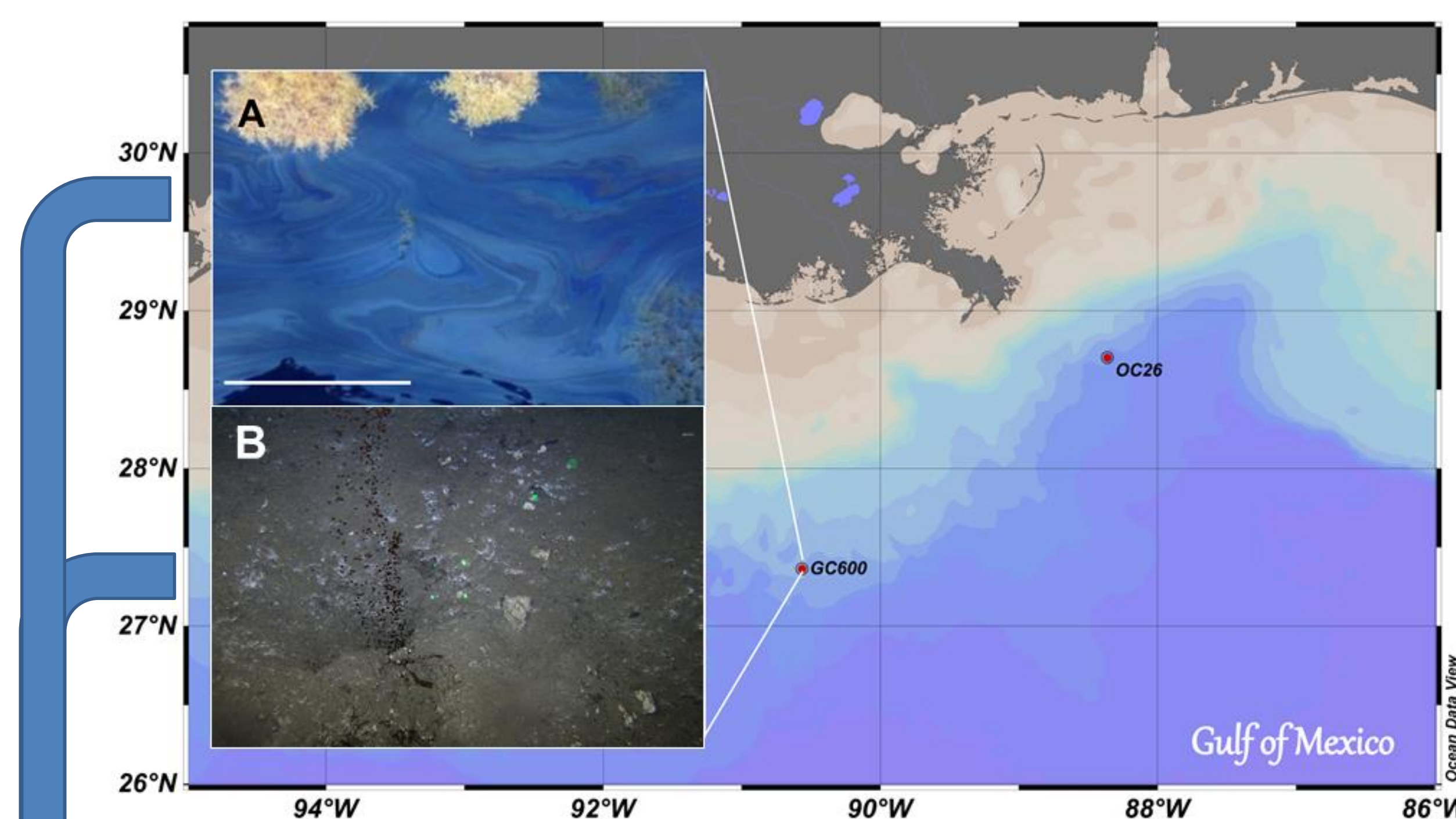
C: Surfactant (dispersant) application at the surface and at depth (BP spill 2010)



Hypothesis: Polysaccharides produced in the course of primary oil degradation stimulate activities of secondary consumers.

Strategy: Determine spectrum and rates of polysaccharide hydrolysis in natural assemblages incubated with oil (WAF vs chemically dispersed WAF).

2. Sampling site and experimental set-up



GC600:

One of the largest natural oil and gas seep of the Green Canyon oil reservoir; ~200 miles off the Louisiana coast.

