Co-Limitation of Phytoplankton by Light and Multiple Nutrients

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and many others in European research teams of

MERLIM, CARUSO, IRONAGES

Koninklijk Nederlands Instituut voor Onderzoek der Zee
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Europese Unie
European Union

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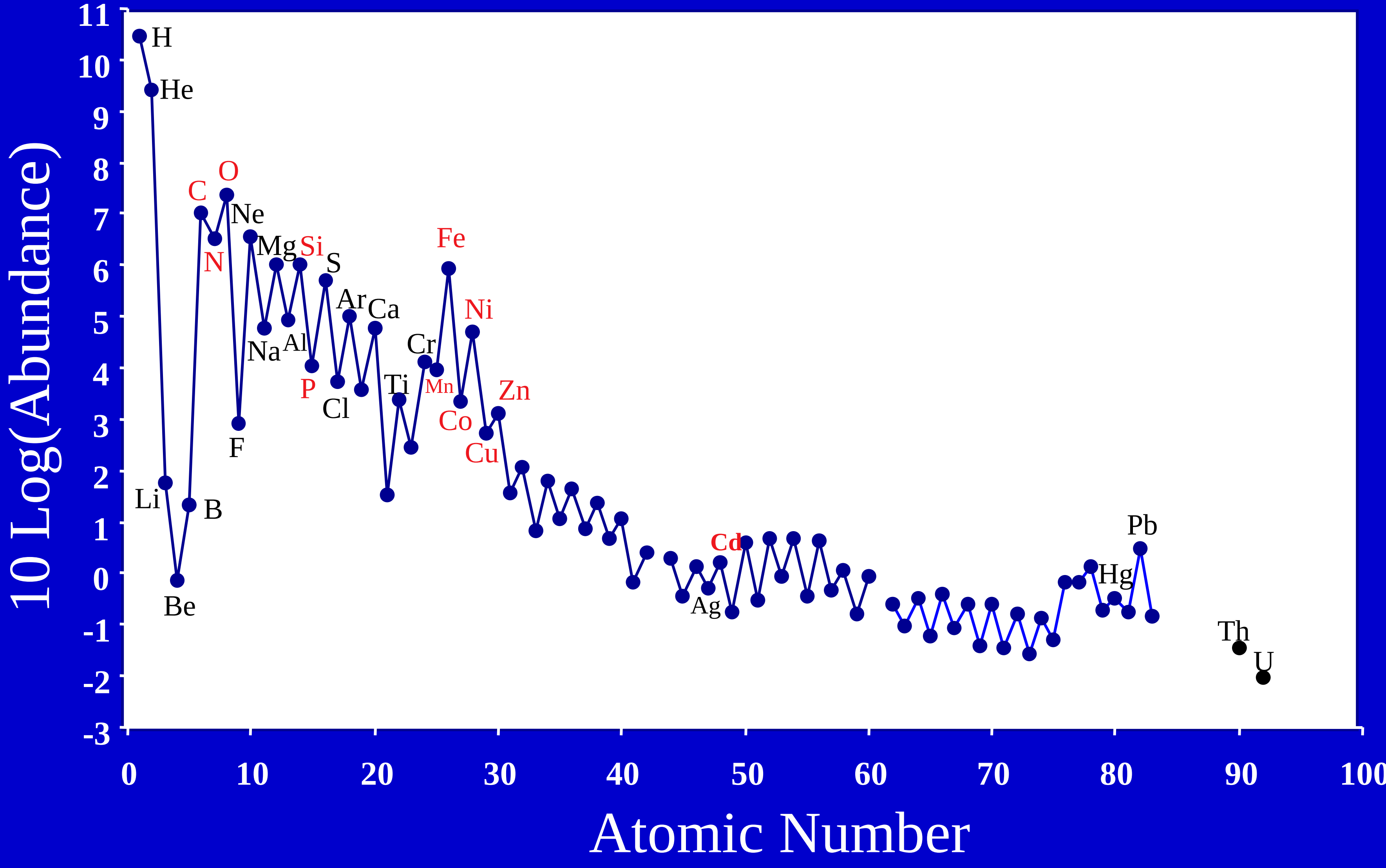
Abundance of Chemical Elements



Major Bio-Elements

Abundances versus one million Si atoms

Carbon	10×10^6
Nitrogen	3×10^6
Silicon	1×10^6
Phosphorus	1×10^4
Iron	0.9×10^6

Metals Abundance & Biological Evolution

Mn 9550	**Fe** 900000	**Co** 2250	**Ni** 49300	**Cu** 522	**Zn** 1260	
				Ag 0.49	**Cd ?** 1.61	
					Hg 0.34	**Pb** 315

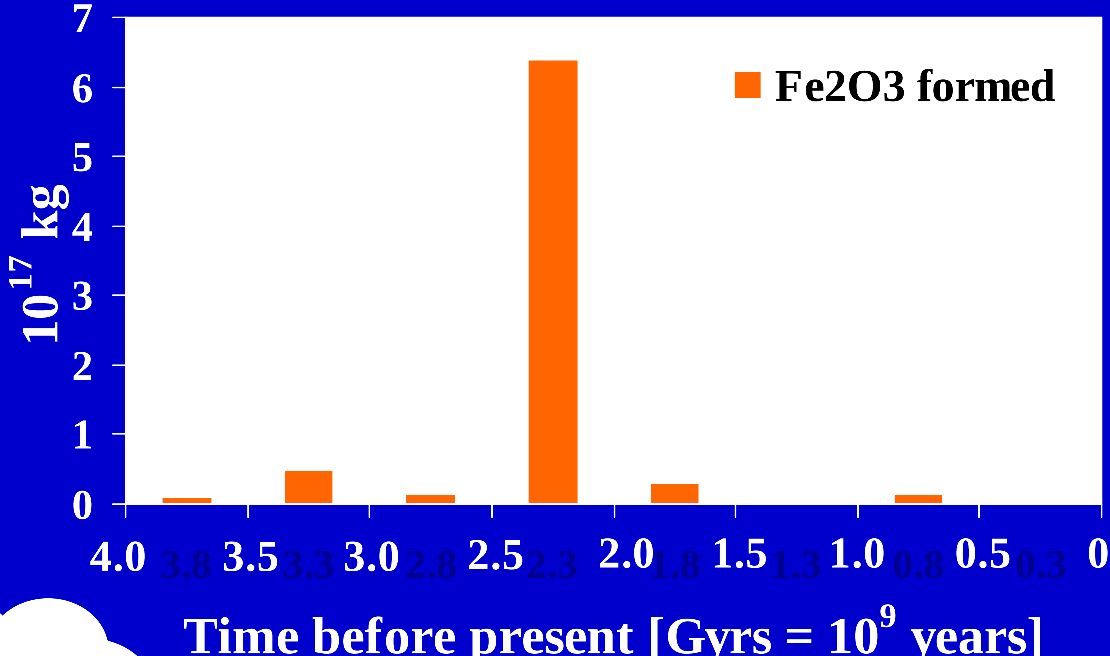
numbers of atoms versus 1 million Si atoms

Evolution used abundant metals: essential

Low abundant metals no bio-functions: toxic

Photosynthetic Oxygen Captured in Iron Formations

$$4\ \mathrm{Fe(II)}_{\mathbf{dissolved}} + 3\ \mathrm{O}_2 \longrightarrow 2\ (\mathrm{Fe_2O_3})_{\mathbf{deposit}}$$



photosynthesis

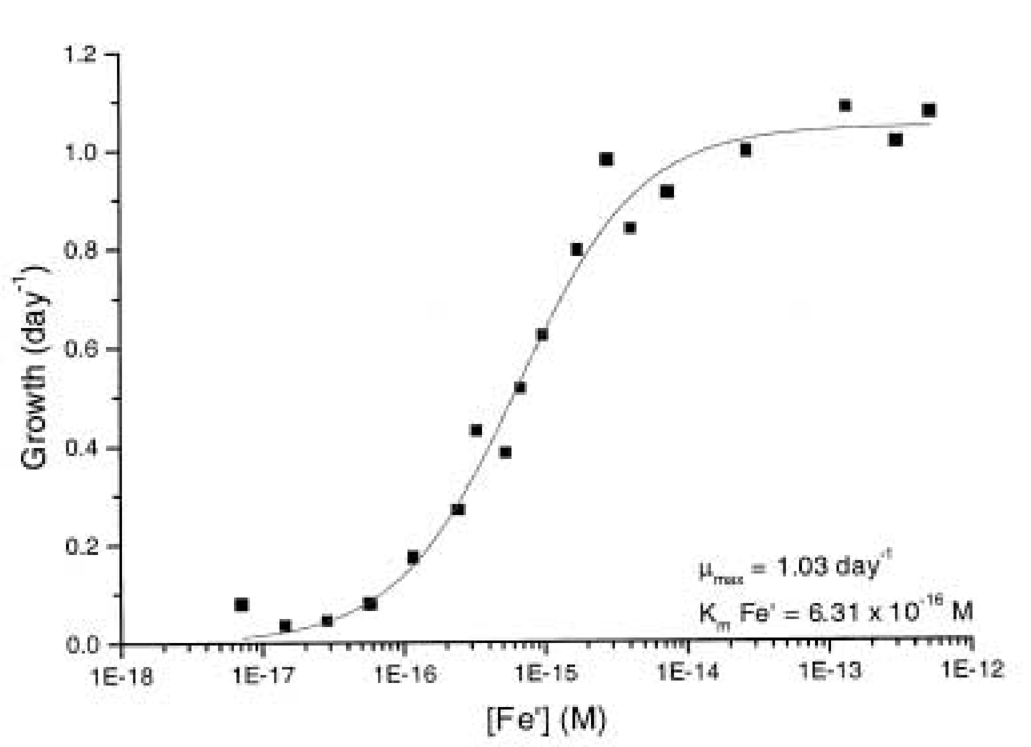
oxygen in the air

Dumb algae took away their own iron supply

2. Concepts of limitation

$$\frac{\mu}{\mu_{max}} = \frac{[nutrient]}{(K_{nutrient} + [nutrient])}$$

Michaelis, M. & Menten, M.L. (1913) Kinetics of invertase action. Zeitschrift f. Biochemie, 49, 333.
Monod, J. (1942) Recherches sur la croissance des cultures bacteriennes. Paris, Herrmann.



Emiliania huxleyi
in pristine natural seawater

driven into iron limitation by siderophore addition

Timmermans et al., in prep.

Multiple Limitations in Real Ocean

$$\frac{\mu}{\mu_{max}} = \{(1-\exp(aI/K_{max})\}\left\{\frac{[N]}{(K_N + [N])}\right\}\left\{\frac{[P]}{(K_P+[P])}\right\}\left\{\frac{[Fe]}{(K_{Fe}+[Fe])}\right\}\left\{\frac{[Si]}{(K_{Si}+[Si])}\right\}$$

growth light nitrate phosphate iron silicate

Moreover terms for Mn, Cu, Zn, Co to be included as well !?

