Studying ocean biogeochemistry, one cell at a time

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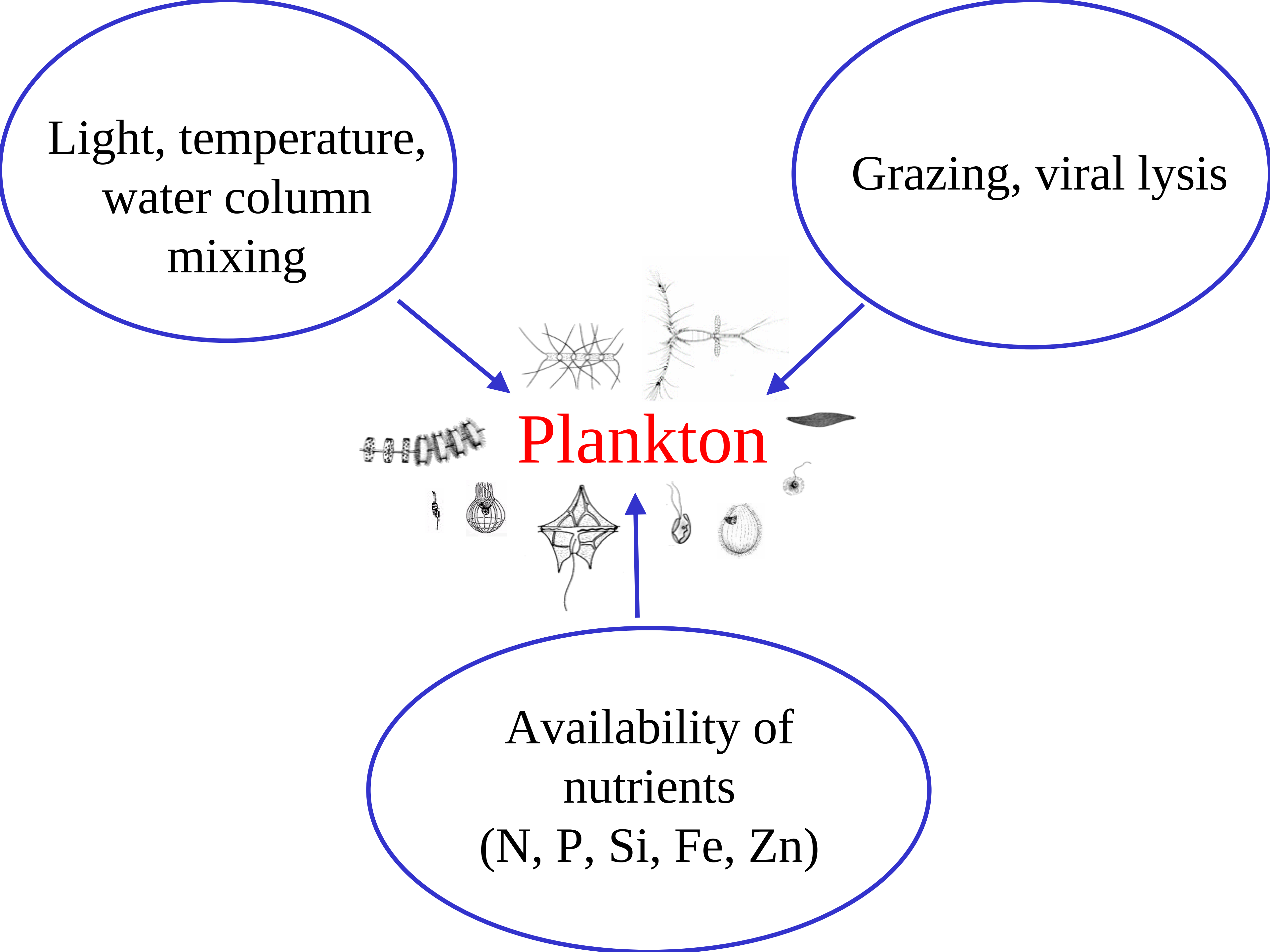
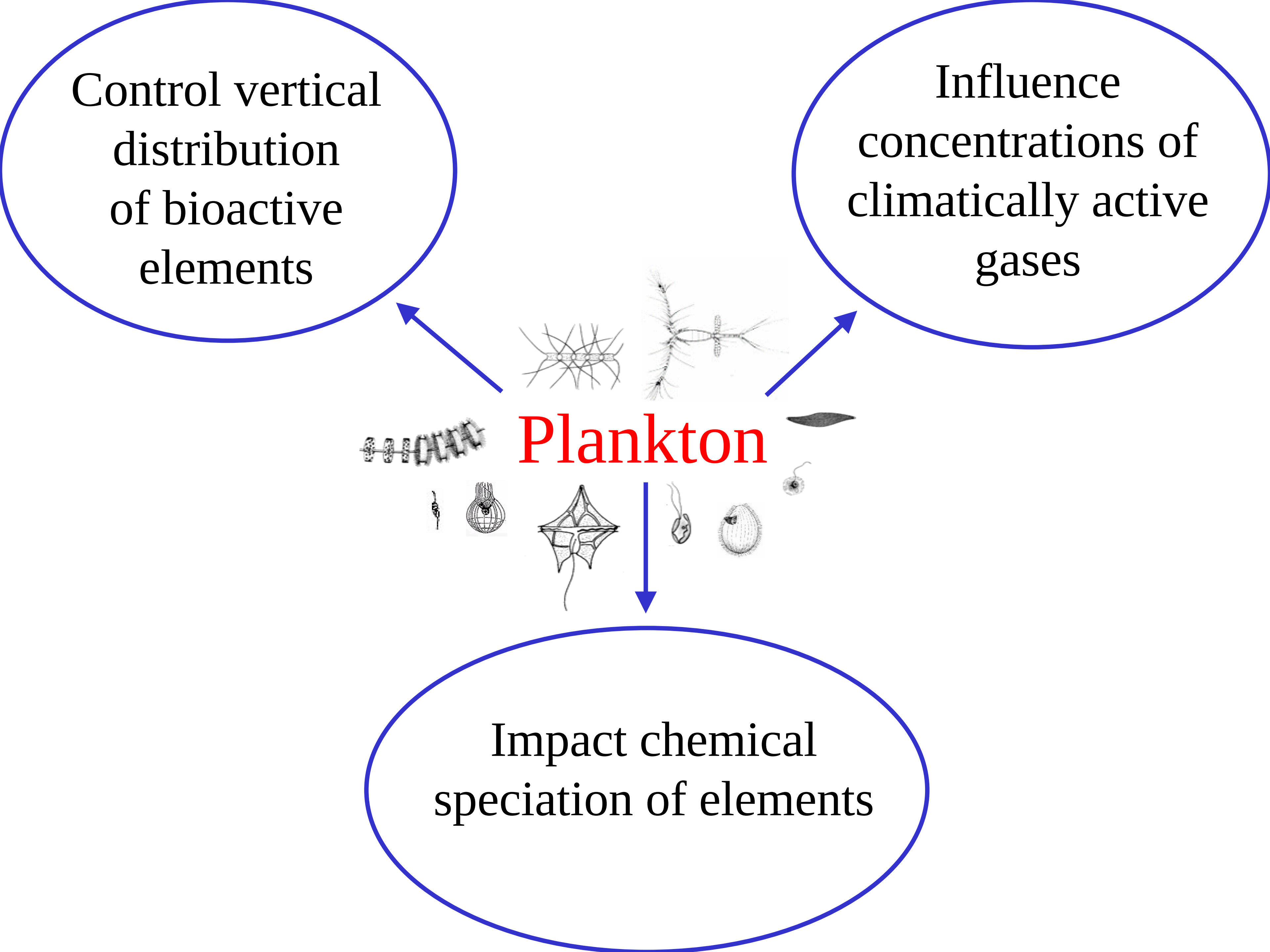
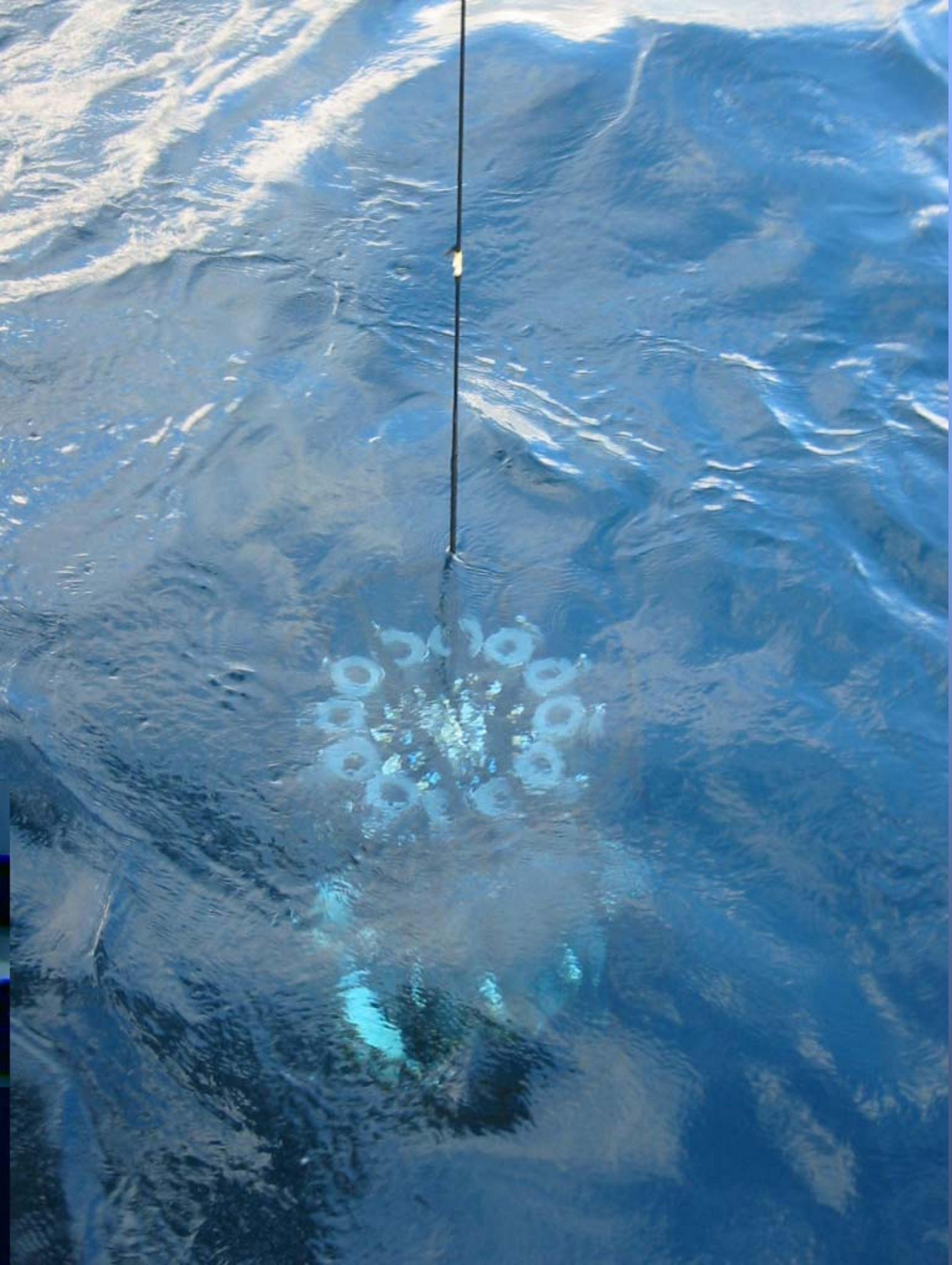

Light, temperature,
water column
mixing
Grazing, viral lysis
Plankton
Availability of
nutrients
(N, P, Si, Fe, Zn)


Control vertical distribution of bioactive elements
Influence concentrations of climatically active gases
Plankton
Impact chemical speciation of elements

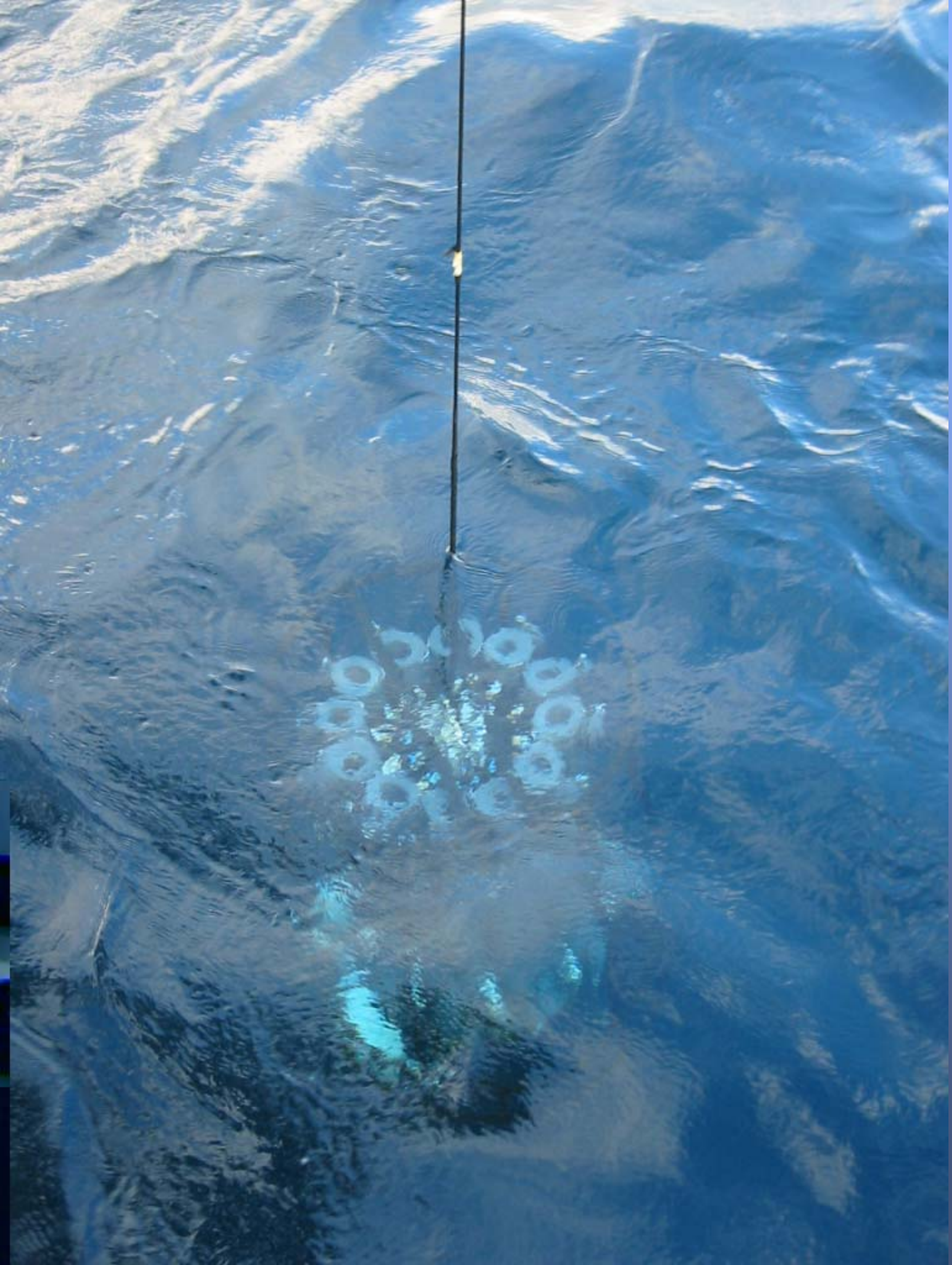
Plankton impact ocean biogeochemistry primarily by **accumulating elements** during growth and releasing them during degradation (often in different chemical forms).

Therefore, measurements of **plankton elemental composition** are needed to understand ocean biogeochemistry.



Outline

1. Importance of metal quotas to ocean biogeochemistry
2. A single-cell approach
3. Case study 1: Southern Ocean
4. Case study 2: Equatorial Pacific Ocean
5. Case study 3: Eddies in the Sargasso Sea



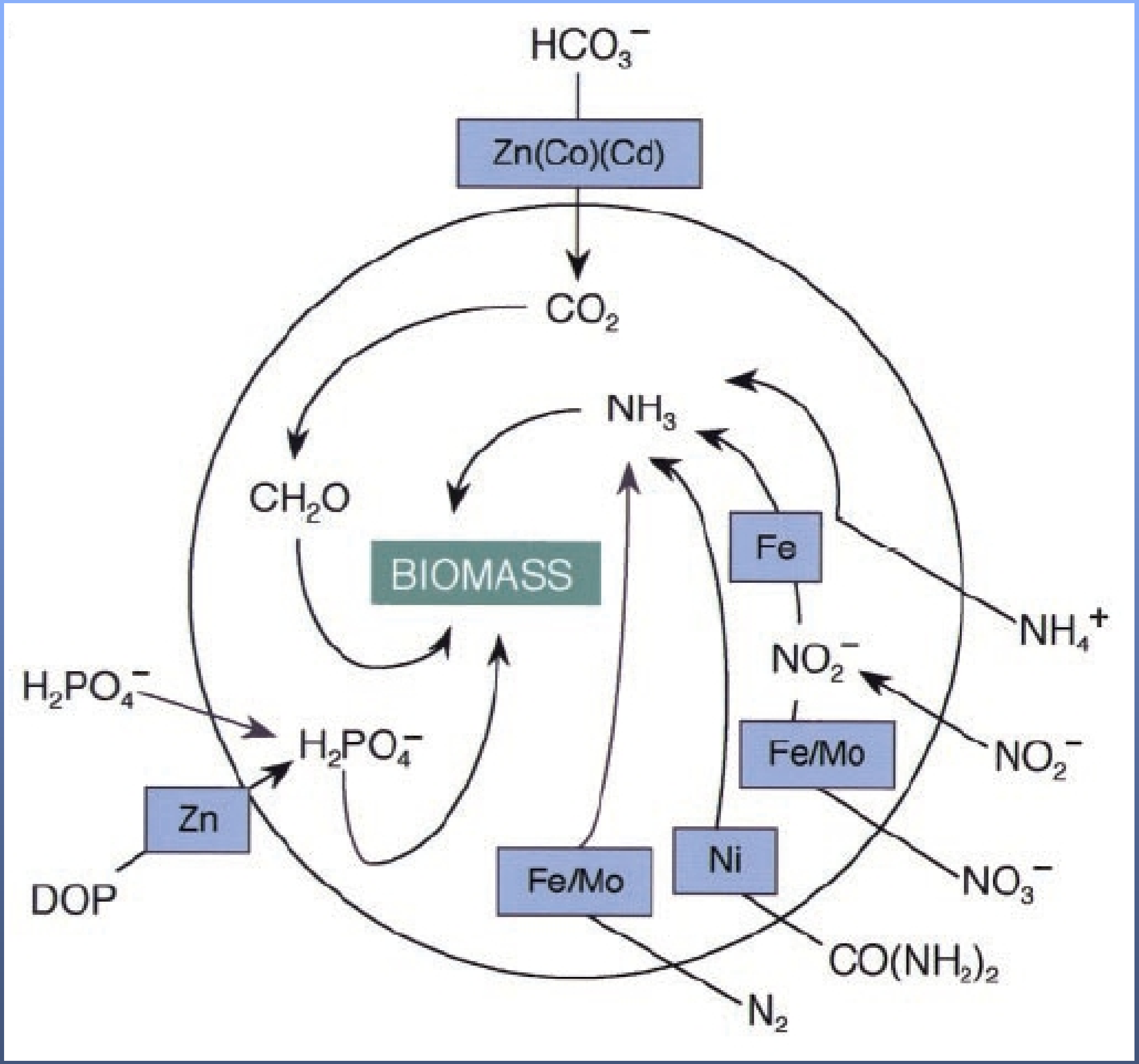
Outline

1. Importance of metal quotas to ocean biogeochemistry

Metalloproteins in phytoplankton

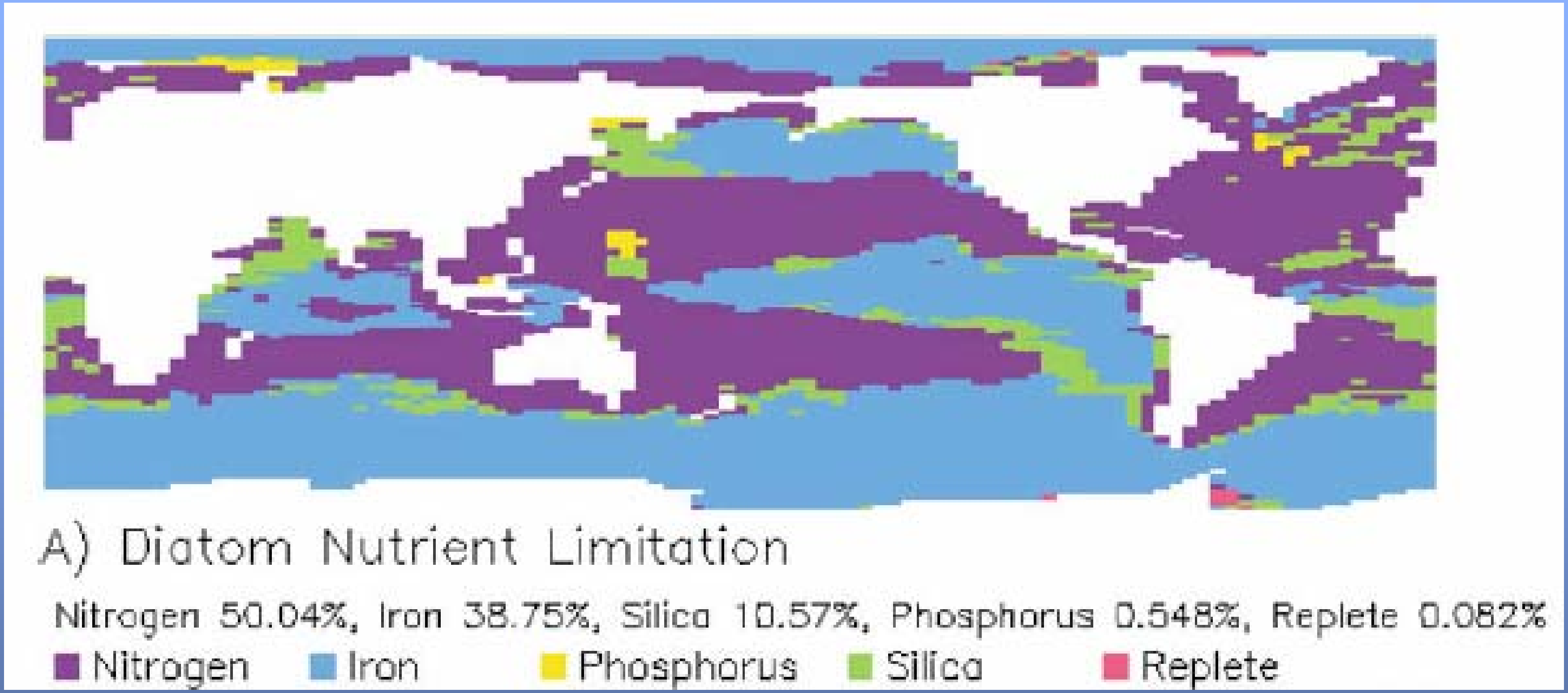
Metal	Compound	Function
Mn	O_2-evolving enzyme	Oxidize H_2O to O_2 during photosyn.
	Superoxide dismutase	Convert $O_2{\cdot}^-$ to H_2O_2
Fe	Cytochromes	Electron transport in photosyn./respiration
	Ferredoxin	Electron transport in photosyn./N-fixation
	Fe-S proteins	Electron transport in photosyn./respiration
	Nitrate reductase	NO_3^- assimilation
	Chelatase	Porphyrin and phycobiliprotein synthesis
	Nitrogenase	N fixation
Cu	Plastocyanin	Photosynthesis electron transport
	Cytochrome *c* oxidase	Mitochondrial electron transport
Zn	Carbonic anhydrase	Hydration and dehydration of CO_2
	Alkaline phosphatase	Hydrolysis of phosphate esters
	DNA/RNA polymerase	Nucleic acid replication/transcription
Co	Vitamin B_{12}	Carbon and H transfer reactions
Ni	Urease	Hydrolysis of urea
Mo	Nitrogenase	Nitrogen fixation
	Nitrate reductase	Nitrate reduction to ammonia