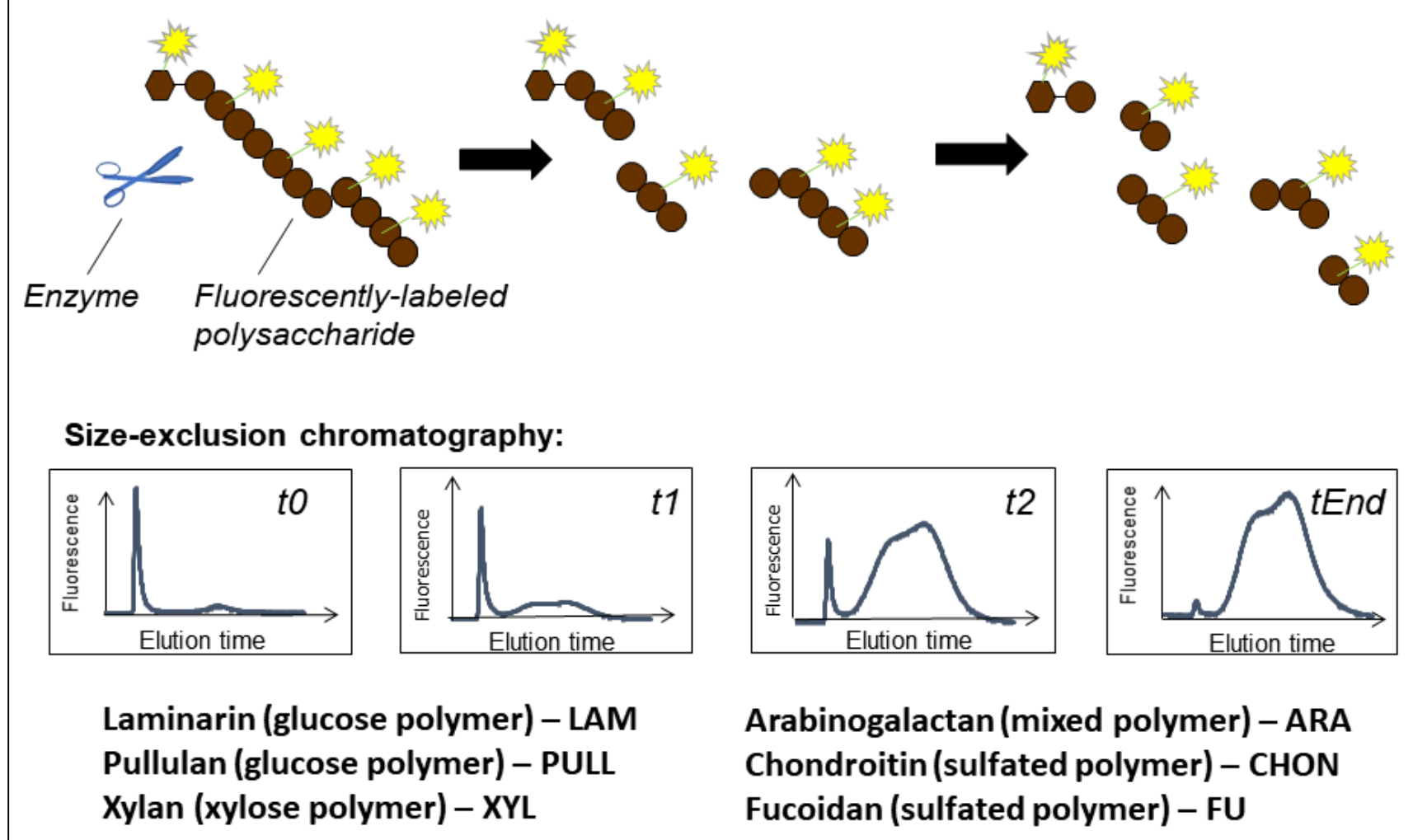
A: Thin oil slick at the surface;
B: Oil bubbling out of the seafloor; picture taken by Ian MacDonald (FSU), scales: 30 cm.