- Caveats
  - static (steady state) equation applied to dynamic wax and wane of plankton blooms
  - limitations presumed independent while within living cell they are all interacting

de Baar and Boyd (2000) JGOFS Midterm Synthesis Book

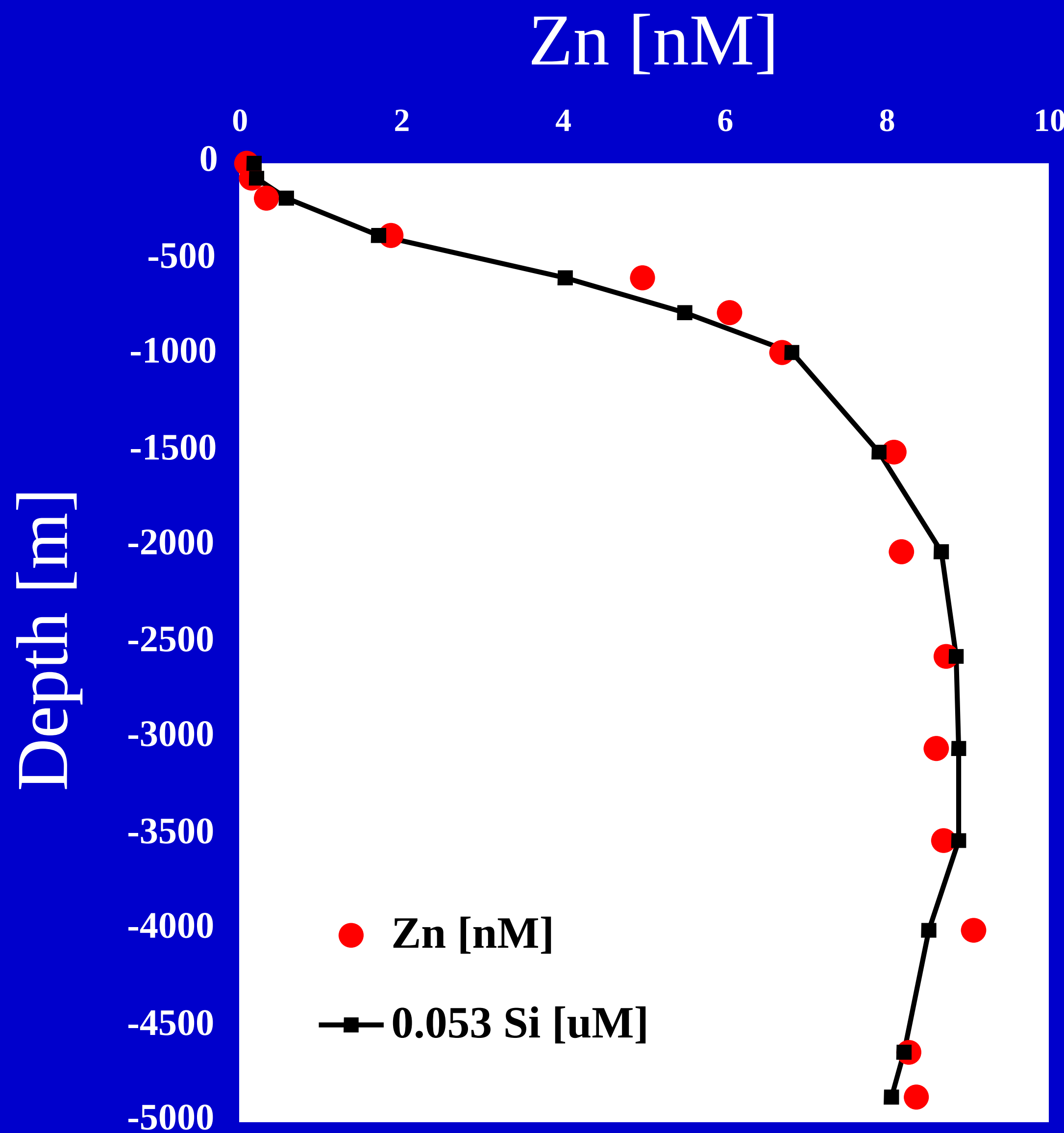
Some examples of interactions within the plant cell

- Iron-light co-limitation
  - electron transfer in photosystems
- Iron essential for nitrate uptake
  - nitrate reductase, nitrite reductase
- Zinc - bicarbonate co-limitation
  - carbonic anhydrase

3. Observations in the Sea

- Zn and silicate
- Cd and phosphate
- Cu and Ag and silicate
- Fractionations Zn/Cd and Cu/Ag
- Anomalies of major nutrients

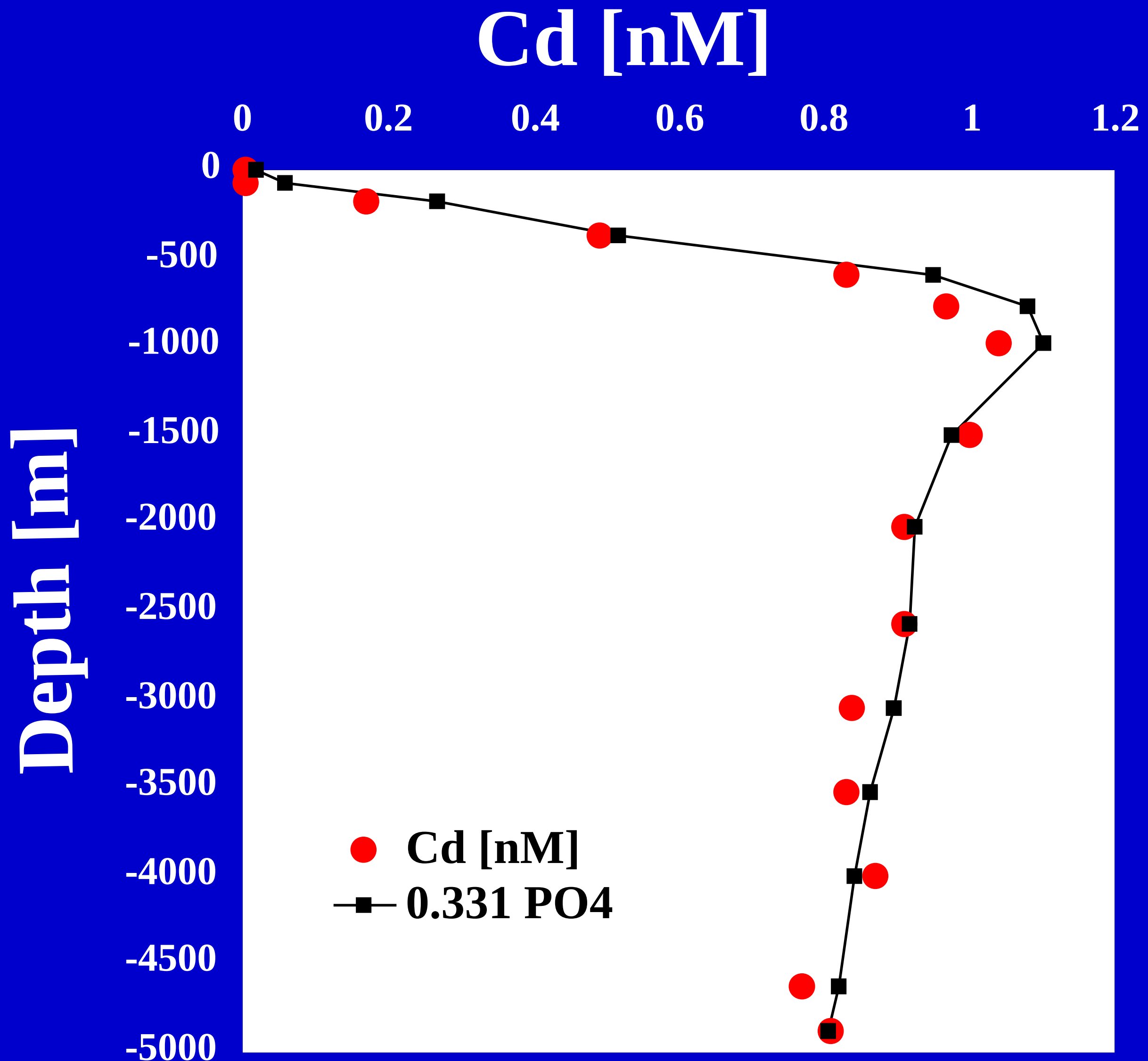
Zinc resembles Silicate



North Pacific Ocean (33°N, 145°W)

Bruland (1980)

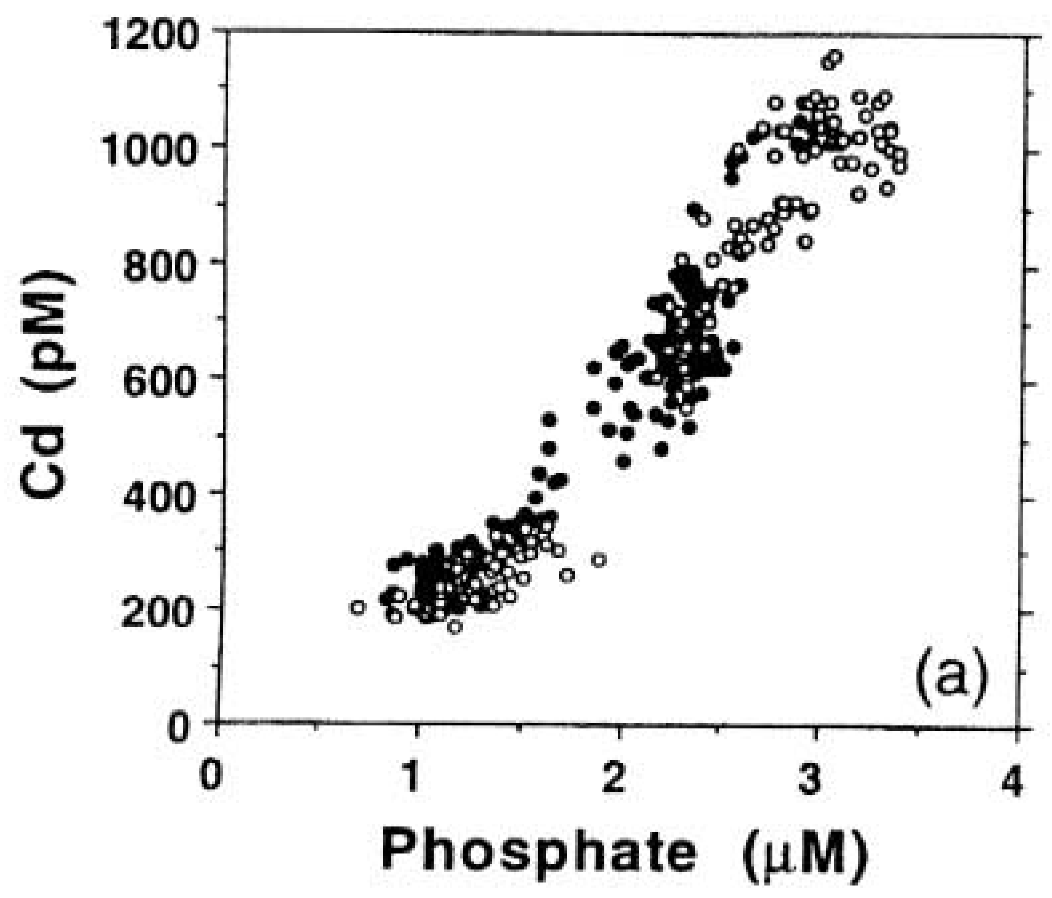
Cadmium resembles Phosphate



North Pacific Ocean (33°N, 145°W)

Bruland (1980)

Improved accuracy of both Cd and PO4 is crucial for further progress



Global Cd/phosphate dataset for waters >1000m depth.

Open circles deBaar et al. (1994);

Filled circles new data Loscher etal (1998).