Role of trace metals in C, N and P biogeochemistry



(Morel & Price 2003)

Iron limitation in the ocean



(Moore et al. 2002)

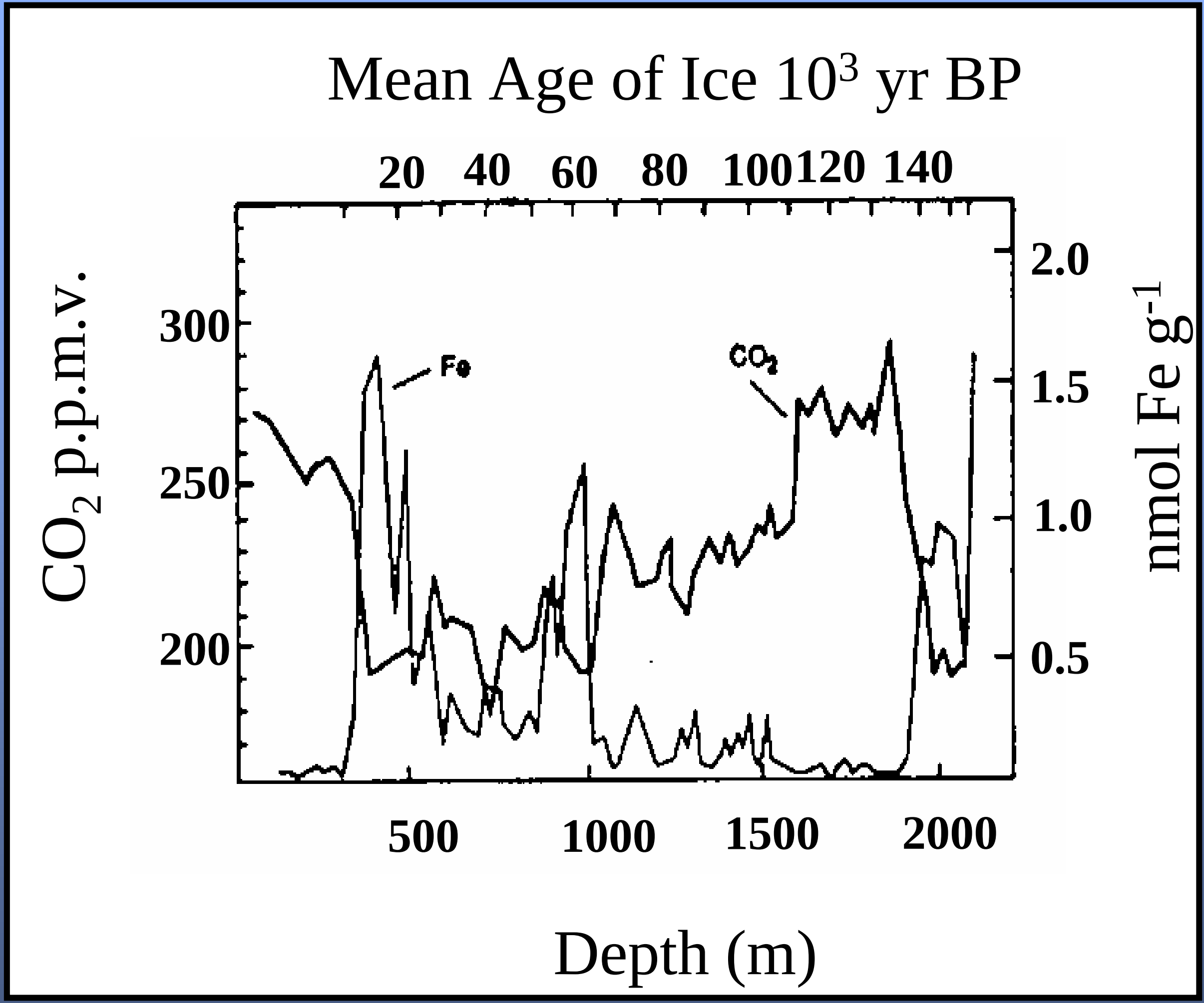
Equatorial Pacific:

Largest oceanic source of CO_2 to the atmosphere

Southern Ocean:

Region of upwelling and bottom water formation

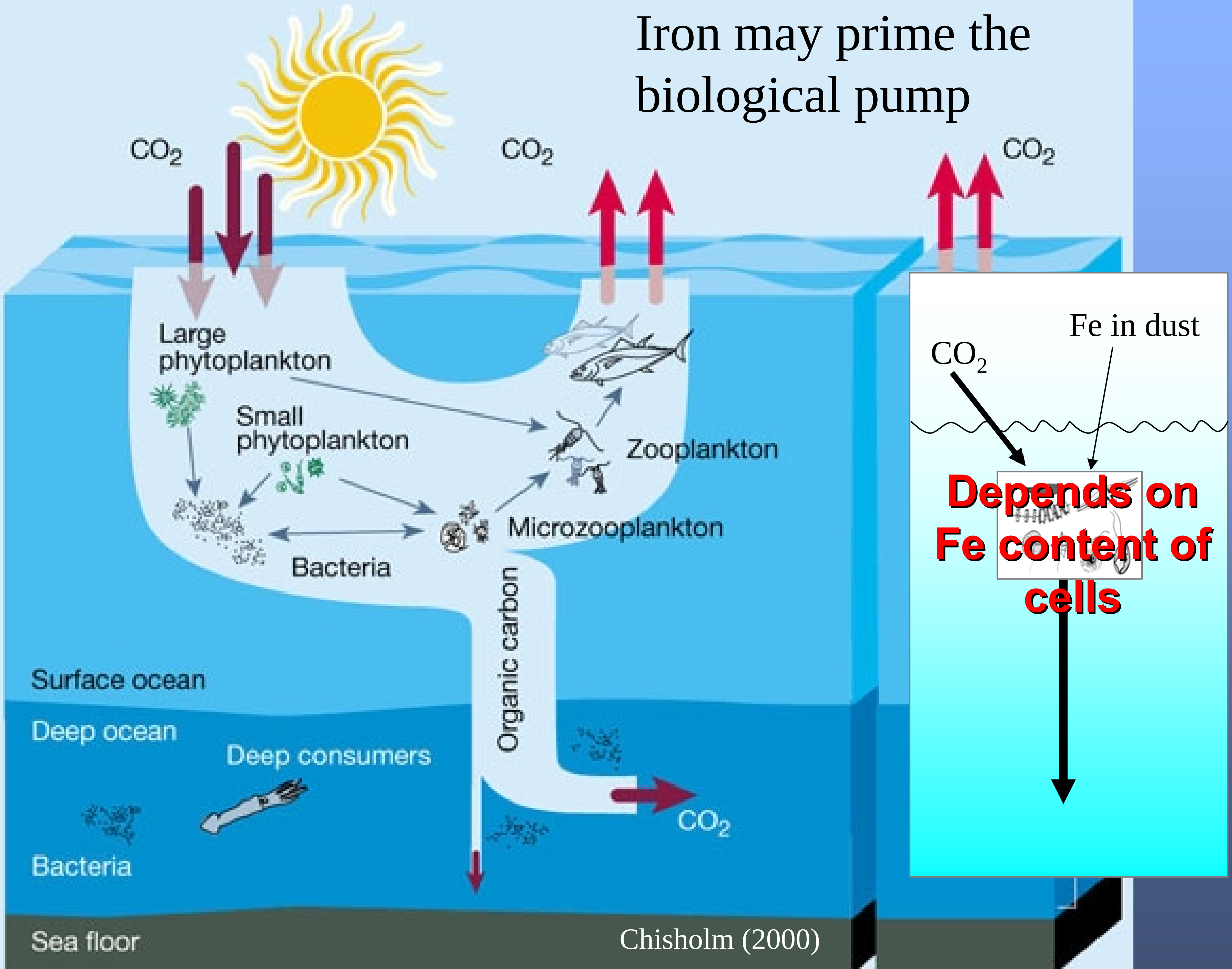
The Iron Hypothesis



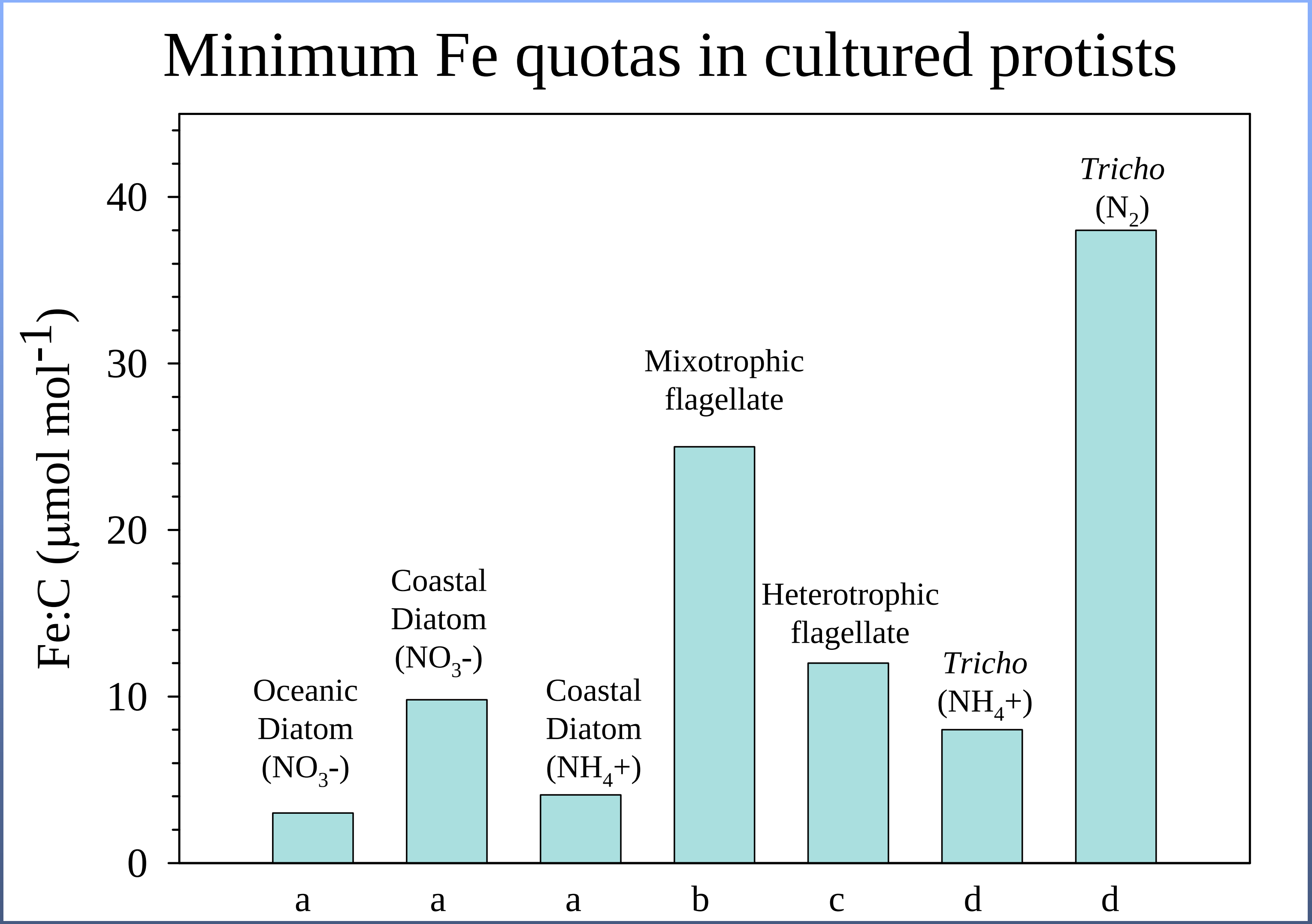
(Martin 1990)

Inverse correlation of Fe and CO_2 in Antarctic ice

Iron may prime the biological pump



How much Fe do phytoplankton need?



References

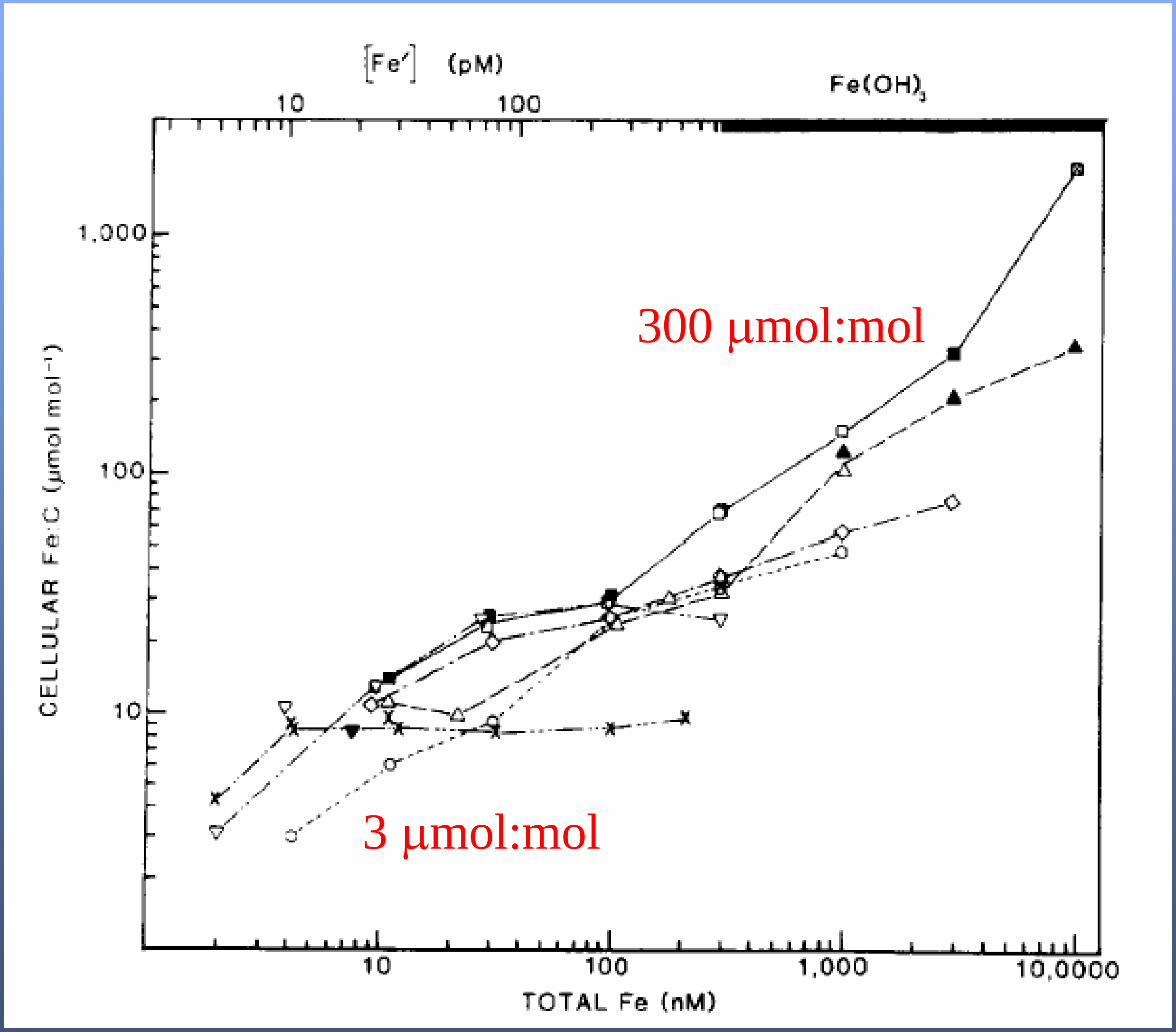
a. Maldonado & Price, 1996

b. Maranger et al., 1998

c. Chase & Price, 1997

d. Kustka et al., 2003

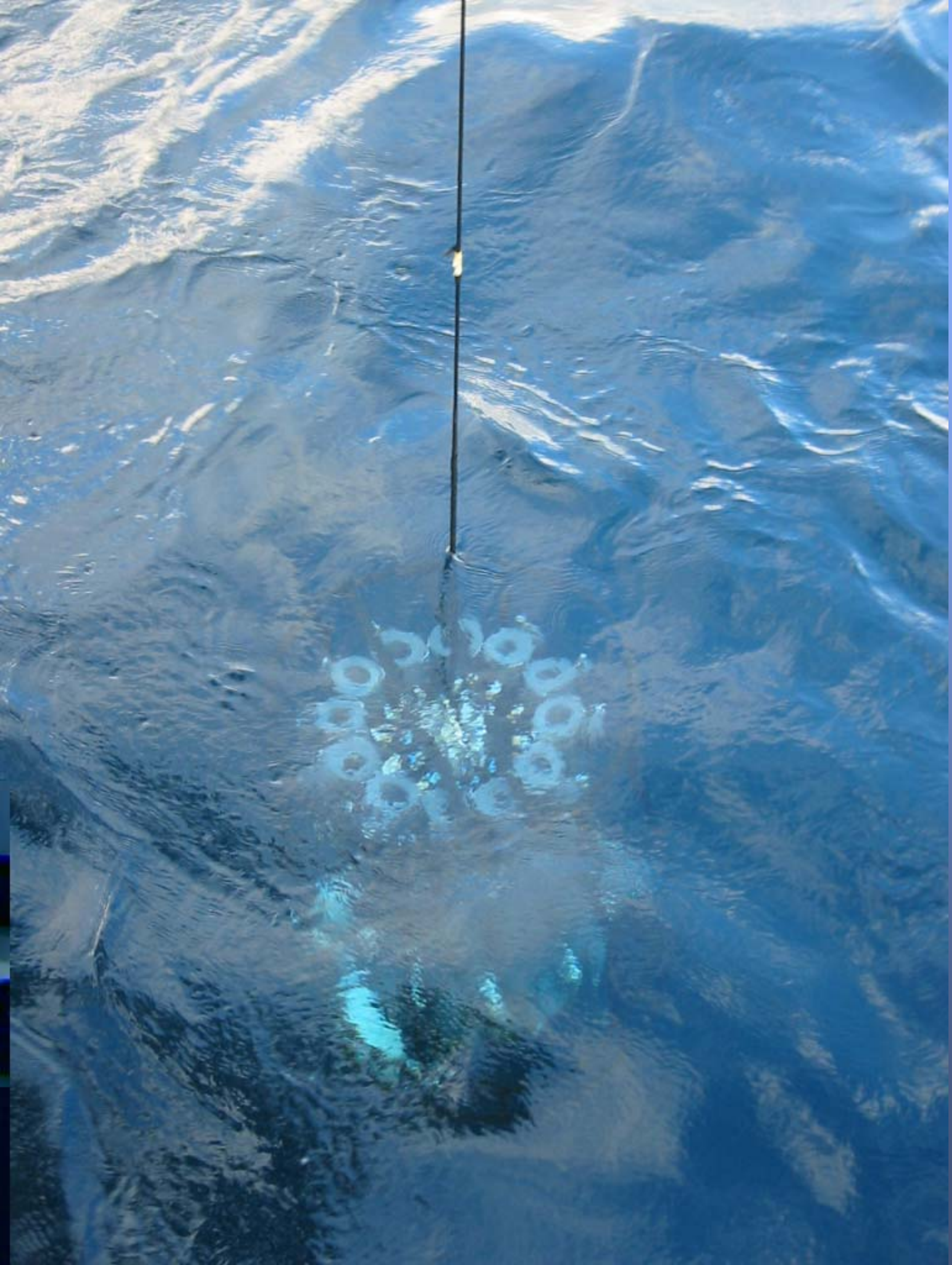
Phytoplankton capable of 'luxury' iron uptake and storage



(Sunda and Huntsman 1995)

- *E. huxleyii*
- *T. weissflogii*
- *T. pseudonana*
- *P. minimum*

- Fe quotas span 2 orders of magnitude



Outline

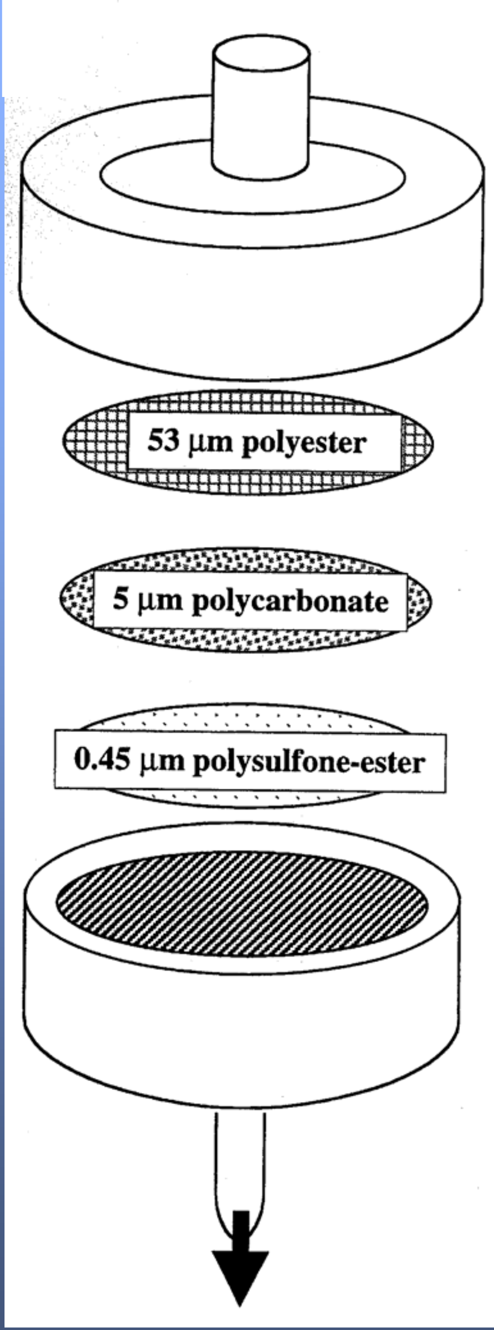
1. Importance of metal quotas to ocean biogeochemistry
2. A single-cell approach

Bulk measurements of metal quotas

- Cells concentrated on filter membranes for analysis by AAS/ICP-MS
- Radiotracer incubations of natural samples or lab cultures followed by filtration and counting

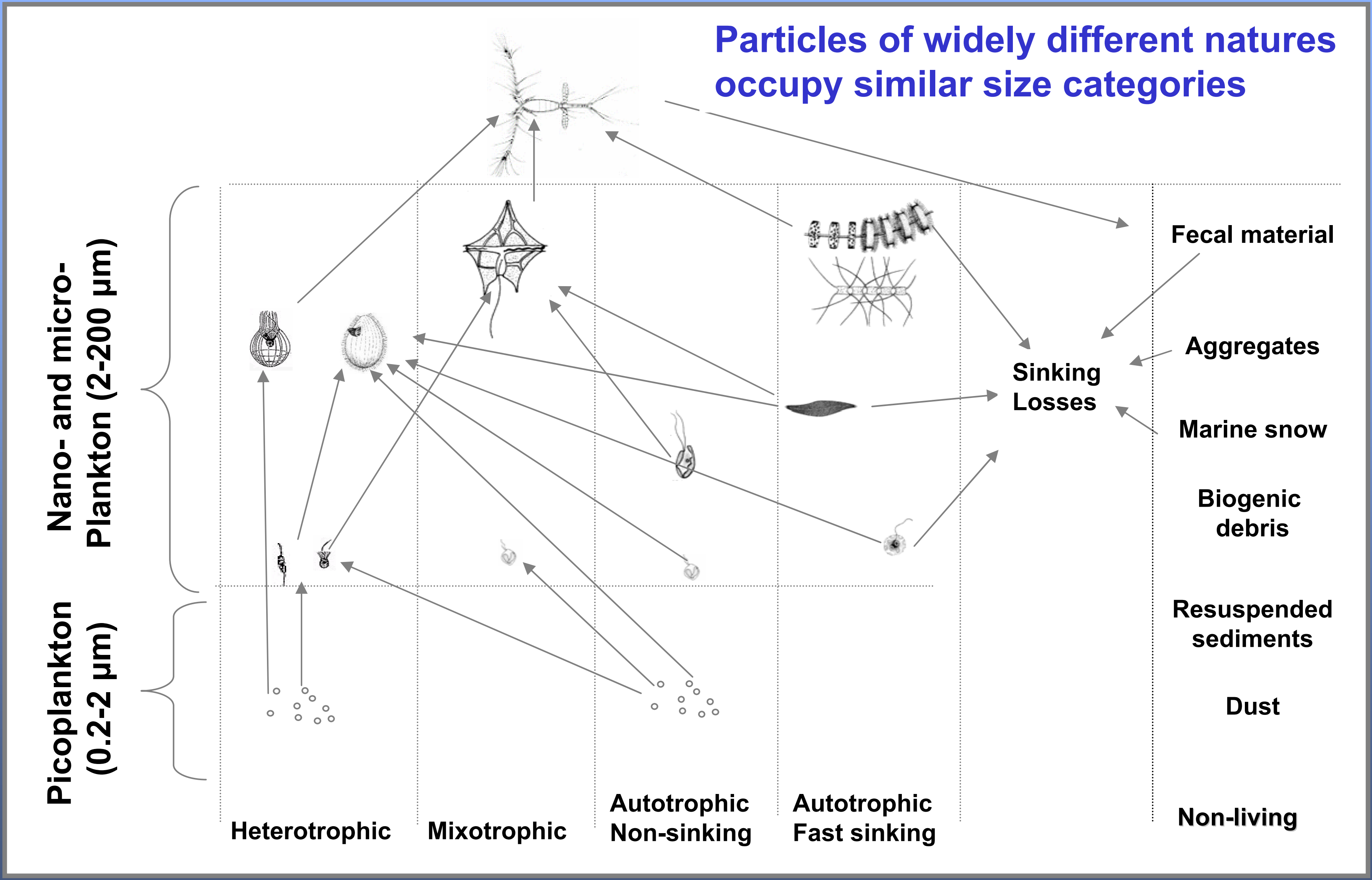
however….