A: Surface water (1 week)
whole water control WAF Dispersant CEWAF

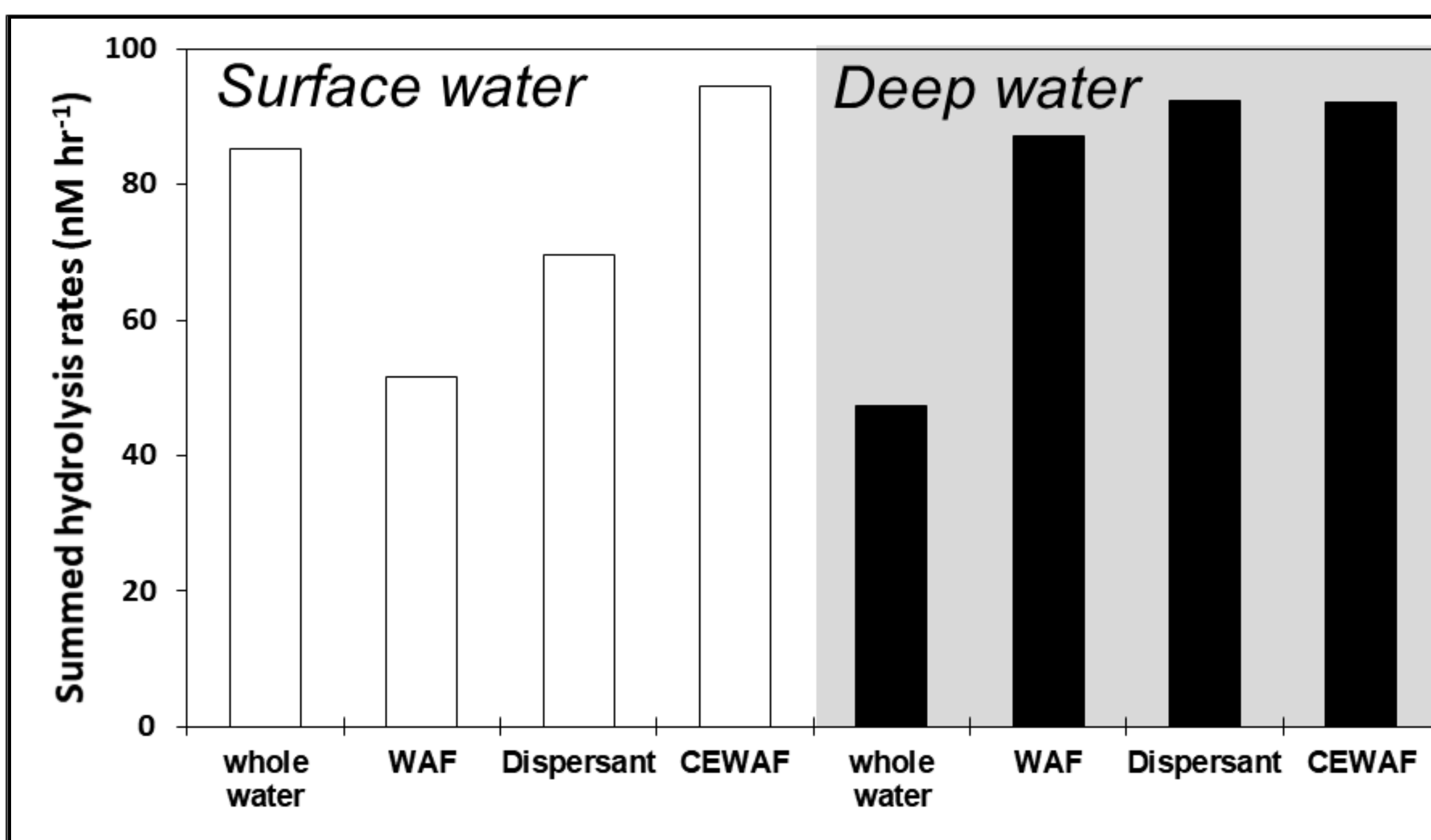
B: Deep water (6 weeks)
whole water control WAF Dispersant CEWAF

Treatment ID	Description
whole water	Whole water plus control water (1:8.5 v/v)
control	Filtered (0.2-µm) and pasteurized (2hr at 80°C) whole water
WAF	Water accommodated fraction (WAF) of oil into whole water (1:10 v/v)
Dispersant	Corexit 9500 into whole water (1:100 v/v)
CEWAF	Corexit 9500 into WAF (1:66 v/v) into whole water (1:500 v/v)

Fluorescently-labeled polysaccharides as substrates for hydrolytic enzymes



3. Results and discussion



❖ Enhanced polysaccharide hydrolysis in *Deep water* oil and dispersant treatments relative to whole water;

❖ In contrast to *Deep water*, oil and dispersant treatments in *Surface water* show lower (WAF, Dispersant) and similar (CEWAF) hydrolysis rates relative to whole water;

❖ Spectrum of polysaccharide hydrolyzing enzymes broader and more variable in *Surface water* compared to *Deep water*;

❖ LAM and XYL hydrolysis generally highest; note hydrolysis of FU (*Surface water*) and CHON (*Deep water*) → transparent exopolymer particles (TEP)

One-way ANOVA:

	Surface water				Deep water			
	Whole	WAF	Dispersant	CEWAF	Whole	WAF	Dispersant	CEWAF
LAM	A	AB		B	B	B	B	B
PULL	B	B		B	D	B	C	C
XYL	A	A	n.s.	A	n.s.	C	A	A
ARA	B	n.d.	n.s.	B	n.d.	n.d.	n.d.	n.d.
CHON	AB	n.d.		B	A	B	A	A
FU	AB	AB		B	n.d.	n.d.	n.d.	n.d.