Loscher, vander Meer, de Baar, Saager, de Jong (1998) The global Cd/phosphate relationship in deep ocean waters and the need for accuracy. Mar Chem., 59, 87-93

Biological function for Cd after all

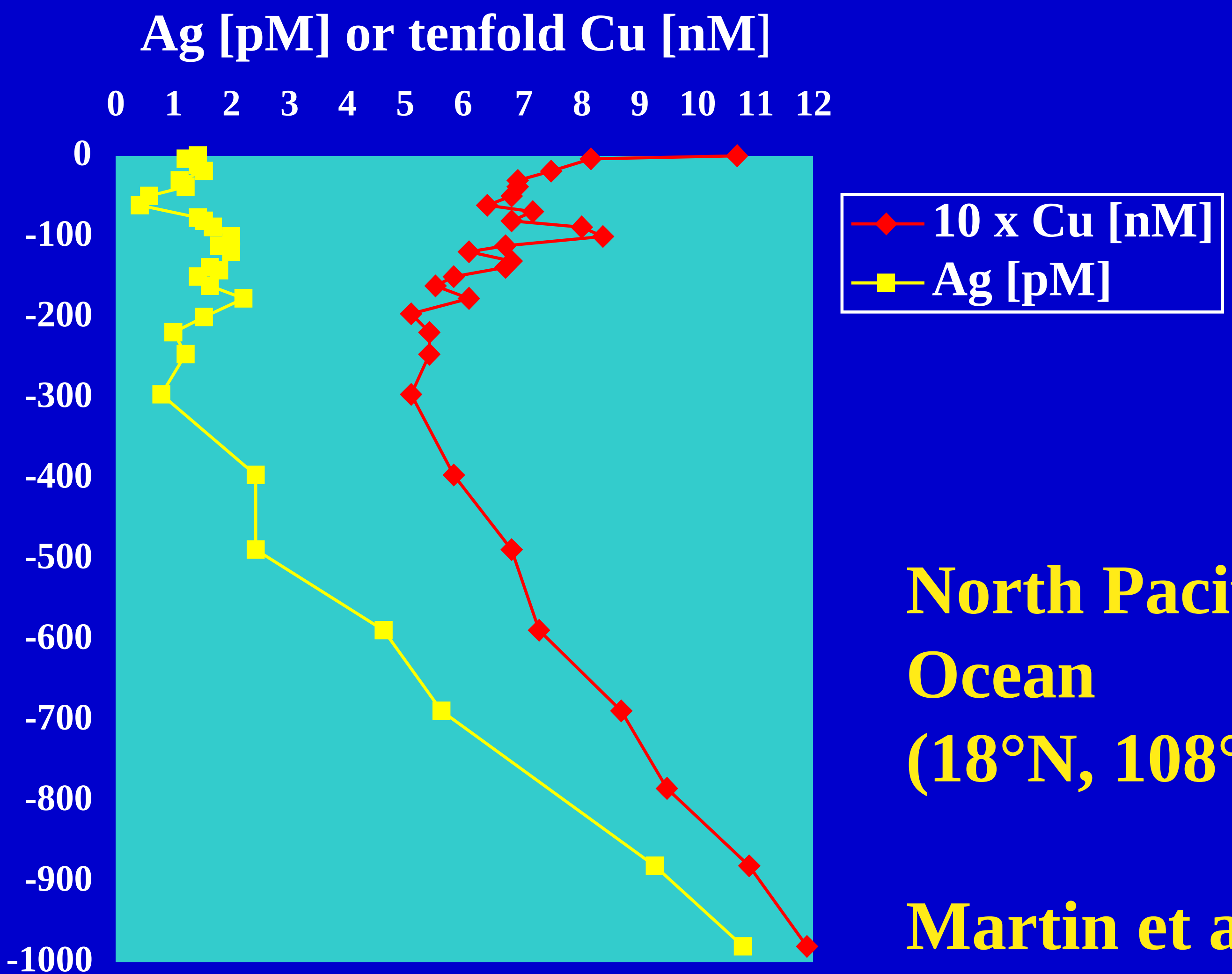
- Replacement of Zn by Cd in marine phytoplankton. Lee and Morel, Mar.Ecol.Prog.Ser., 127, 305-309, 1995
- A biological function for Cd in marine diatoms. Lane and Morel, Proc. Nat.Acad.Sci., 97, 4627-463, 2000

join the Green Party

carbonic anhydrase

Mn 9550	**Fe** 900000	**Co** 2250	**Ni** 49300	**Cu** 522	**Zn** 1260	
				Ag 0.49	**Cd** 1.61	
					Hg 0.34	**Pb** 315

Silver (Ag) resembles Copper (Cu)



North Pacific Ocean (18°N, 108°W)

Martin et al. (1983)

Ag has better correlation with Si

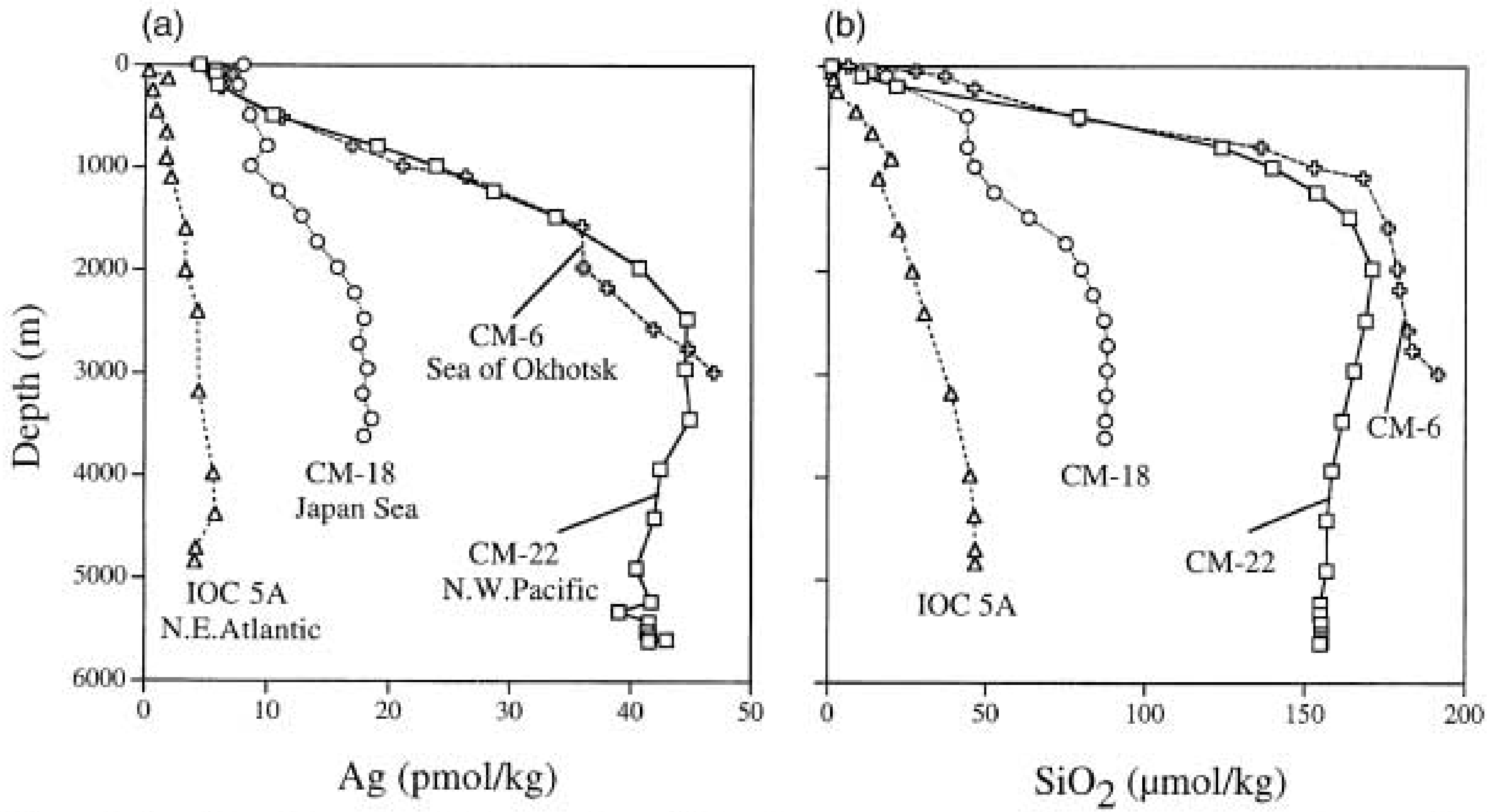


Fig. 5. The vertical profiles of dissolved Ag (a) and reactive Si (b) for different oceanic basins (the squares for the Northwest Pacific; circles for the Japan Sea; pluses for Sea of Okhotsk). The North Atlantic data indicated by triangles are based on the IOC station 5A (24°N, 23°W), after Flegal et al. (1995).

Zhang, Amakawa & Nozaki (2001) Mar. Chem., 75, 151

Worldwide correlation Ag and Si

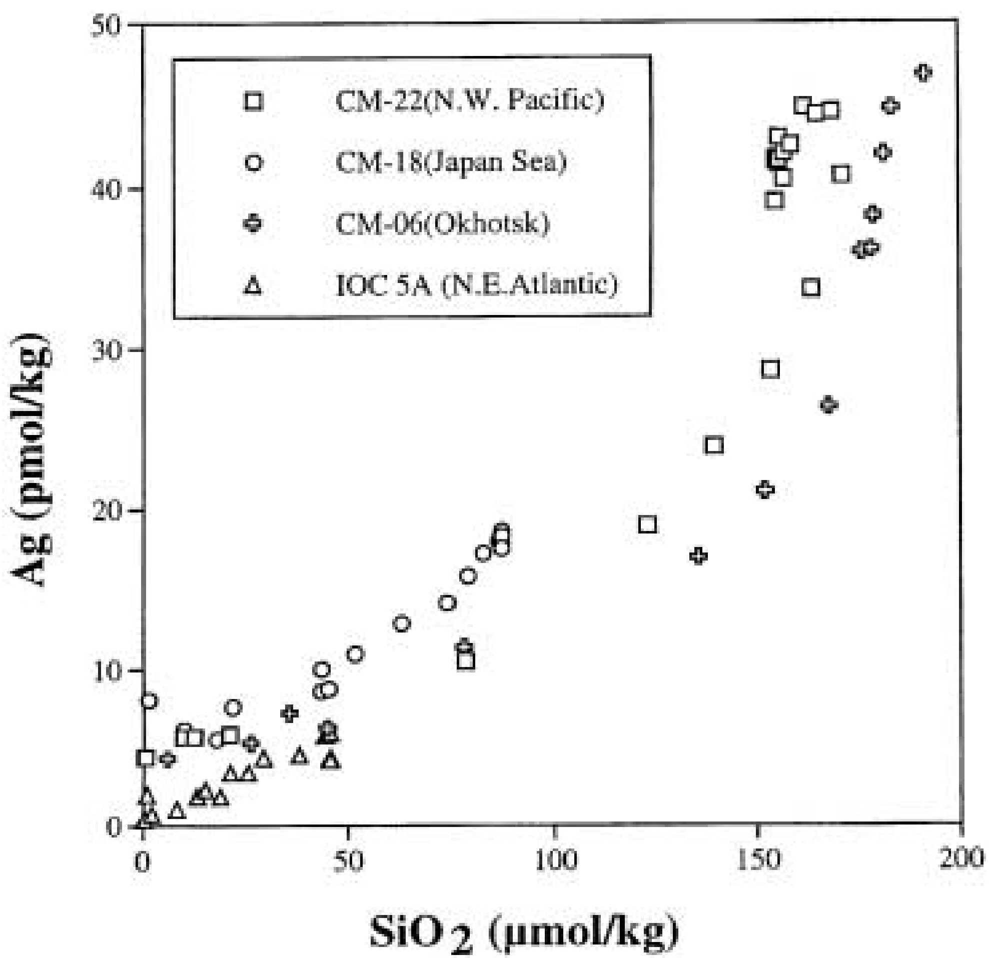


Fig. 6. Correlation between dissolved Si and Ag.