- Delicate cells may burst during filtration
- Cannot distinguish between similarly-sized cells and abiotic particles
- Cannot distinguish between cells of different taxonomy or trophic function



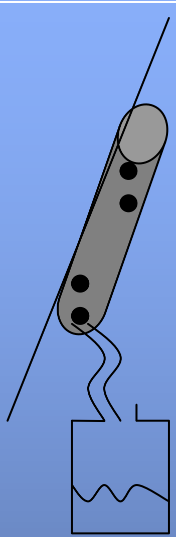
(Cullen & Sherrell 1999)

Limitations of bulk size-fractionation

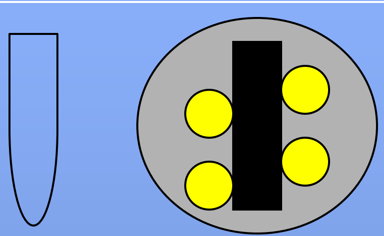


Taxonomy influences biogeochemistry

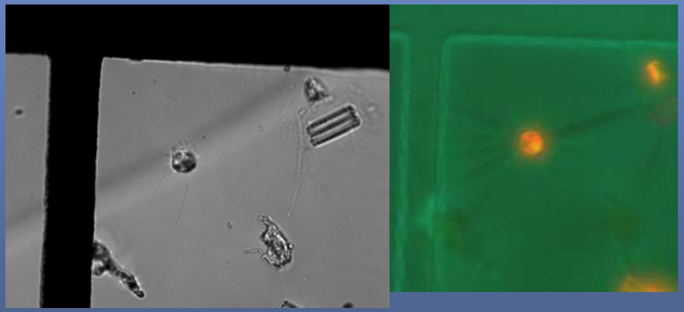
Synchrotron x-ray fluorescence microscopy



1. Collection: trace metal 'clean' water collection with Teflon-lined bottles

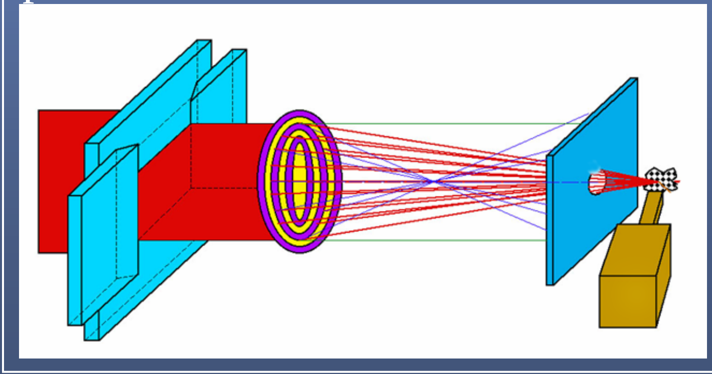


2. Mounting: samples preserved and centrifuged onto gold TEM grids. Grids rinsed with Milli-Q and dried in laminar flow hood

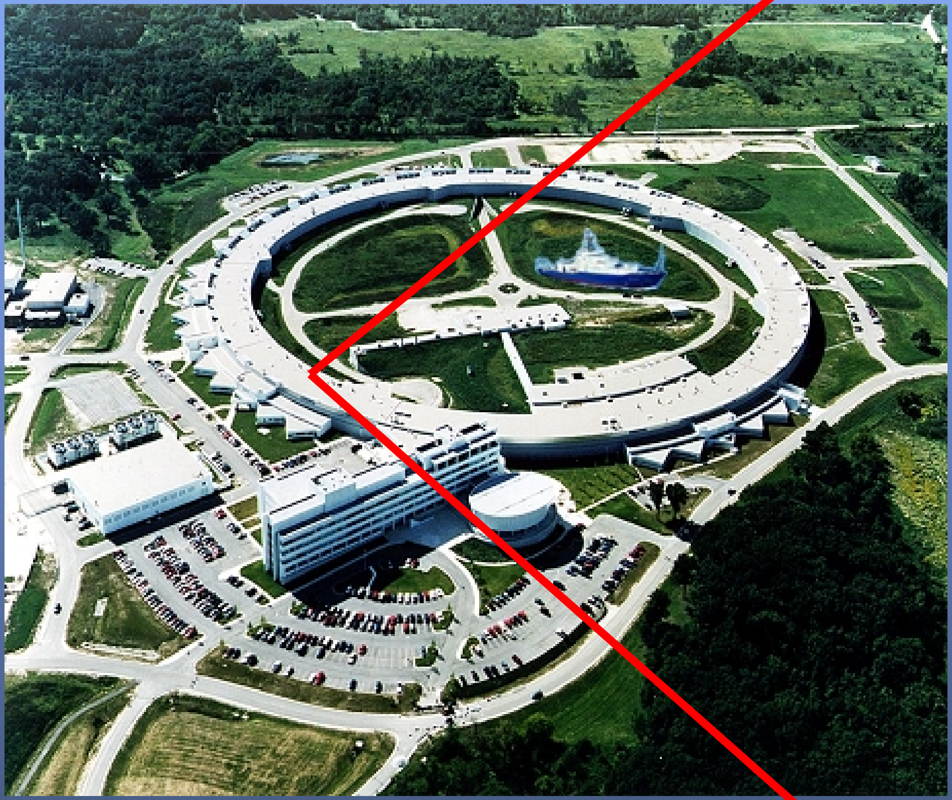


3. Targeting: grids examined with light and epifluorescence microscopy. Kinematic stage used to map locations of cellular targets

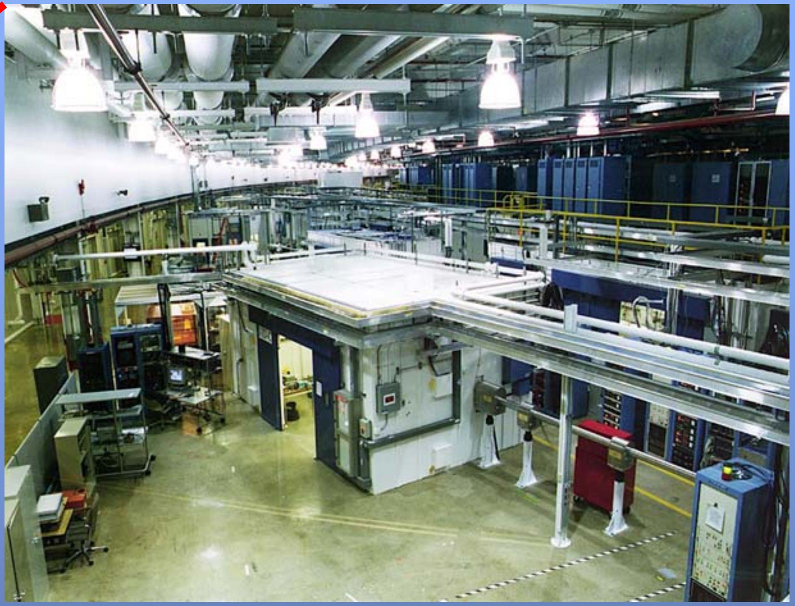
4. SXRF: target raster scanned with incident X rays. Excited x-ray fluorescence spectra recorded at each pixel

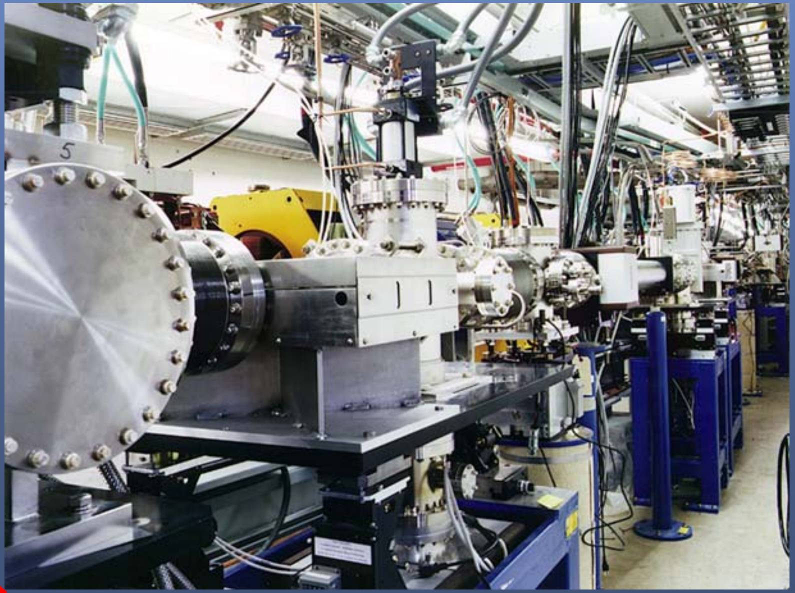


Synchrotron x-ray fluorescence microscopy

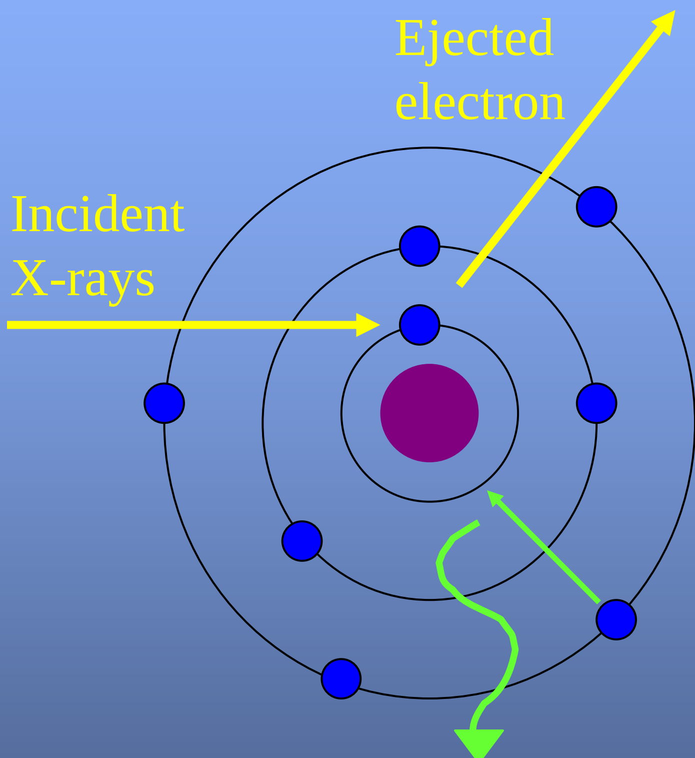


Advanced Photon Source
Argonne National Laboratory

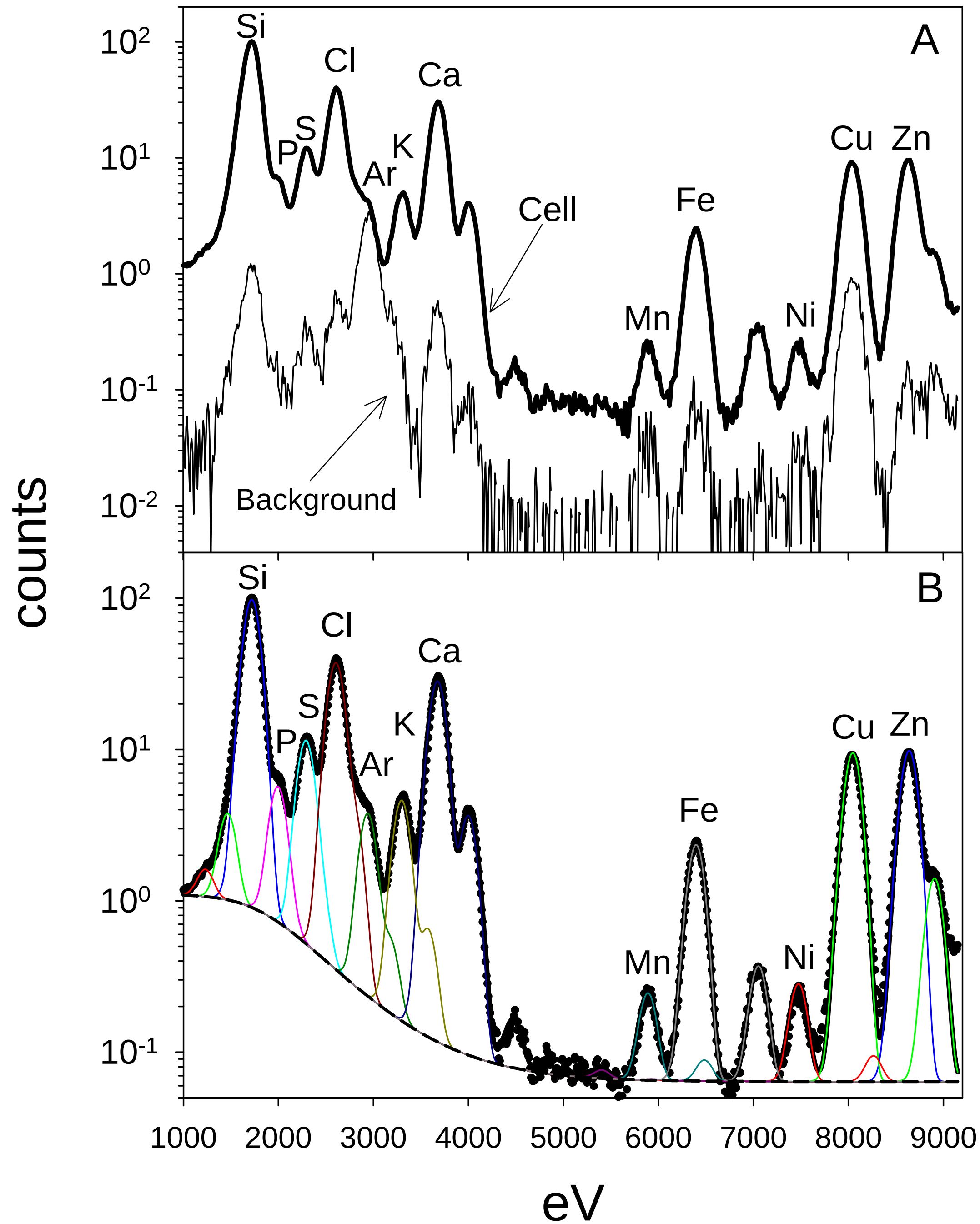




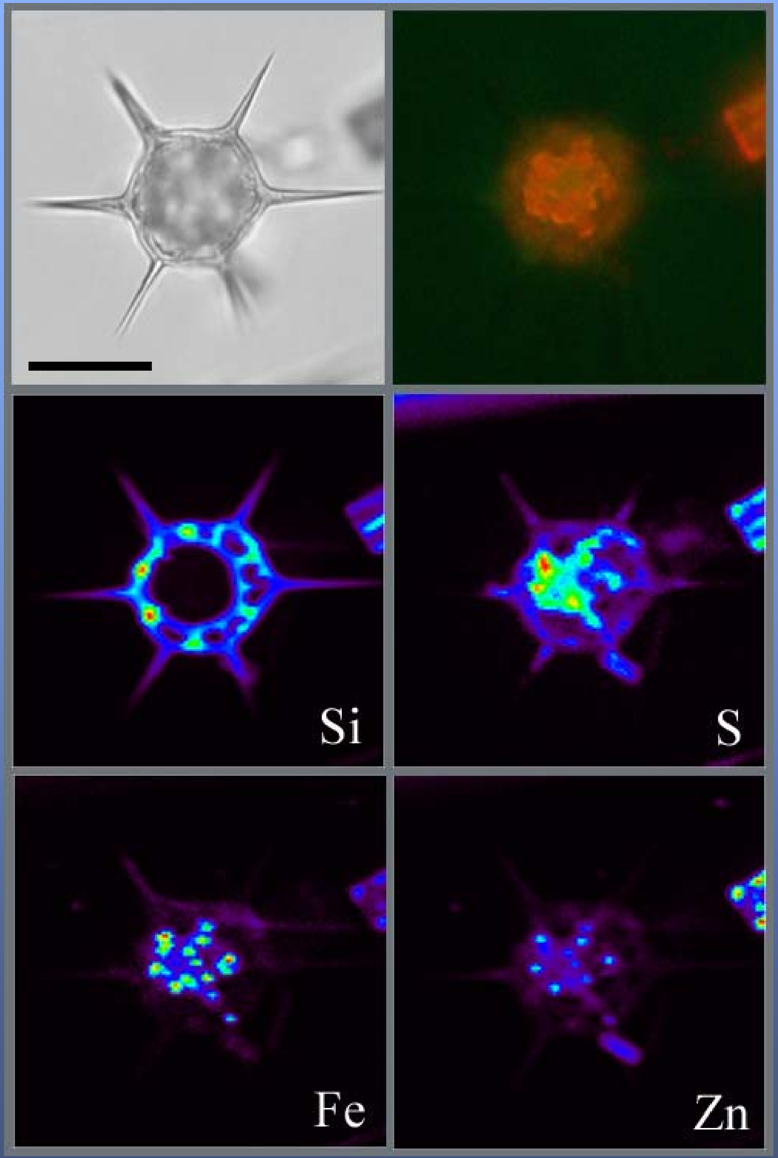
X-ray fluorescence



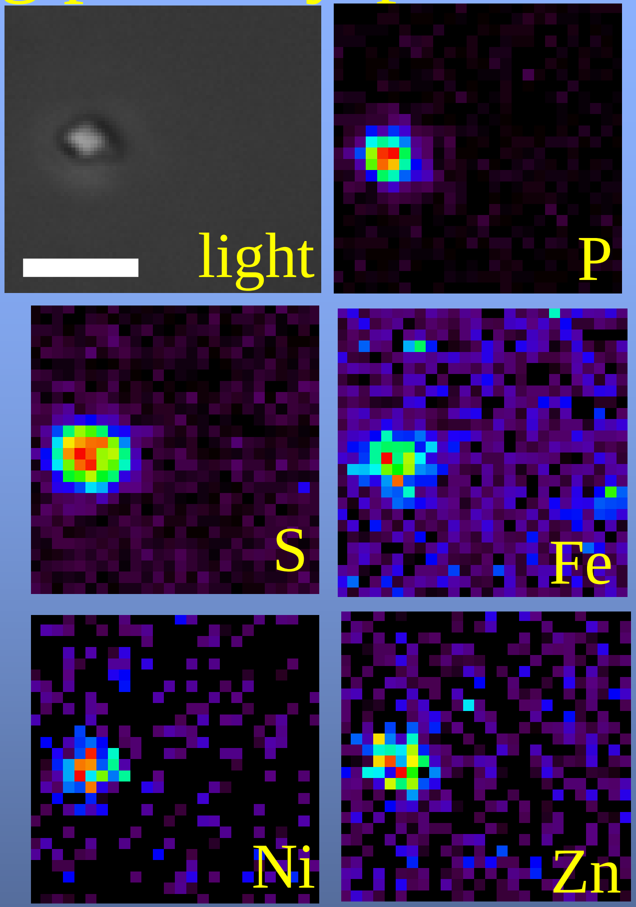
Fluorescence photons emitted when electron from outer orbital fills vacancy



Element maps generated using pixel-by-pixel fitting



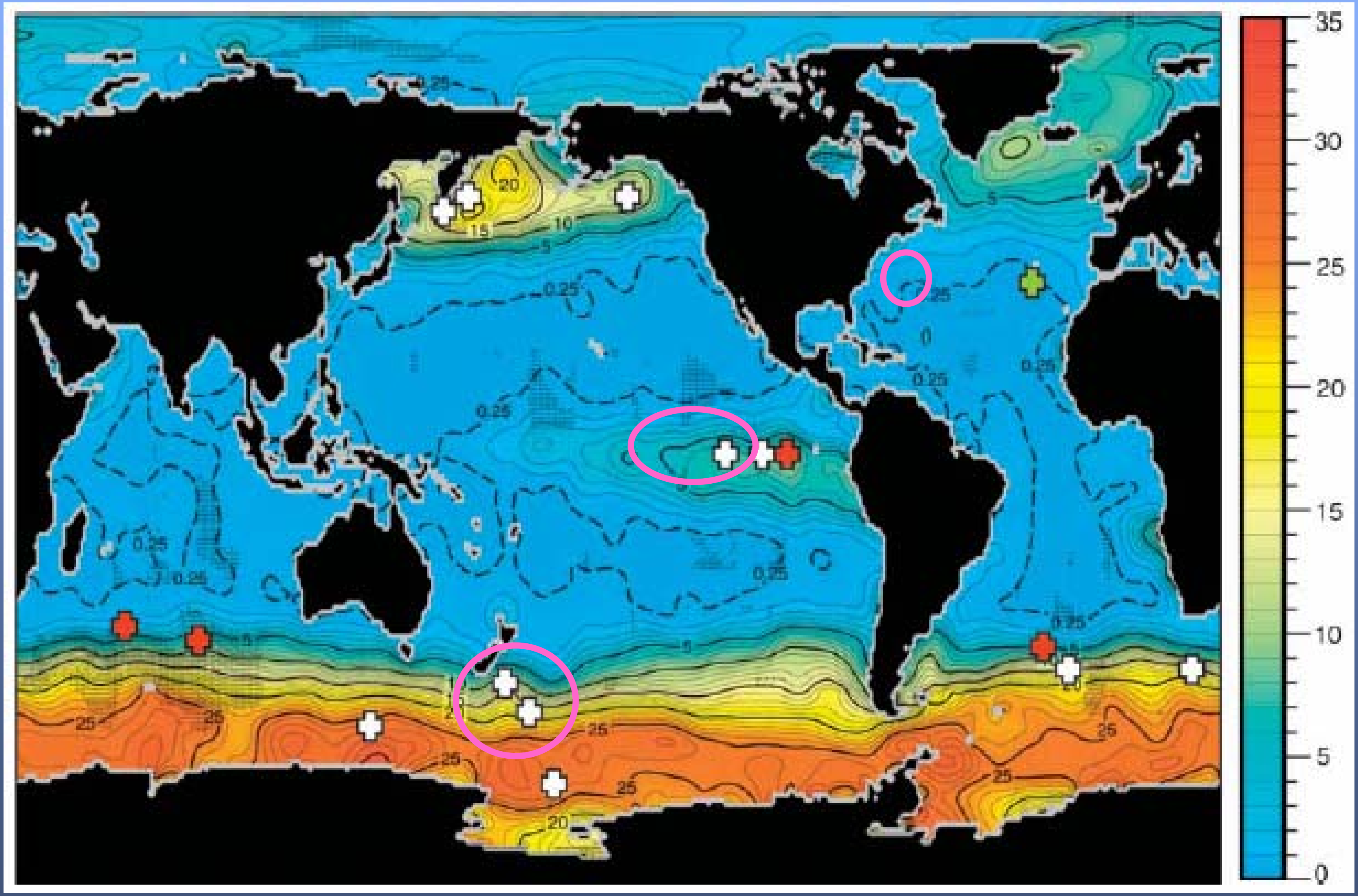
Silicoflagellate (~20 µm)



