ALFRED-WEGENER-INSTITUT
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Isotopes in Ocean Sciences II

Florian Koch
Alfred Wegner Institut
Biogeowissenschaften
"ECOTRACE"

IsoSIM Summer School
25th-30th of July 2016
Schmitten, Germany

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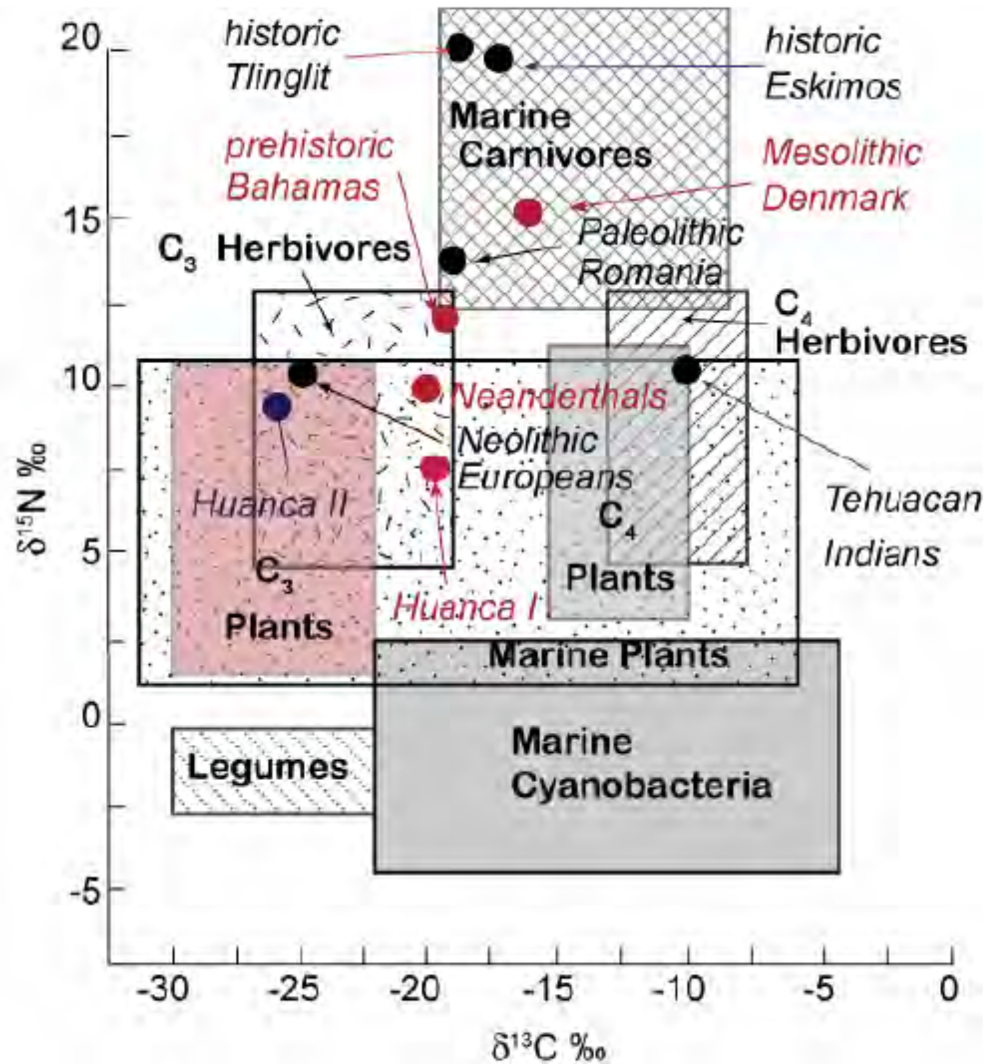
Isotopic fingerprinting in Archeology

- Collected from bone collagen.
- Tehuacan Indians probably depended heavily on maize.
- In Peru Indians the diet shifted from a mix of C4,C3 to mainly C3.
- Paleodiet indicator!



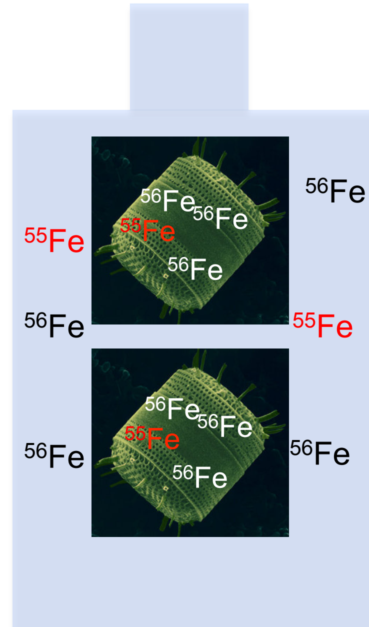
DeNiro and Epstein, 1981

Application: Radioisotopes as tracers

- Besides the dating of processes (through their decay), radioisotopes can be used as a 'tag' to measure uptake of nutrients.

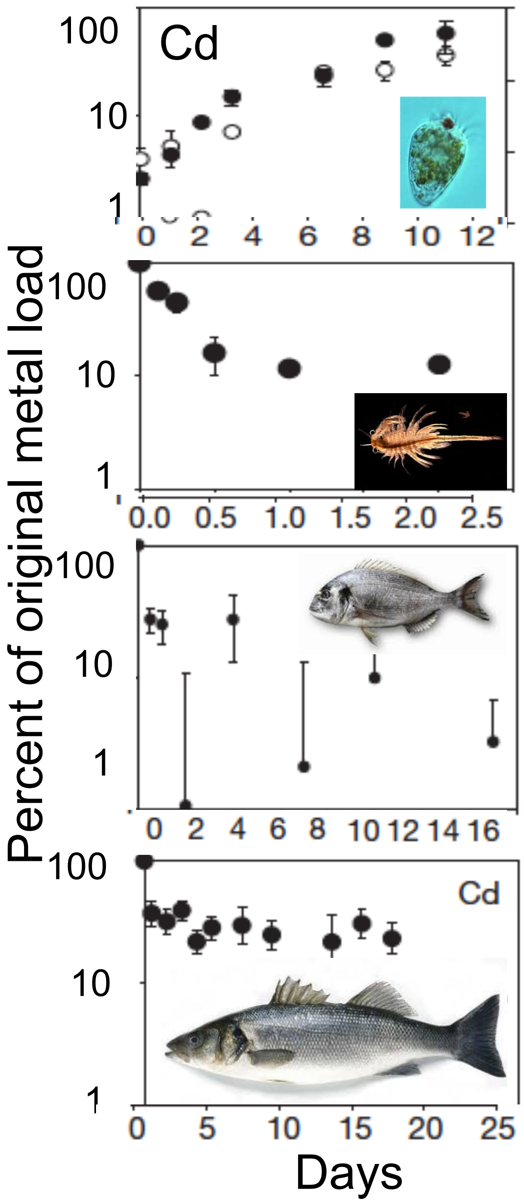
- More sensitive than stable isotopes and more importantly need less material.

- Important to consider the ambient concentrations of the compound at interest
  - Michaelis Mentin kinetics
  - Need to add <10% in order to not affect kinetics.

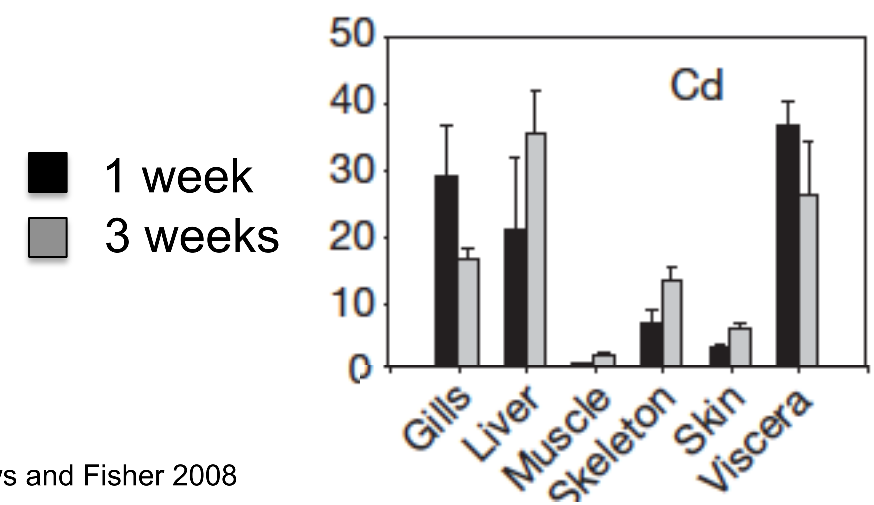


$$\frac{A_{55Fe} \text{ on filter (plankton)}}{A_{55Fe} \text{ added to water}} \times \frac{[\text{Fediss}] + [\text{Fe added}]}{\text{Incubation time}} = \rho\,[\text{Fe}]\,t^{-1}$$

Tracking heavy metal contamination



- We are able to follow heavy metals through the food chain
- Indicator for potential to bio accumulate
- Also are able to see where in the body they are deposited



Matthews and Fisher 2008

Back to phytoplankton…….

- The role of vitamins in phytoplankton ecology.
- Harmful algal blooms and vitamins.

Phytoplankton growth depends on:

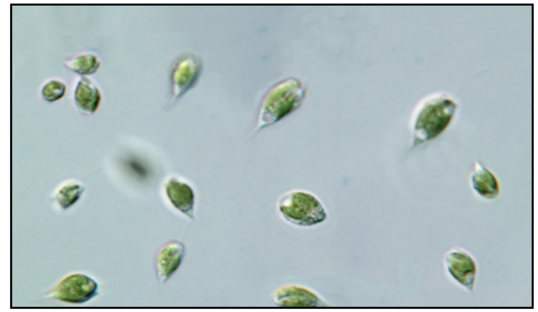
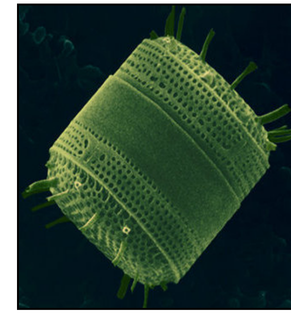
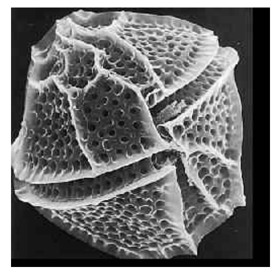
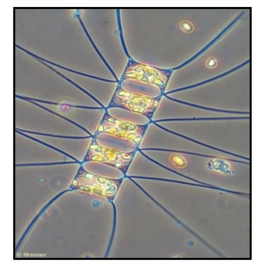
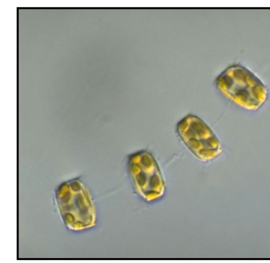
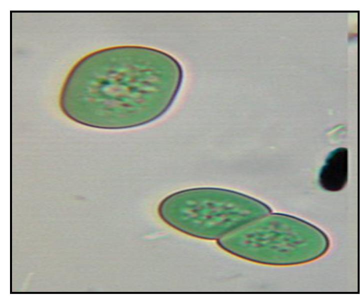
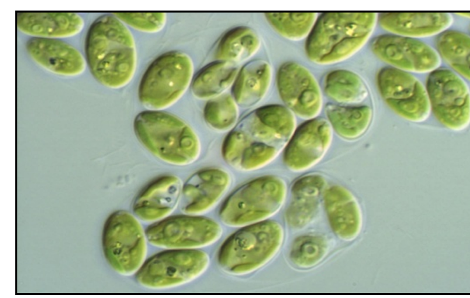
- Light and temperature
- Nutrients
  - Macronutrients:

    N, P, Si

  - Micronutrients:

    Fe, Zn, Cu, Mn, Mo, Co

  - Vitamins
    - B_{12} (cobalamin),
    - B_1 (thiamin)
    - B_7 (biotin)

Vitamin B_{12}: *Who needs it, who makes it?*

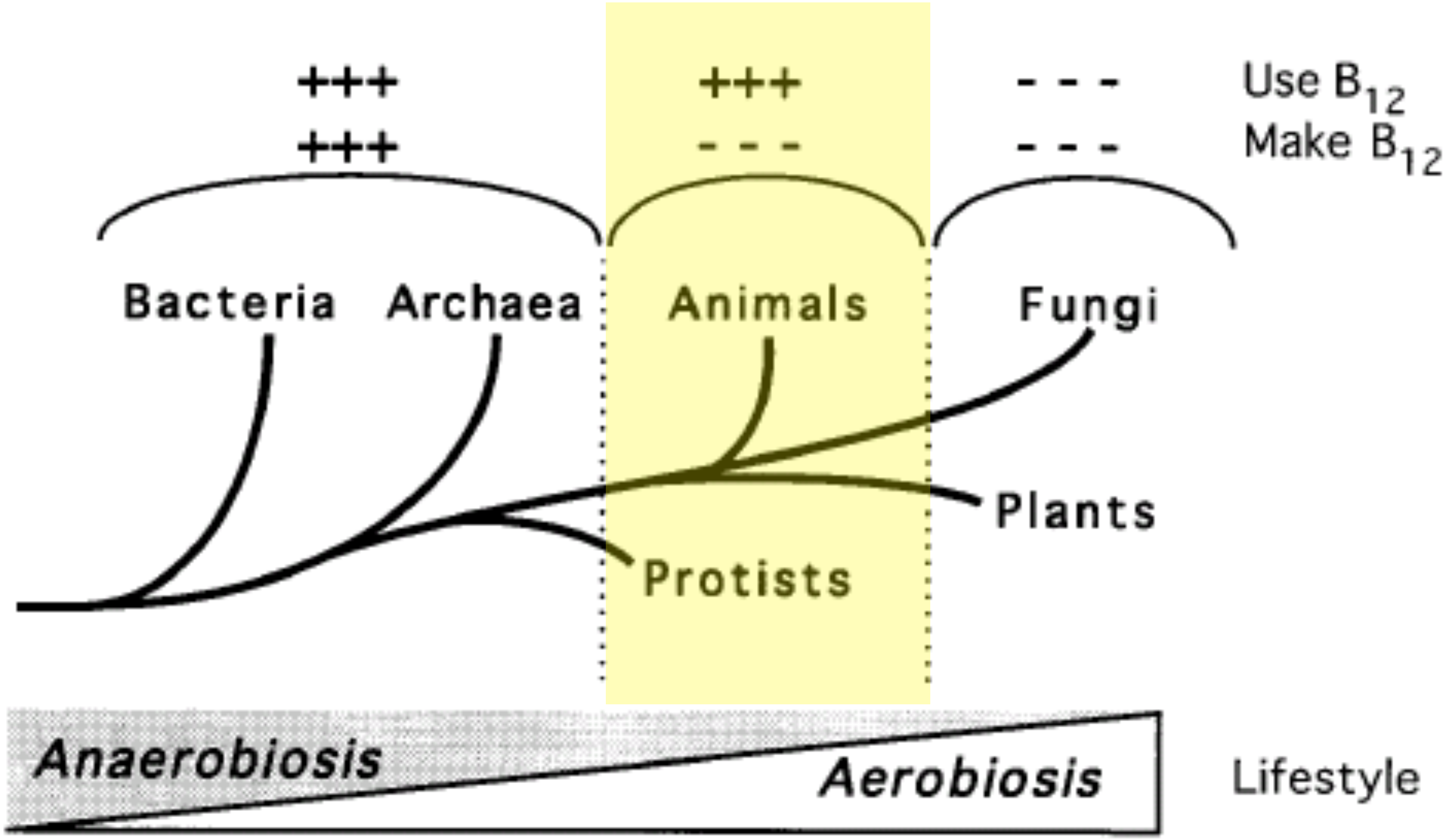
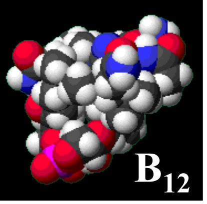
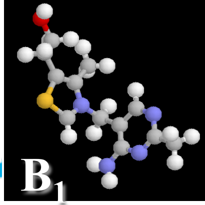


Figure 3 Distribution of cobalamin synthesis and use among living forms. Wedges designate in a general way the evolutionary and current importance of oxygen to organisms in each group.