Ag/Si ratio increases from
~1.2 10^{-6} in Atlantic
to
~2.7 10^{-6} in Pacific

Zhang, Amakawa & Nozaki (2001) Mar. Chem., 75, 151

Fractionations Cu/Ag and Zn/Cd

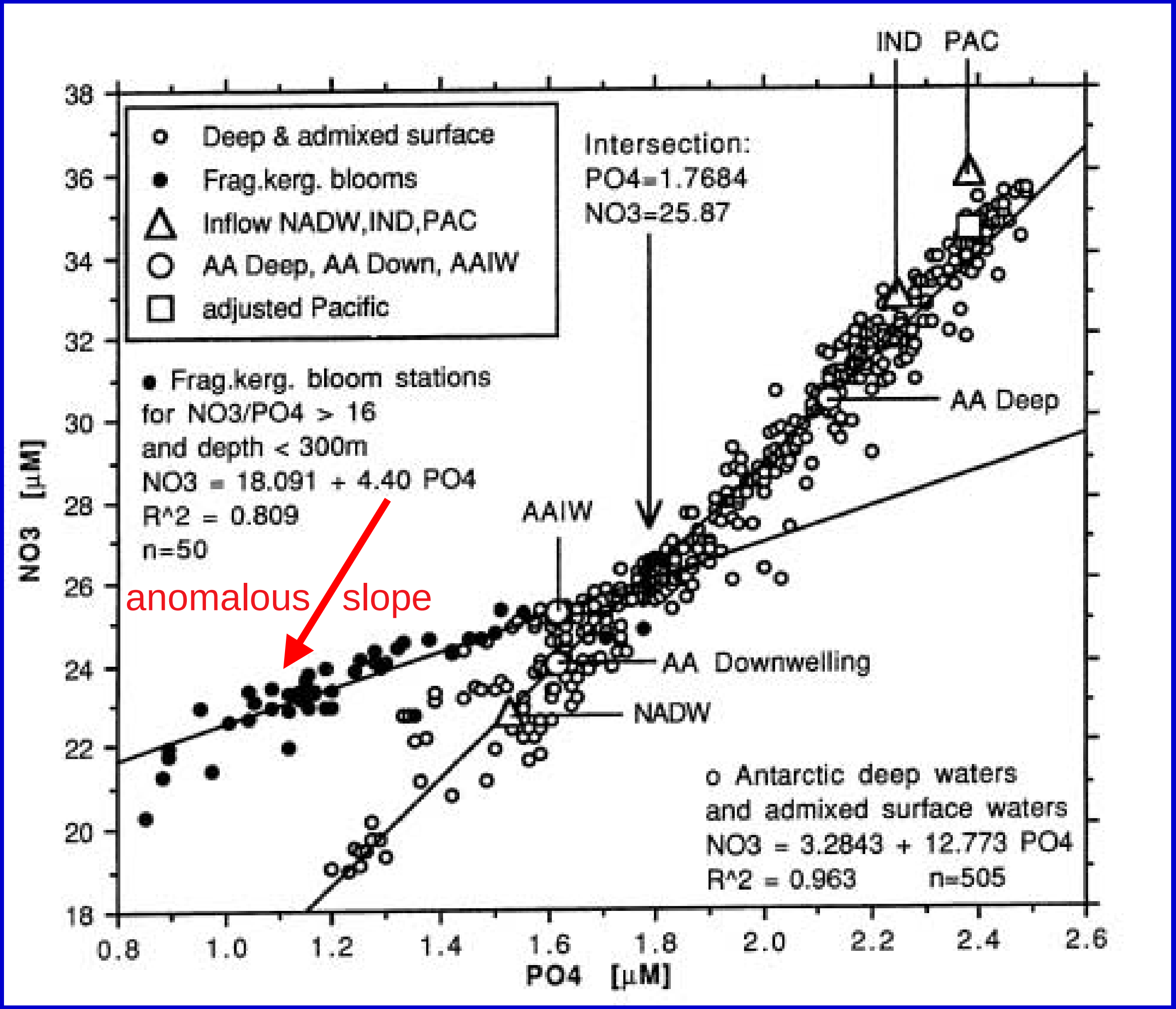
Periodic Table	**Group 1b Cu /Ag**	**Group 2b Zn / Cd**
Crustal abundance ratio	~1060	~780
Oceanic waters ratio	~8 ± 3	~91
Fractionation factor	~130	~8.6

Mn 9550	**Fe** 900000	**Co** 2250	**Ni** 49300	**Cu** 522	**Zn** 1260	
				Ag 0.49	**Cd** 1.61	
					Hg 0.34	**Pb** 315

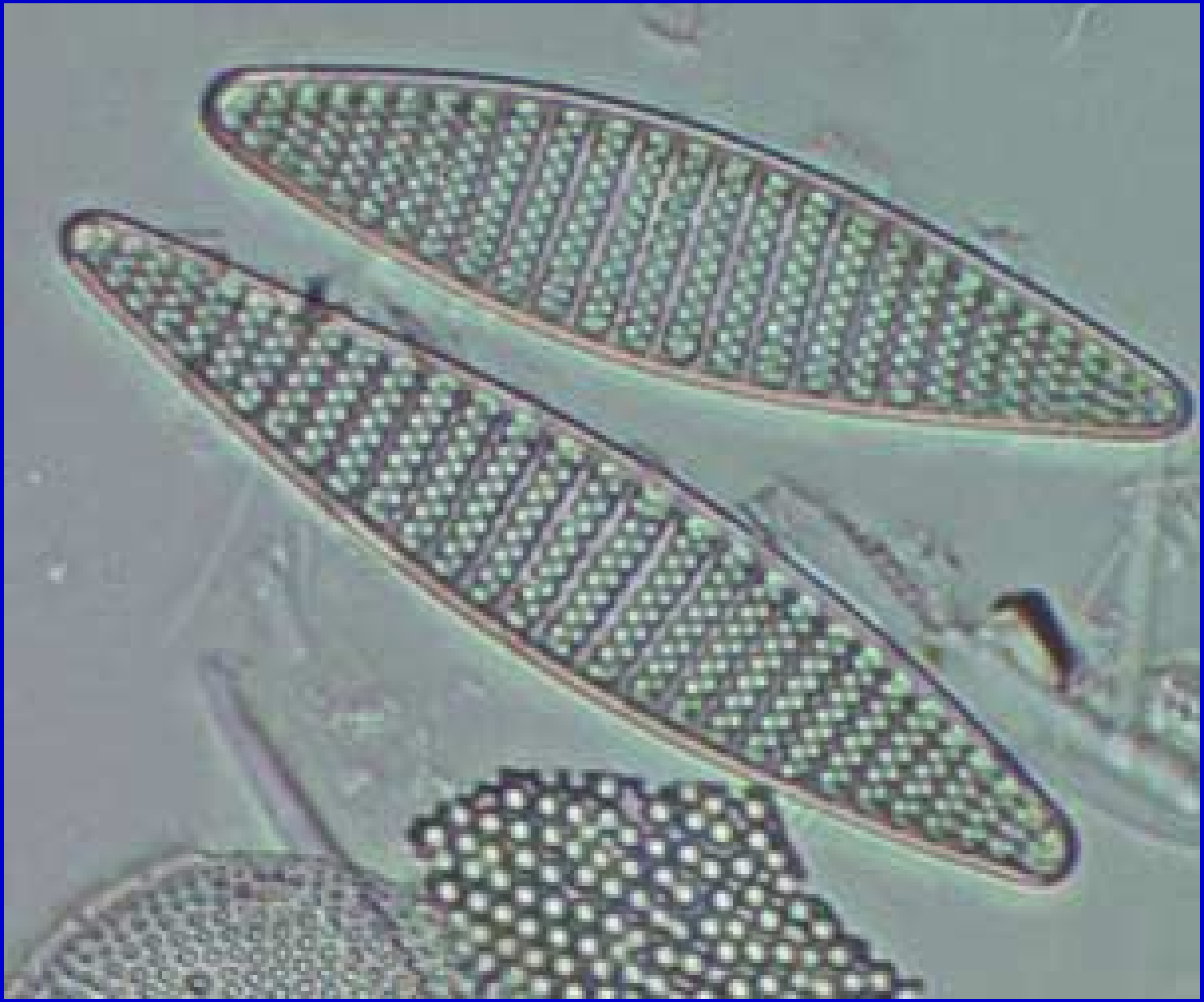
First row 'real biometals' have shorter ocean residence time than second row 'abiotic' metals

(Also differences inorganic speciation)

Nutrient anomalies *Fragilariopsis kerguelensis* blooms



Deep Sea Research II, 44, 229-260 (1997)



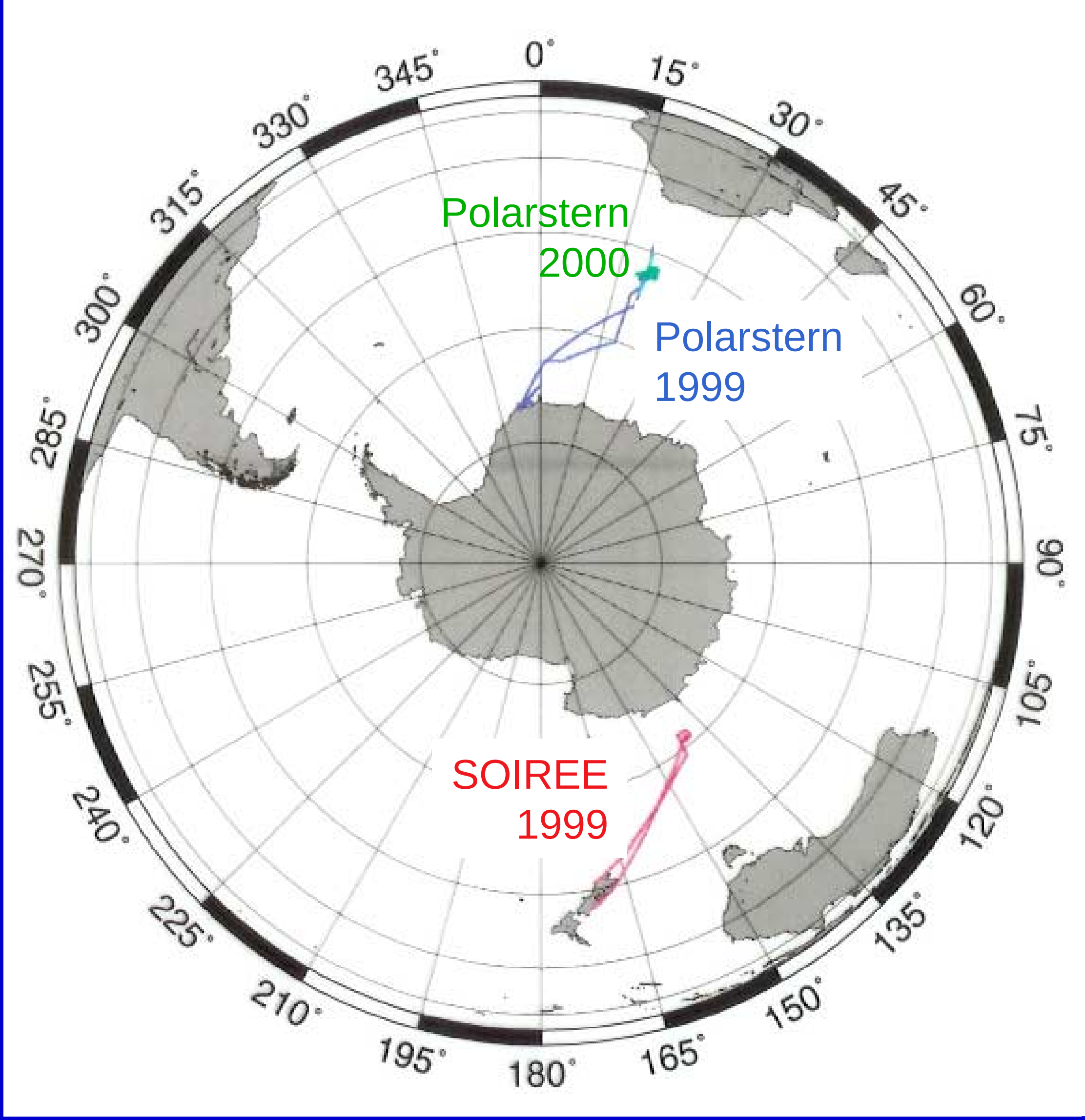
Fragilariopsis kerguelensis with heavily silicified armor ‘pantzer’

More Fe co-limitations major nutrients

Study	Fe-deplete	Fe-replete
Southern Ocean (Takeda, 1998)		
plankton community	Si/N=2.3	Si/N = 0.95
	N/P = 12	N/P = 14
Chaetoceros dichaeta	Si/N = 1.9	Si/N = 0.7
Nitzschia sp.	Si/N = 2.1	Si/N = 1.2
California upwelling (Hutchins et al., 1998)		
plankton community	Si/N = 1.6	Si/N = 0.8
	Si/N = 2.7	Si/N = 1.0
	Si/N = 3.0	Si/N = 1.0