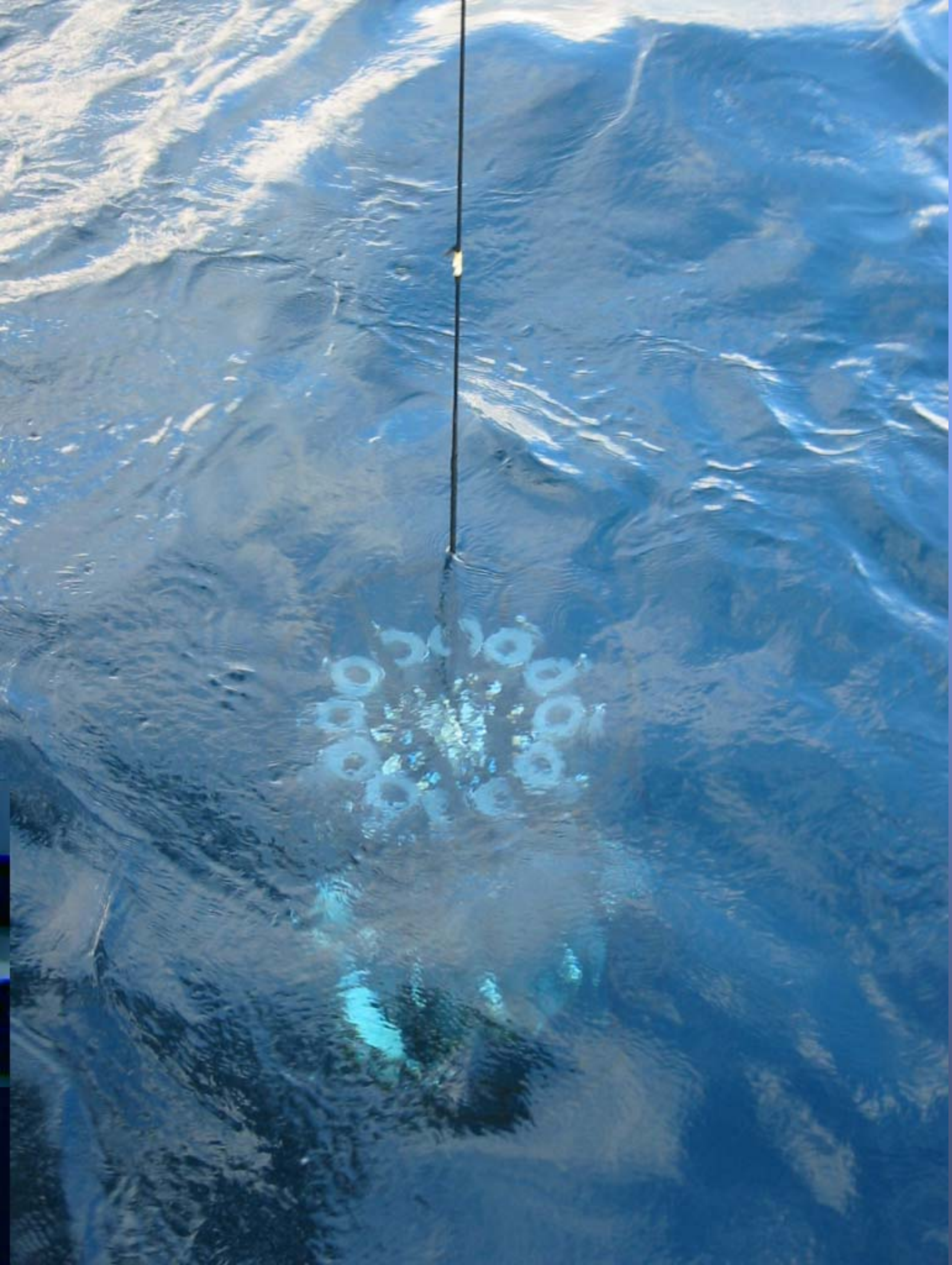
Autotrophic picoplankton (~1 µm)

C calculated from P, S, or cell volume.

How can this approach be used to understand ocean biogeochemistry?



(Boyd et al. 2007)



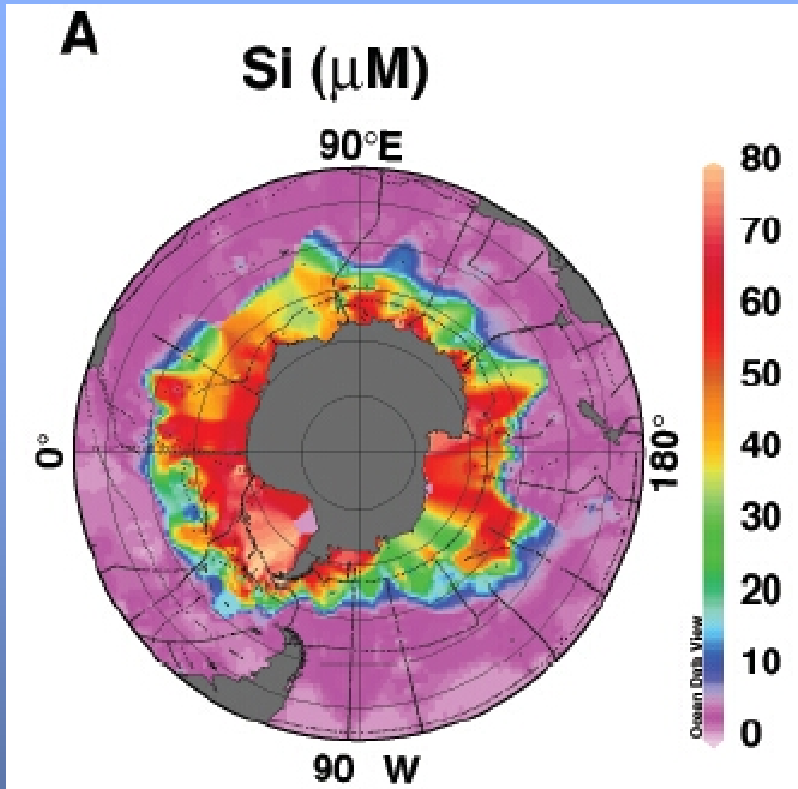
Outline

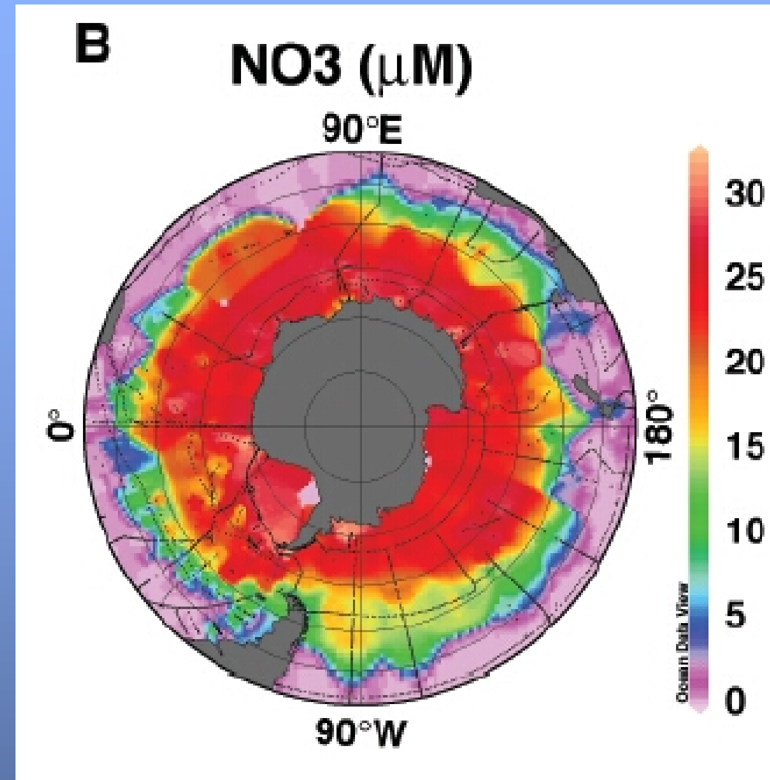
1. Importance of metal quotas to ocean biogeochemistry

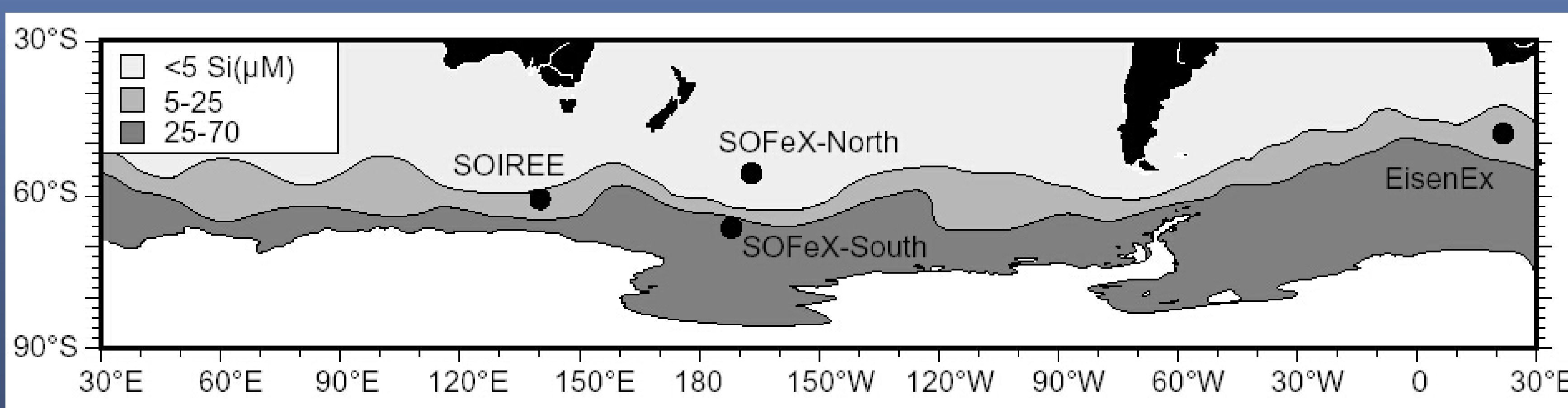
2. A single-cell approach

3. Case study 1: Southern Ocean

Southern Ocean Iron Experiment







Southern Ocean Iron Experiment (SOFeX)

Guiding questions:

What are the metal quotas of plankton in the Southern Ocean?

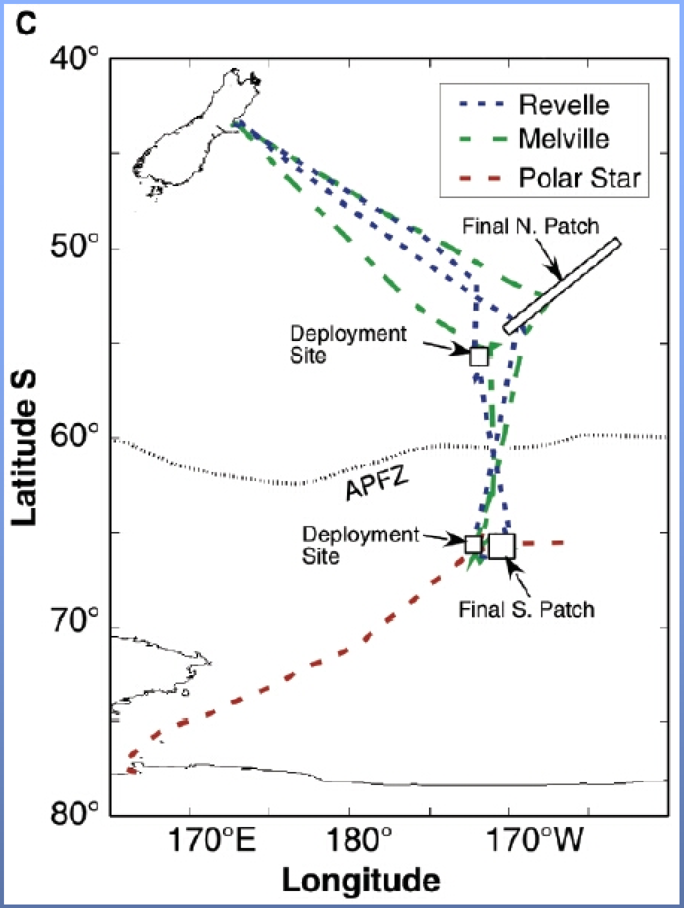
How do metal quotas respond to inputs of Fe?

Are there significant differences between taxa?

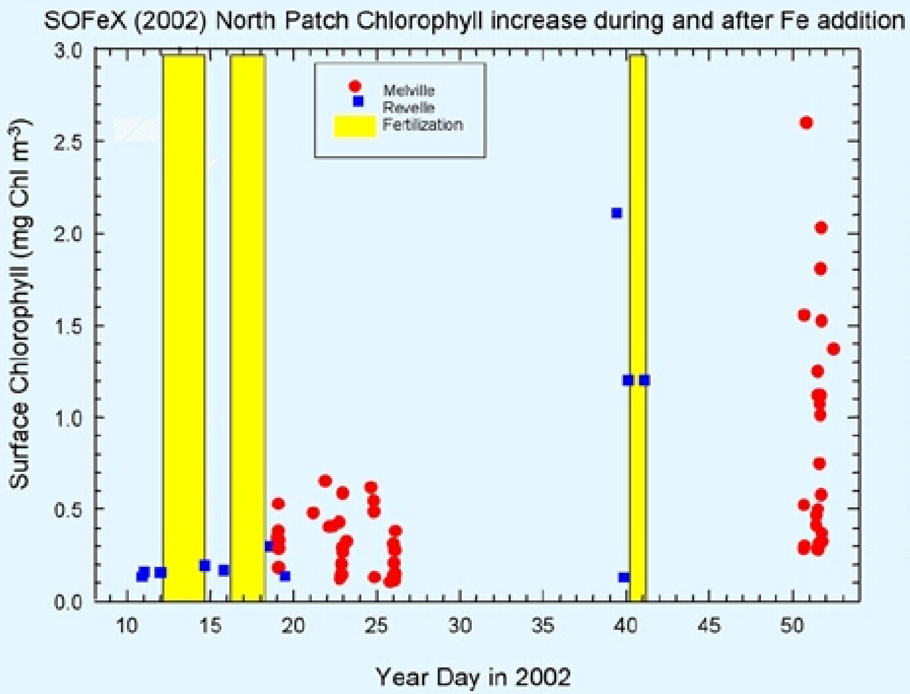
What do these data suggest about the biogeochemical fate of the bloom?

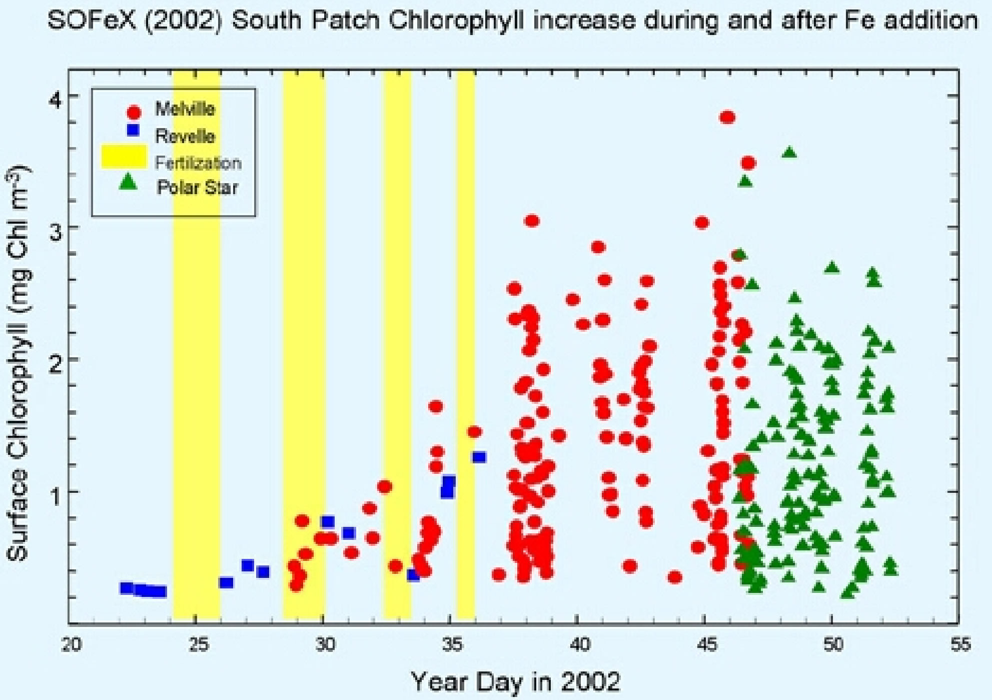
SOFeX cruise plan

- Austral summer: Jan-Feb
- Fertilized two patches of water:
  - North: high N, P; low Si
  - South: high N, P, Si
- Plankton samples collected inside and outside each patch for x-ray fluorescence analysis