Roth et al. 1996



Phytoplankton and vitamins



- Phytoplankton requiring an extracellular source of vitamins are deemed auxotrophs.
- Essential for multiple biosynthetic pathways including methionine and DNA synthesis. Fenech 2002; Croft et al 2005, Helliwell et al. 2011, Bertrand and Allen 2012
- Non-auxotrophs synthesize their own vitamins (most prokaryotes) ***or*** have vitamin-independent metabolic pathways (some eukaryotes).
- Many prokaryotes have been shown to produce vitamins and thus are a potentially important source of this micronutrient to the ocean. Palenik et al. 2003; Rocap et al. 2003; Vitreschak et al. 2003; Croft 2005
- Other sources include release of vitamins via microbial processes (viral lysis, zooplankton grazing,cell lysis) Provasoli 1963

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More than half of all phytoplankton surveyed require one or more of the B-vitamins

TABLE 1. Vitamin requirements of the individual species detailed in the supplemental material compiled under the different algal groups[a]

Phylum	No. of species surveyed	No. of species requiring:		
		Cobalamin	Thiamine	Biotin
Chlorophyta	148	44	19	0
Rhodophyta	13	12	0	0
Cryptophyta	6	5	5	1
Dinophyta	27	24	7	7
Euglenophyta	15	13	11	1
Haptophyta	17	10	14	0
Heterokontophyta	80	47	11	5
Total	306	155	67	14

Croft et al. 2006

Pioneering vitamin work

- Past studies have concluded vitamin concentrations may affect phytoplankton growth and species composition; basis for vitamin 'bioassay'

- Cellular requirements were deemed low
  - 3-24 molecules B_{12} μm^{-3} of cell
  - Low K_S (~ 0.1 – 2 pM)

- Led to dismissal of vitamins as 'ecologically irrelevant'. Droop 2007

Droop 1957, Nature

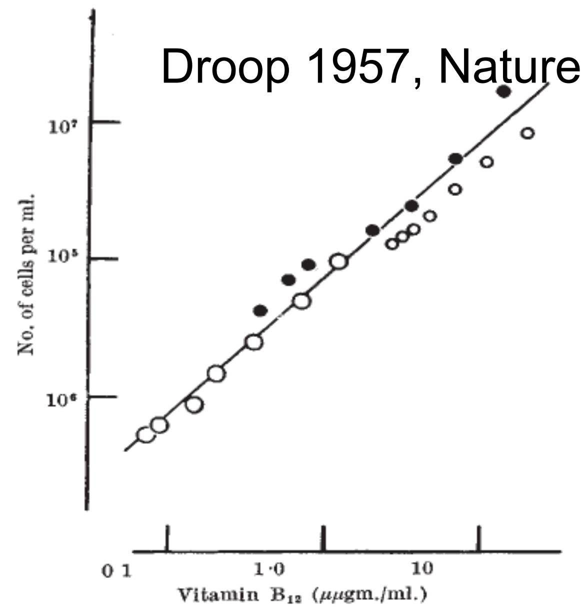
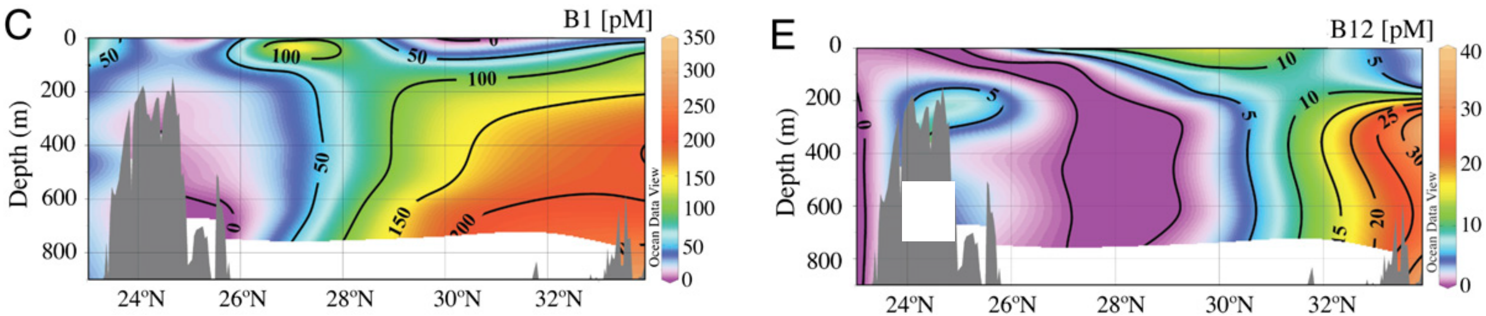


Fig. 1. Response of *Monochrysis lutheri* to vitamin B_{12}. Small open circles, 1954 experiment (ref. 2); small closed circles, 1955 experiment (ref. 3); large open circles, 1957 experiment

Renewed interest in vitamins

- Method to measure vitamins directly.
  - $[B_{12}] = 0.1 - 20\ pmol\ L^{-1}$
    $[B_1] = 0.2 - 120\ pmol\ L^{-1}$

Sañudo-Wilhemly et al. 2012



- The experimental enrichment of sea water with vitamin B_{12} has been shown to increase phytoplankton biomass in NY estuaries and HNLC regions of the Southern Ocean.

- No study has measured pelagic, plankton vitamin uptake rates in any marine ecosystem.

- Phytoplankton benefiting from elevated B-vitamin concentrations are unknown.

Research Questions:

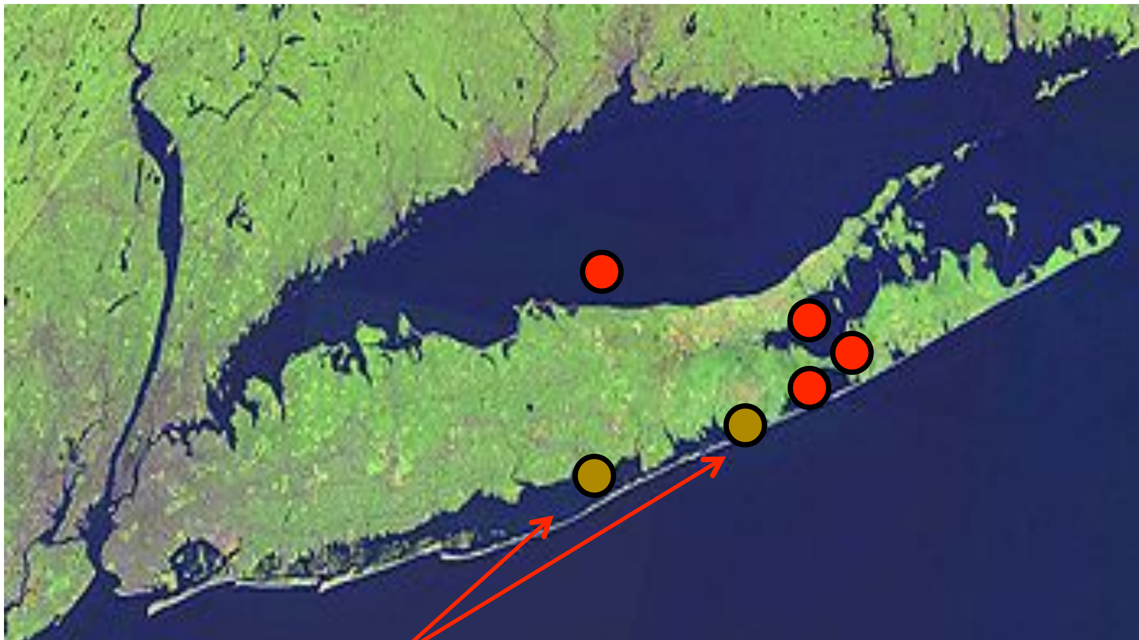
- Who are the primary vitamin utilizers in marine systems?

- How does vitamin limitation impact phytoplankton species composition and succession, particularly in high latitude HNLC regions and with regards to HABs?

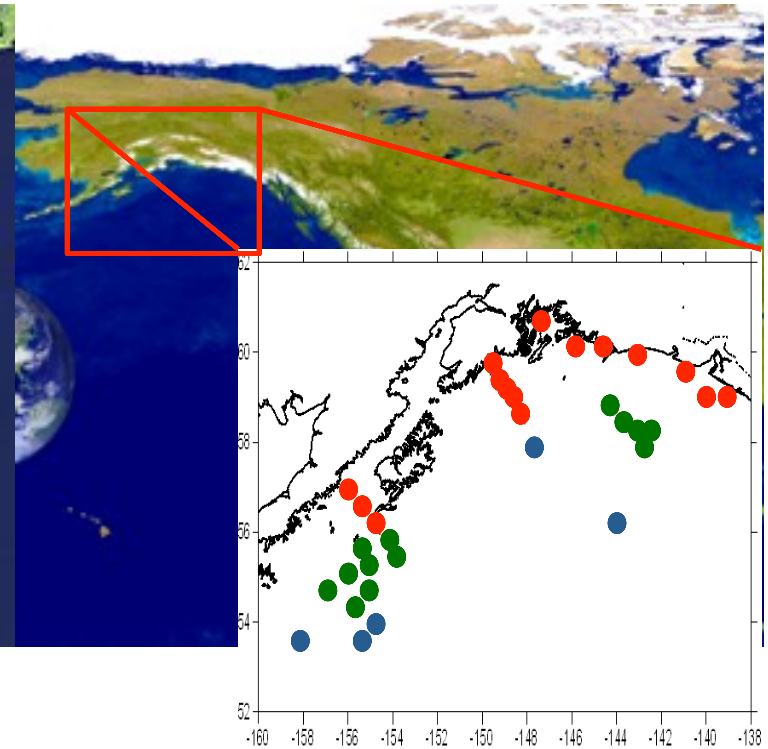
- How do vitamins influence ocean productivity and the carbon cycle?

Study sites

New York Estuaries



Gulf of Alaska



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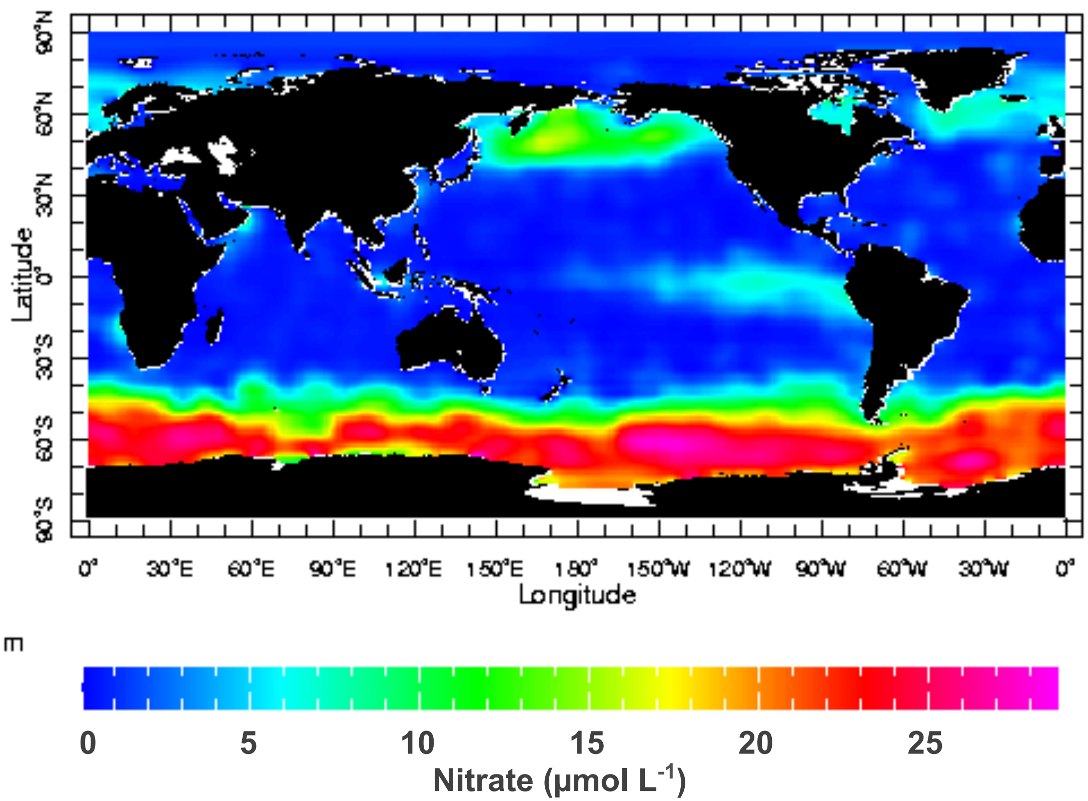
High Nutrient Low Chlorophyll



- Plenty of macronutrients and light but very low phytoplankton.
- ‘HNLC’ mystery until 1980s.
- 2 hypothesis:
  - Top down control
  - Bottom up control
- John Martin proved in late 1980s that Fe limits phytoplankton growth in large areas of the worlds oceans.

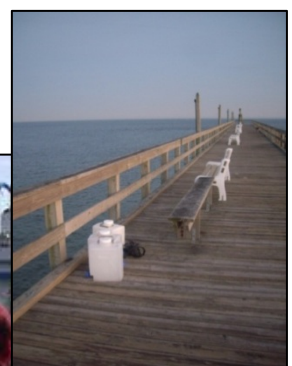
Trace Metal Clean Techniques

- Ensure samples are not contaminated by ship or other metal sources.
  - Fe and Zn are biggest issues.
- Specialized equipment. (Teflon, plastics)
- Clean rooms.
- ULTRA clean techniques.

Time Series Field Measurements

- Plankton biomass and community
- composition (size fractionated chl *a*, microscopy, flow cytometry).
- Vitamin concentrations (B_1 & B_{12}).
- Primary productivity (^{14}C).
- Size fractionated vitamin uptake rates
  - ^{57}Co-labeled vitamin B_{12}
  - ^{3}H-labeled vitamin B_1





Vitamin Enrichment Experiments

- Triplicate polycarbonate bottles amended with:
  - **10 nM Fe, 10μM N, 100pM B_{12}** and combinations thereof
- Establish plankton community response

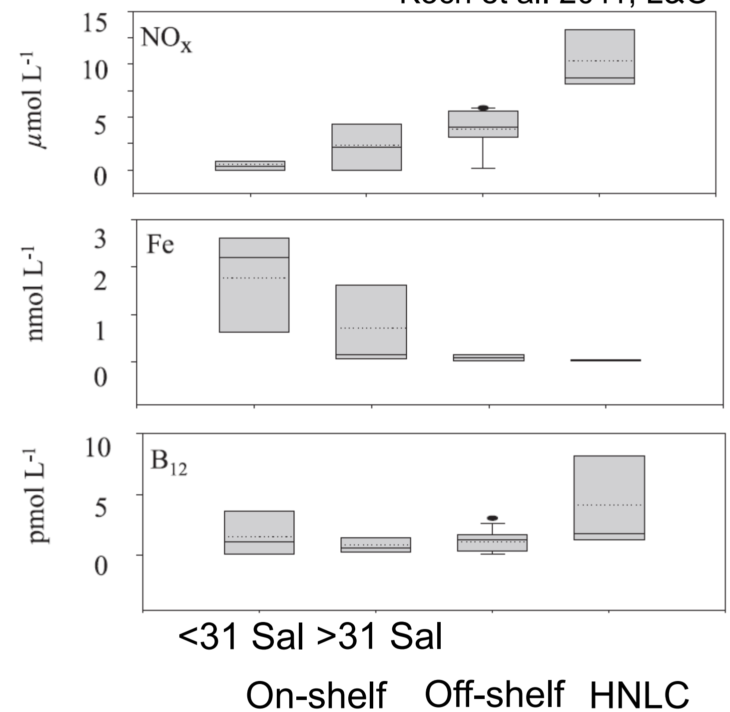
Culture Experiments

- Culturing of various harmful algae under vitamin replete and deplete conditions.
- Used radiotracers to establish kinetics constants and cellular quotas.
  - K_s, V_{max} and Q_{cell}

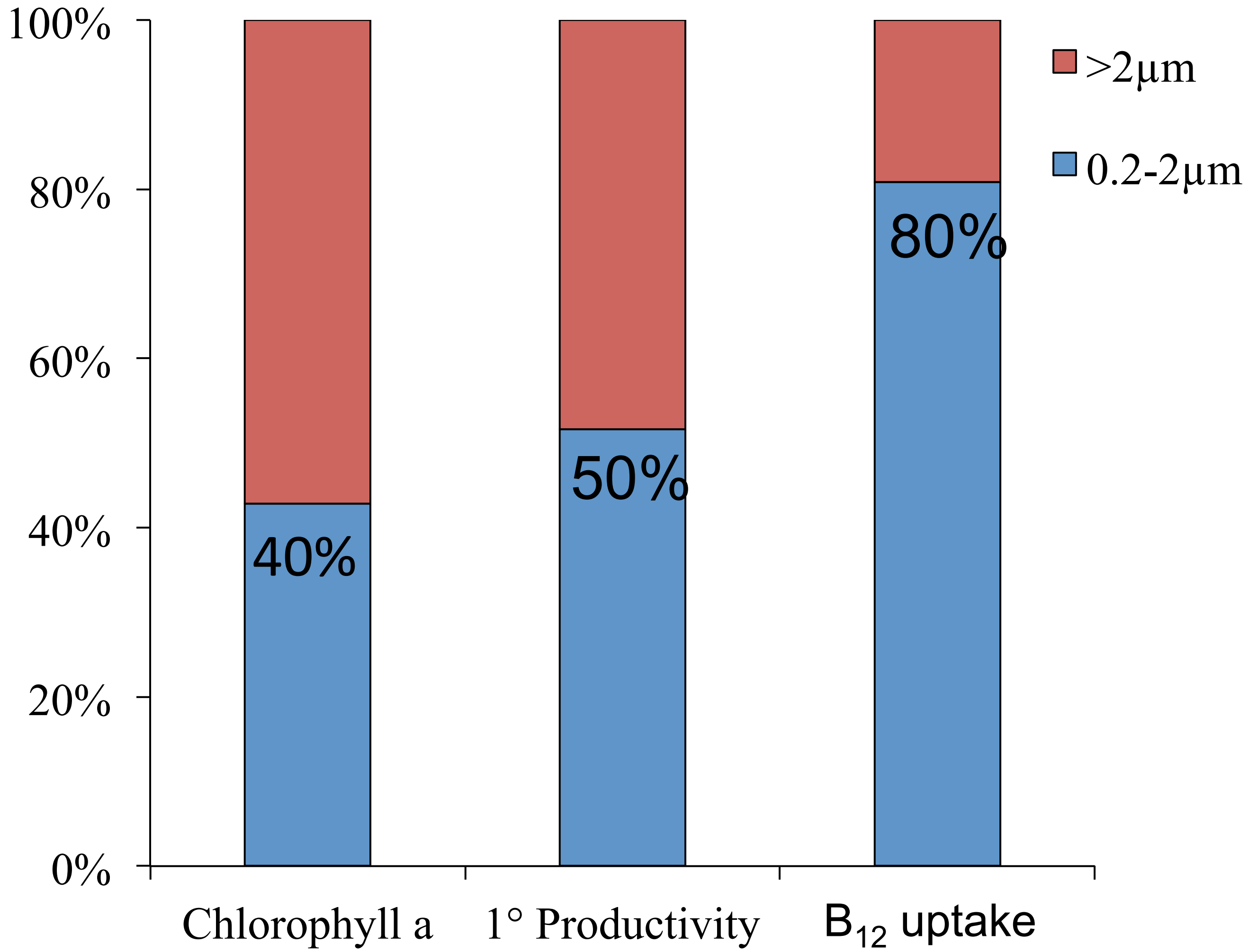
Spatial trends in nutrients , B_{12} and iron in the Gulf of Alaska