Uptake by blooms in Ross Sea	Diatoms	Phaeocystis
Arrigo et al. (1999)	N/P = 9.5	N/P = ~19

Three more recent cases of nitrate anomalies in *Fragilariopsis* blooms



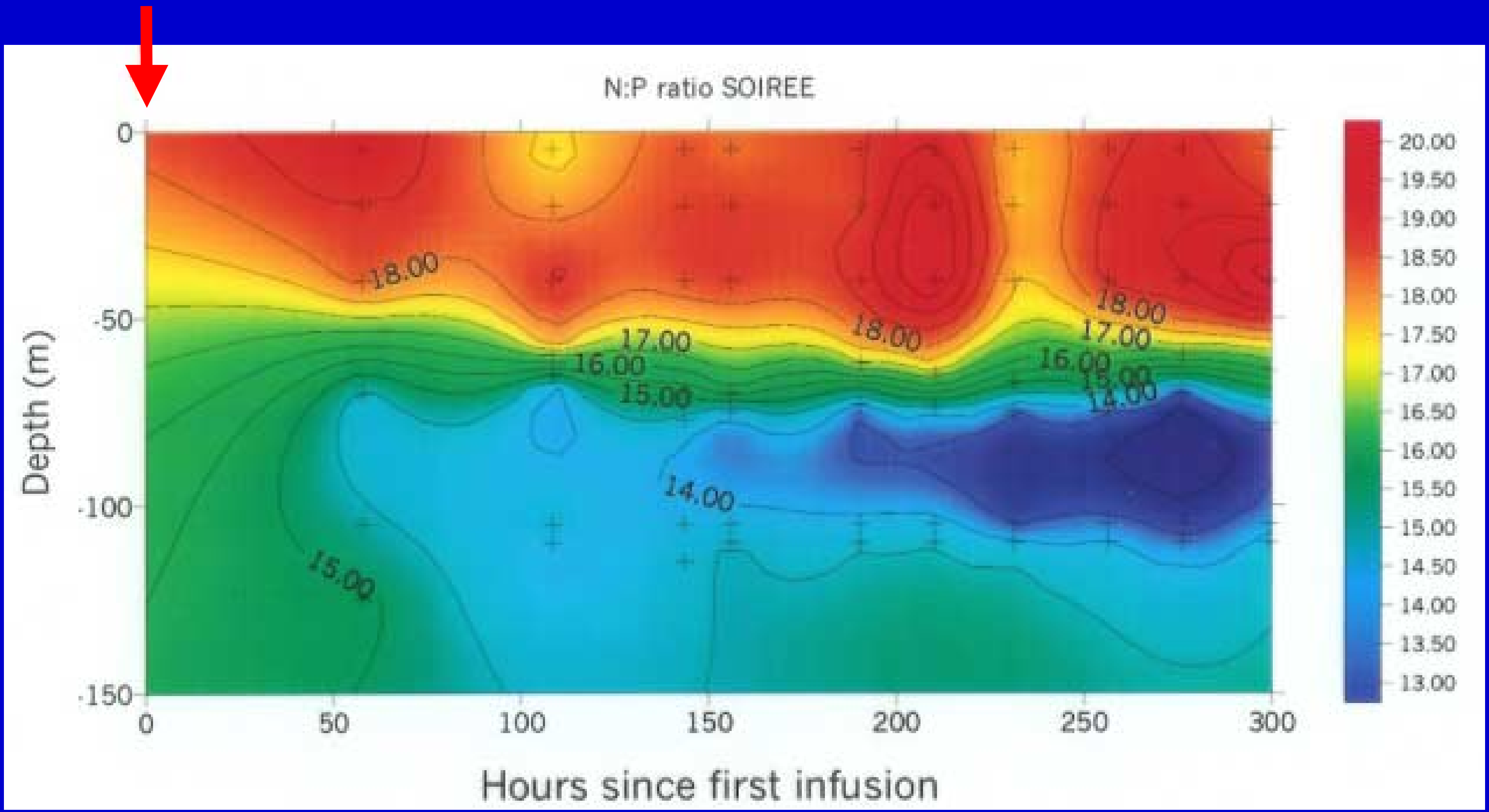
Polarstern 2000

Polarstern 1999

SOIREE 1999

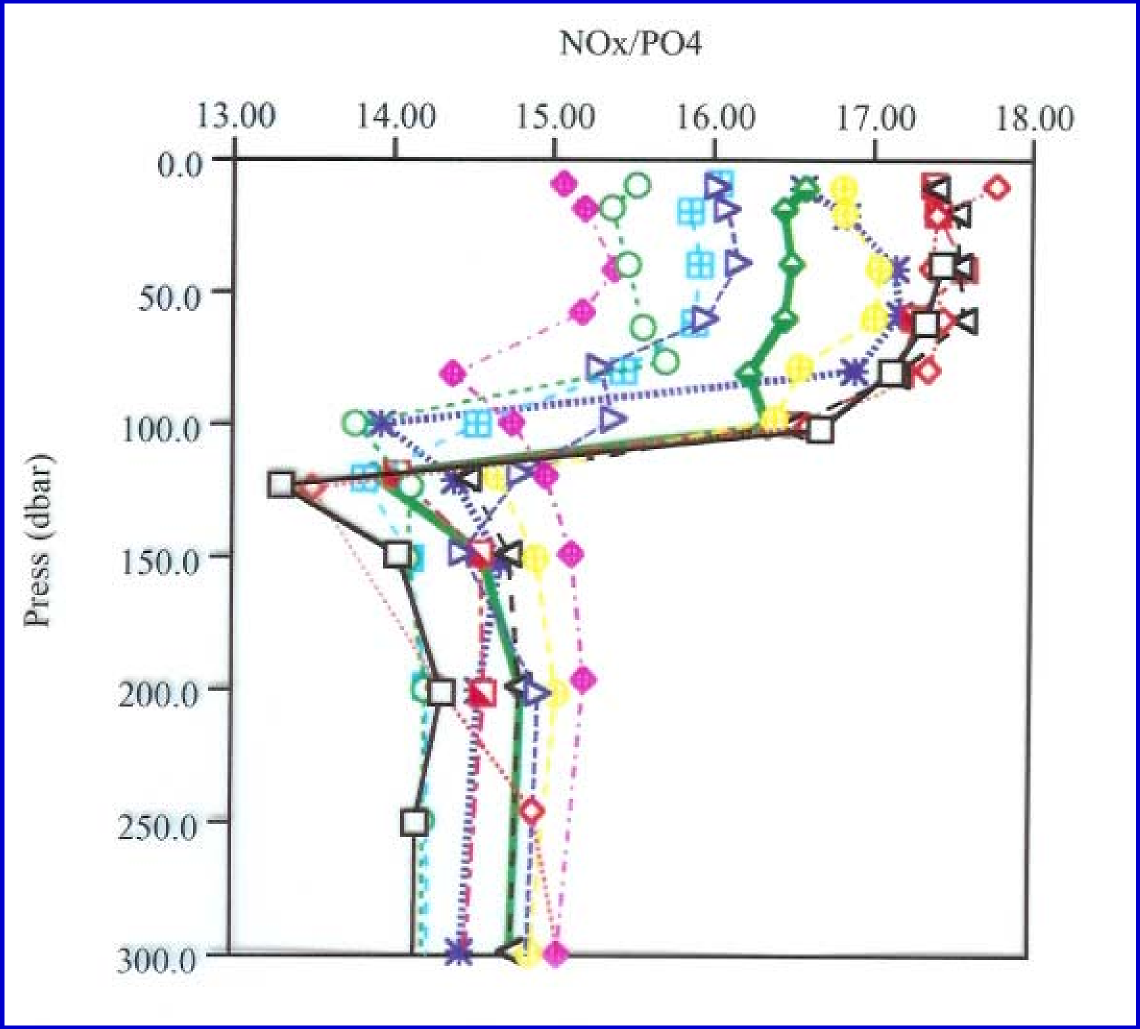
February 1999 SOIREE Nutrient Anomalies: *Fragilariopsis kerguelensis* strikes again

end of bloom season

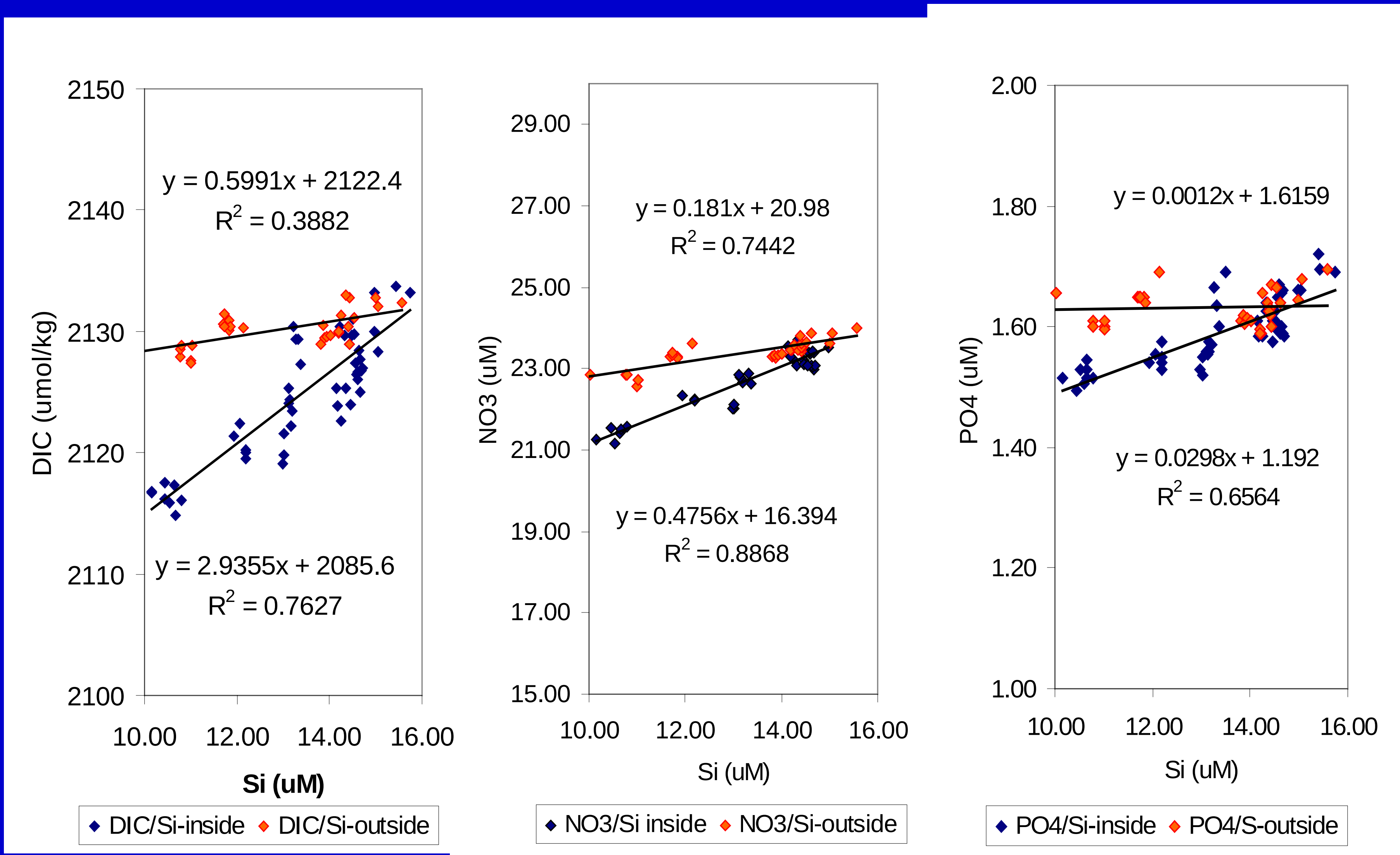


Nutrients data courtesy Stuart Pickmere, NIWA, New Zealand

Polarstern 1999 survey cruise: NOx/PO4 anomalies at stations dominated by Fragilariopsis