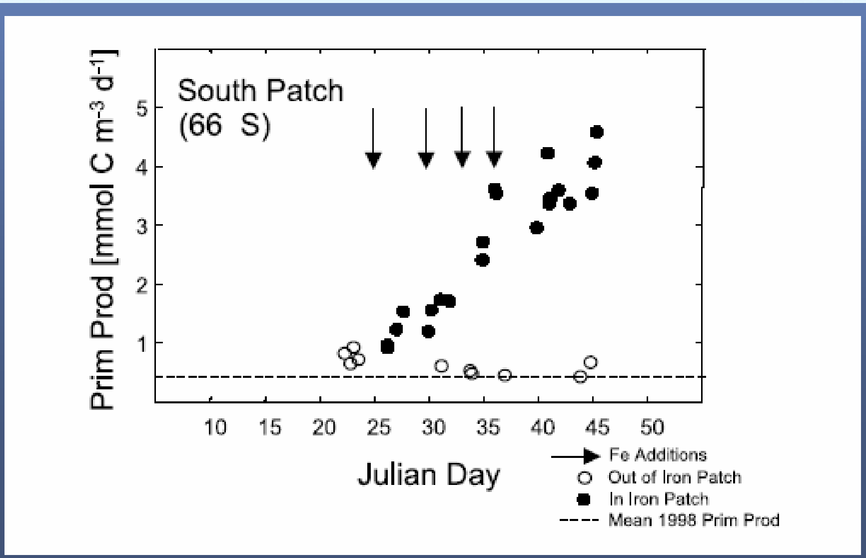


Response of phytoplankton community

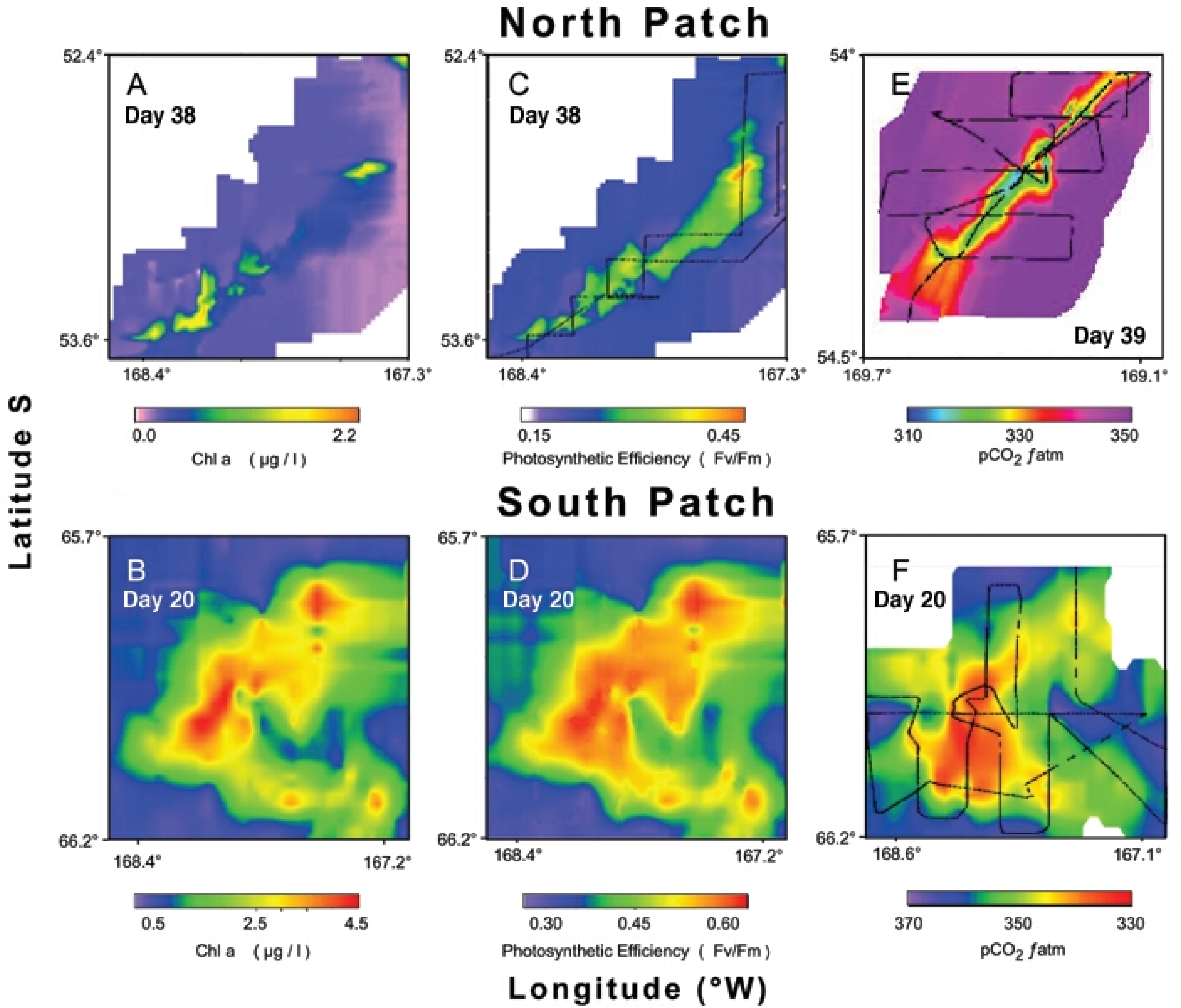




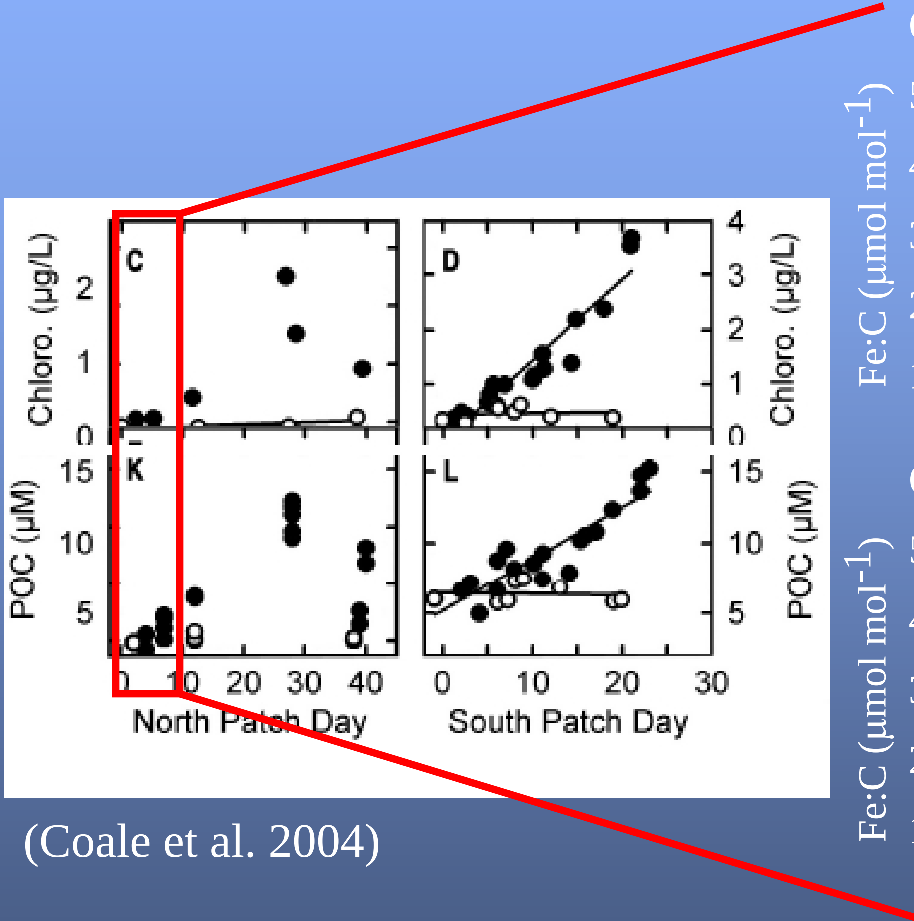
North



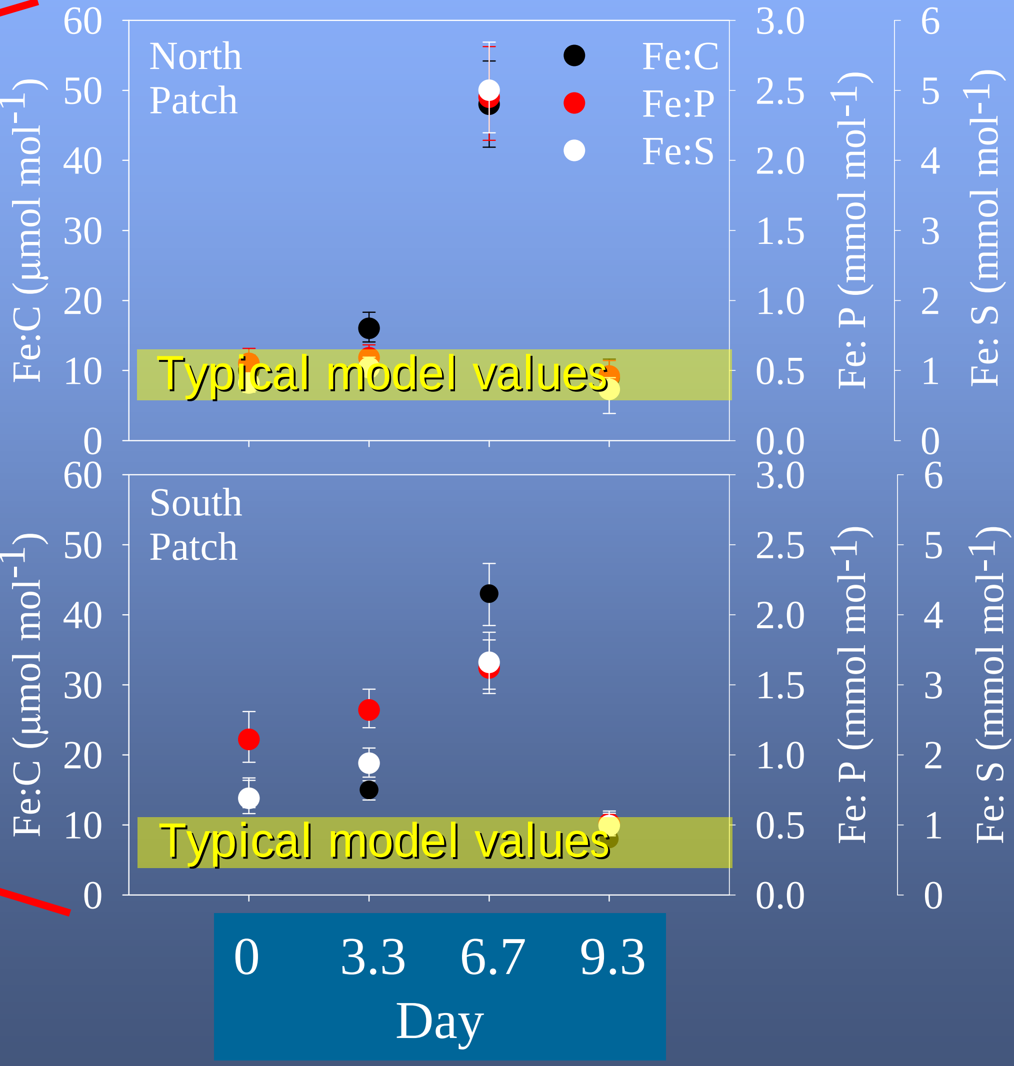
South


North Patch
A
Day 38
C
Day 38
E
Day 39
52.4°
53.6°
168.4°
167.3°
54°
54.5°
169.7°
169.1°
0.0
2.2
Chl a (µg / l)
0.15
0.45
Photosynthetic Efficiency (Fv/Fm)
310
330
350
pCO2 ƒatm
South Patch
B
Day 20
D
Day 20
F
Day 20
65.7°
66.2°
168.4°
167.2°
168.6°
167.1°
0.5
2.5
4.5
Chl a (µg / l)
0.30
0.45
0.60
Photosynthetic Efficiency (Fv/Fm)
370
350
330
pCO2 ƒatm
Latitude S
Longitude (°W)

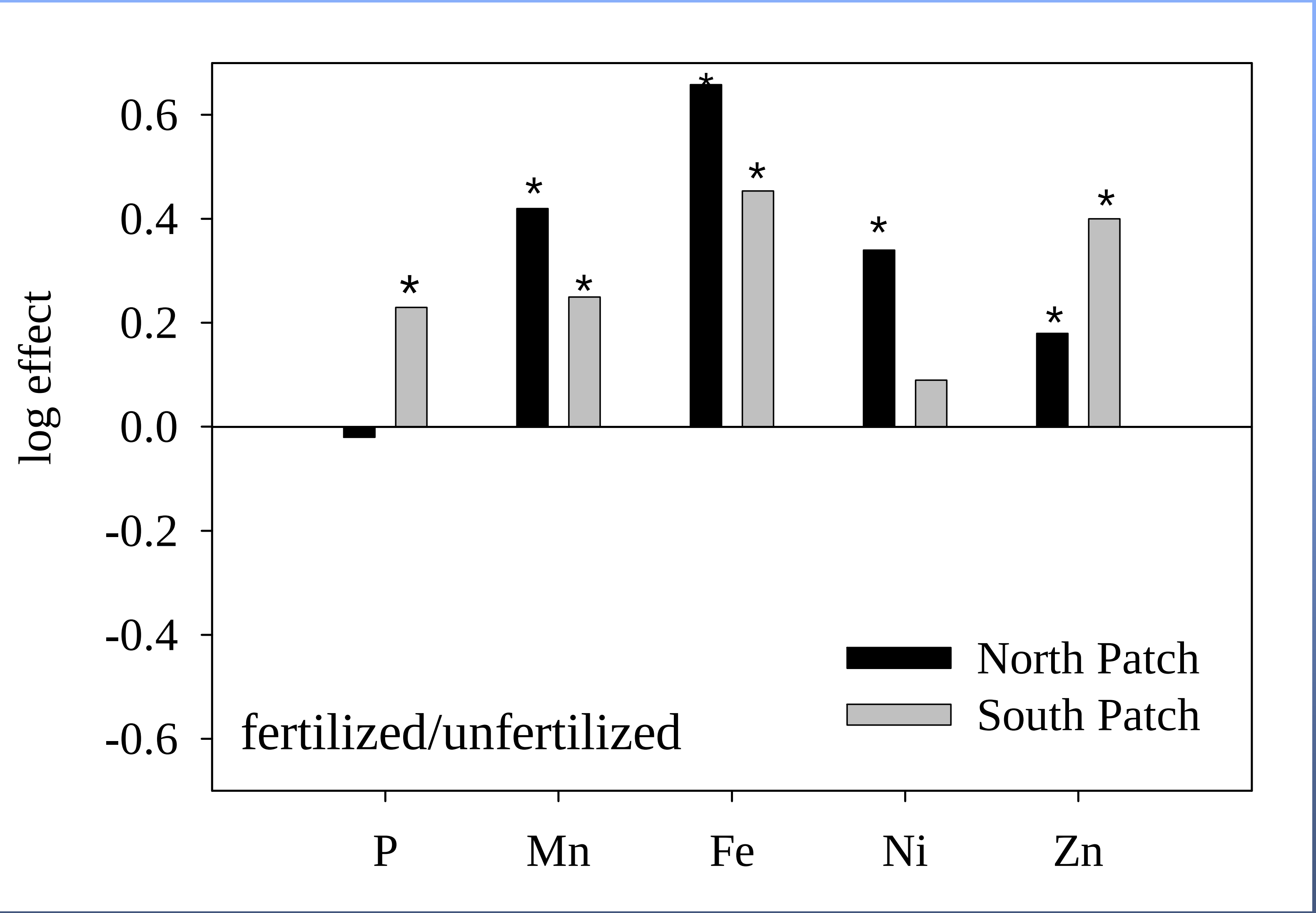
Cellular response to iron fertilization



(Coale et al. 2004)

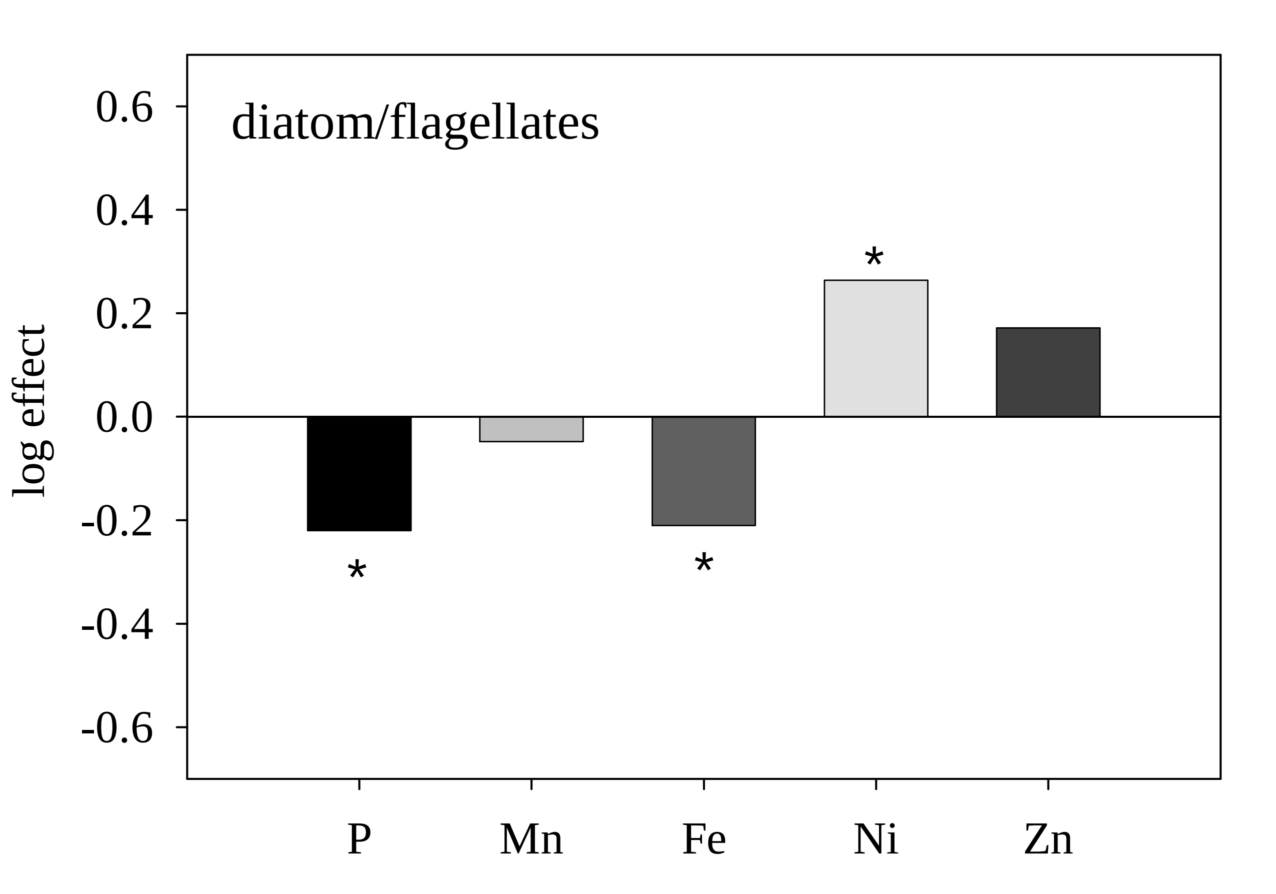


Other elements also increased after fertilization



(Twining et al. 2004)

Element quotas varied between taxonomic groups



(Twining et al. 2004)

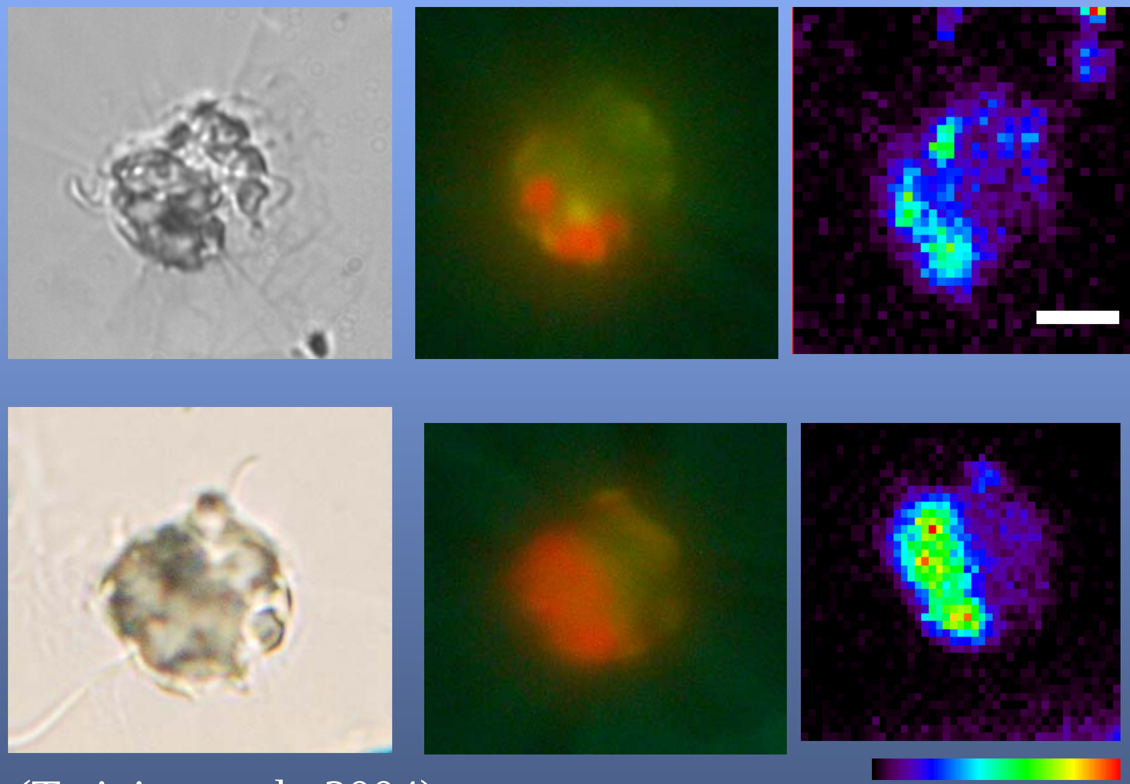
- P and Fe enriched in flagellated cells
- Ni and Zn enriched in diatoms

Iron appeared to accumulate internally

Light micrograph | Chl *a* fluorescence | Fe content

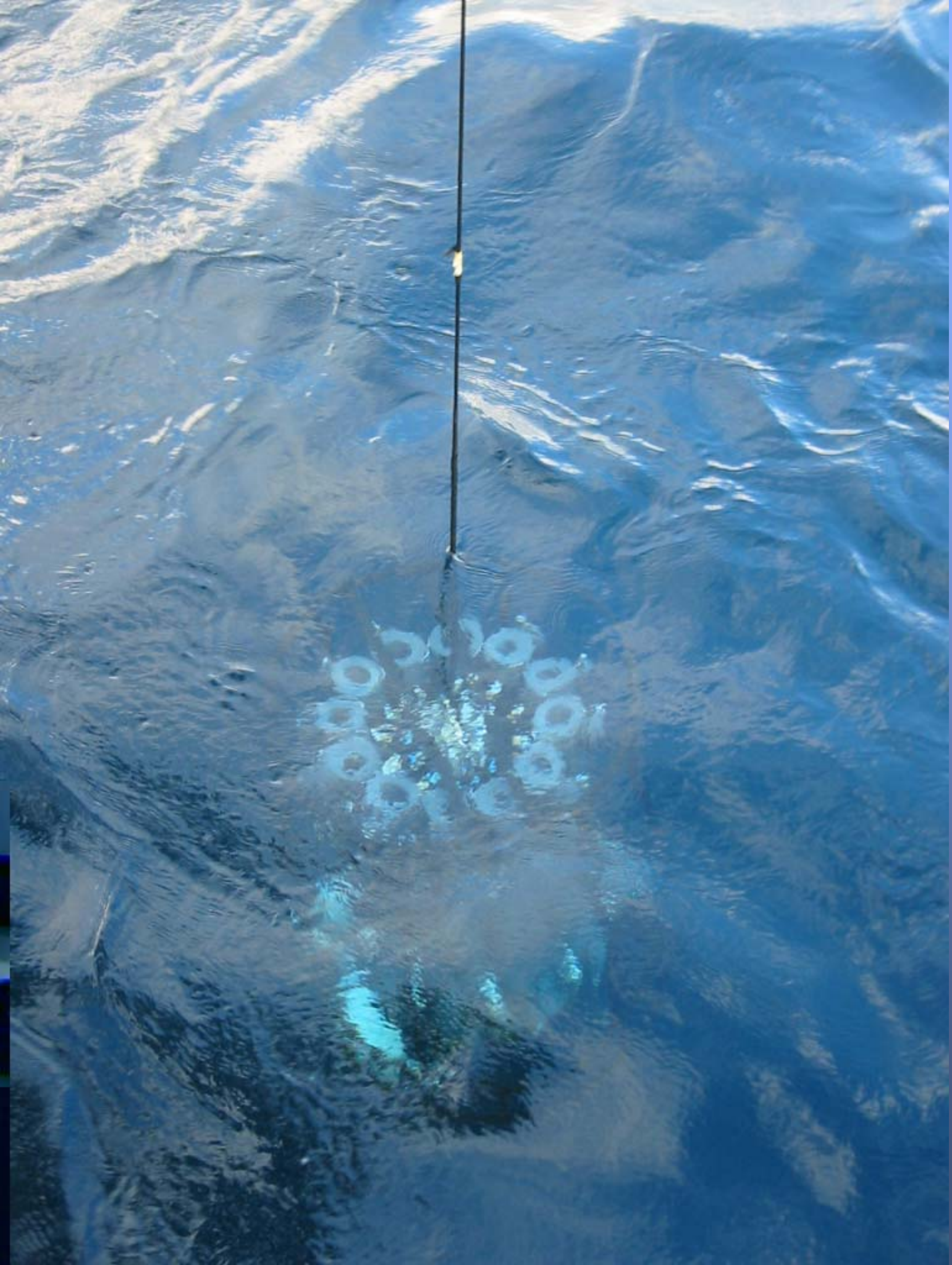
Out

In



(Twining et al., 2004)

Areal concentration



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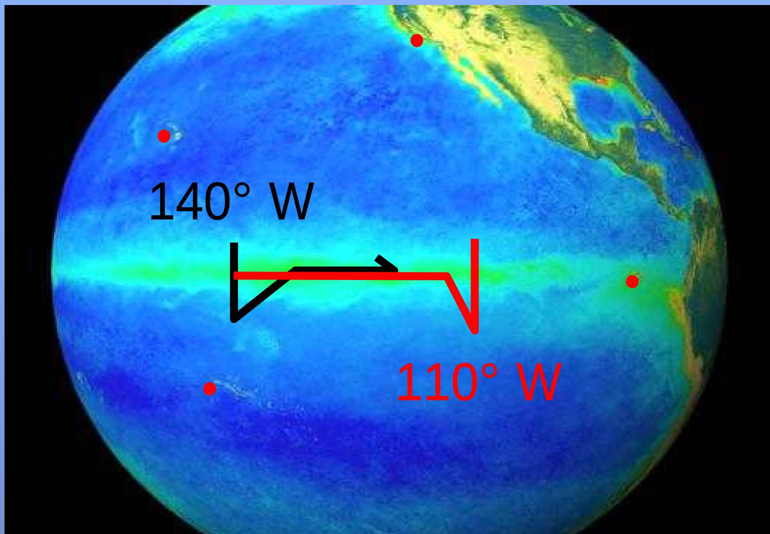
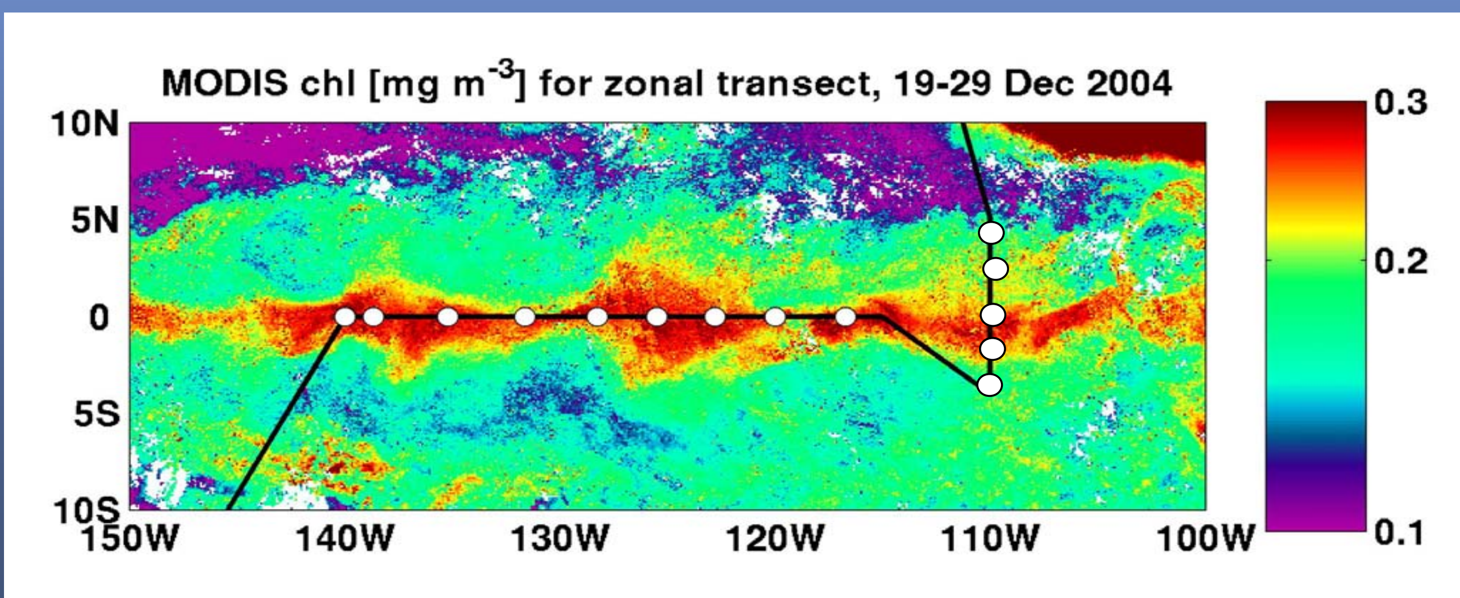
4. Case study 2: Equatorial Pacific Ocean