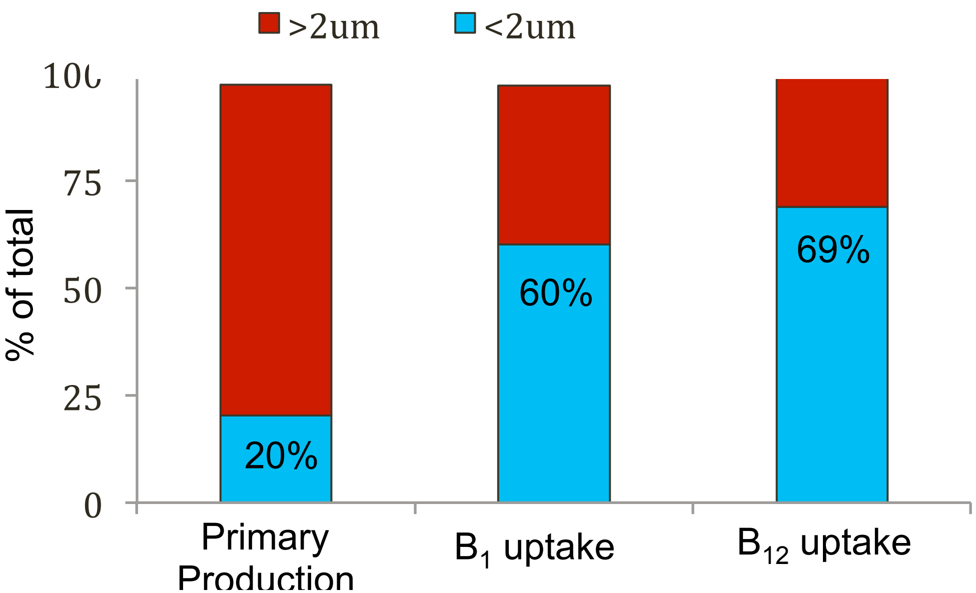
Koch et al. 2011, L&O

- Vitamin B_{12} behaved like a macronutrient, high in HNLC, low near shore.
- Vitamin B_{12} was negatively correlated with iron concentrations.
- Primary production was lowest in HNLC.
- Highest B_{12} utilization/lowest turnover times in HNLC regions.



Size distribution of phytoplankton, 1° production, and B_{12} uptake



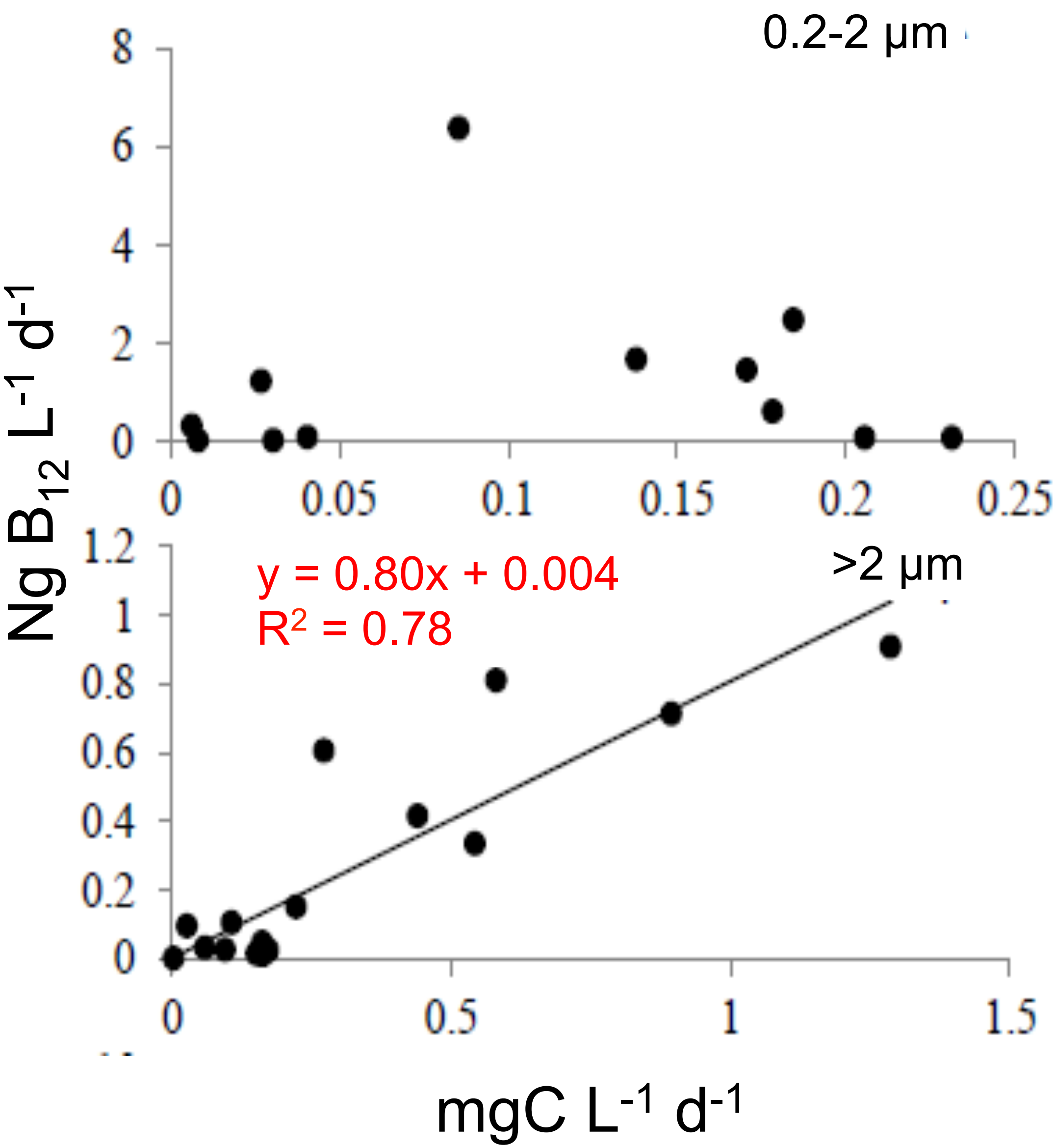


- Picoplankton (< 2µm) account for the majority of vitamin B_1 and B_{12} uptake in marine environments.

- Picoplankton < 2µm also have a high carbon specific vitamin demand.

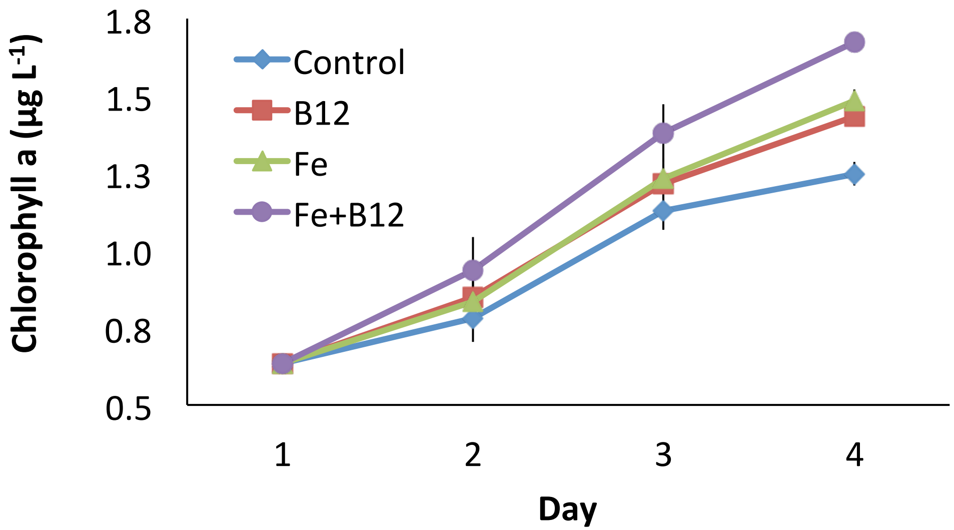
Koch et al. 2012, FMICB

Who uses vitamins?

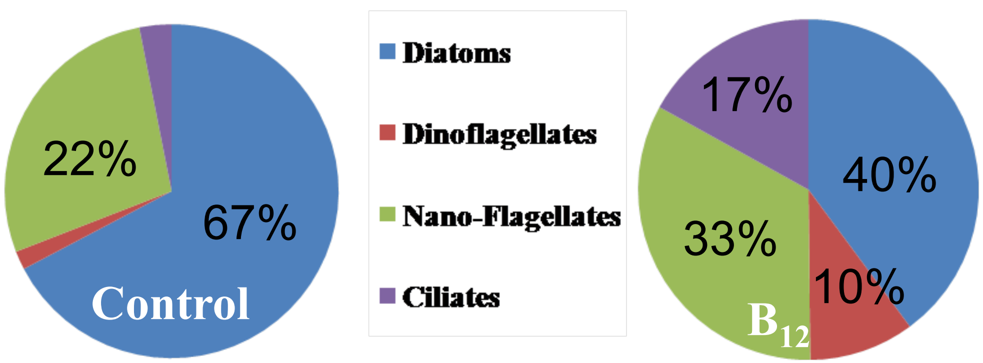


Koch et al. 2012, FMICB

- No correlation in picoplankton
- >2 µm uptake highly correlated with primary production
- Heterotrophic bacteria are the main utilizers of vitamins in marine systems
- Implication: very little of the pM pools are available for larger phytoplankton.



Koch et al. 2011, L&O

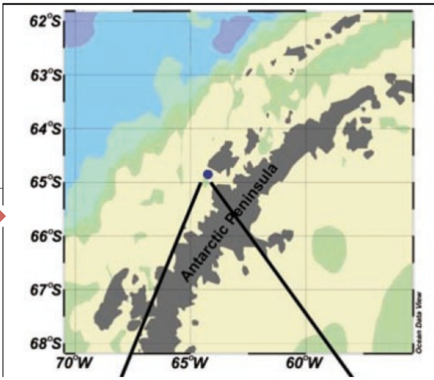


- **B_{12}**
  - Significantly higher total chl. a over control ($p<0.001$).
  - Community change favoring dinoflagellates, autotrophic nanoflagellates, and ciliates.
- **Fe**
  - Significant ($p<0.001$) increase in total chl. a over control.
  - Diatoms increased, all other groups declined.
- **$Fe+B_{12}$**
  - Significantly ($p>0.001$) higher total chl. a then control, B_{12}, and Fe alone.
  - Decrease in diatoms and increase in nanoflagellates and ciliates over Fe alone.

B Vitamins as Regulators of Phytoplankton Dynamics

By C. Panzeca, A. Tovar-Sanchez, S. Agustí, I. Reche, C. M. Duarte, G. T. Taylor, and S. A. Sañudo-Wilhelmy

the American Geophysical Union 87(52)



Limnol. Oceanogr., 52(3), 2007, 1079–1093

Vitamin B_{12} and iron colimitation of phytoplankton growth in the Ross Sea

Erin M. Bertrand[2] *and Mak A. Saito*[1,2]
Marine Chemistry and Geochemistry Department, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

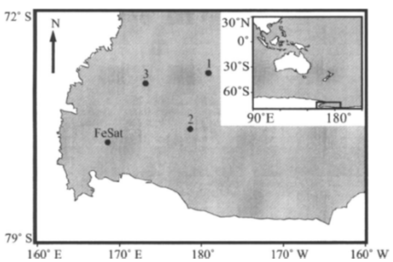
Julie M. Rose
Department of Biological Sciences, University of Southern California, Los Angeles, California 90089

Christina R. Riesselman
Department of Geological and Environmental Sciences, Stanford University, Stanford, California 94305

Maeve C. Lohan
School of Earth, Ocean and Environmental Sciences, University of Plymouth, Drake Circus, Plymouth PL4 8AA, United Kingdom

Abigail E. Noble
Marine Chemistry and Geochemistry Department, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

Peter A. Lee and Giacomo R. DiTullio
Grice Marine Laboratory, College of Charleston, Charleston, South Carolina 29412



frontiers in MICROBIOLOGY
ORIGINAL RESEARCH ARTICLE
published: 15 August 2011
doi: 10.3389/fmicb.2011.00160

Iron limitation of a springtime bacterial and phytoplankton community in the Ross Sea: implications for vitamin B_{12} nutrition

Erin M. Bertrand[1], Mak A. Saito[2]*, Peter A. Lee[3], Robert B. Dunbar[4], Peter N. Sedwick[5] and Giacomo R. DiTullio[6]

Low bacterial abundances, metabolic rates and high microbial cycling in HNLC areas may leads to the observed vitamin limitation in polar regions.

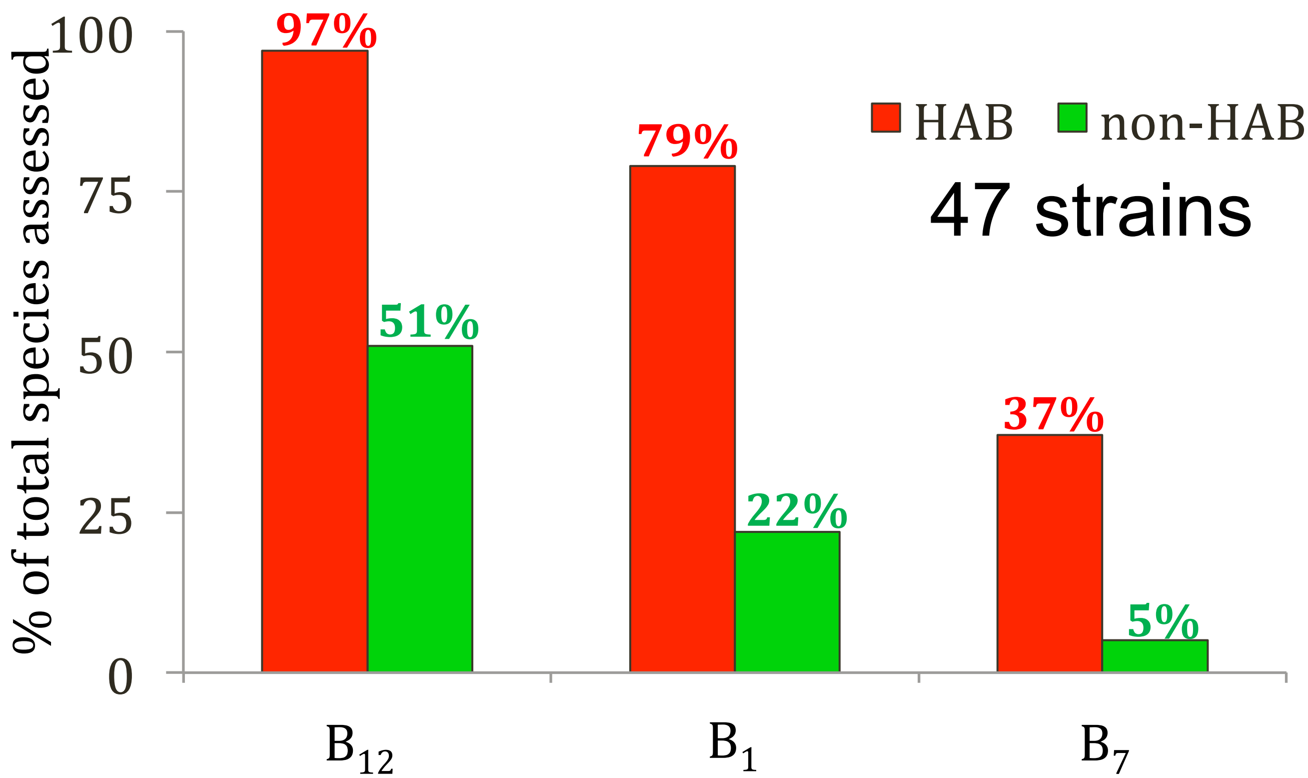
Outline

- The role of nutrients/vitamins in phytoplankton ecology.
- Harmful algal blooms and vitamins.