Polarstern (2000) *in situ* Fe enrichment



Bozec, Bakker, de Baar, Thomas, Bellerby and Watson (2003) submitted

Polarstern (2000) *in situ* Fe enrichment

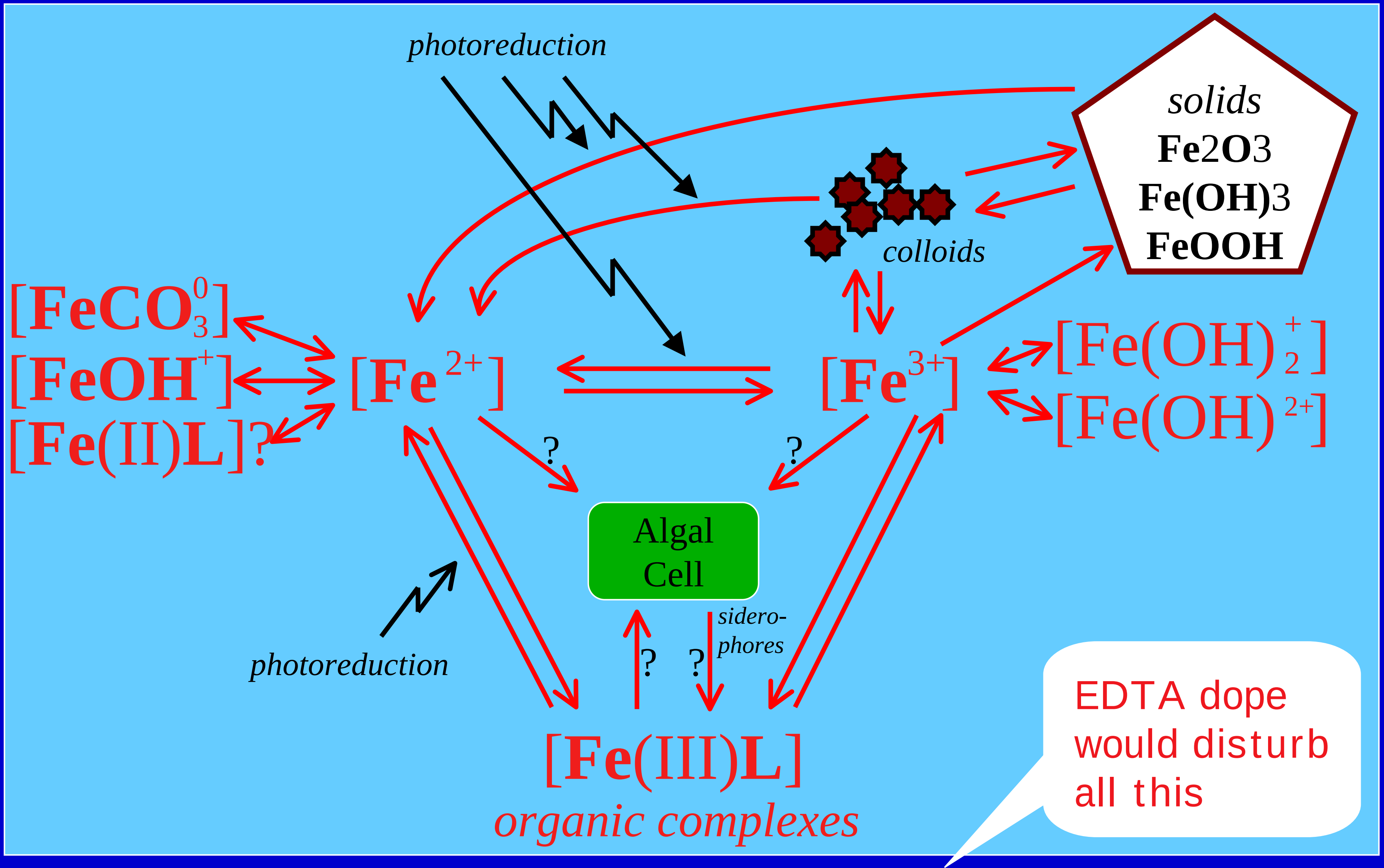
	Polarstern (2000)	Ironex II (1994)	Redfield (1934)	Takeda (1998)	
				-Fe	+Fe
ΔC/ΔP	82	90 + 5	106		
ΔC/ΔN	5.9	6.2 + 0.2	6.6		
ΔN/ΔP	12	14.3 + 0.2	16	12	14
ΔC/ΔSi	2.9	5.1 + 0.3			
ΔSi/ΔN	2.1			2.3	0.9
	in the patch plankton community	in the patch plankton community (Steinberg & Millero, 1998)		in bottles plankton community	

Bozec, Bakker, de Baar, Thomas, Bellerby and Watson (2003) submitted

4. Growth Experiments

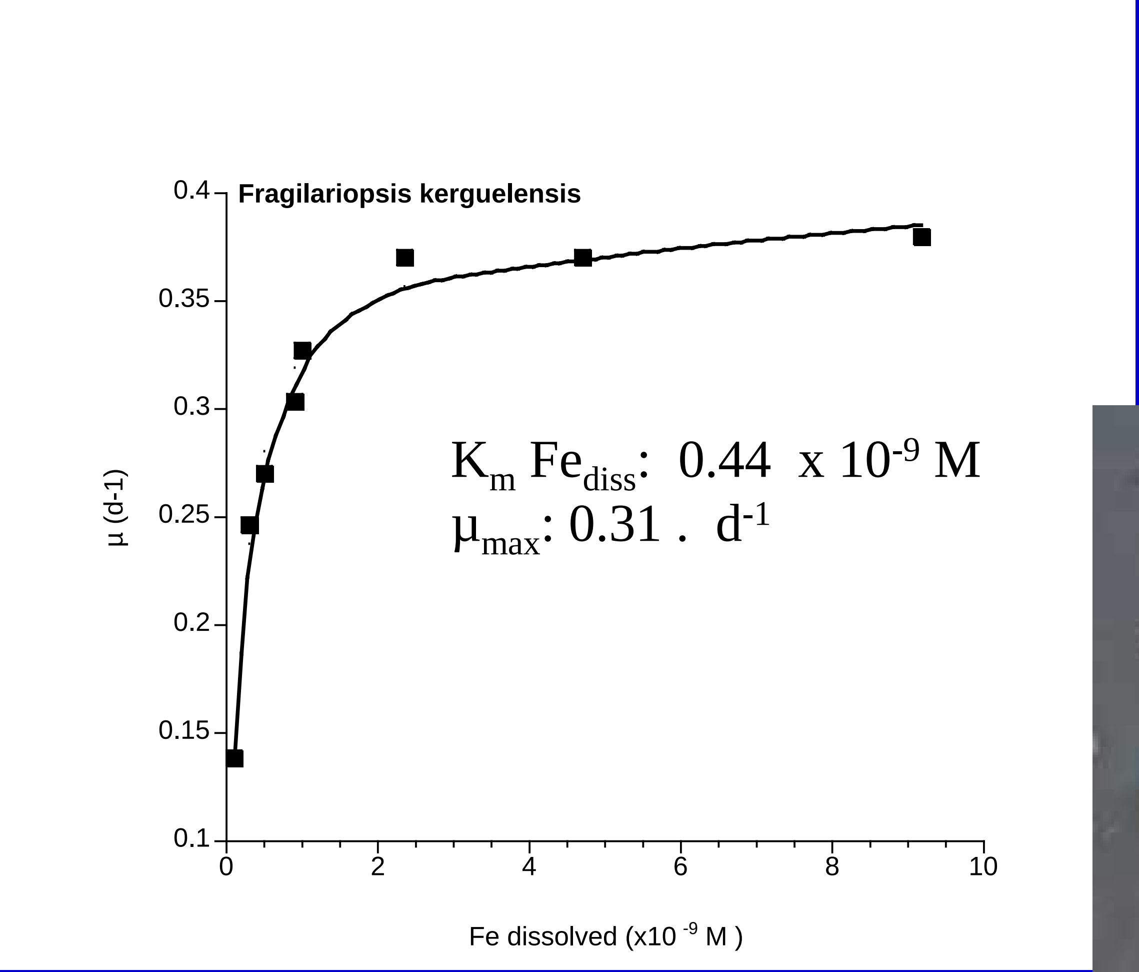
- Pristine natural seawater medium
- Fragilariopsis kerguelensis
- Diatoms are Forever
  - light & Fe co-limitation
  - small versus large *Chaetoceros sp.*
- Zn-HCO_3 co-limitation *Emiliania huxleyi*

Different forms of Fe in seawater



Gerringa, de Baar and Timmermans (2000), *Marine Chemistry*, 68, 335-346

Fragilariopsis kerguelensis in natural Antarctic seawater



80 μm

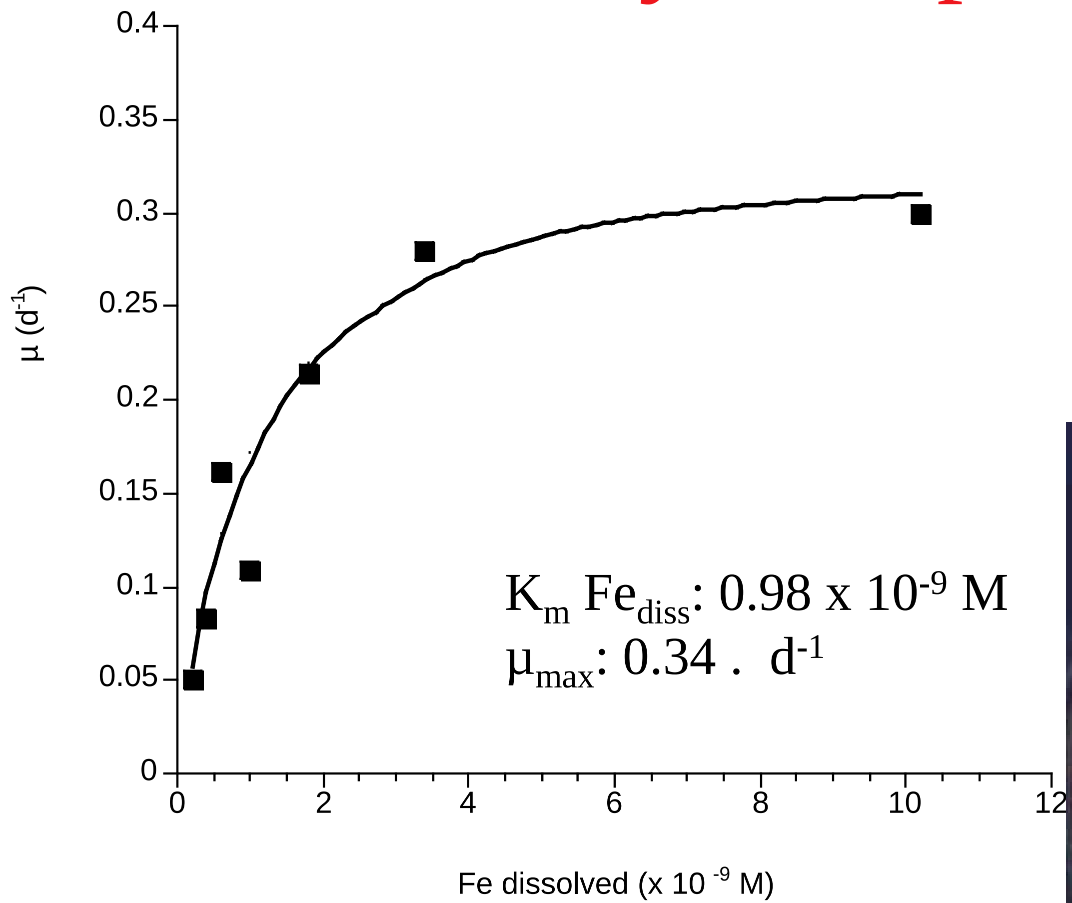
Timmermans, van der Wagt, de Baar, in prep.