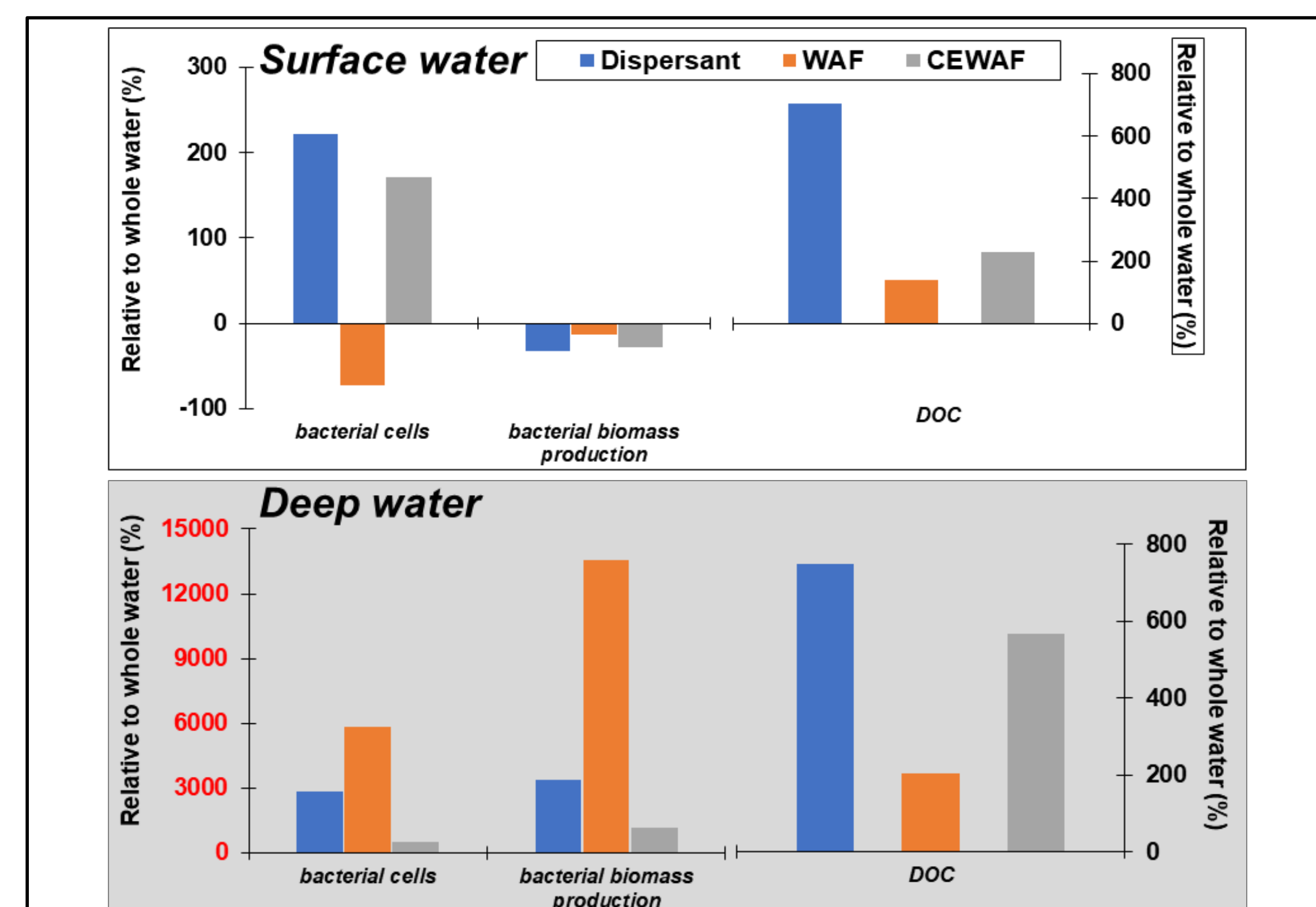
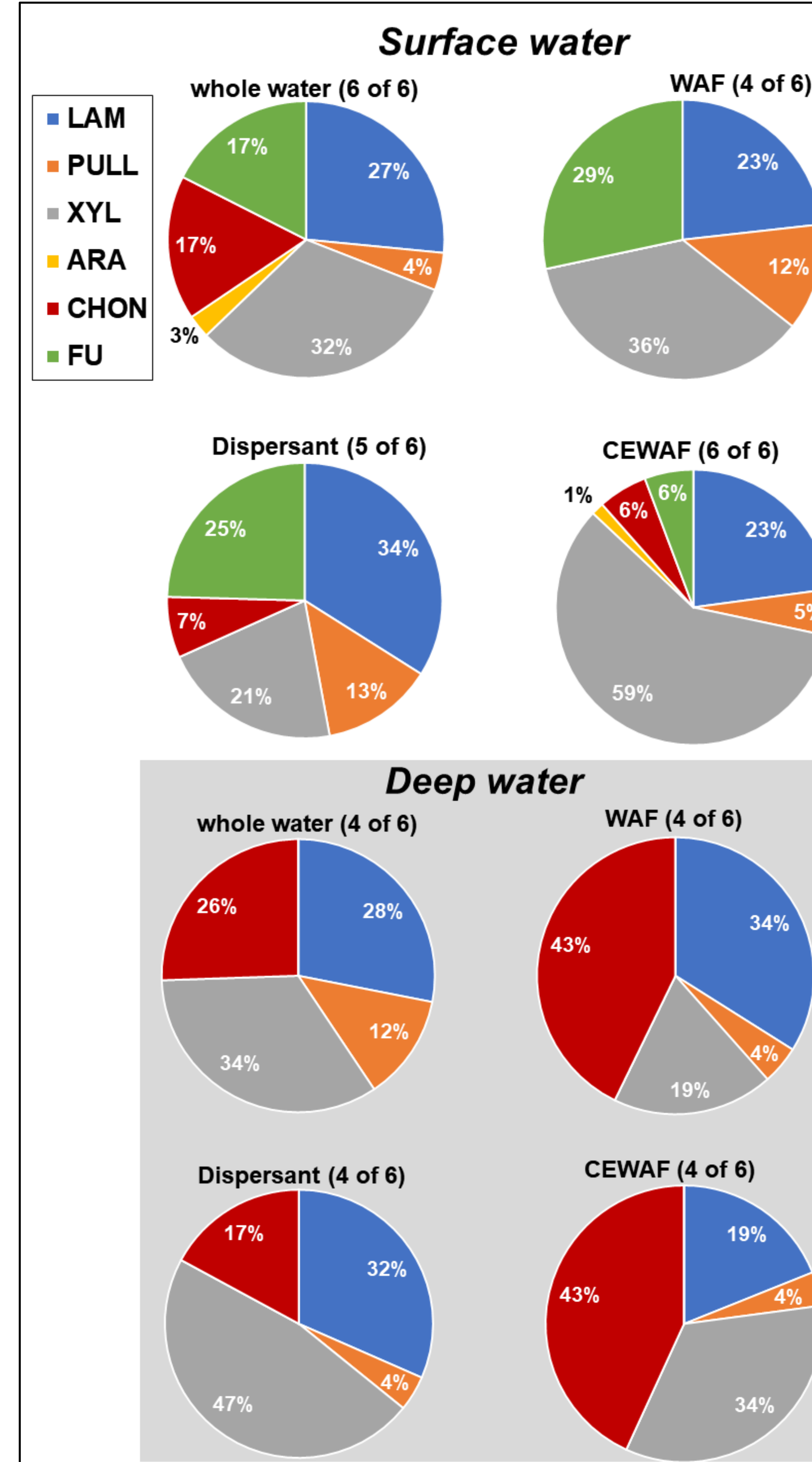
n.s. means not significant ($p < 0.05$); n.d. means not detectable

Conclusions:

- Stimulation of secondary consumers in *Deep water* fuels higher trophic levels in the deep Gulf;
- Oil-degradation network in *Surface water* more complex (protozoen predators; see cell counts in WAF treatment);
- *Surface and Deep water*: Chemical dispersant acts as carbon source for secondary consumers → pathway for contaminants into food webs

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Cited literature: Gutierrez et al. (2013), *PLoS ONE* 8(6): e67717. doi:10.1371/journal.pone.0067717; Head et al. (2006), *Nature Reviews* 4, 173-182; Kleindienst et al. (2015), *PNAS* 112 (48) 14900-14905



Additional mesocosm results from Kleindienst et al. (2015) – *Deep water* - and Malkin et al. (in prep.) – *Surface water*.