Most harmful algal bloom species are vitamin B_1 and B_{12} auxotrophs

Ying Zhong Tang, Florian Koch, and Christopher J. Gobler[1]
School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, NY 11794-5000

PNAS



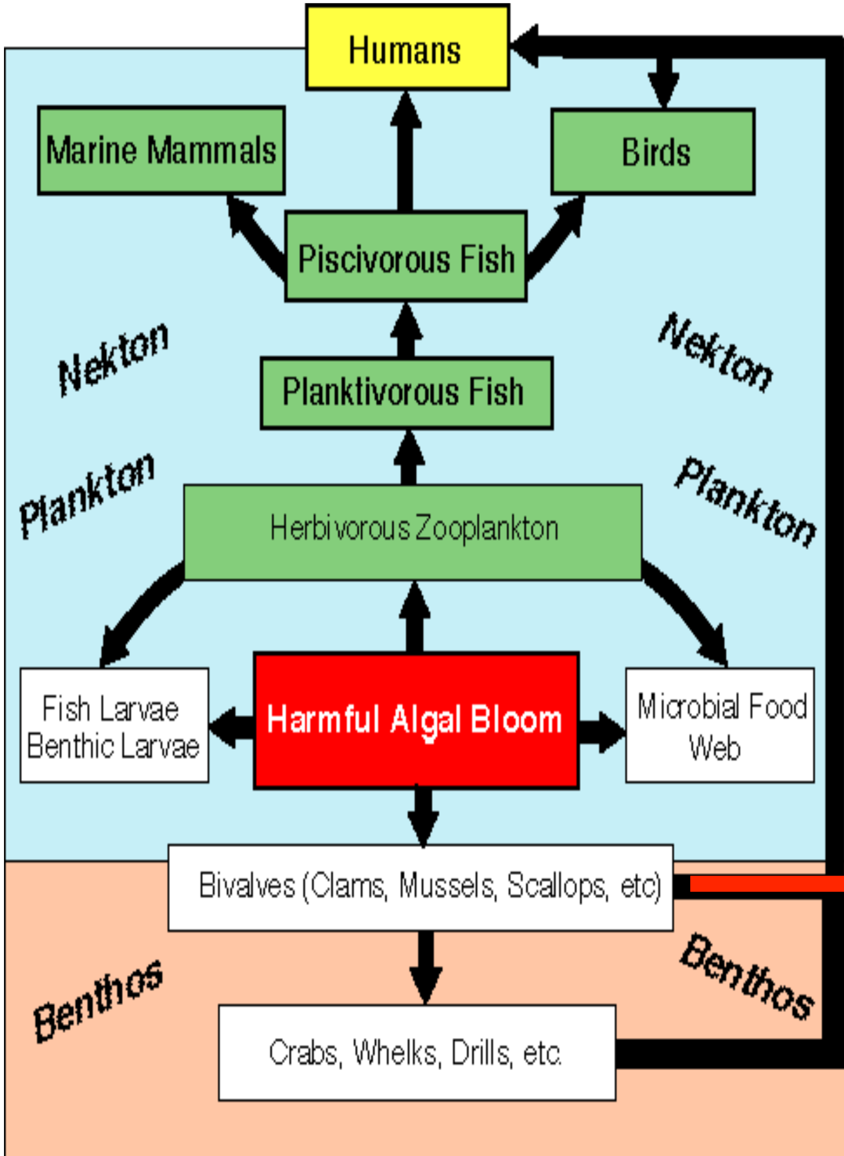
- Vitamins have been hypothesized to play a key role in the in the occurrence of harmful algal blooms (HABs; Carlucci 1970; Hunter and Provasoli 1964; Steward et al. 1967; Collier 1969) but this has not been investigated in decades.

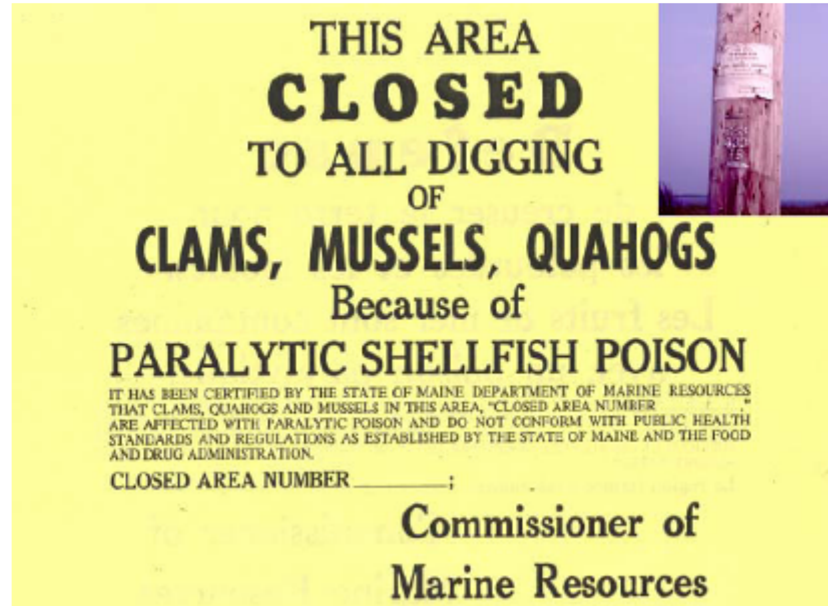
Growth of an harmful algal species to densities which negatively impact an ecosystem =

Harmful Algal Bloom (HAB)

Of the thousands known phytoplankton species, several dozen are known to be *harmful* to marine ecosystems.

Many HABs contain toxic compounds which can impact all levels of marine food webs, including humans.





Ecosystem disruption by algal blooms: EDABs

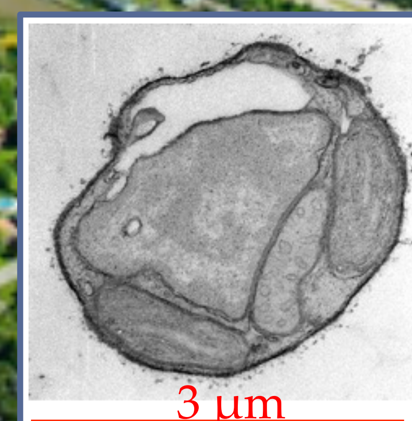
Vitamins and *Aureococcus anophagefferens*

- Pelagophyte.

- Blooms in eastern US, South Africa, and China. Gobler et al. 2005, Zhang et al. 2012,

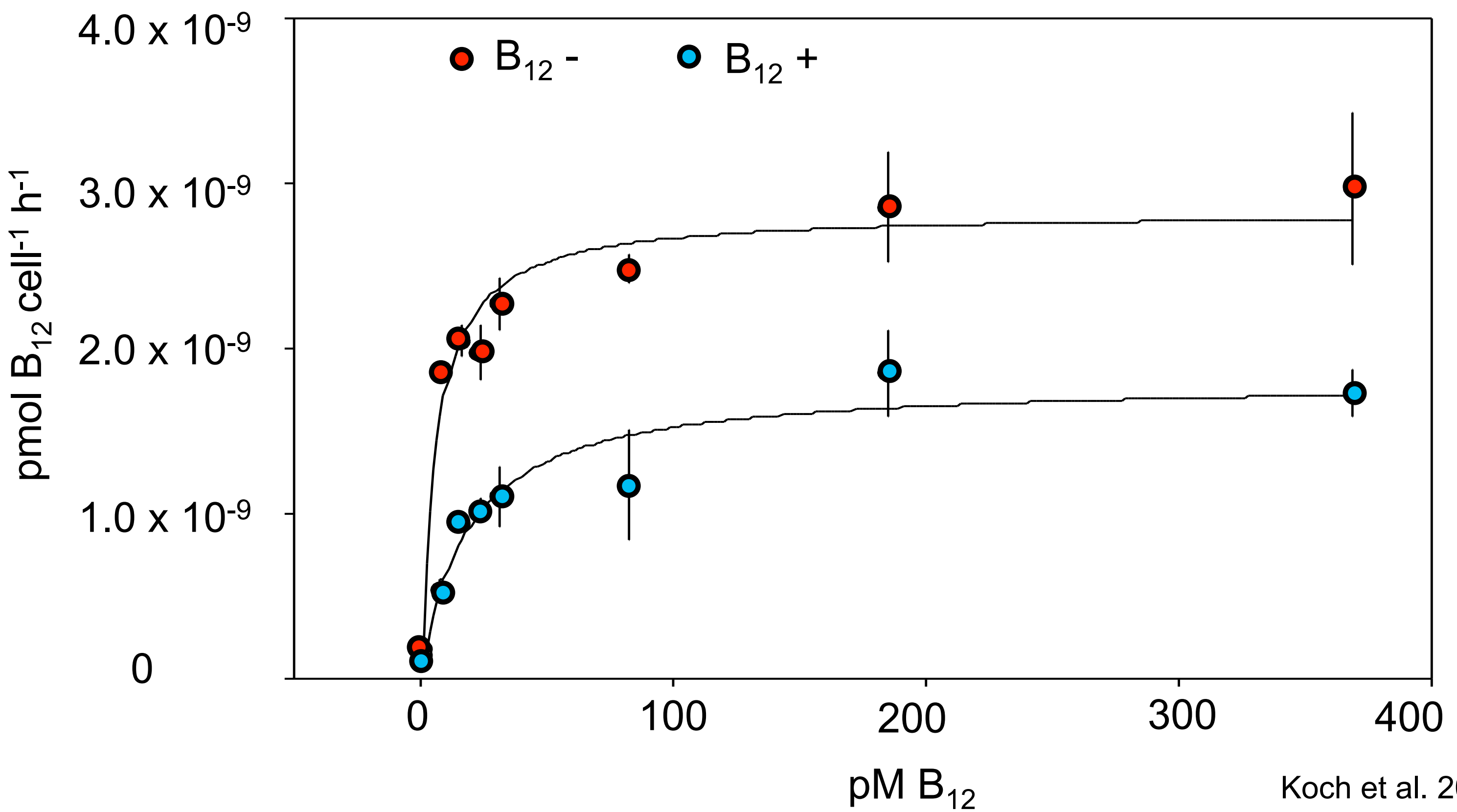
- Vitamin B_{12} auxotrophy confirmed by culture studies and genomic analysis. Gobler et al. 2011, Tang et al. 2010,

- Vitamin B_1 auxotrophy confirmed by culture studies. Tang et al. 2010



Picture courtesy of the Suffolk County Health Department

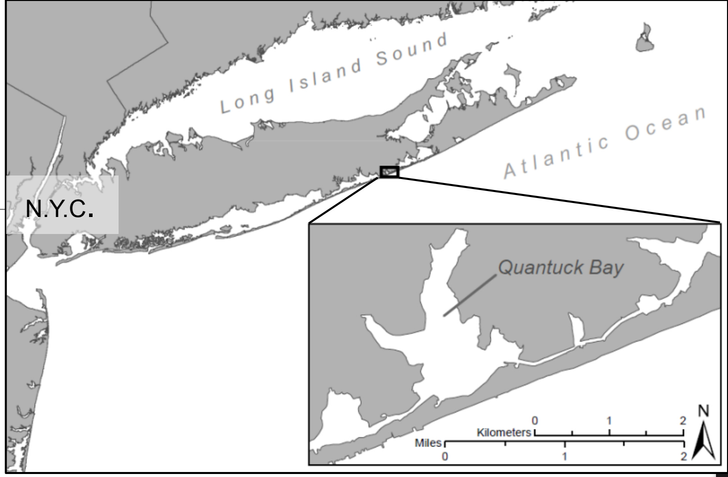
Vitamin B_{12} uptake by *A. anophagefferens*

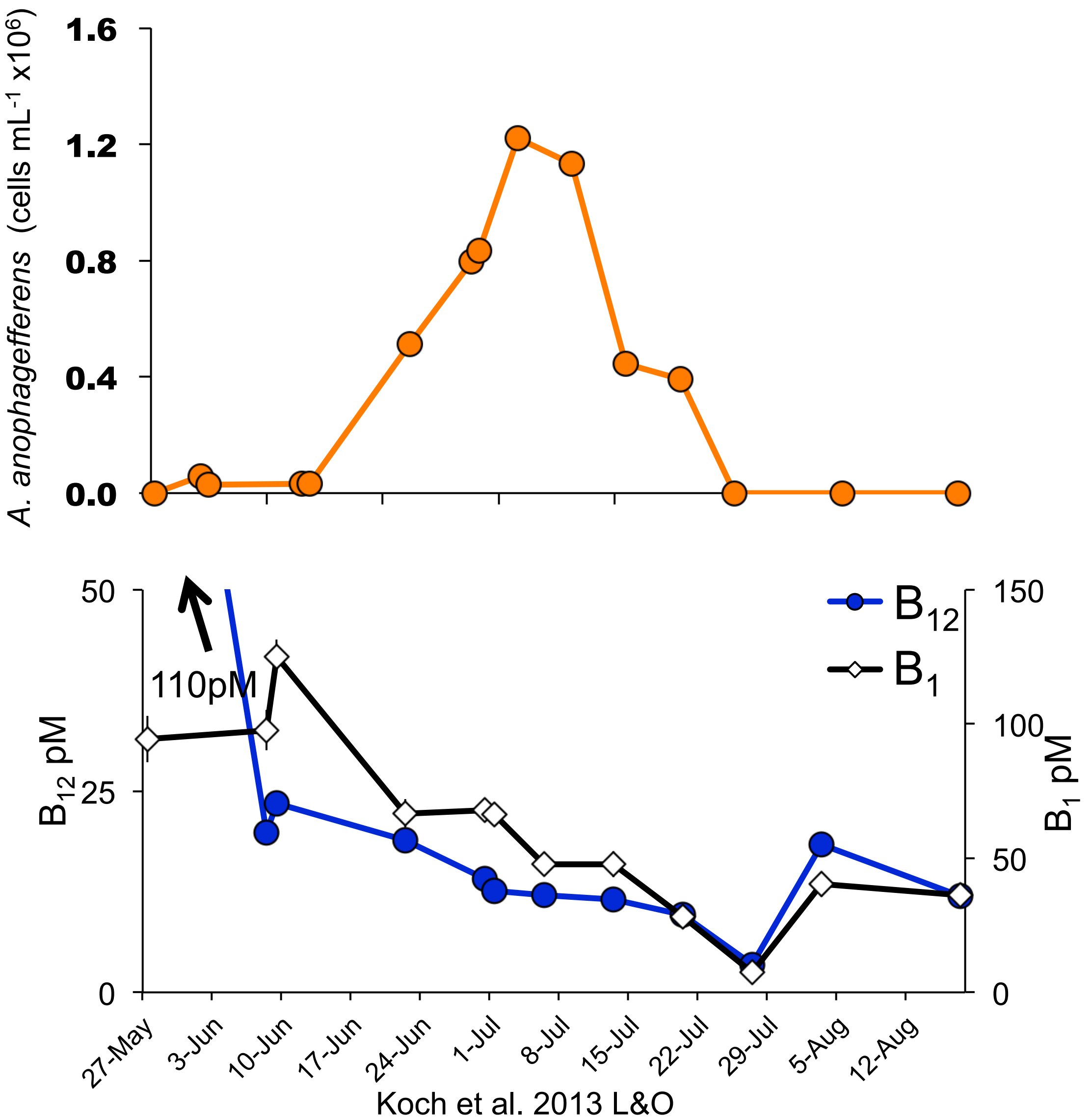


Koch et al. 2013, L&O

Half saturation of uptake	**5.55 - 20.8 pM**
Mean concentration in NY estuaries	20.3 ± 4.39 pM

Brown Tide Bloom, Quantuck Bay, 2009





B_1 and B_{12} are drawn down from 125pM (B_1) and 110 pM (B_{12}) to <5pM.