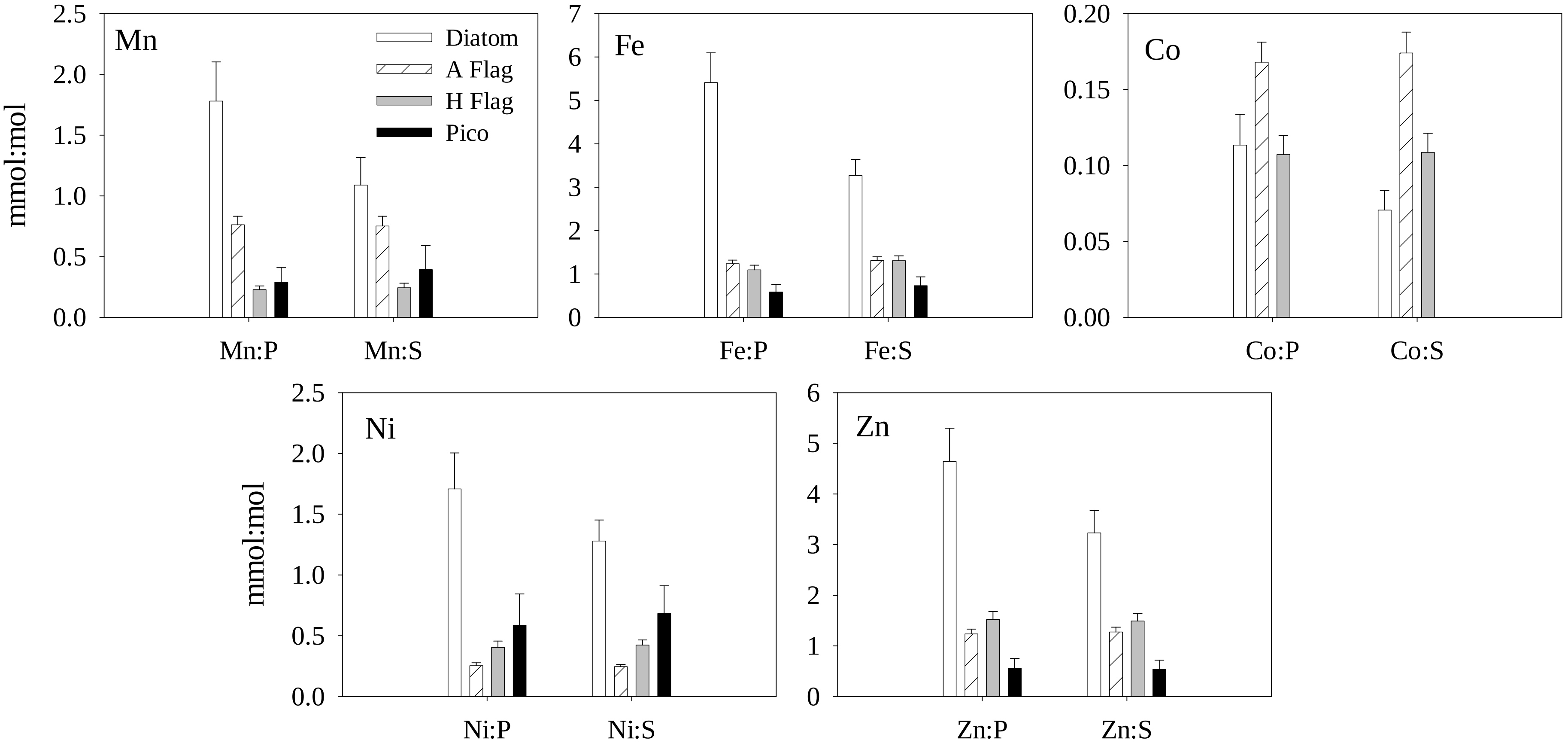
Equatorial Biocomplexity Cruise



- Collected samples from surface mixed layer (20 m)
- Cells analyzed with SXRF for Si, P, S, Mn, Fe, Co, Ni, and Zn
- Quotas compared in different plankton taxa
- Fe and Si added to deckboard grow-out experiments

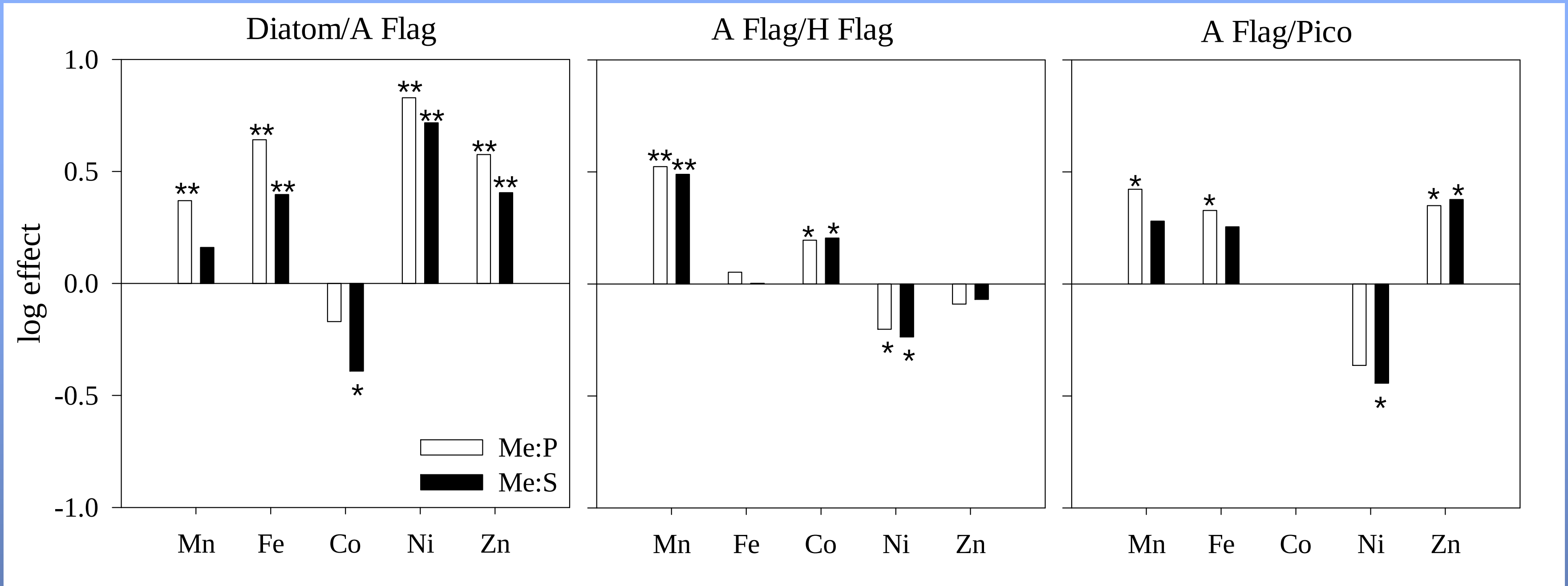


Significant differences in metal stoichiometries of 4 taxa



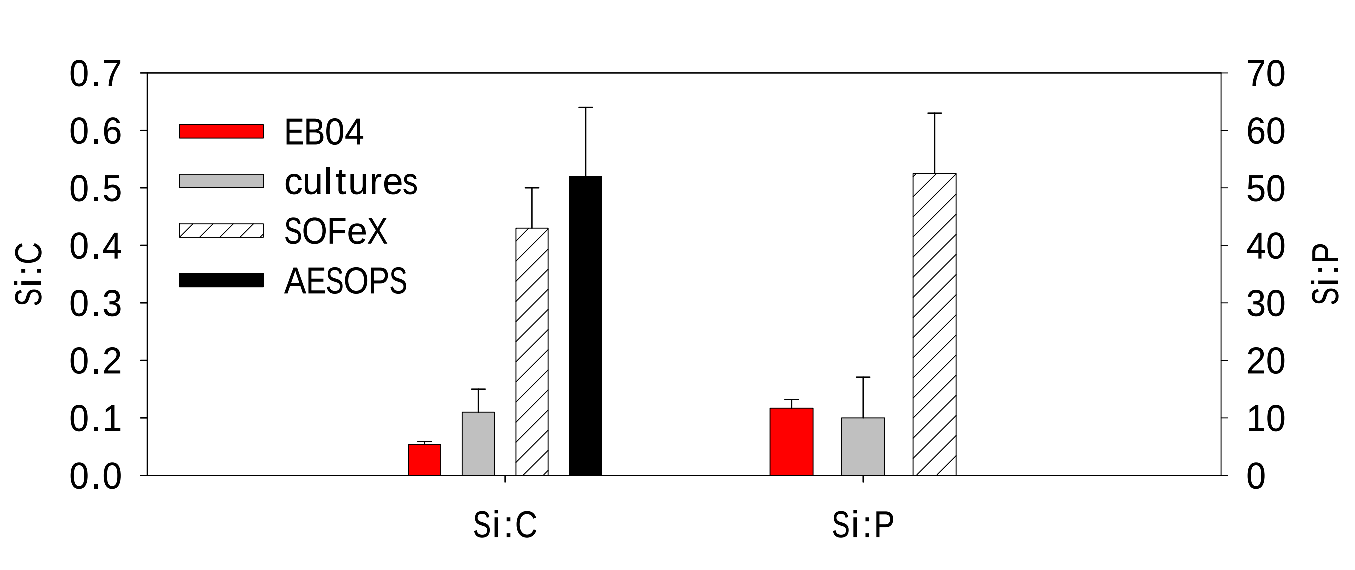
(Twining et al. 2010)

Pairwise group comparisons



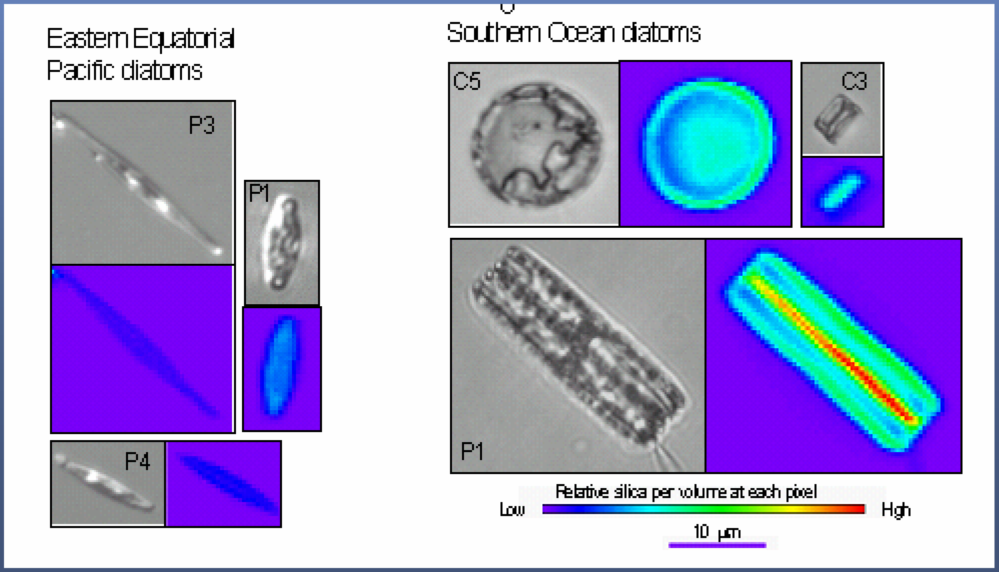
- Diatoms enriched in Mn, Fe, Ni and Zn compared to non-diatoms
- Autotrophs enriched in Mn and Co, depleted in Ni, compared to heterotrophs
- Picoplankton enriched in Ni and depleted in Zn compared to autotrophic flagellates

EqPac diatoms are lightly silicified compared to Southern Ocean diatoms



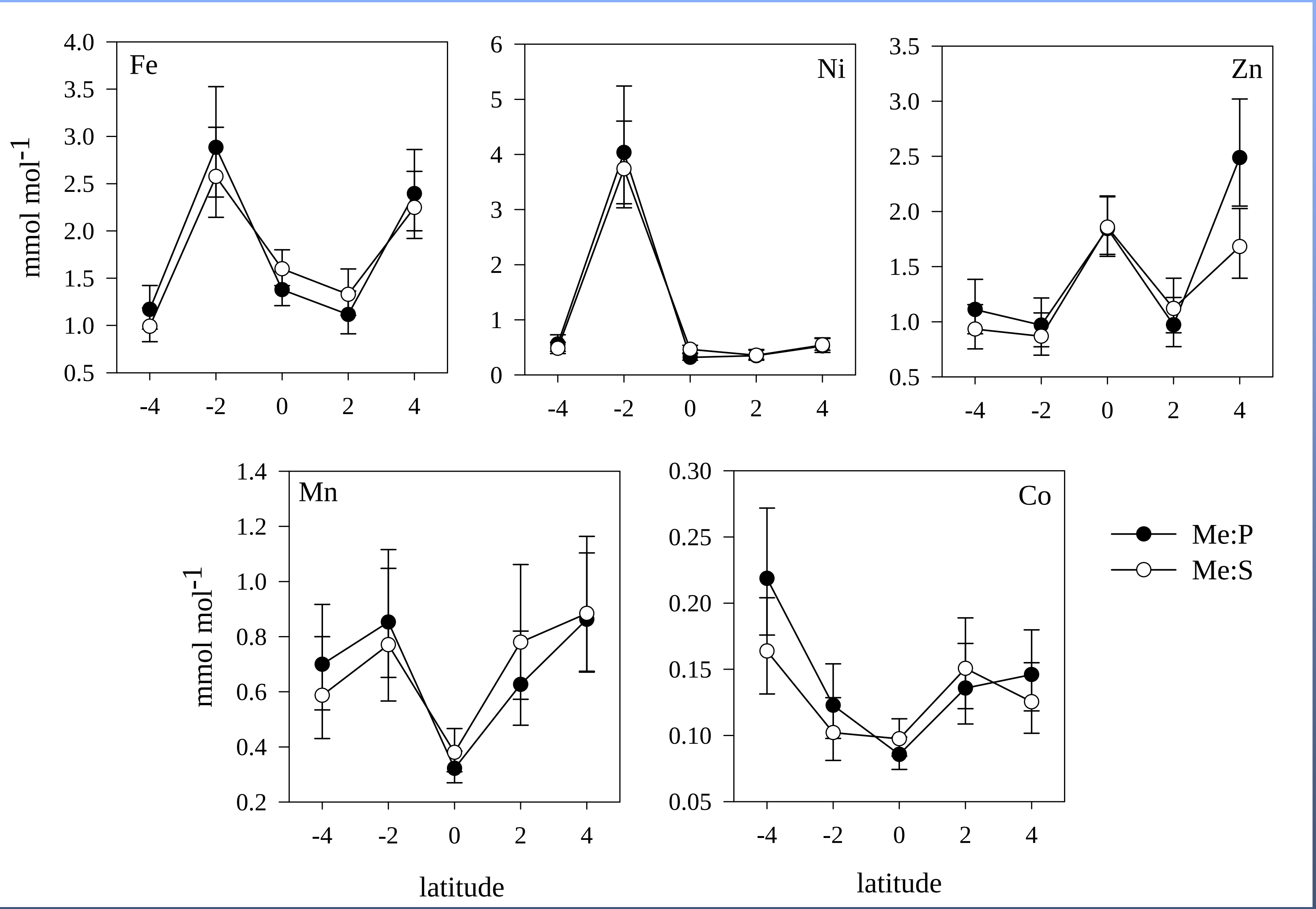
$[Si(OH)_4]$

EqPac — <5 µM
Southern Ocean — 60 µM



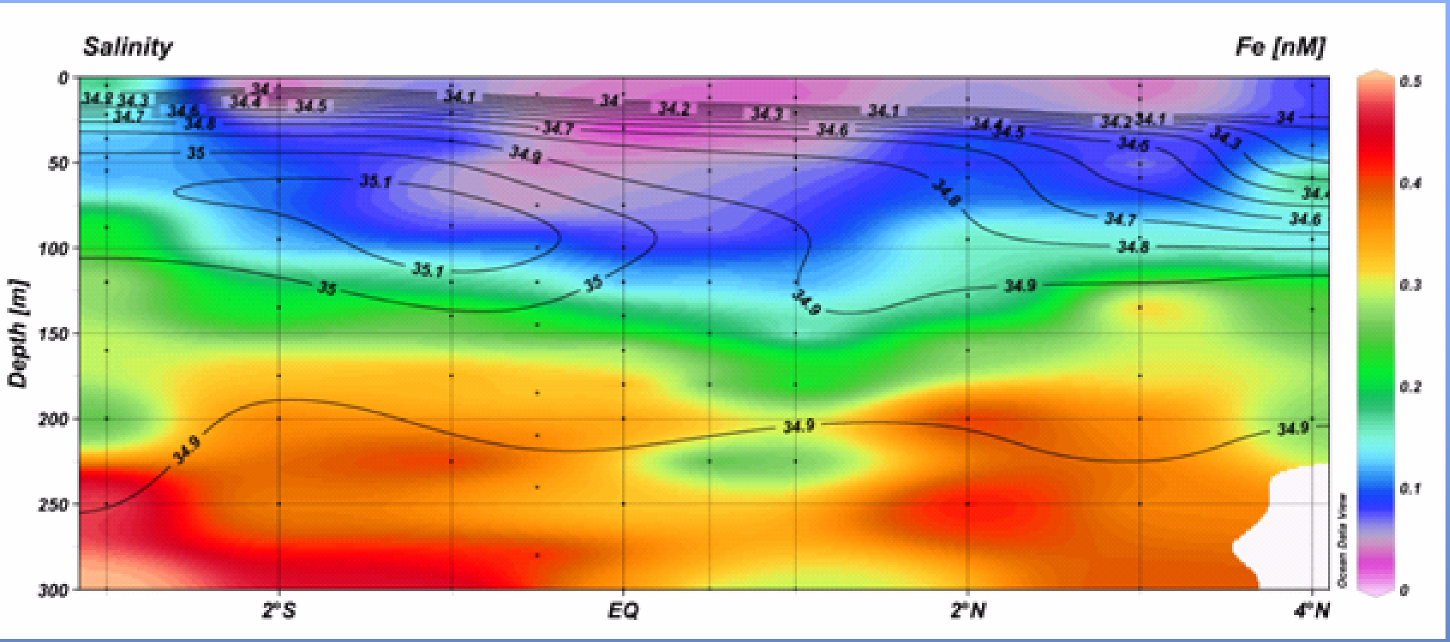
(Baines et al. 2010)

Metal quotas across the equator at 110ºW

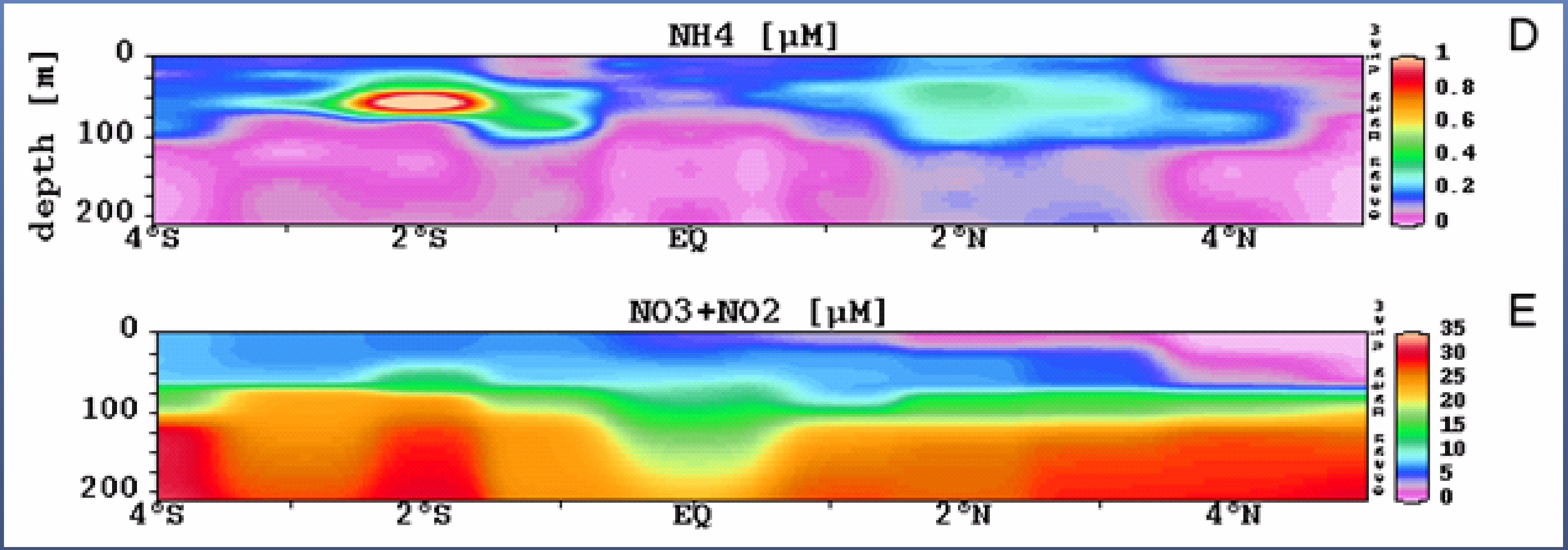


(Twining et al. 2010)

Nutrients across the equator at 110°W

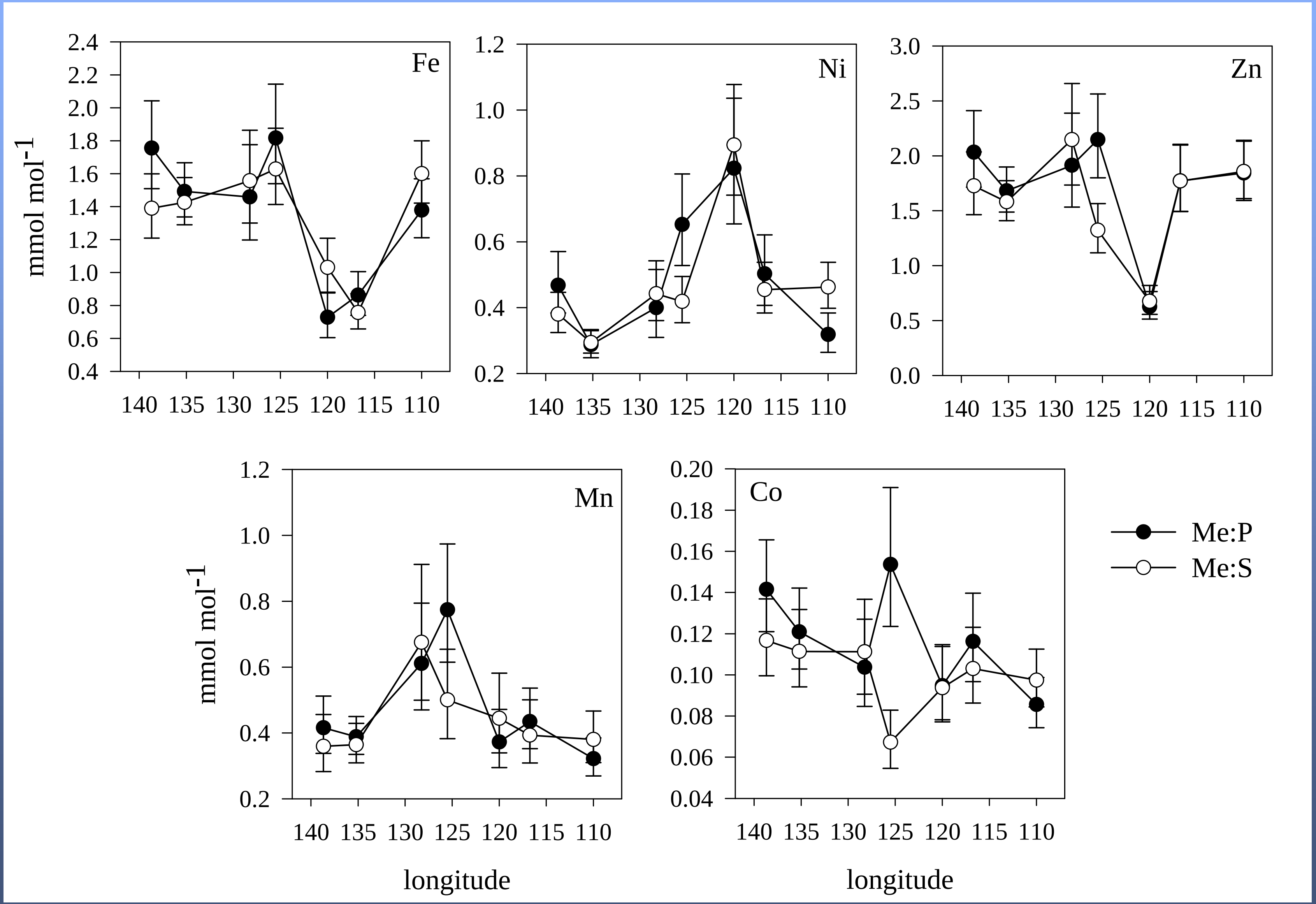


(Kaupp et al. 2010)