Nutrients Stoichiometry of *Fragilariopsis kerguelensis*

Ratio	Southern Ocean	Incubations	
	Fe-deplete	Fe-deplete	Fe-replete
Si : N		7.7	2.5
N : P	~ 5 $\pm$ 1	~ 5 $\pm$ 1	~12 $\pm$ 2

heavily silicified *Frag.kerguelensis* has higher Si/N ratio

Actinocyclus sp.



Klaas Timmermans et al., in prep.

Elemental composition in relation to Fe_{diss}

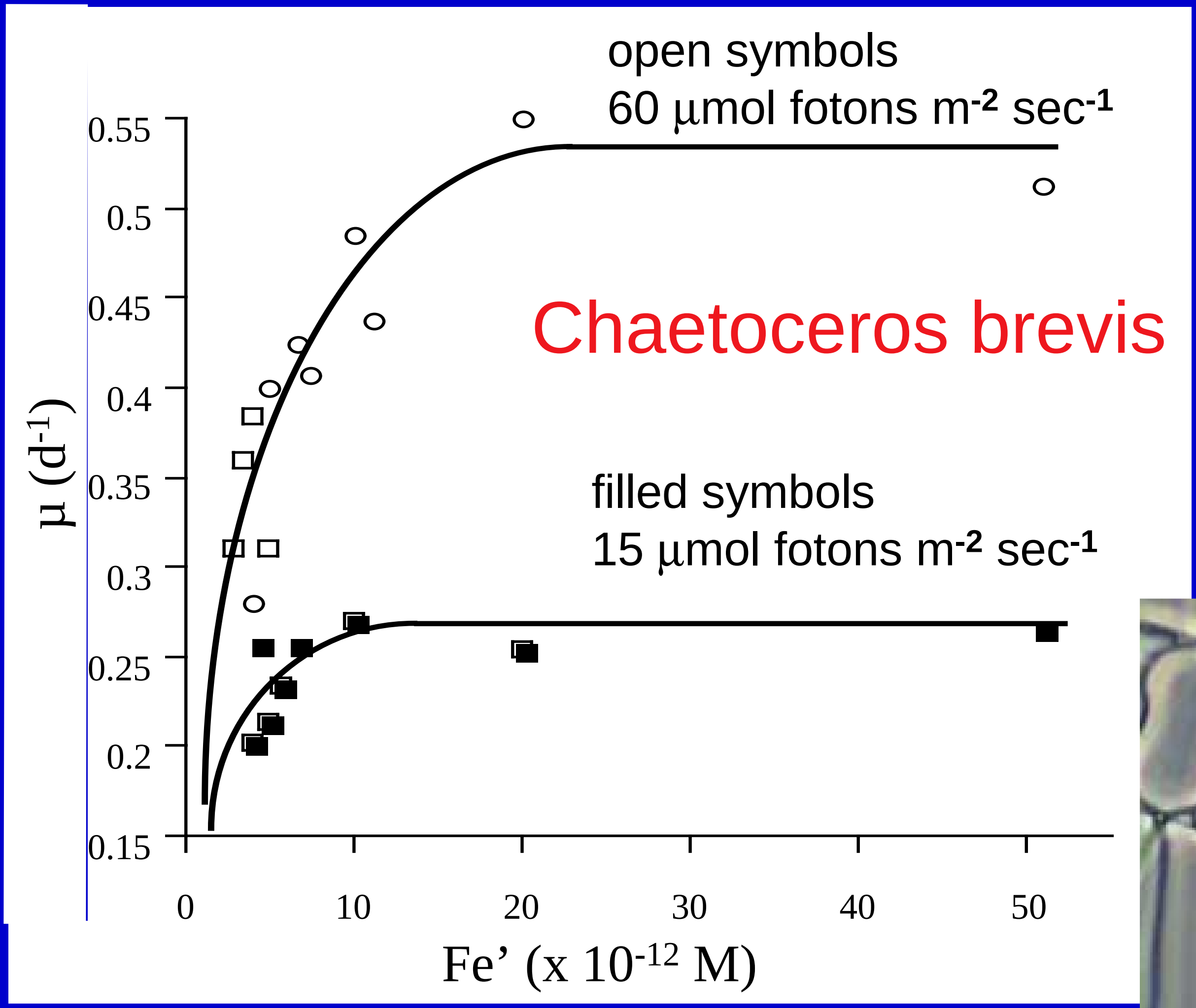
mol per liter cell volume

Actinocyclus sp.

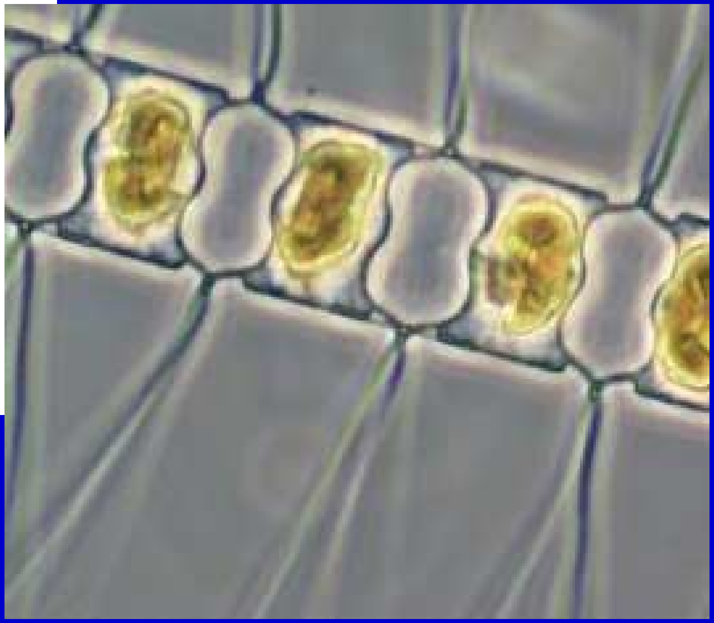
Fe_{diss} ($\times 10^{-9}$ M)	Si	N	P	Si : N	N:P
0.25	18.25	0.69	1.48	27	0.47
0.45	17.25	0.75	1.50	23	0.50
0.65	9.88	0.56	1.38	18	0.41
1.05	5.69	0.59	0.78	10	0.76
1.85	4.02	0.52	0.52	8	1.00
3.45	3.66	0.53	0.63	7	0.85
10.45	2.36	0.61	0.33	4	1.86

Klaas Timmermans et al., in prep.

Light and Fe co-limitation



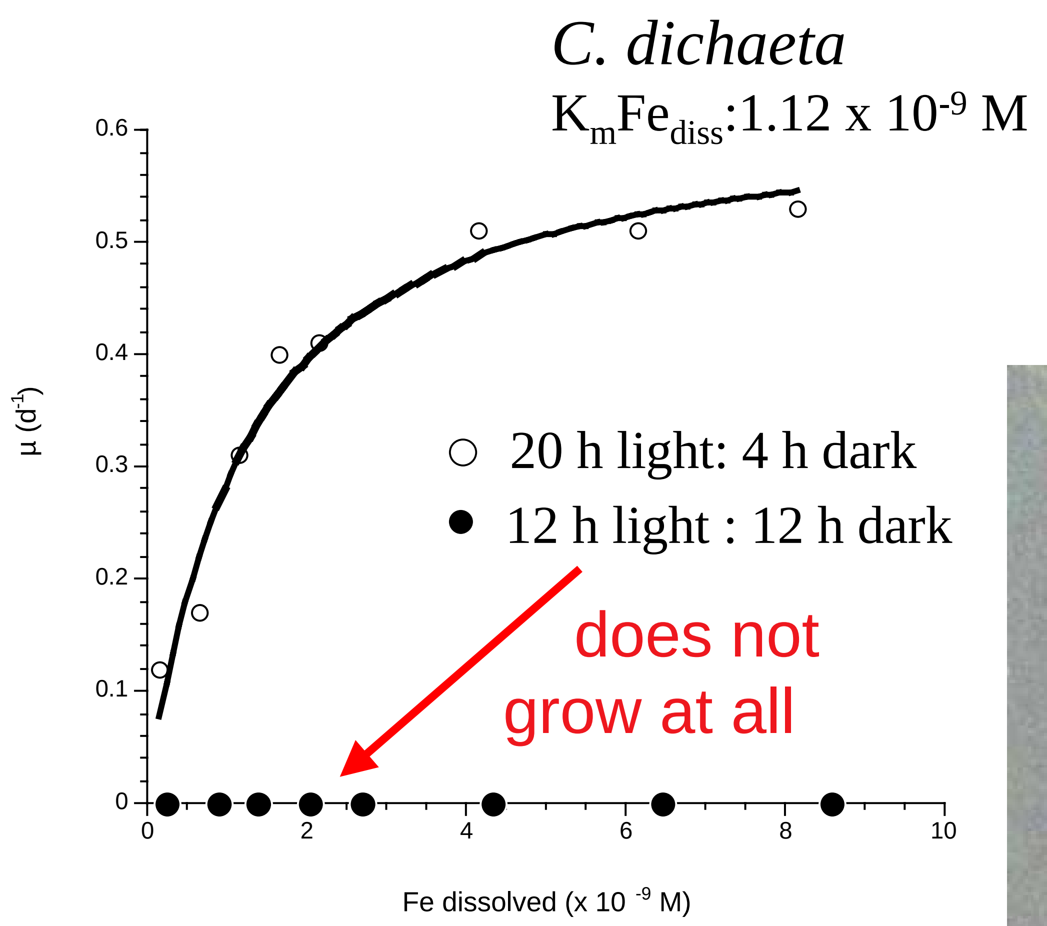
<u>single</u> cells
4 - 6 µm diameter (small)



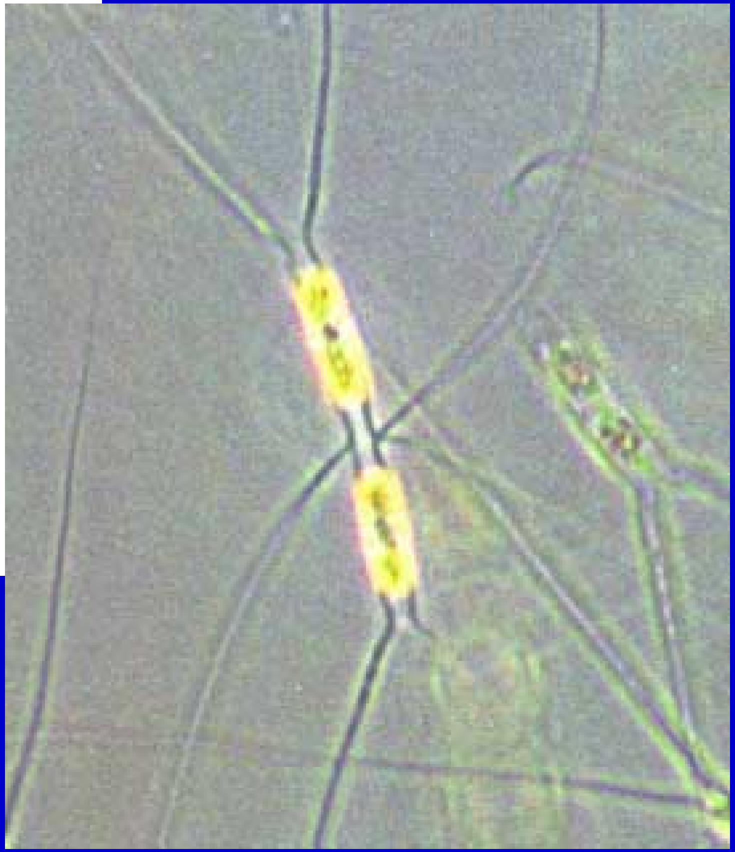
NIOZ

Timmermans et al. (2001), MEPS 287 - 297.