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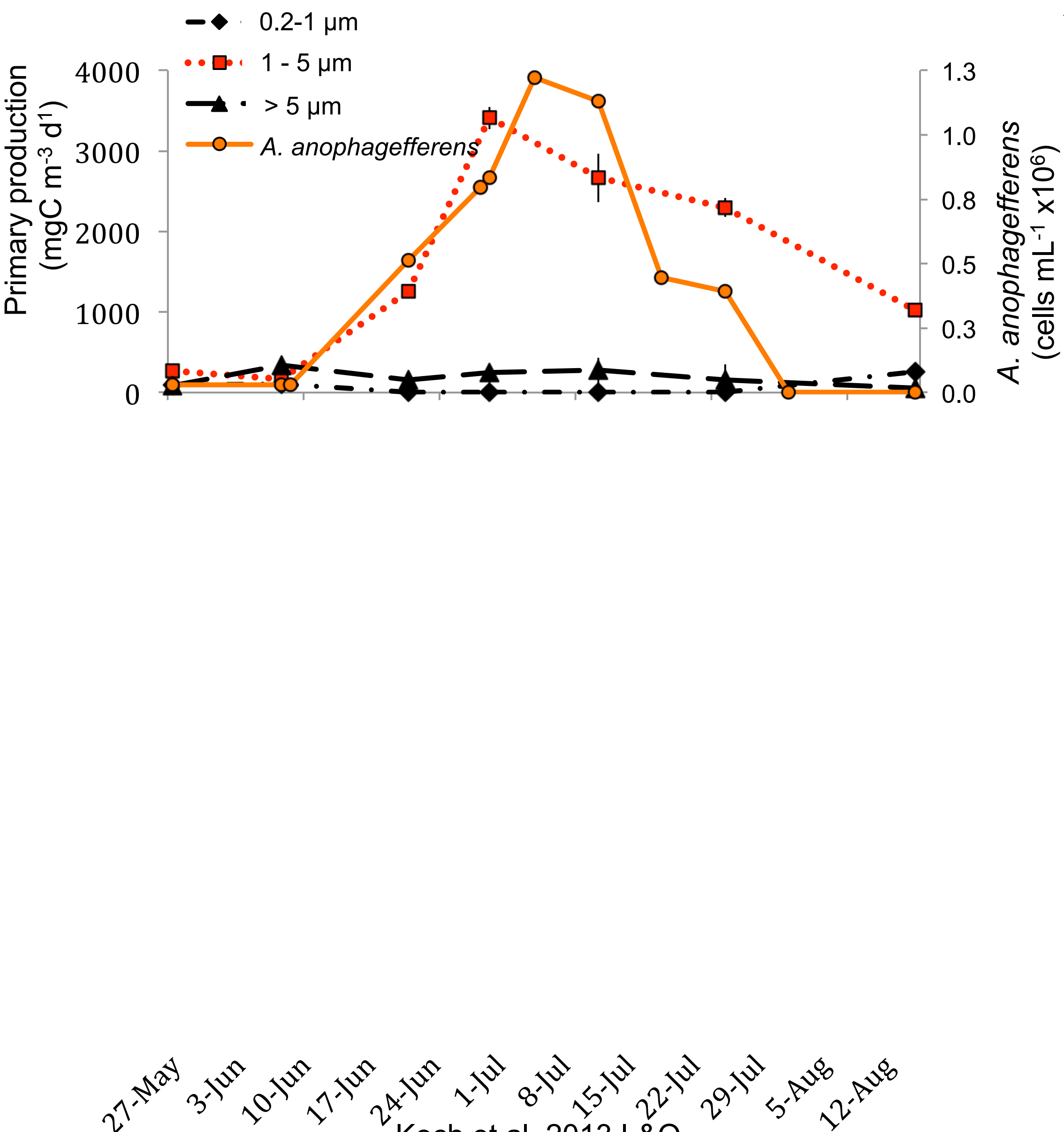
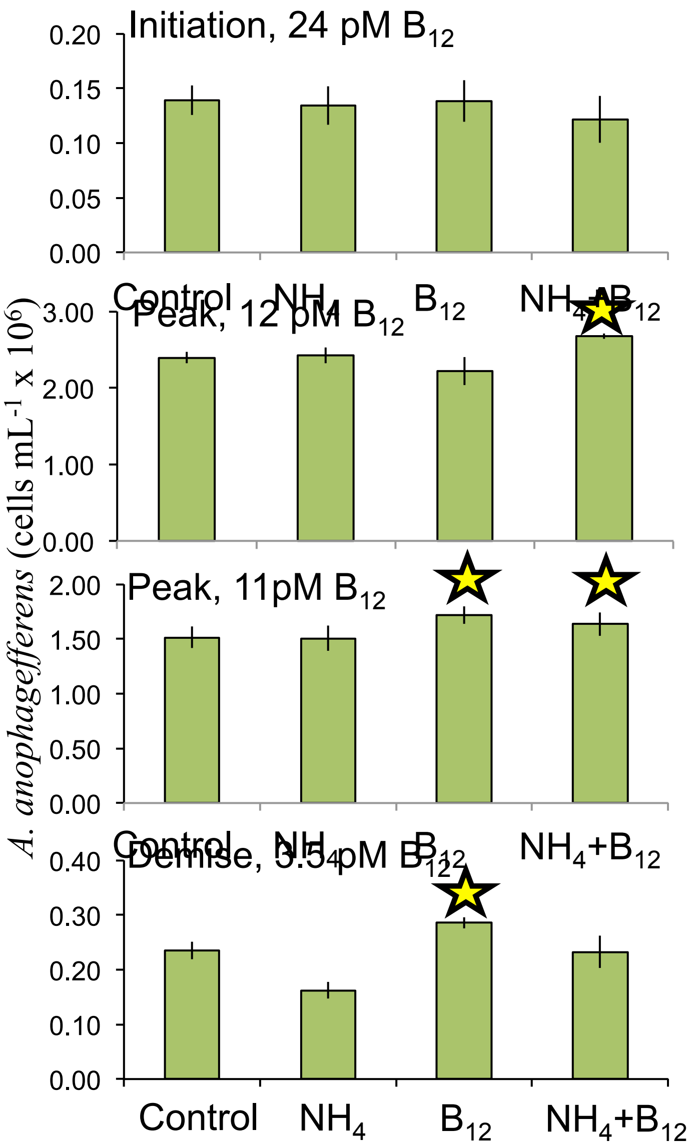
Brown Tide bloom, Quantuck Bay, 2009

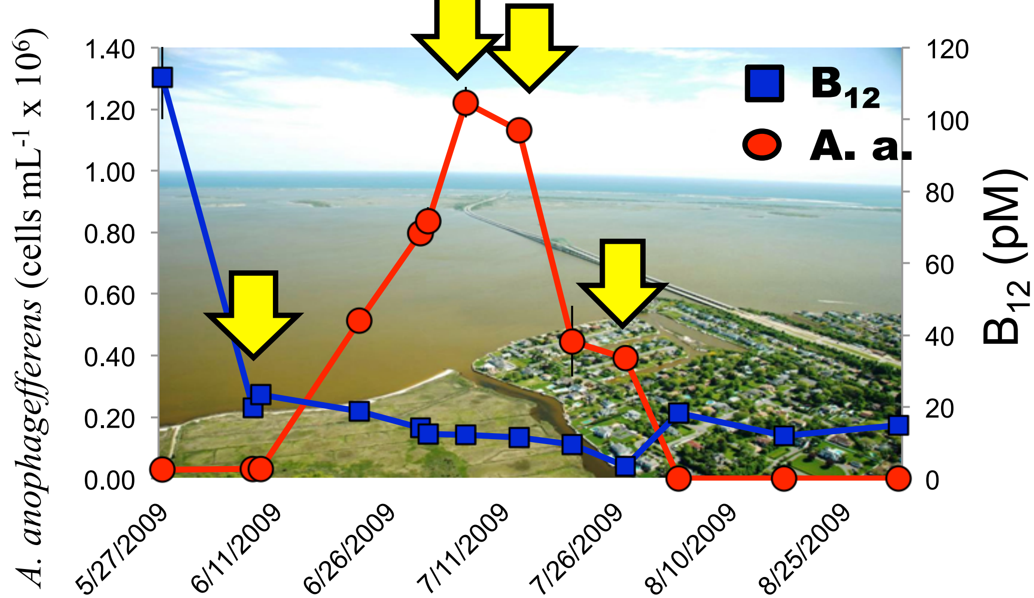


Koch et al. 2013 L&O

- Most **primary production** in the 1-5 µm size fraction (*A. anophagefferens* = 1-3µm).
- Vitamin $\mathbf{B_1}$ utilization was primarily in the 1-5 µm size fraction and highest during the peak of the bloom.
- Vitamin $\mathbf{B_{12}}$ utilization was shared between the 0.2-1 and 1-5 µm size fractions and highest during the peak of the bloom.
- **Pools turning over daily**
  - $\mathbf{B_{12}}$ **= 13h**
  - B_1 = 62h

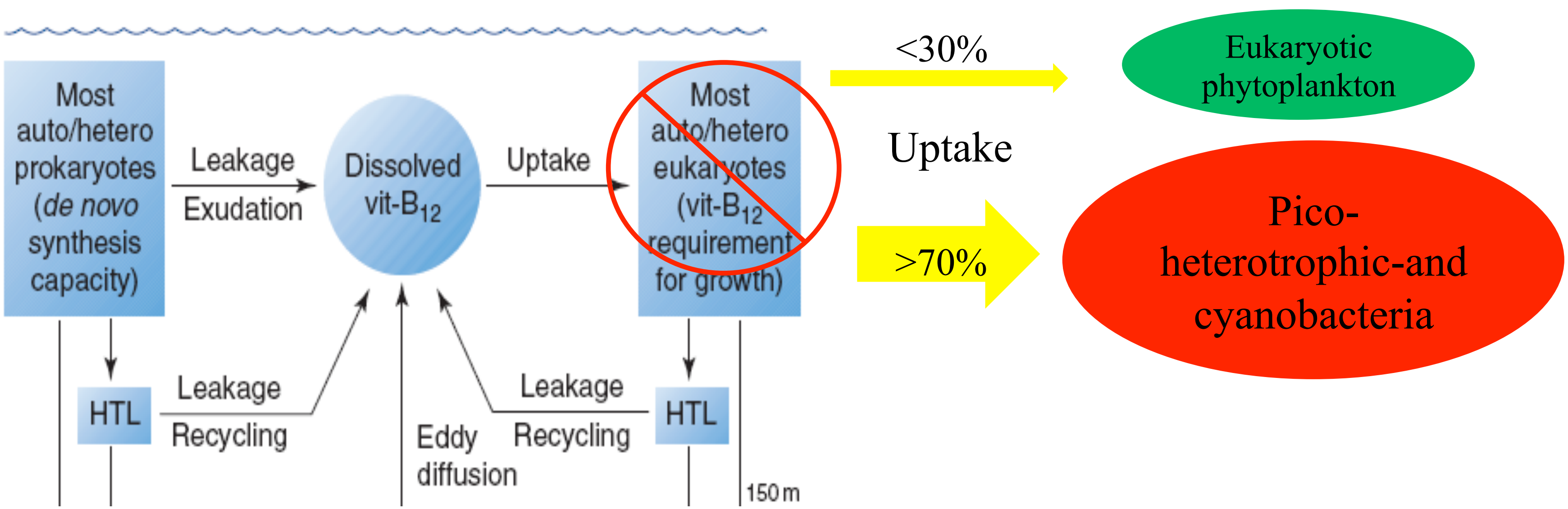
Effects of B_{12} on *Aureococcus*, Quantuck Bay 2009





- *A. anophagefferens* nutritionally replete during initiation of bloom.
- Vitamin B_{12} and B_{12} co-limitation with NH_4 during the peak of the bloom.
- Vitamin B_{12} limitation during the demise of the bloom

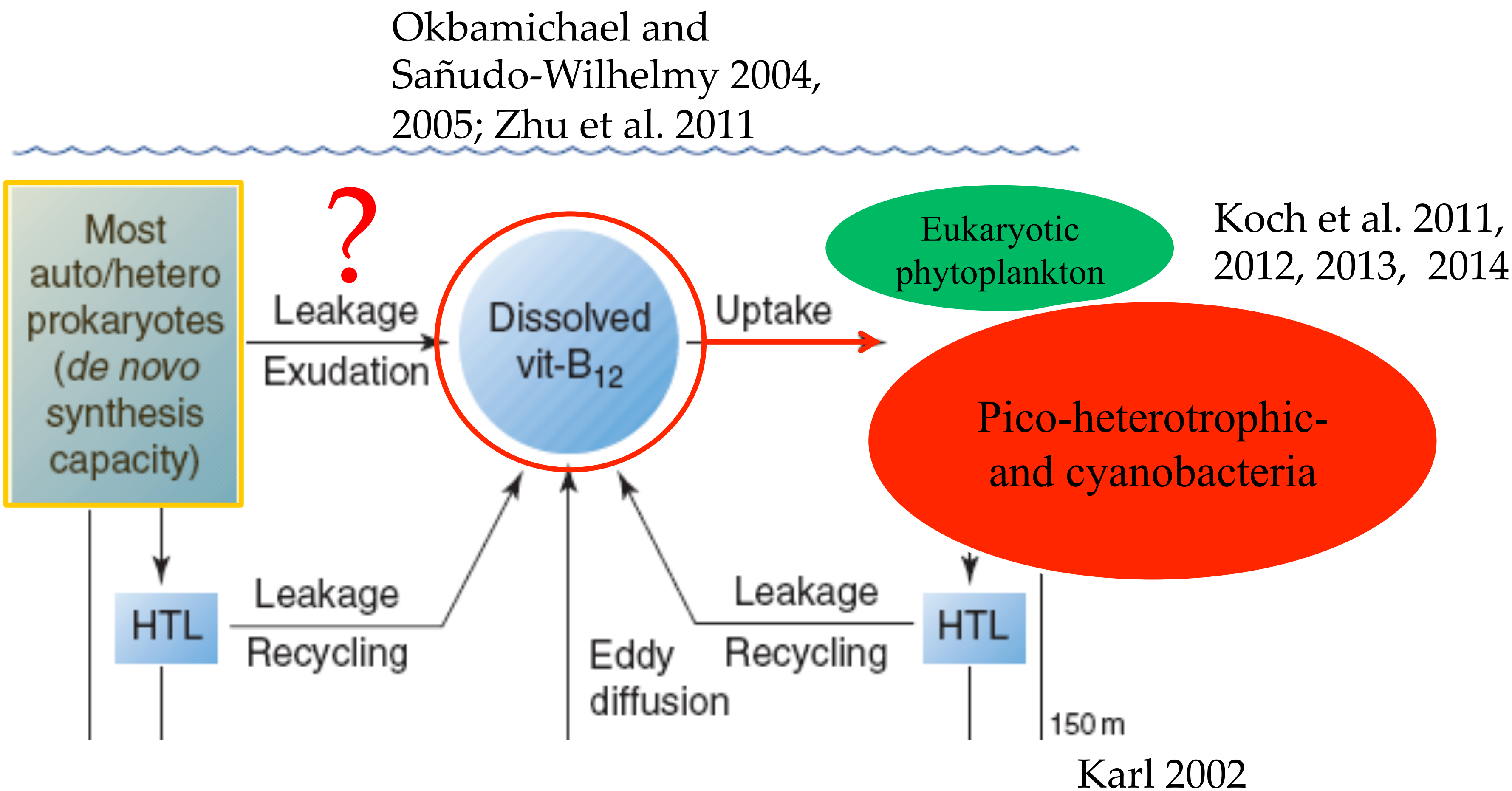
A revised notion of vitamin cycling



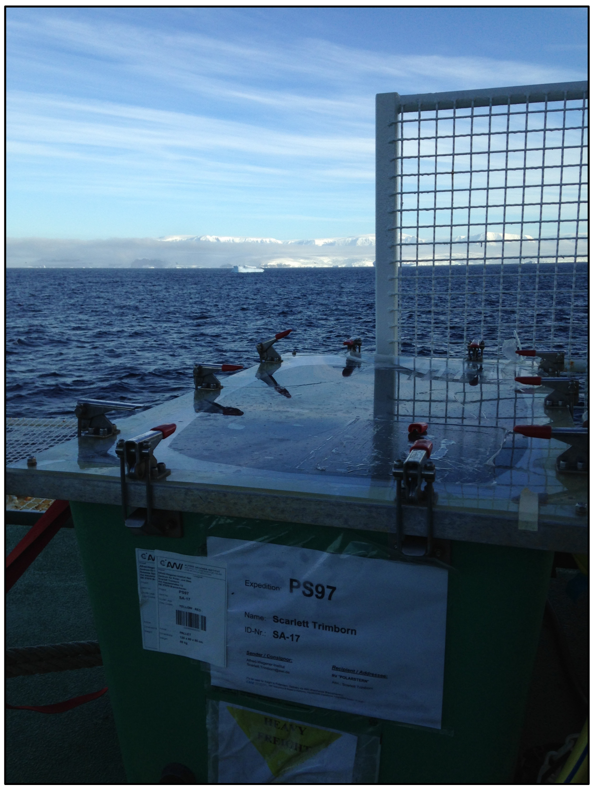
Karl 2002

"Vitamin B_{12} requirement in marine pelagic algae (are) so low that oceanic and coastal concentrations of the vitamin would usually be sufficient to sustain the populations that occur" -Droop, 2007

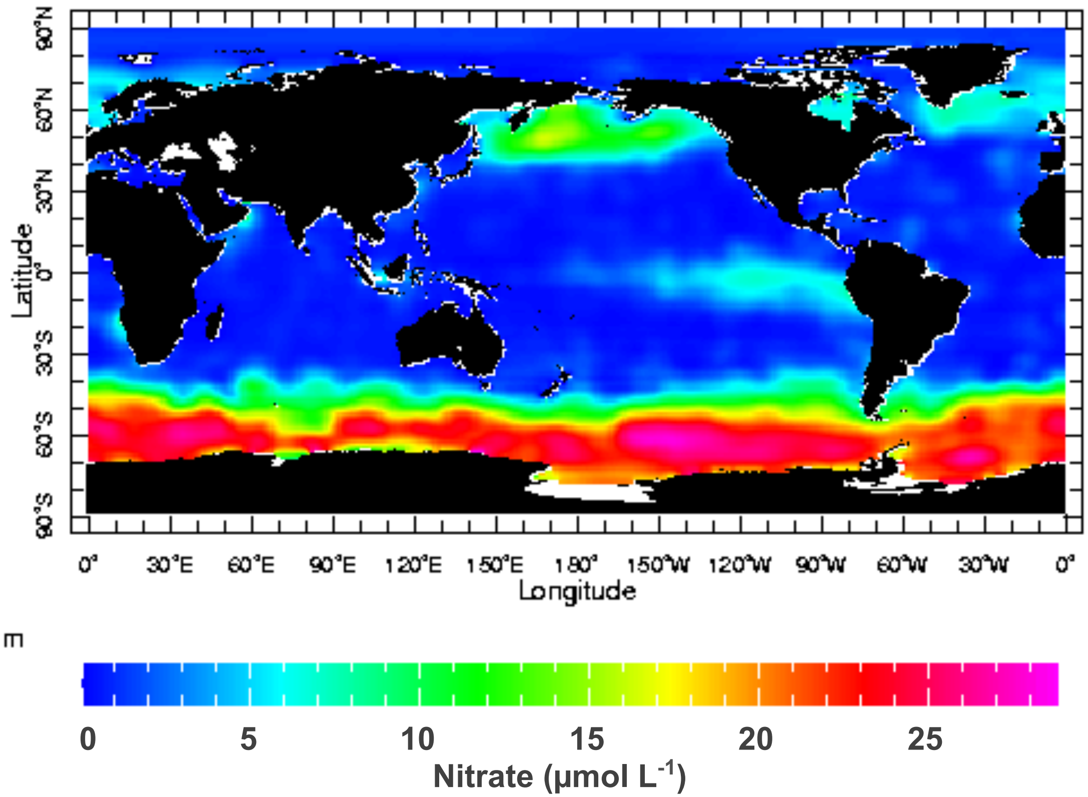
Vitamin production



Using a simple but novel approach to elucidate the dynamics and effects of Iron, Zinc, Cobalt and Vitamin B_{12} cycling on the plankton communities in the Polar Ocean



Using tracers to study trace element cycling in the Southern Ocean



- Strong influence on global carbon cycle
- 40% uptake of anthropogenic CO2
- 20% of global marine primary production
- In large parts limited by trace elements (Fe, vitamins).

Goals

- To investigate the effects of trace metal limitation on the physiology and composition of plankton communities both in the lab and in the field.

- To understand the relative importance of removal and production/recycling mechanisms for Fe, Zn, Co, Vitamin B_{12}, and the key players responsible.