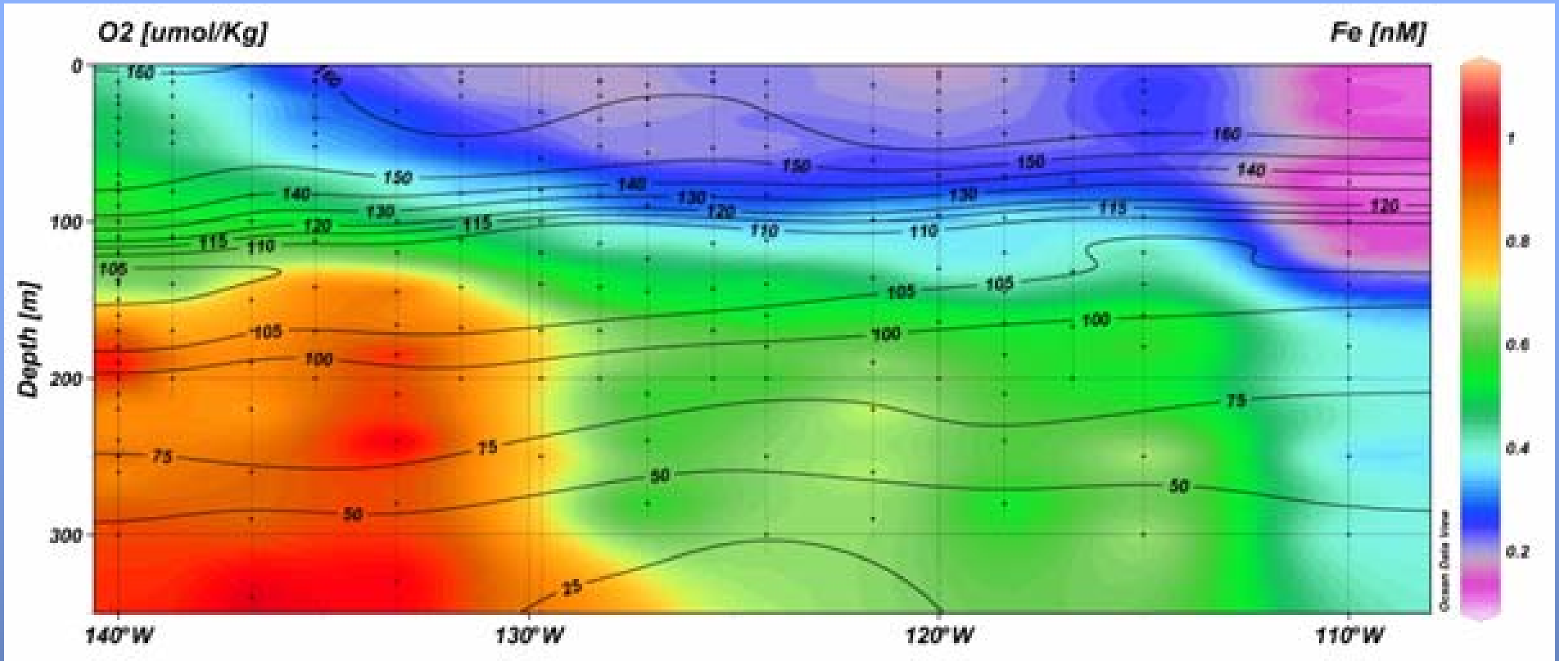
(Strutton et al. 2010)

Metal quotas along the equator between 140ºW and 110ºW

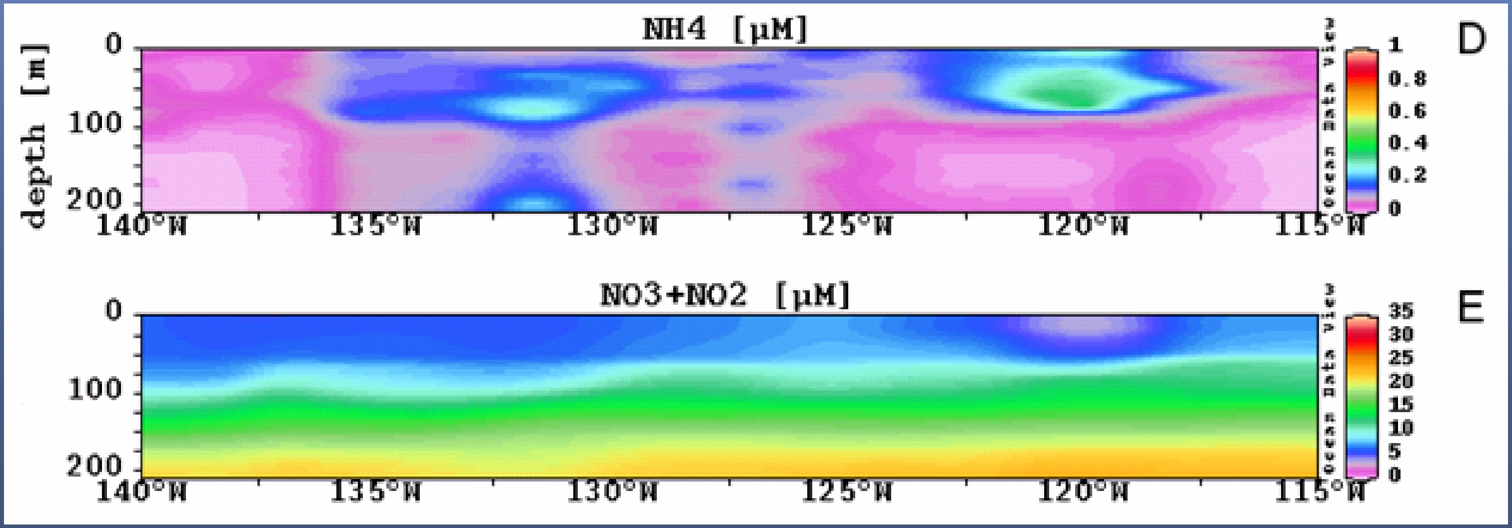


(Twining et al. 2010)

Nutrients along the equator: 140ºW to 110ºW

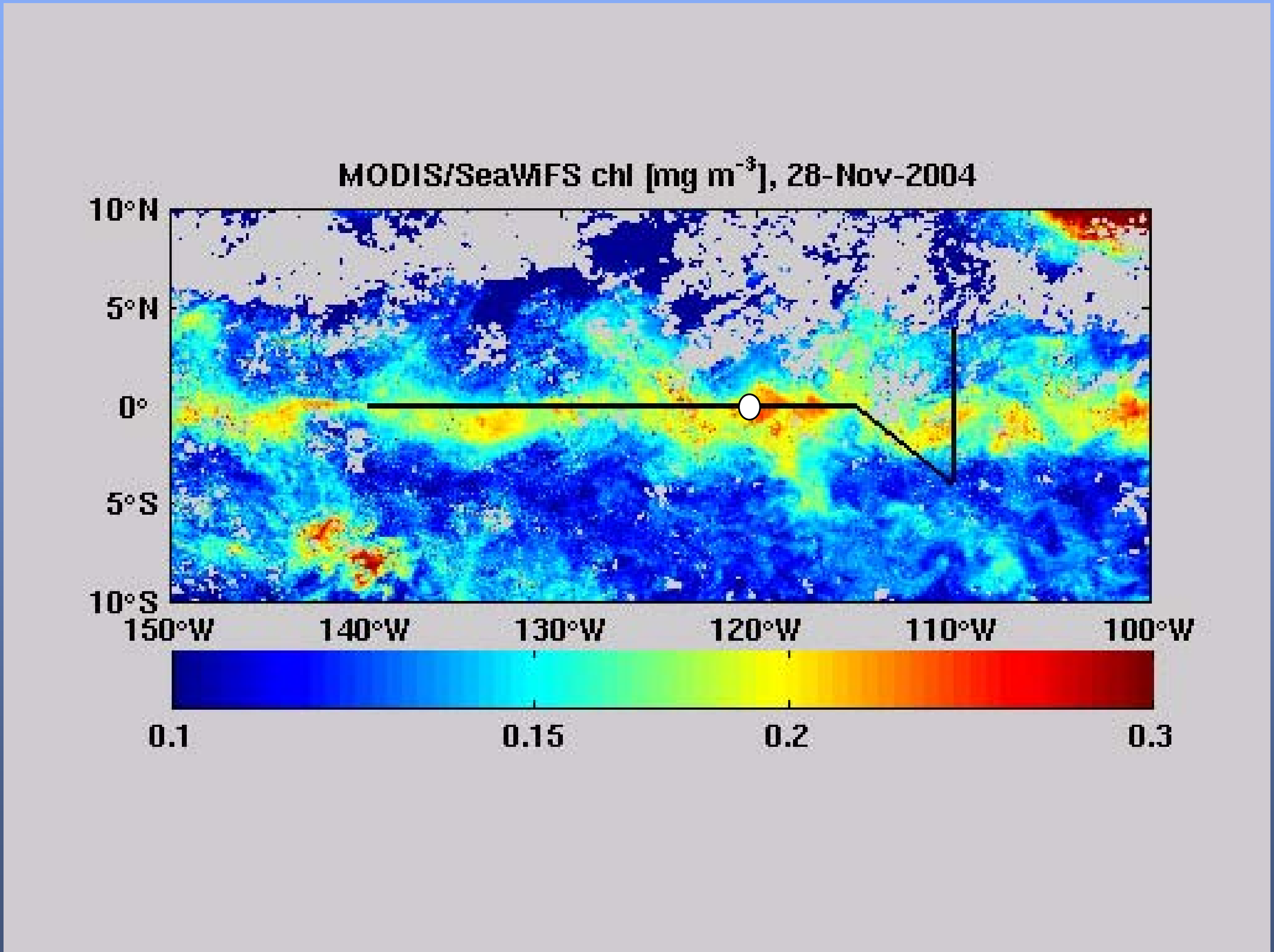


(Kaupp et al. 2010)



(Strutton et al. 2010)

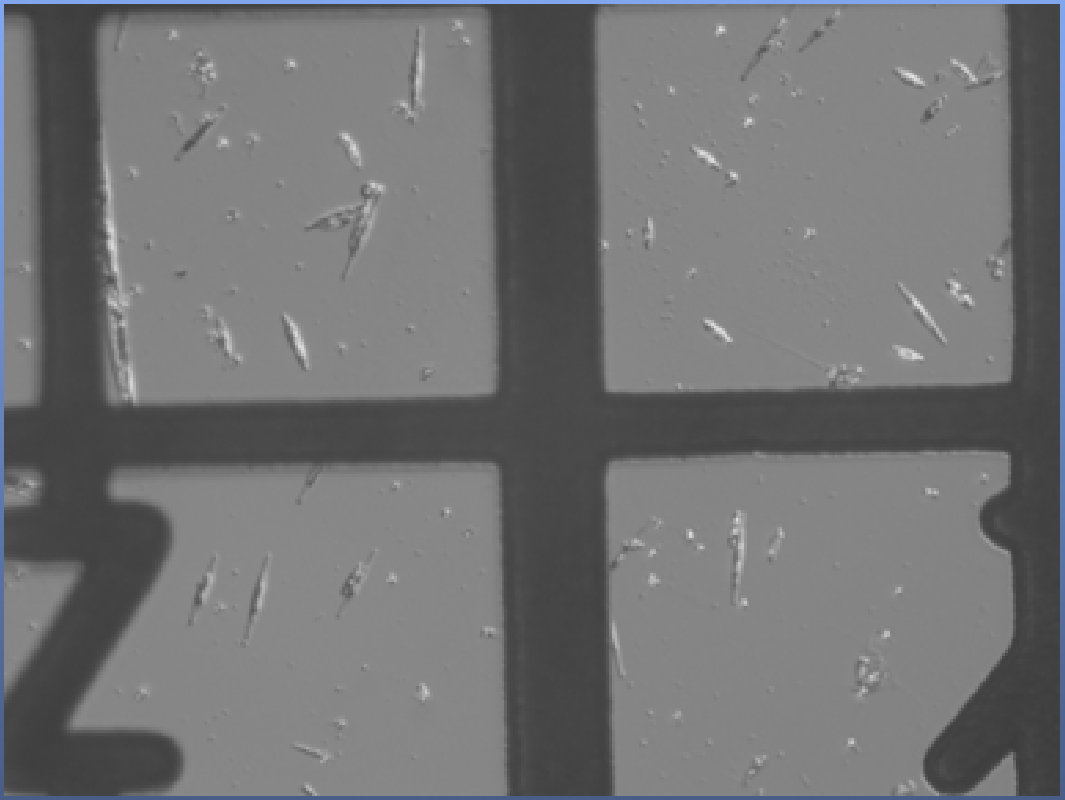
Plankton may be responding to the passage of a tropical instability wave



(P. Strutton, unpublished)

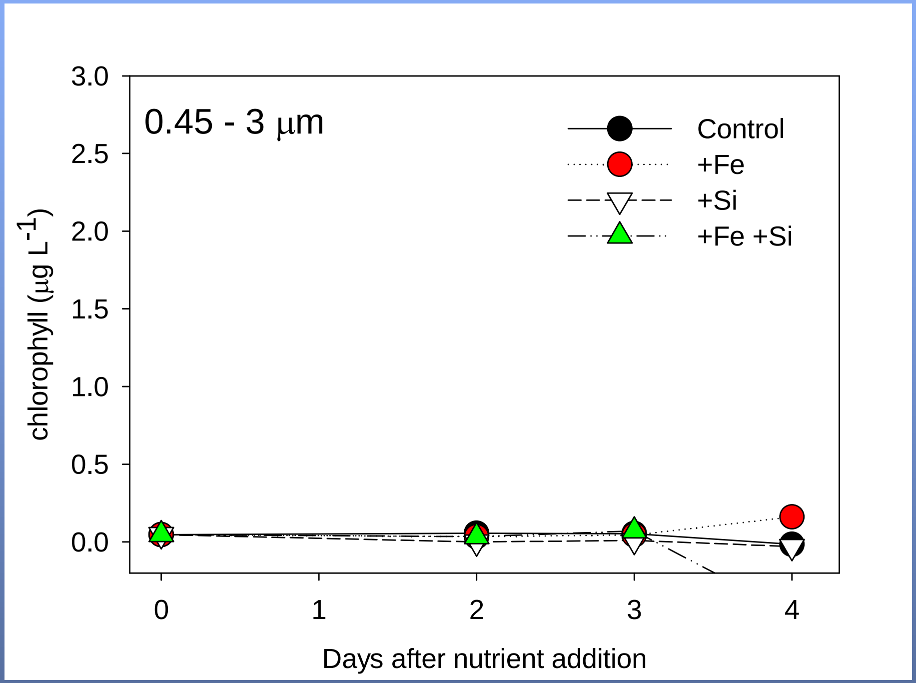
Response of diatoms to Fe and Si additions in carboys

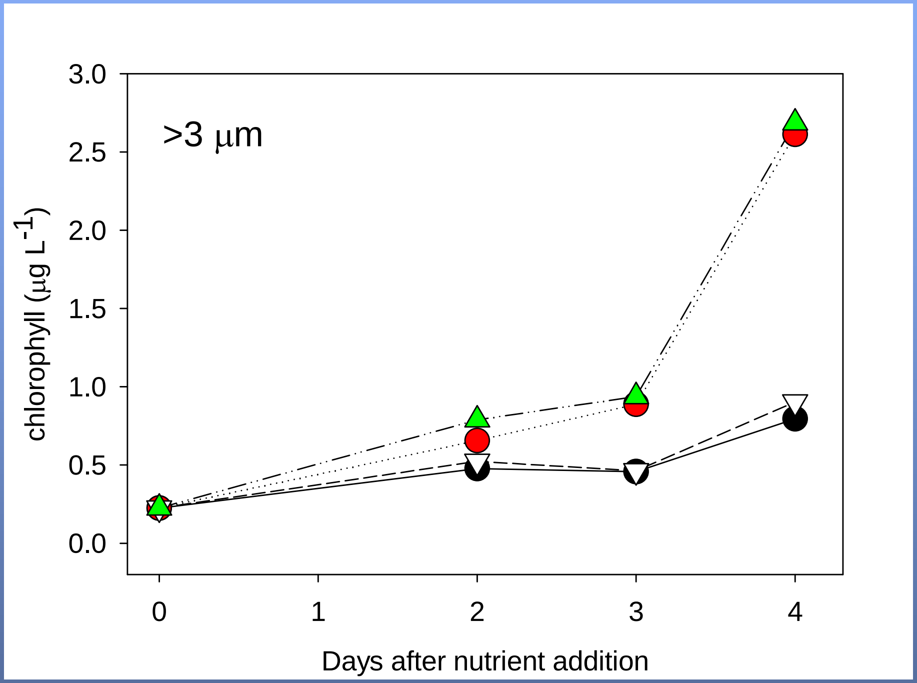
- 20-L shipboard carboy experiments
- Unamended control, +Fe, +Si treatments
- 96-hr grow out
- Small pennate diatoms analyzed with SXRF



100 µm

Phytoplankton growth response to added Fe



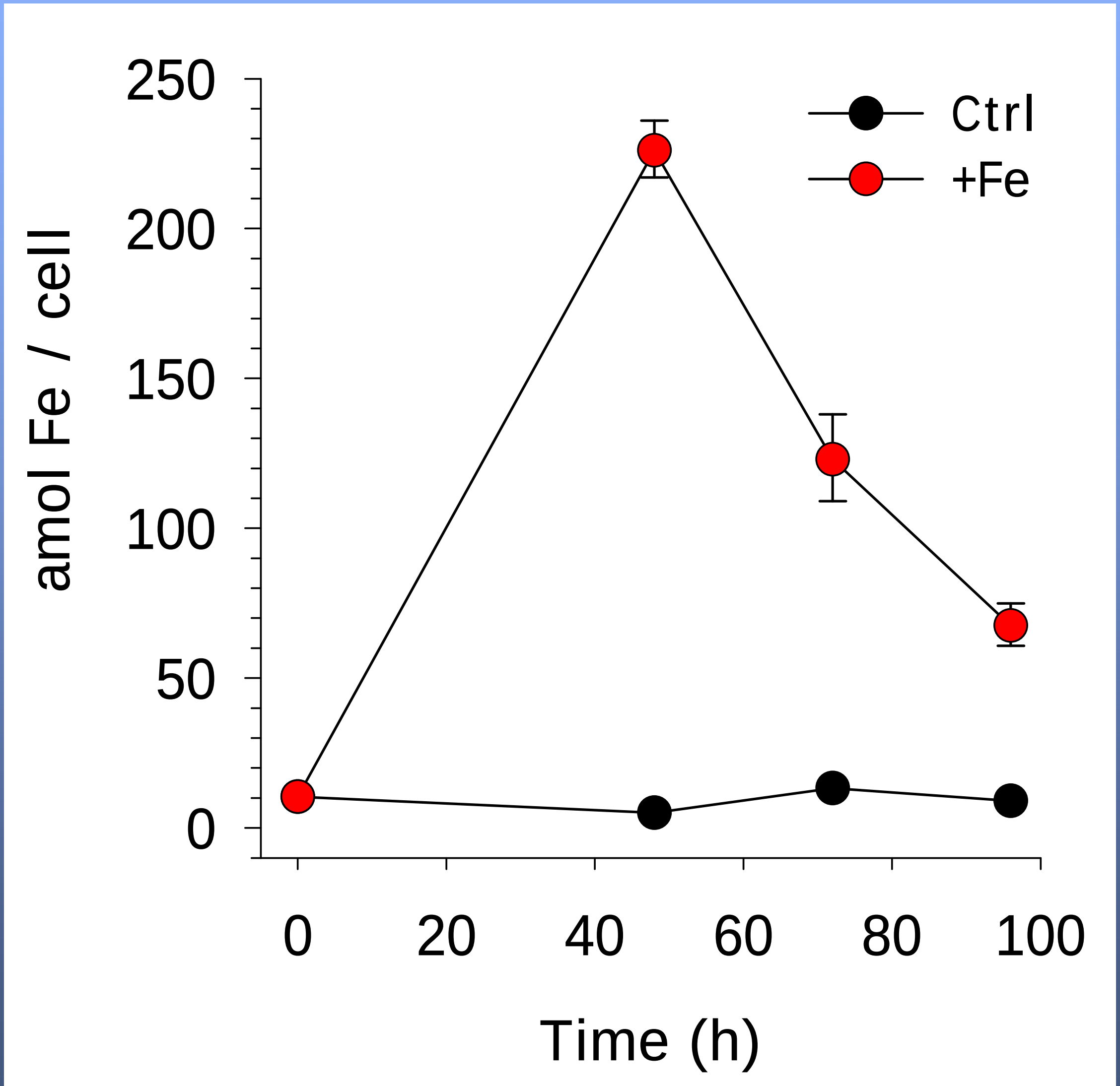


(Brzezinski et al. 2010)

Smaller phytoplankton don't appear to be limited by Fe.

Diatoms respond to added Fe but not to Si.