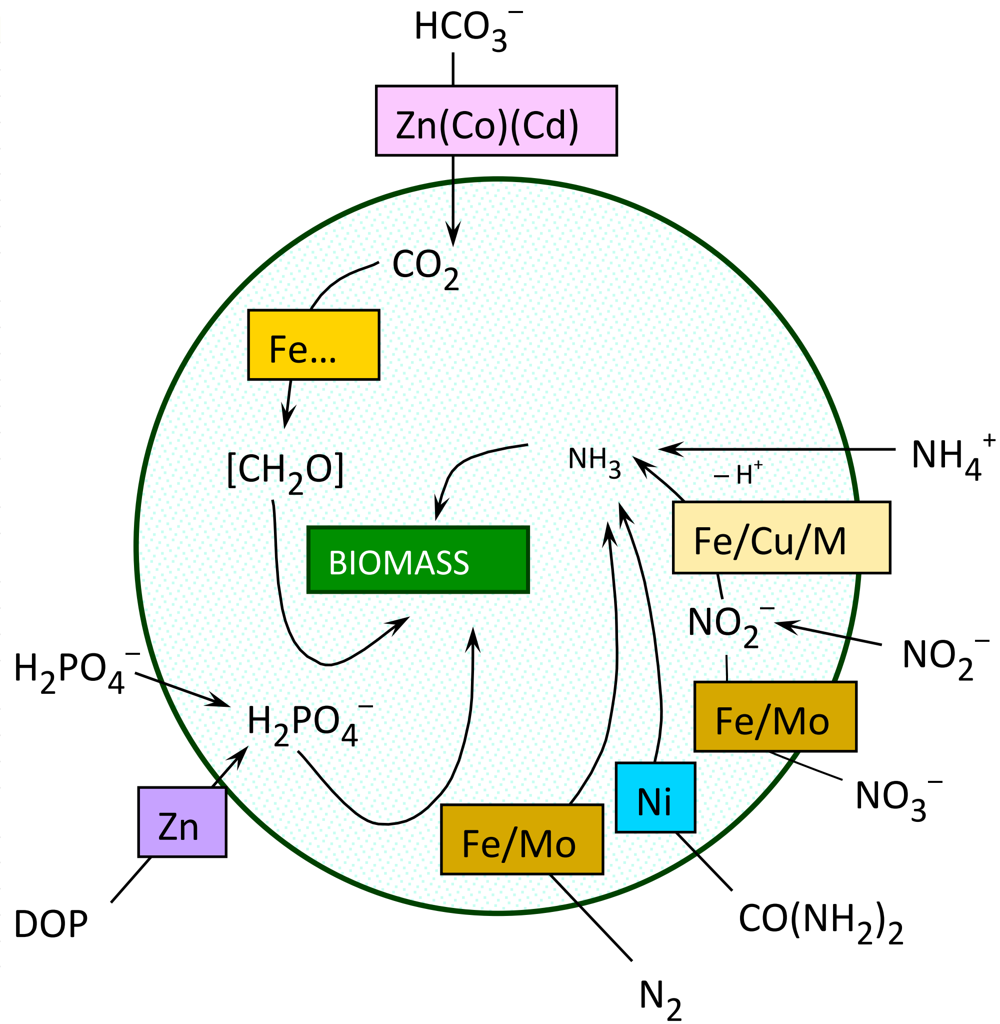
This will help explain observed limitations and co-limitations of plankton communities in the field

Trace metal and vitamin requirement. What is it used for?

Table 1 Common metalloproteins present within marine phytoplankton

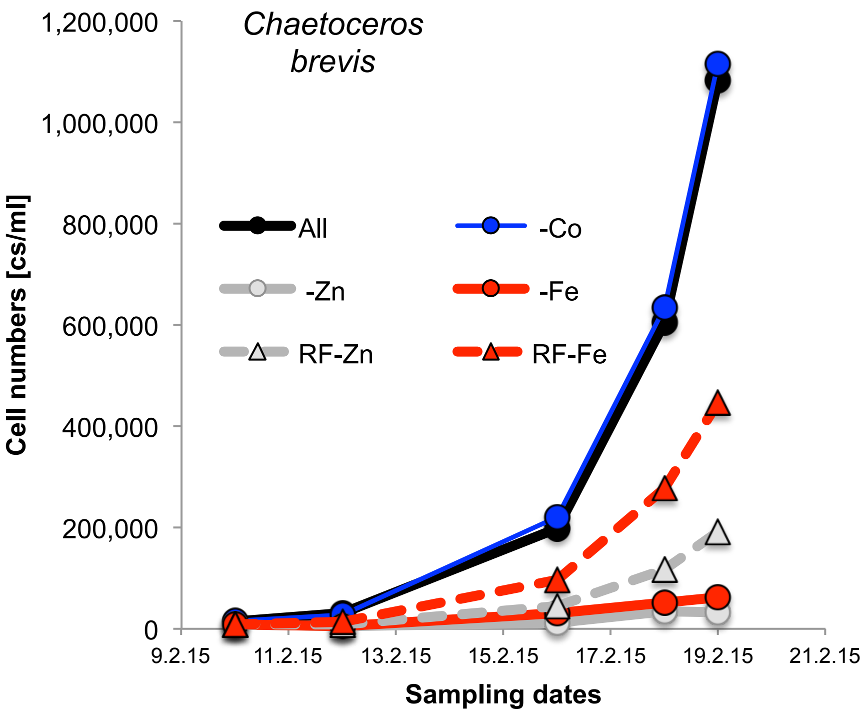
Metal	Protein(s)	Function(s)
Fe	Cytochromes	Electron transport in photosynthesis and respiration
	Ferredoxin	Electron transport in photosynthesis and N fixation
	Other Fe-S proteins	Electron transport in photosynthesis and respiration
	Nitrate and nitrite reductase	Conversion of nitrate to ammonia
	Chelatase	Porphyrin and phycobiliprotein synthesis
	Nitrogenase	N fixation
	Catalase	Conversion of hydrogen peroxide to water
	Peroxidase	Reduction of reactive oxygen species
	Superoxide dismutase	Disproportionation of superoxide to hydrogen peroxide and O_2
Zn	Carbonic anhydrase	Hydration and dehydration of carbon dioxide
	Alkaline phosphatase	Hydrolysis of phosphate esters
	RNA polymerase	Nucleic acid replication and transcription
	tRNA synthetase	Synthesis of tRNA
	Reverse transcriptase	Synthesis of single-stranded DNA from RNA
	Carboxypeptidase	Hydrolysis of peptide bonds
	Superoxide dismutase	Disproportionation of superoxide to hydrogen peroxide and O_2
Mn	O_2-evolving enzyme	Oxidation of water during photosynthesis
	Superoxide dismutase	Disproportionation of superoxide to hydrogen peroxide and O_2
	Arginase	Hydrolysis of arginine to ornithine and urea
	Phosphotransferases	Phosphorylation reactions
Ni	Urease	Hydrolysis of urea
	Superoxide dismutase	Disproportionation of superoxide to hydrogen peroxide and O_2
Cu	Plastocyanin	Photosynthesis electron transport
	Cytochrome oxidase	Mitochondrial electron transport
	Ascorbate oxidase	Ascorbic acid oxidation and reduction
	Superoxide dismutase	Disproportionation of superoxide to hydrogen peroxide and O_2
	Multicopper ferroxidase	High-affinity transmembrane Fe transport
Co	Vitamin B_{12}[a]	C and H transfer reactions
Cd	Carbonic anhydrase[b]	Hydration and dehydration of carbon dioxide
Mo	Nitrate reductase	Conversion of nitrate to ammonia
	Nitrogenase	N fixation

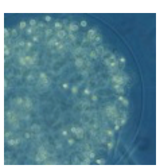
Adapted from Sunda (1988/1989), with additional information from Raven et al. (1999), Frausto da Silva & Williams (2001), and Wolfe-Simon et al. (2005).
[a]Cofactor in a number of enzymes.
[b]Has been found only in diatoms (Price & Morel 1990, Lane & Morel 2000).



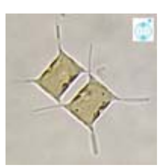
Morel and Price 2003, Michel and Pistorious 2004

What are the effects of trace metal/vitamin limitation on the physiology of different groups?

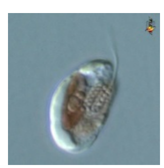




Phaeocystis antarctica



Chaetoceros brevis



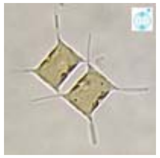
Geminigera sp.

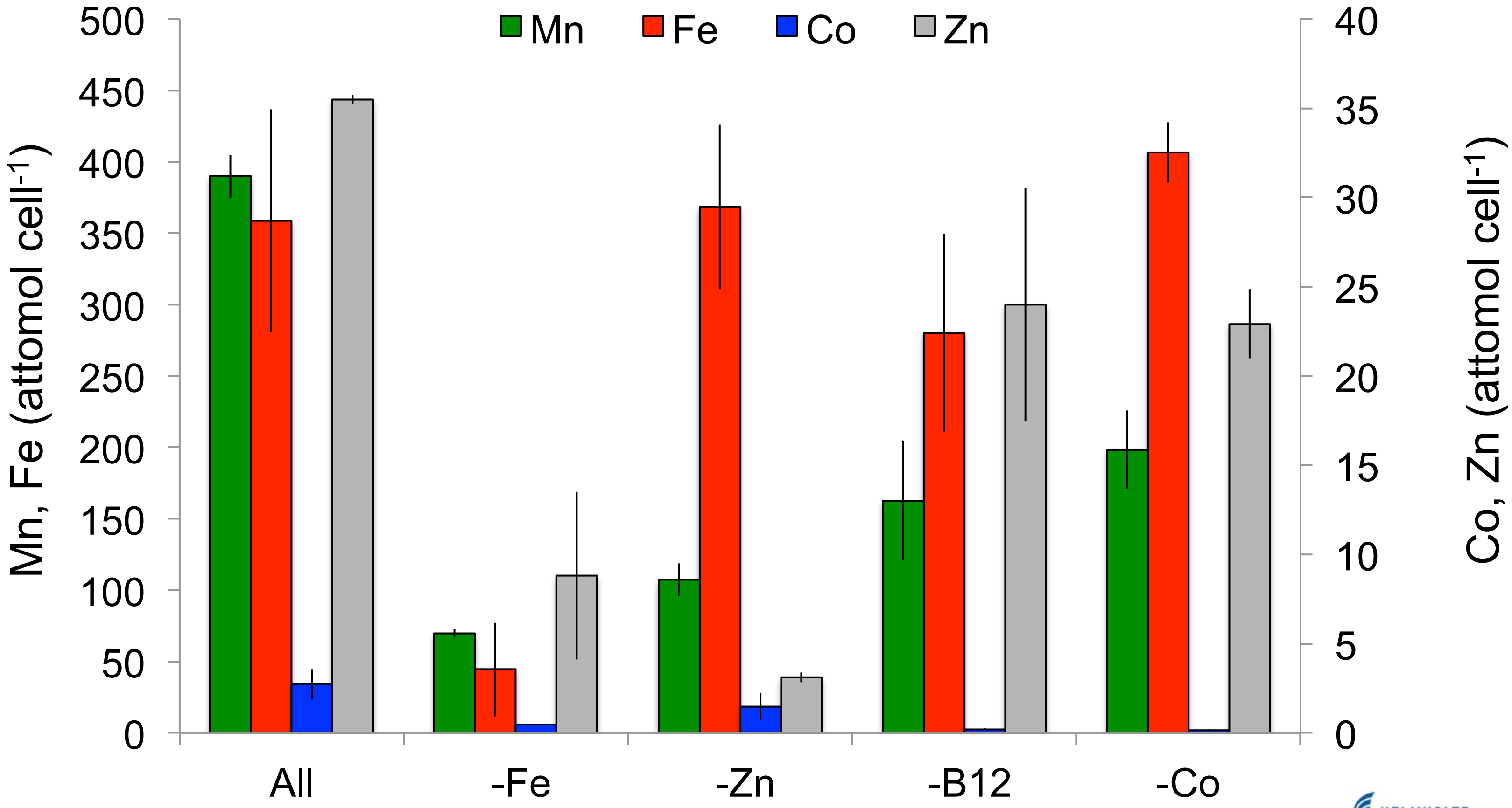
Parameters assessed:

- Photophysiology
- Cellular trace metal contents
- POC/PON
- Pigments
- Biogenic Silica
- Trace metal concentrations and quota
- RNA samples for transcriptomic analysis

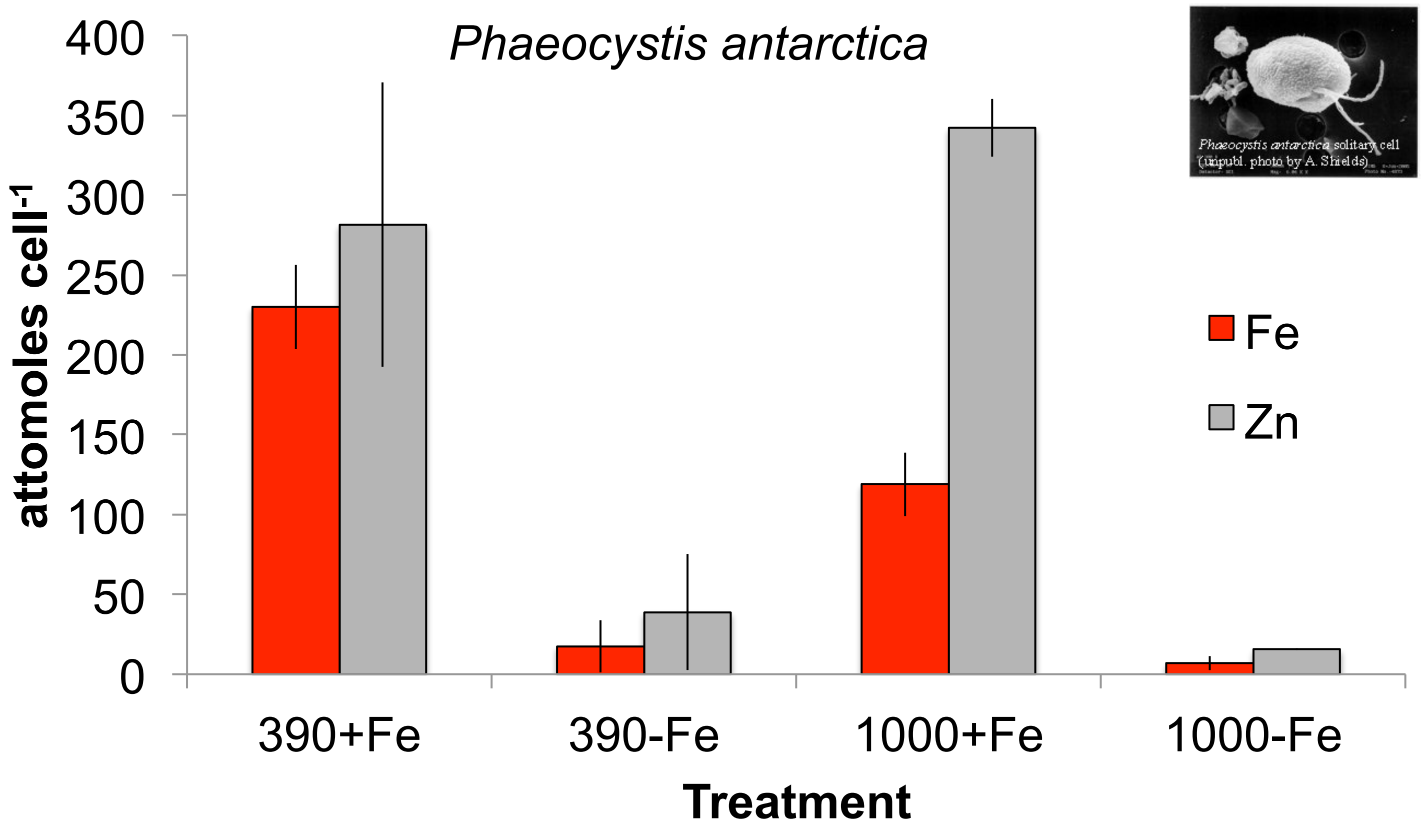
Trace Metal Quota under various limitations

Chaetoceros brevis





What are the effects of trace metal limitation and CO_2 on the physiology of different groups?



Trace Metals and Vitamins are important in many cellular processes of phytoplankton

Table 1 Common metalloproteins present within marine phytoplankton

Metal	Protein(s)	Function(s)
Fe	Cytochromes	Electron transport in photosynthesis and respiration
	Ferredoxin	Electron transport in photosynthesis and N fixation
	Other Fe-S proteins	Electron transport in photosynthesis and respiration
	Nitrate and nitrite reductase	Conversion of nitrate to ammonia
	Chelatase	Porphyrin and phycobiliprotein synthesis
	Nitrogenase	N fixation
	Catalase	Conversion of hydrogen peroxide to water
	Peroxidase	Reduction of reactive oxygen species
	Superoxide dismutase	Disproportionation of superoxide to hydrogen peroxide and O_2
Zn	Carbonic anhydrase	Hydration and dehydration of carbon dioxide
	Alkaline phosphatase	Hydrolysis of phosphate esters
	RNA polymerase	Nucleic acid replication and transcription
	tRNA synthetase	Synthesis of tRNA
	Reverse transcriptase	Synthesis of single-stranded DNA from RNA
	Carboxypeptidase	Hydrolysis of peptide bonds
	Superoxide dismutase	Disproportionation of superoxide to hydrogen peroxide and O_2
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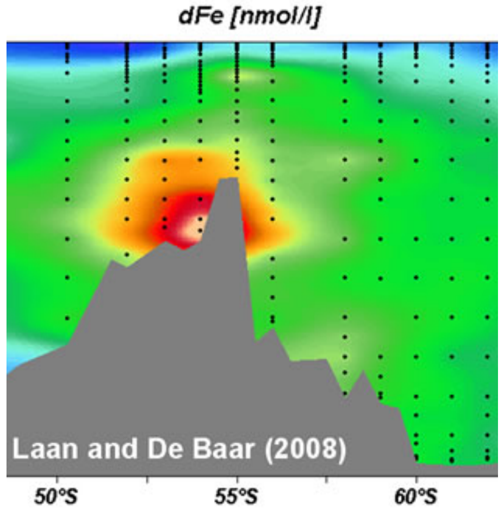
	Superoxide dismutase	Disproportionation of superoxide to hydrogen peroxide and O_2
	Arginase	Hydrolysis of arginine to ornithine and urea
	Phosphotransferases	Phosphorylation reactions
Co	Vitamin B_{12}[a]	C and H transfer reactions

Twining and Baines 2013

Trace metals/vitamins are present at low concentrations (pM-nM)



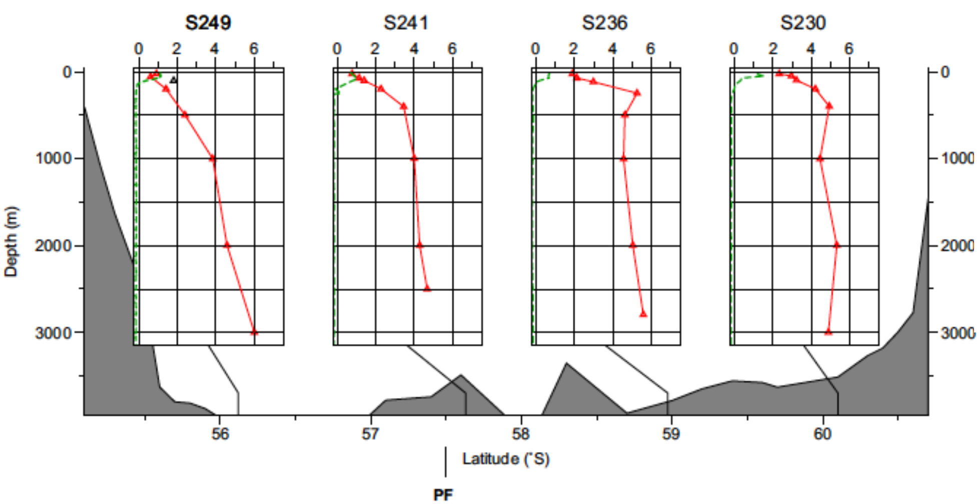
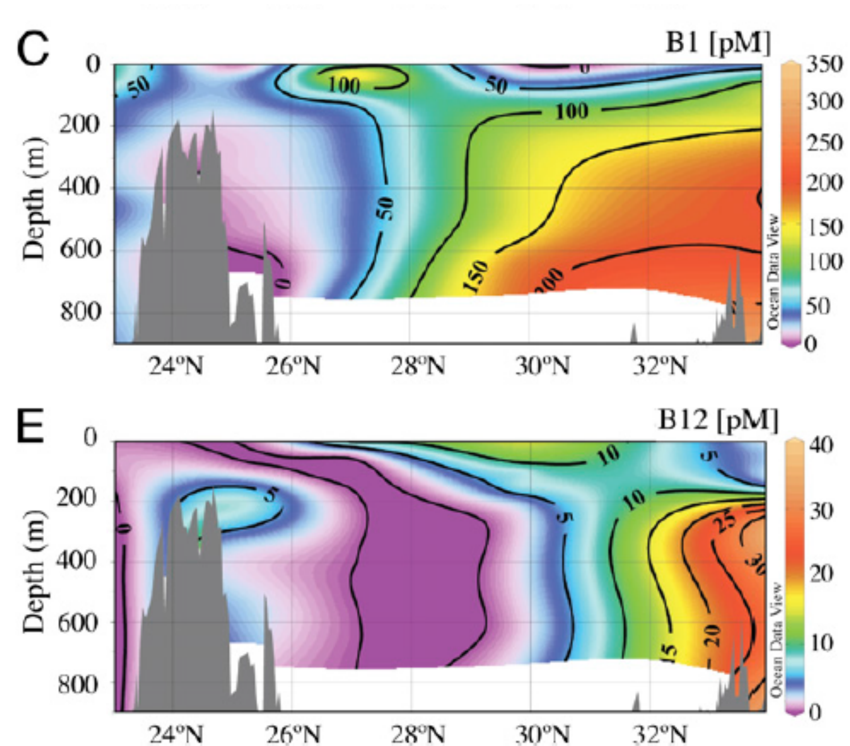


Fig. 5. Distribution of Zn across the Drake Passage during ANTXXIV-3. Croot et al. 2012



Sanudo-Wilhelmy et al. 2012

Trace Metals/Vitamins can limit Primary Productivity

- Fe has been shown to be the primary limiting element in 20% of the worlds oceans.

- A few studies found Zn additions to minimally affect biomass/species composition in polar waters Coale et al. 1991, Schareck et al. 1997, Frank et al. 2000, Coale et al. 2003.

- Co implicated in limiting B_{12} production in North Atlantic Panzeca et al. 2008.

- B_{12} co-limits primary production in the Ross Sea and Antarctic Peninsula (Bertrand et al. 2007, 2014, Panzeca 2006) as well as limiting primary production and shaping community composition in the Gulf of Alaska (Koch et al. 2009) and various coastal ecosystems (Koch et al. 2011, 2012, 2013)

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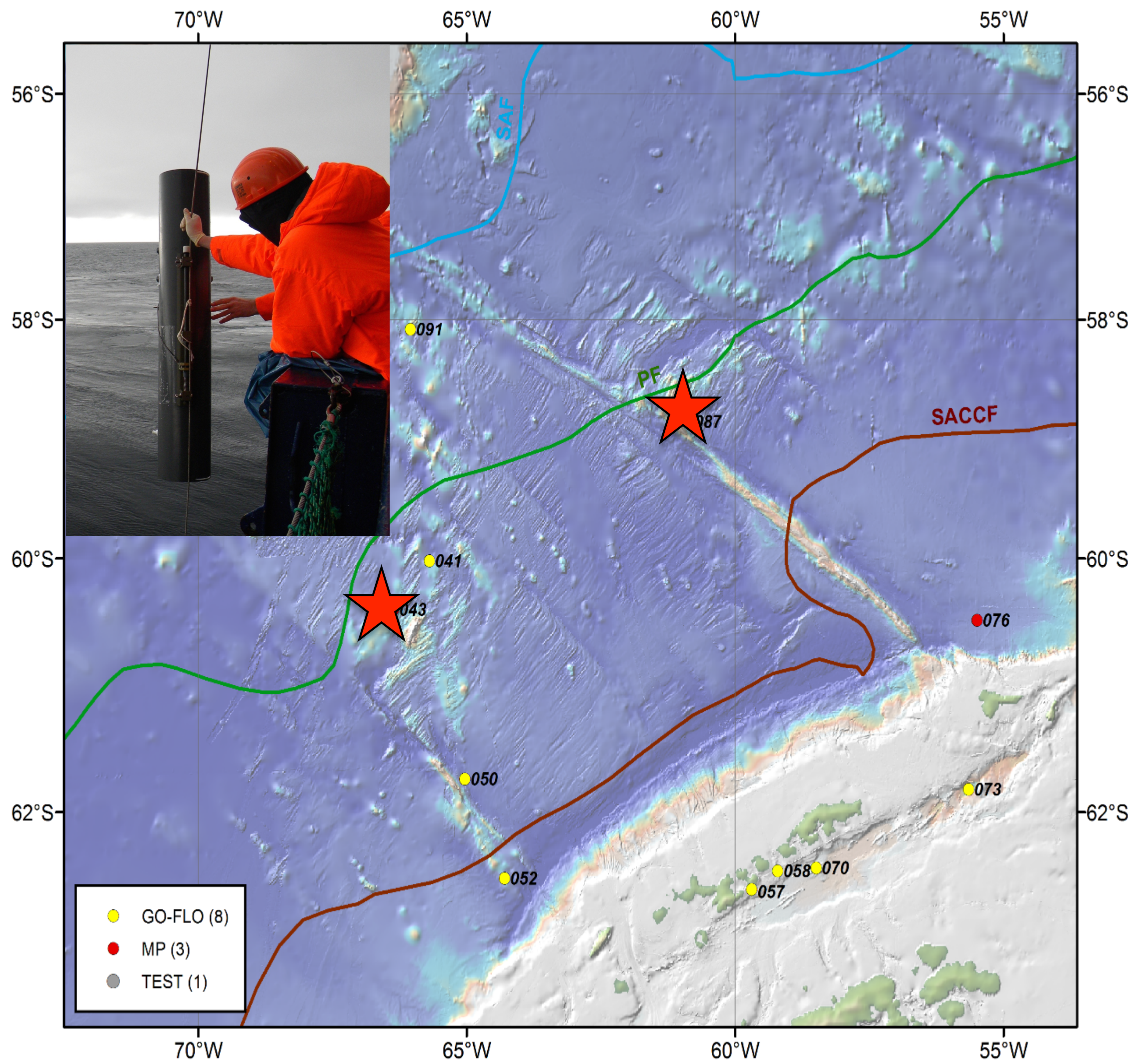
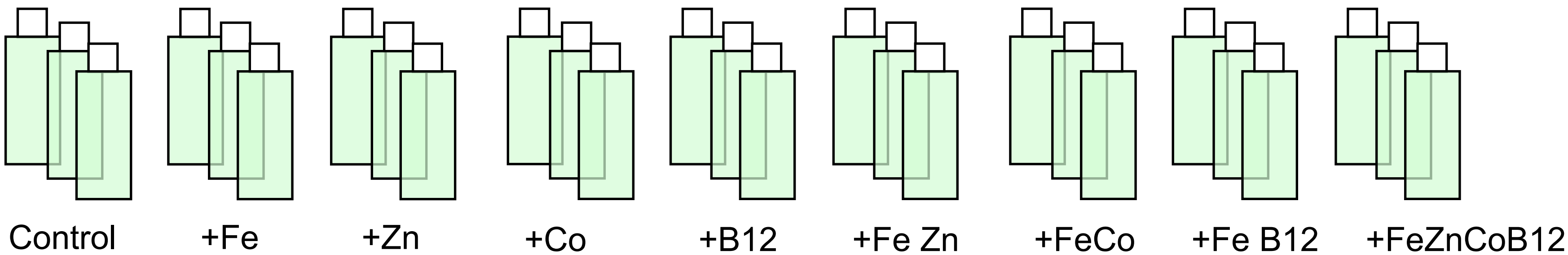
PS97 (16.2.-8.4.2016) PaleoDrake

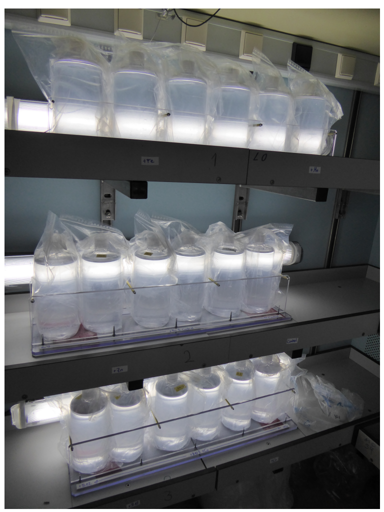


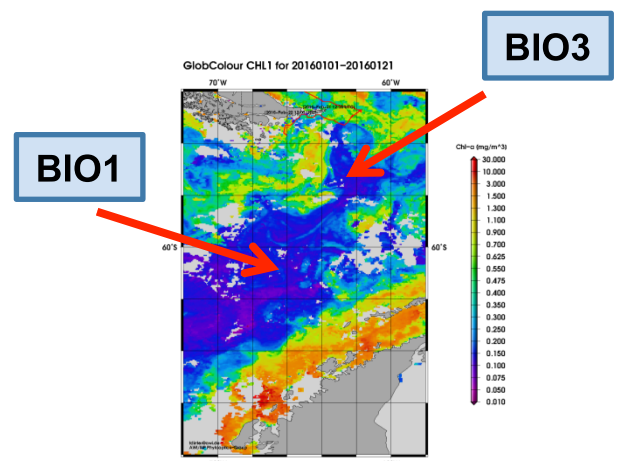
- 11 stations
- 2 long term Incubation Experiments (14 days) ★
- Size fractionated (0.2-2µm and >2µm) uptake of Fe, Zn, Co, B_{12}, Primary Productivity.
- Characterization of plankton community
- Cellular TM contents
- T_0 and T_1 of TM/vitamin concentrations

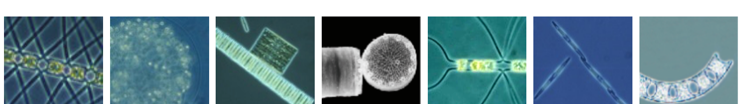
Potential TM/vitamin limitation of the plankton community



Incubation experiments with Fe, Co, Zn and vitamin B_{12} (10-14 days):







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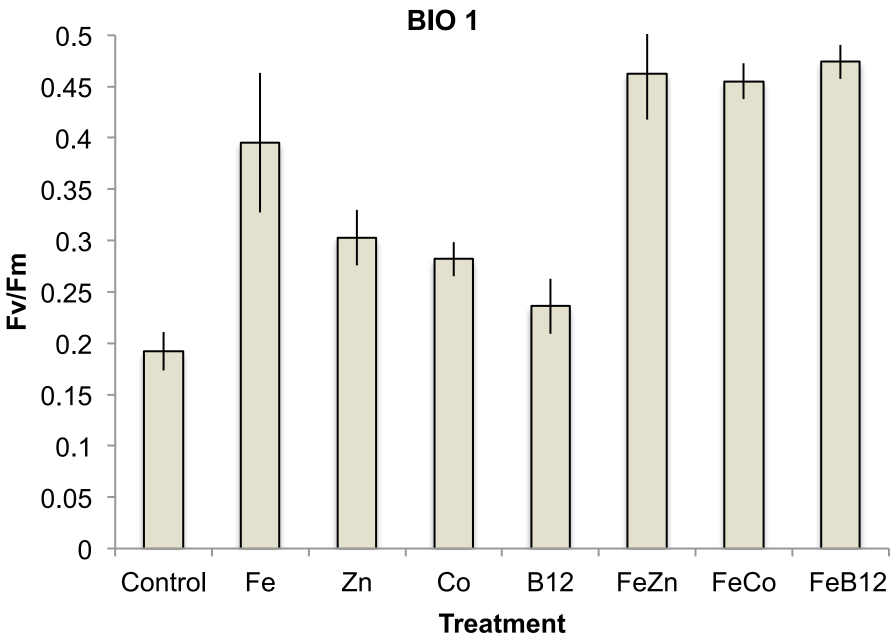
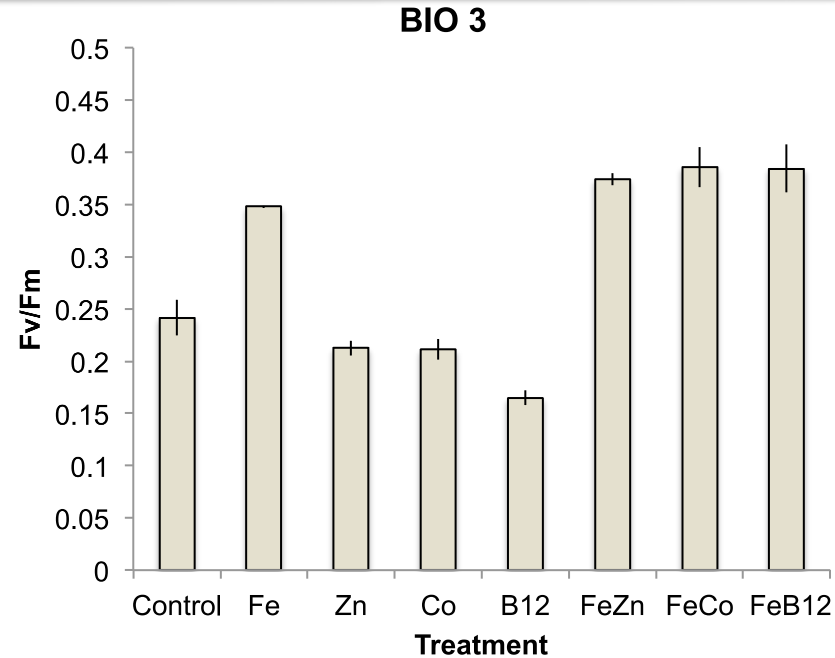
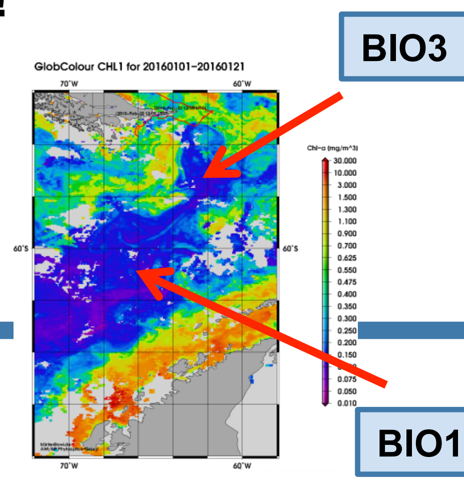
Potential TM/vitamin limitation of the plankton community

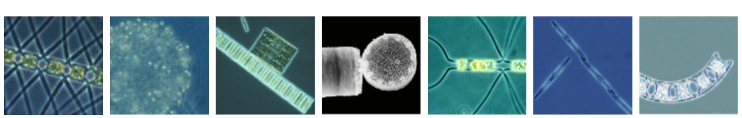




→ Addition of Zn, Co and B_{12} raises the yield for BIO 1!