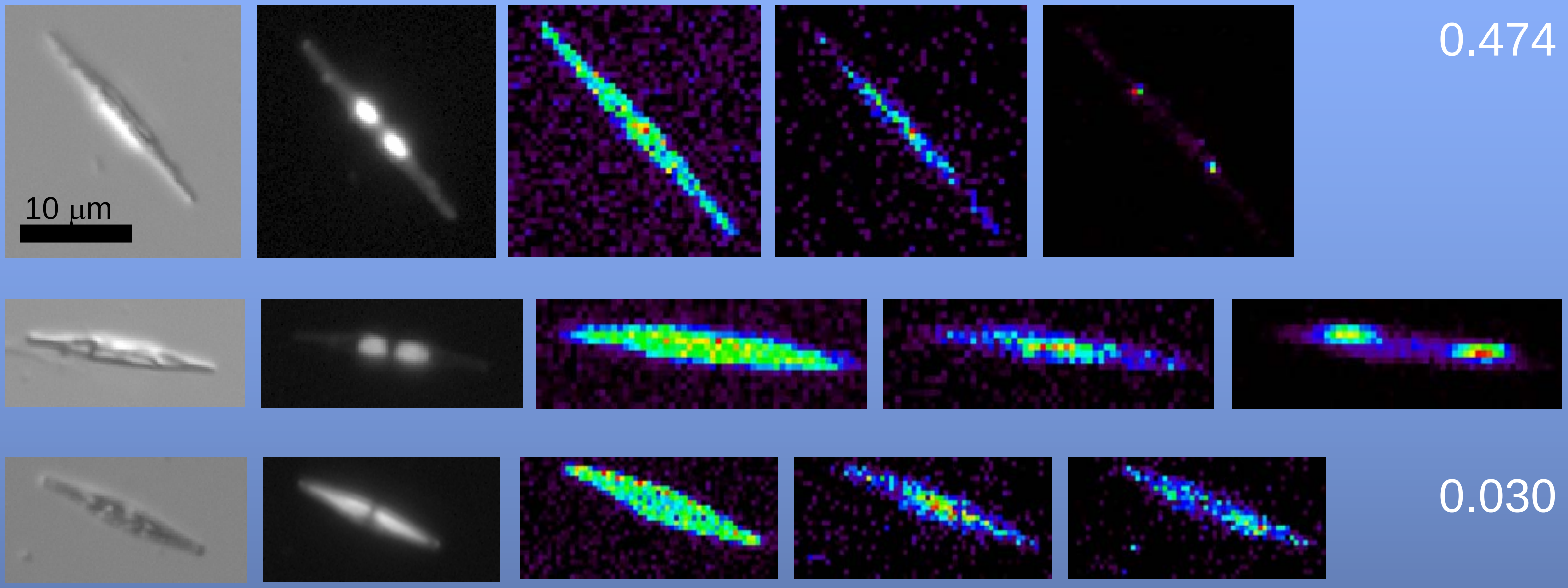
Response of cellular Fe quotas



- Fe quotas in controls constant over course of experiment
- 22-fold increase in Fe quotas 48 h after Fe addition

Localization of accumulated iron

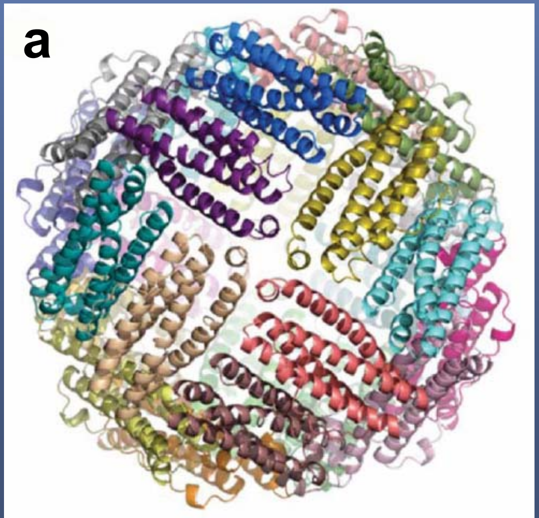
	light	Chl	Si	P	Fe	Max µg/cm²
48 h						0.474
72 h						0.101
96 h						0.030

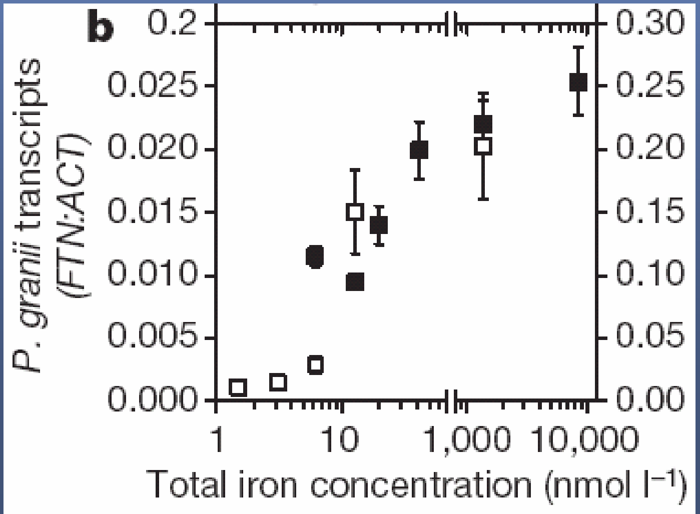


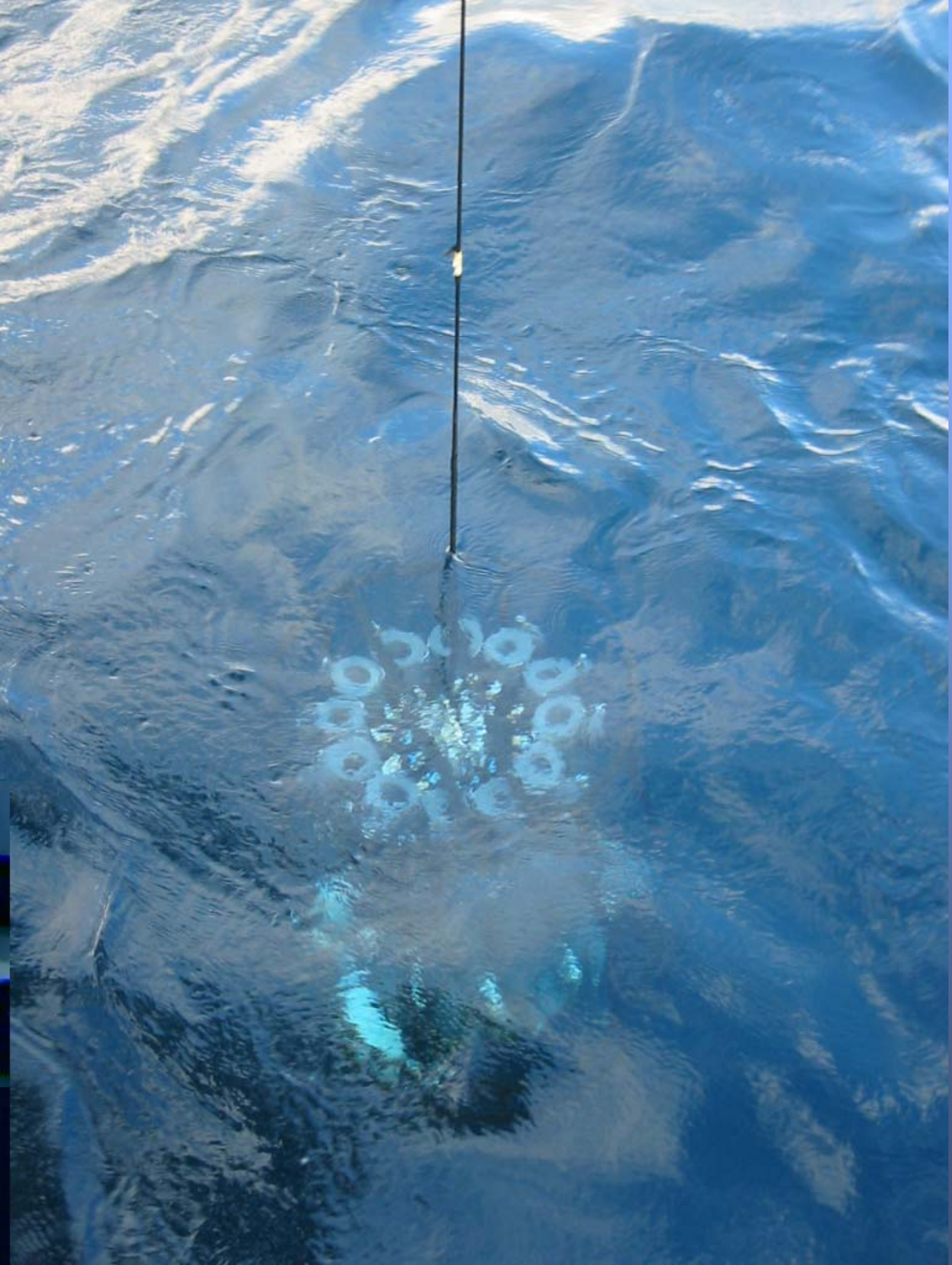
Accumulated Fe was highly localized in storage bodies adjacent to the chloroplasts

Ferritin in diatoms

(Marchetti et al., 2009)



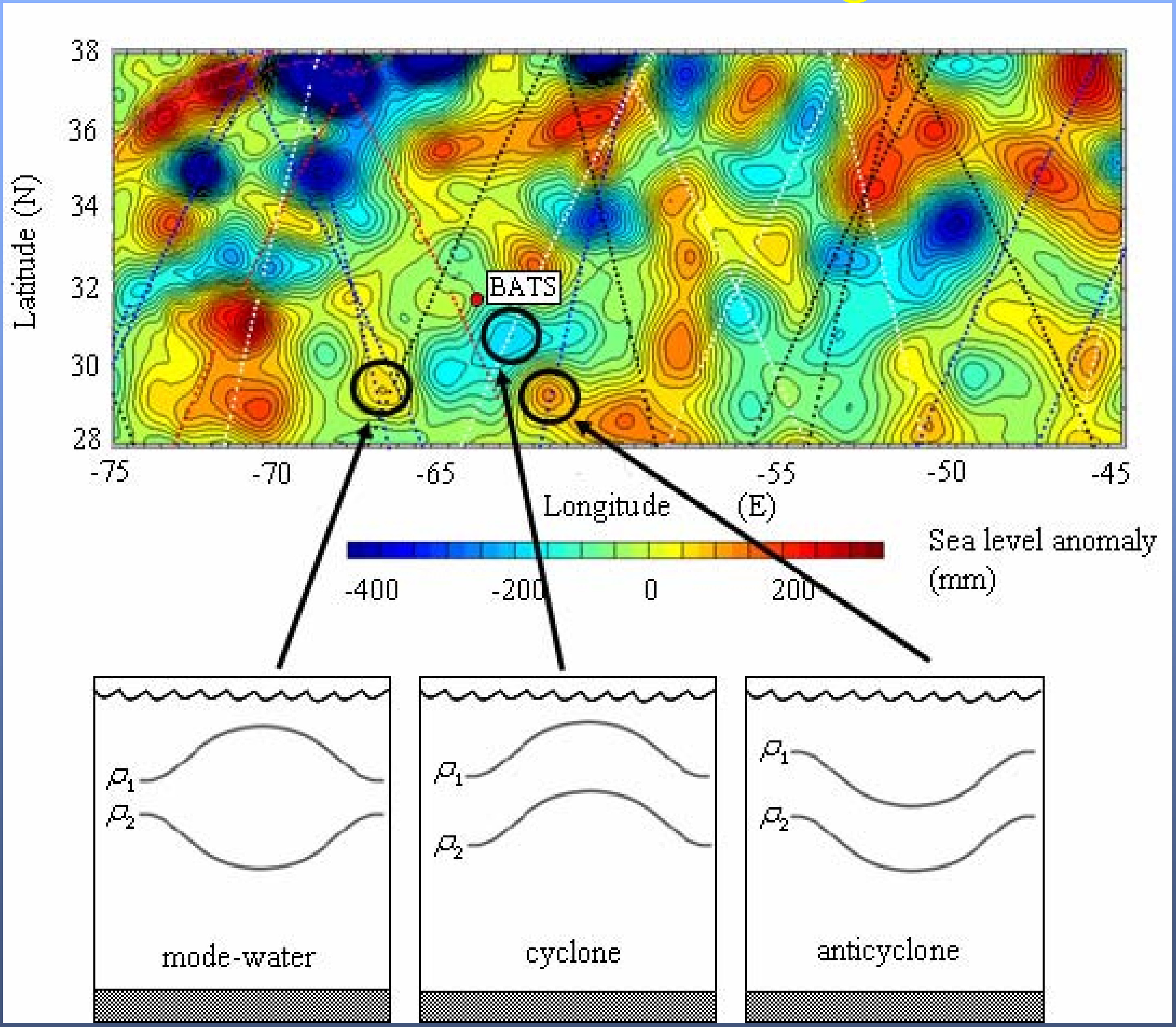




Outline

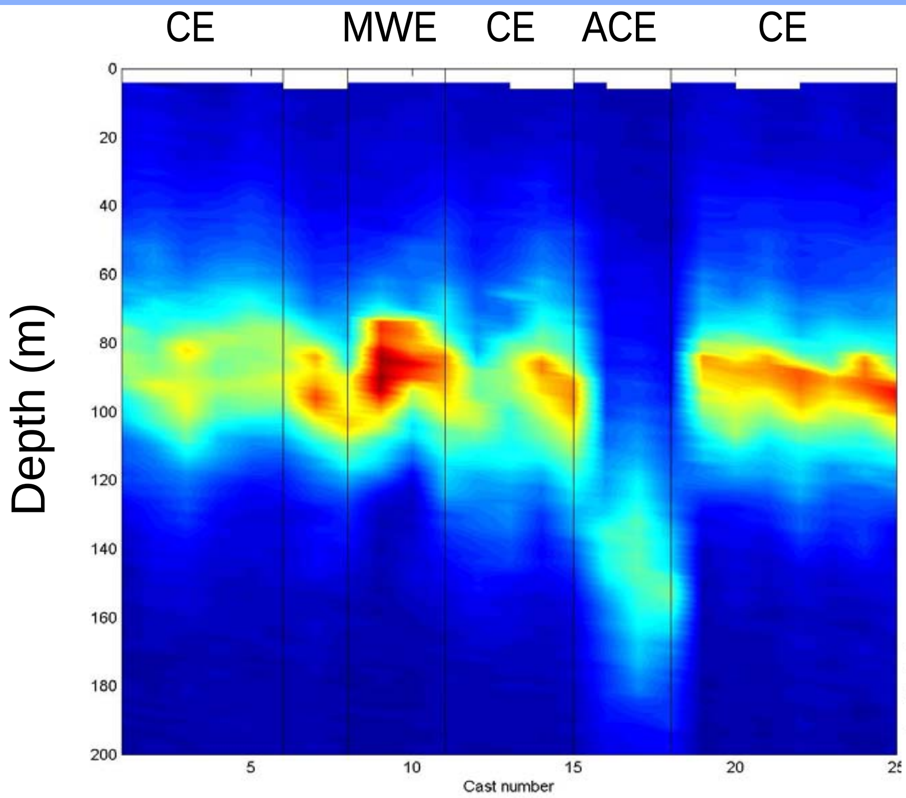
1. Importance of metal quotas to ocean biogeochemistry
2. A single-cell approach
3. Case study 1: Southern Ocean
4. Case study 2: Equatorial Pacific Ocean
5. Case study 3: Eddies in the Sargasso Sea

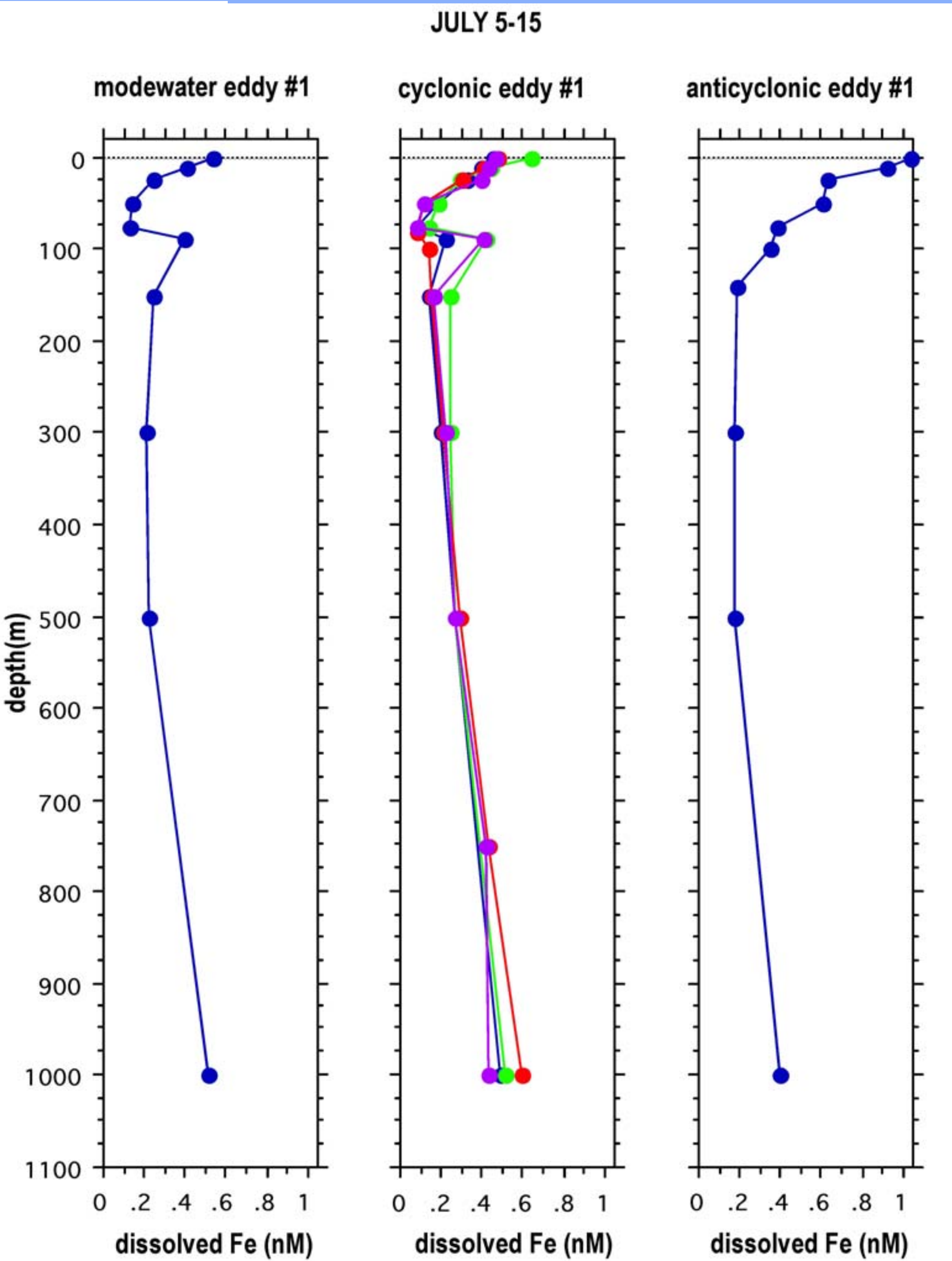
Mesoscale eddies in the Sargasso Sea



(Twining et al. 2010)

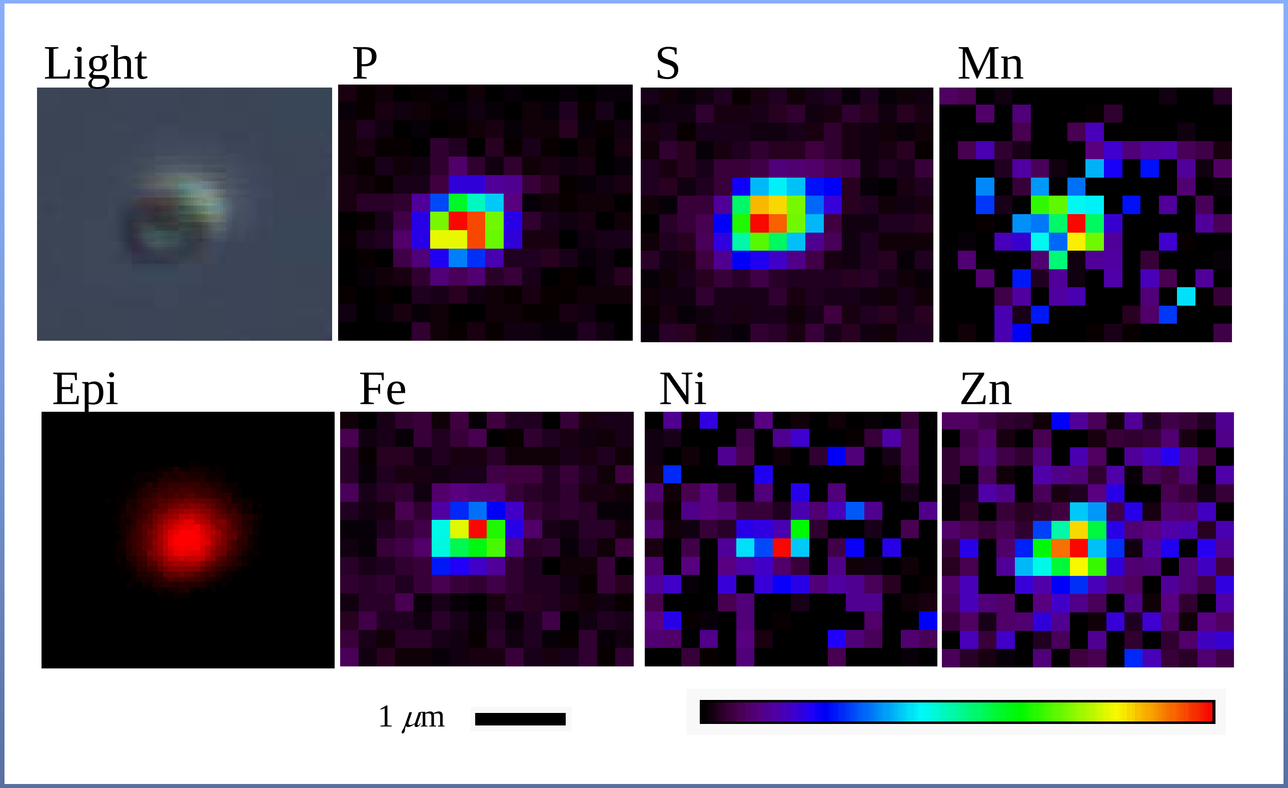
Eddies differ in chlorophyll and Fe profiles





(Twining et al. 2010)

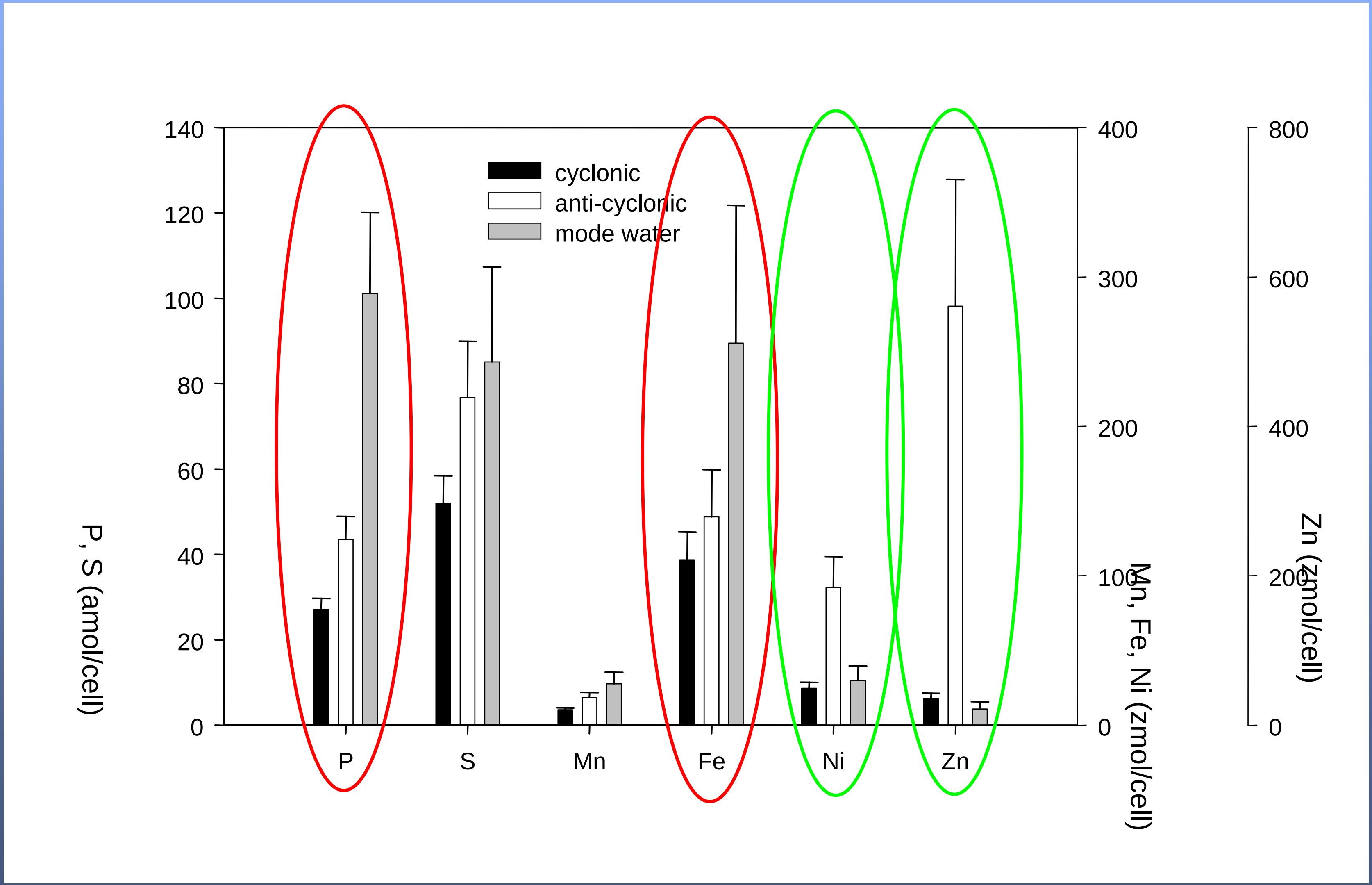
Synechococcus cells were isolated from each eddy



(Twining et al. 2010)

- Cells collected from surface mixed layer and deep chlorophyll maximum layer
- >20 cells from each eddy and depth analyzed with SXRF

Cellular element quotas varied between eddies



(Twining et al. 2010)

Overall Conclusions

1. Single-cell analytical approaches can reveal important information about the biological status of resident cells and biogeochemical functioning of marine communities.

2. Element quotas respond to environmental gradients

3. Element quotas can vary between co-occurring taxonomic groups:
   - -Diatoms have low P, high Ni and Zn
   - -Autotrophs enriched in Mn
   - -Picoplankton/cyanobacteria enriched in Ni, depleted in Zn

4. There may be characteristic element stoichiometries for certain types of plankton communities.

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