→ Fe is the primary limiting trace metal at BIO 3!





Goals

- To understand the physiological effects of trace metal limitation on key phytoplankton groups.

- To understand the relative importance of removal and production/recycling mechanisms for Fe, Zn, Co, Vitamin B_{12}, and the key players responsible.

This will help explain observed limitations and co-limitations of plankton communities in the field

How to measure recycling/production?

Trace metal/vitamin concentrations (ICP-MS and HPLC-MS/MS)

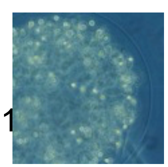
10 µmol l^{-1}

20 µmol l^{-1}

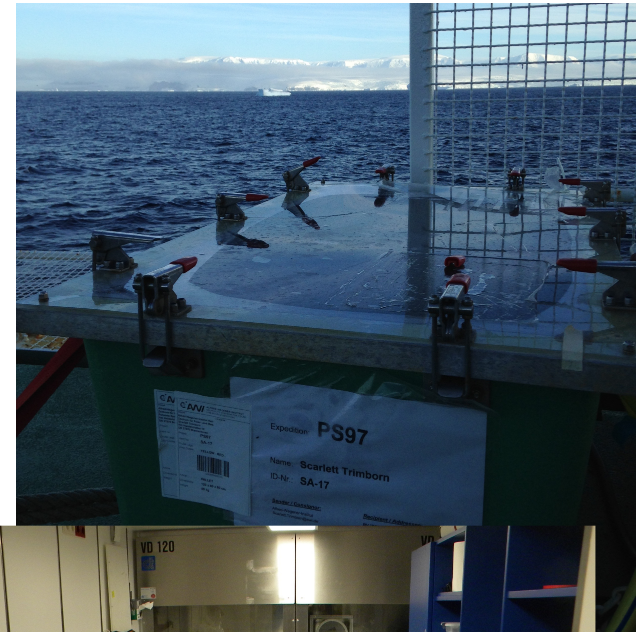
Day 0

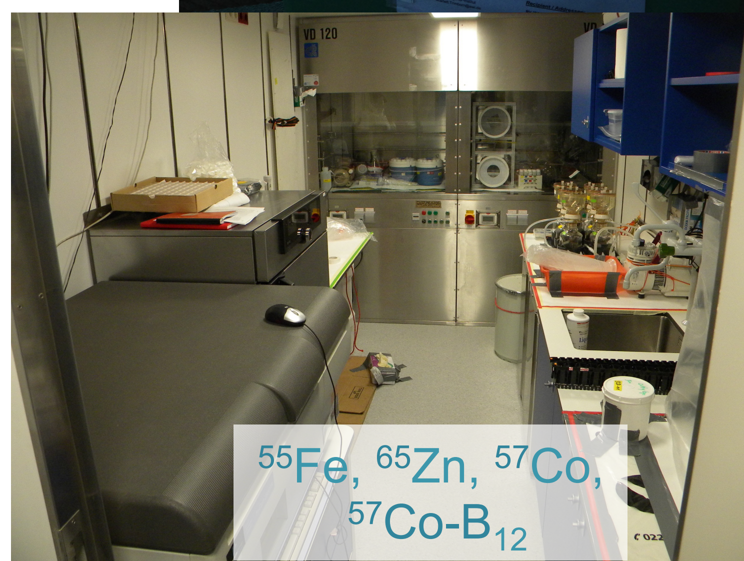
Day 1

Uptake of metal/vitamin (using radioisotopes)



5 µmol l^{-1} d^{-1}





^{55}Fe, ^{65}Zn, ^{57}Co, ^{57}Co-B_{12}

How to measure recycling/production?

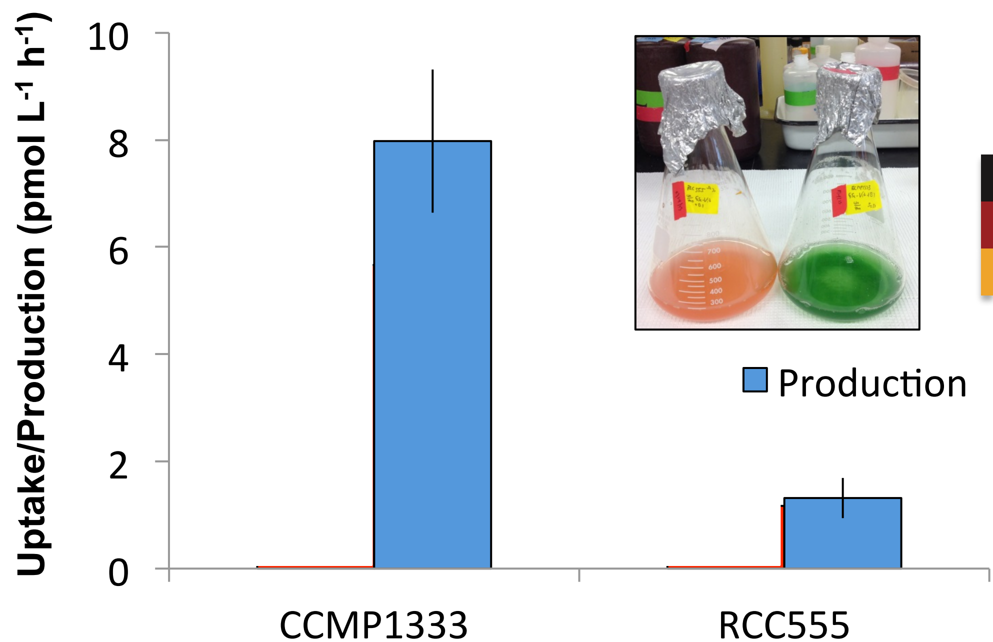
Using a mass balance approach:

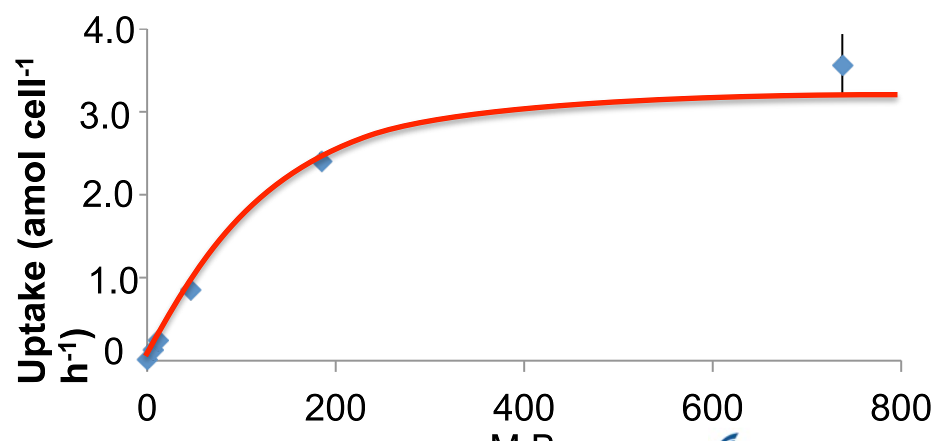
Δ[TM/vitamin]	10 µmol $l^{-1}d^{-1}$
+ρTM/vitamin	5 µmol l^{-1} d^{-1}
Production/Reminieralization	**15 µmol $l^{-1}d^{-1}$**

From this we can obtain uptake and production/recycling rates and calculate turnover times for the various trace metals and vitamins in relation to each other.

Production of B_{12} by cyanobacteria

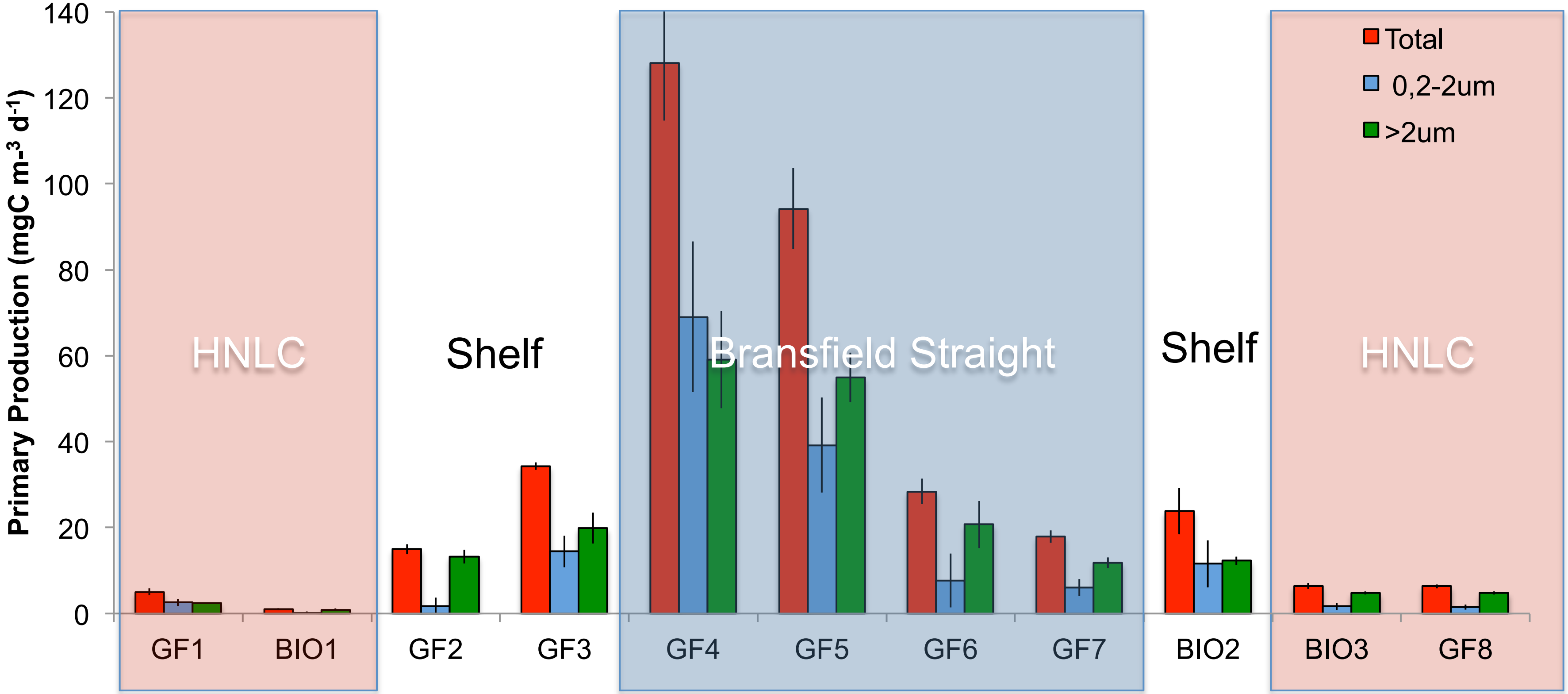
- *Synechococcus* sp.
- Production calculated with a mass balance approach
- Surprise: They also take it up!
- Follows Michaelis Menten kinetics
- Balance of Production and Uptake = source or sink





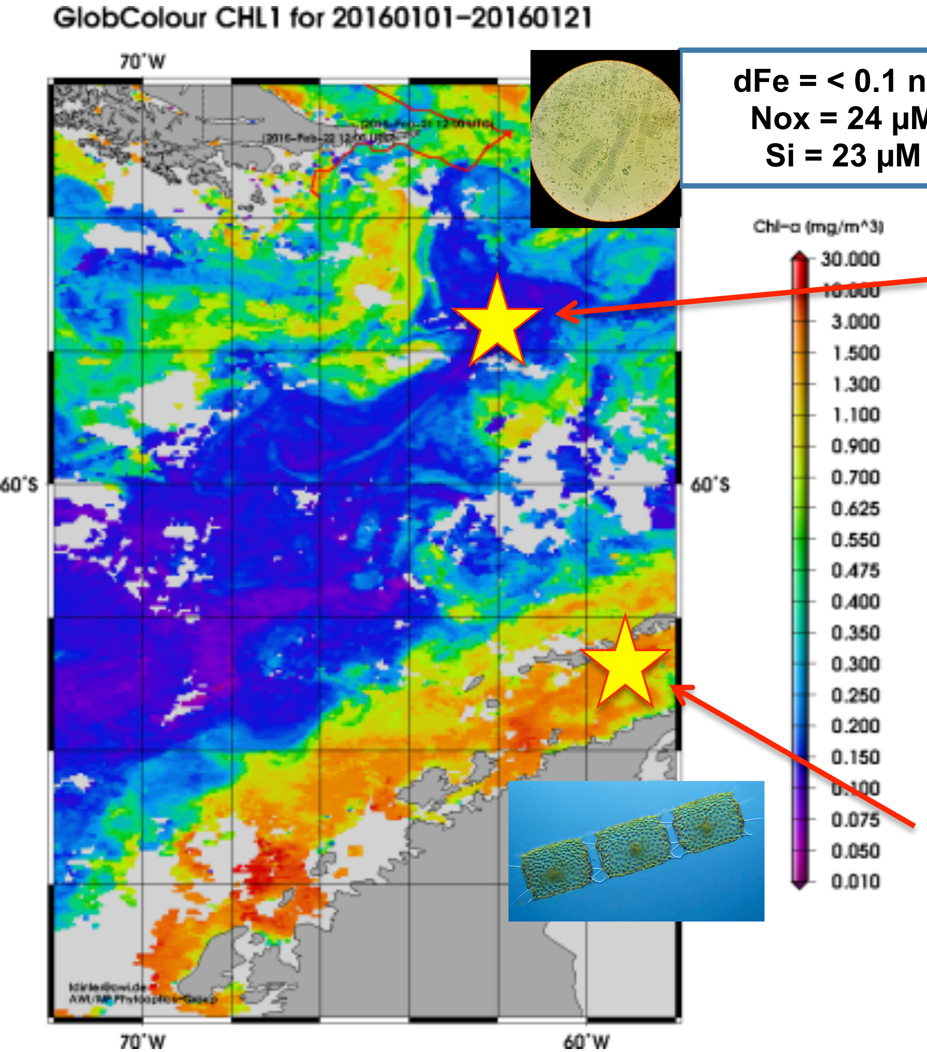
Koch et al. in prep

Primary production PS97

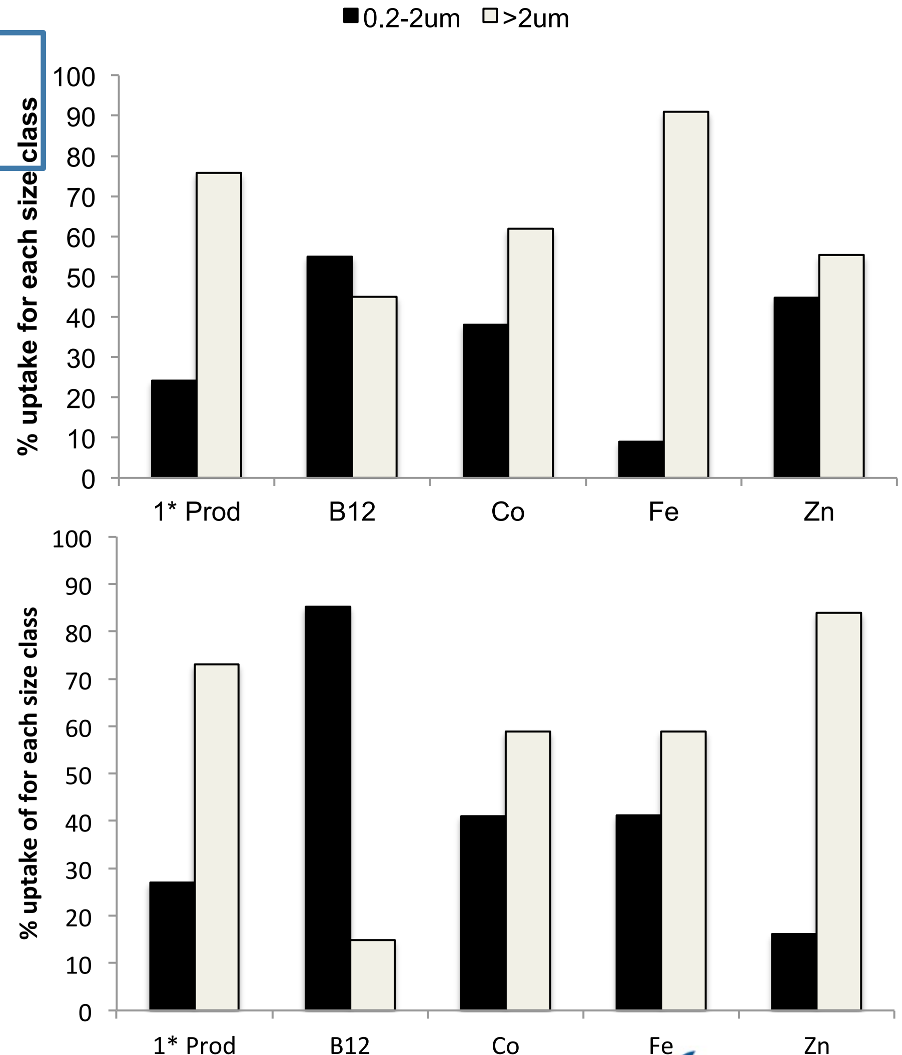


The various regions sampled will shed light on the impacts of the *in situ* plankton community composition on the cycling of essential trace metals and vitamins

Who is using what?



dFe = < 0.1 nM
Nox = 24 µM
Si = 23 µM



Questions?

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