Chaetoceros dichaeta



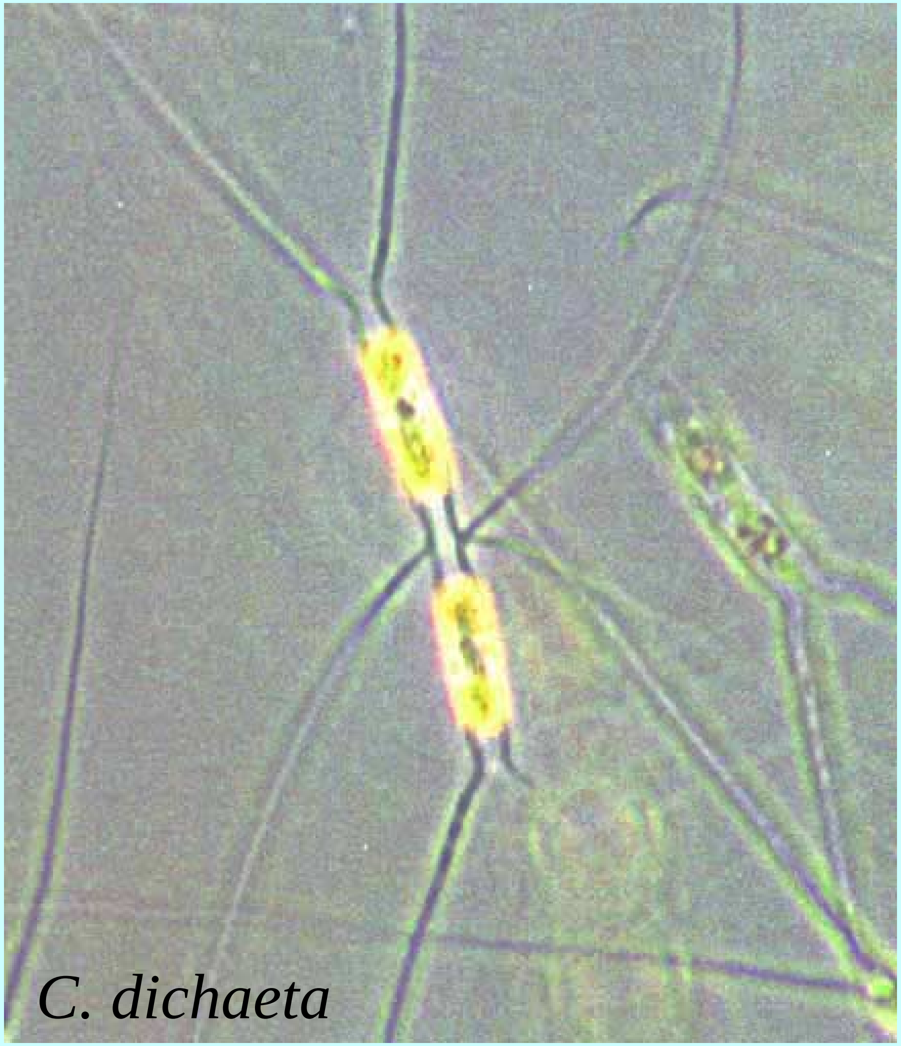
Chain-forming large cells



Timmermans et al. (2001), MEPS 287 - 297.

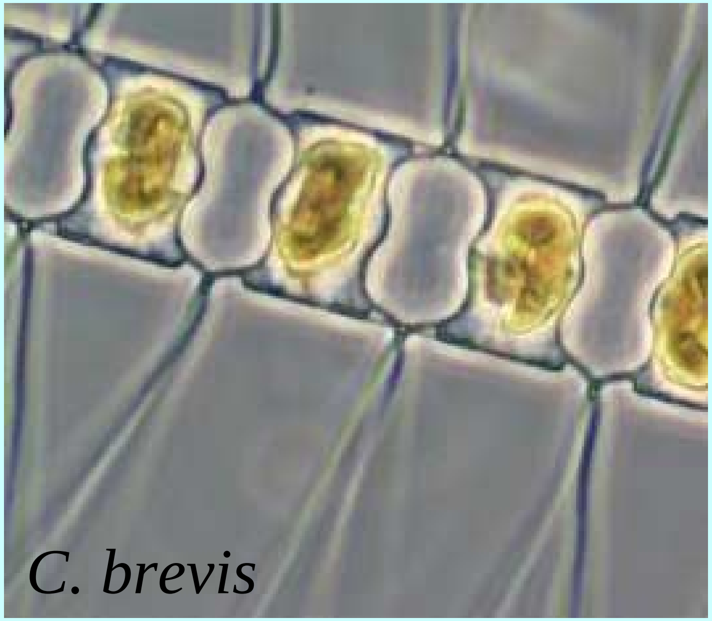
Open Southern Ocean HNLC species

Large versus small
at optimal light levels



chain-forming cells, individual cells
80 µm long, 30 µm width (large)

<u>single</u> cells 4 - 6 µm diameter (small)

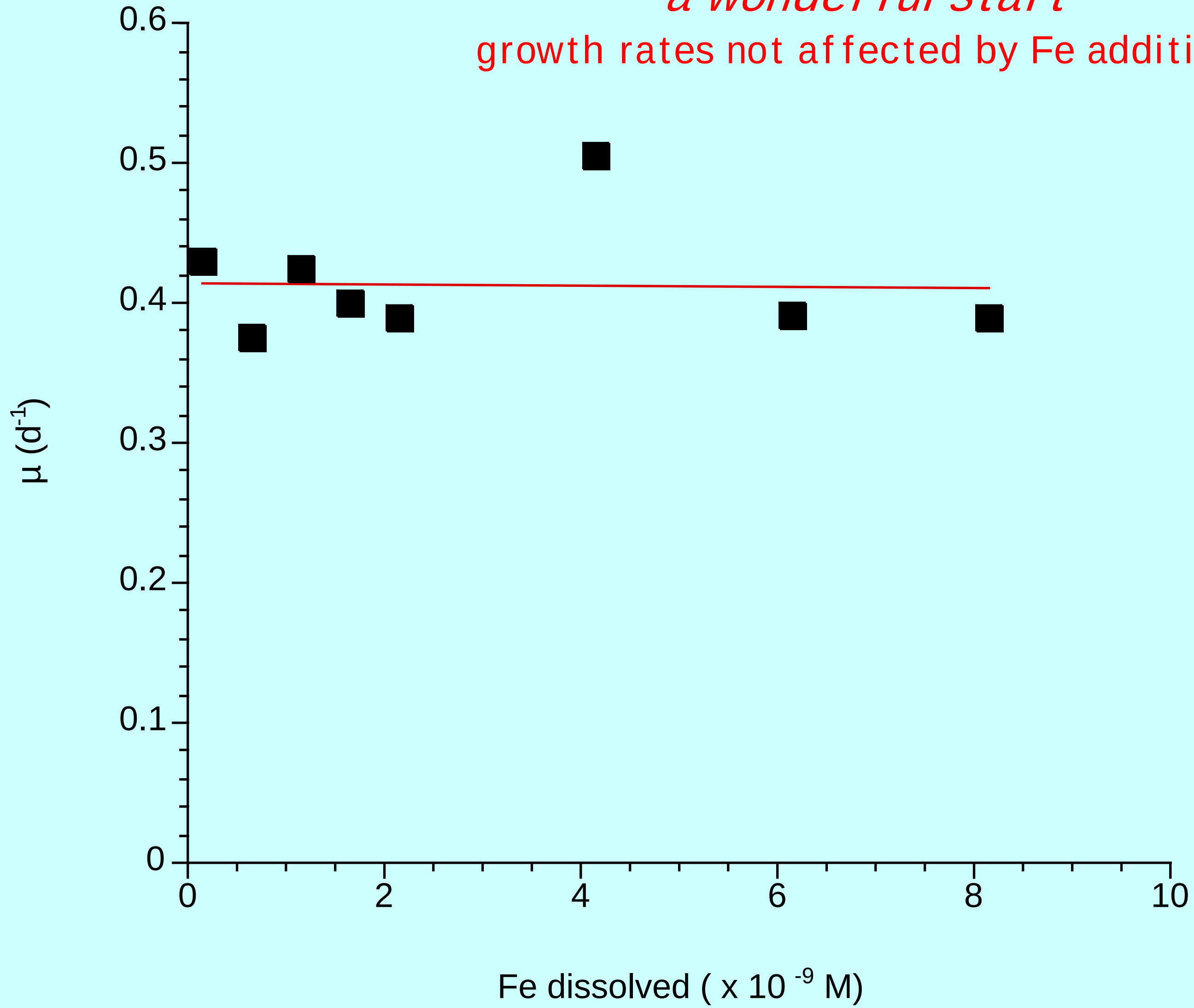


Timmermans et al. Limnol & Oceanogr. 46: 699 - 703.

C. brevis in its pristine Antarctic seawater

"*a wonderful start*"

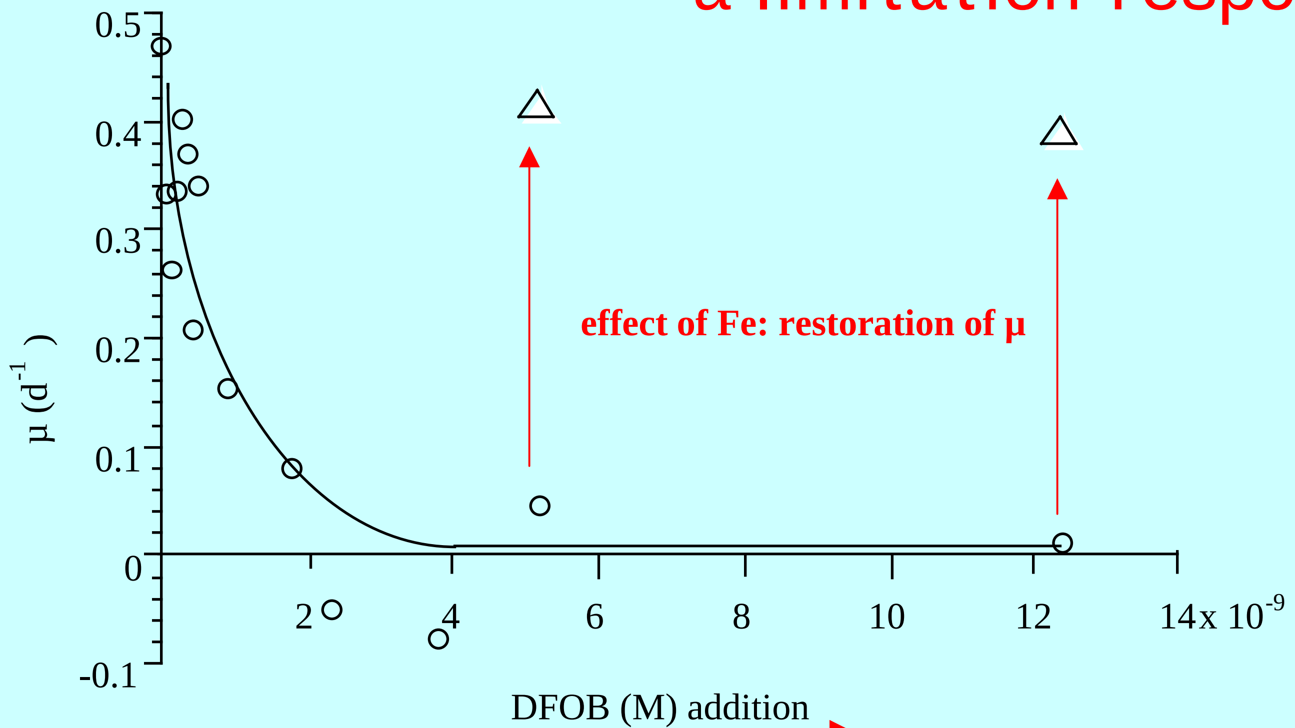
growth rates not affected by Fe additions



Timmermans et al. Limnol & Oceanogr. 46: 699 - 703.

Add DFOB siderophore to tie down the iron

C. brevis, it works….
a limitation response

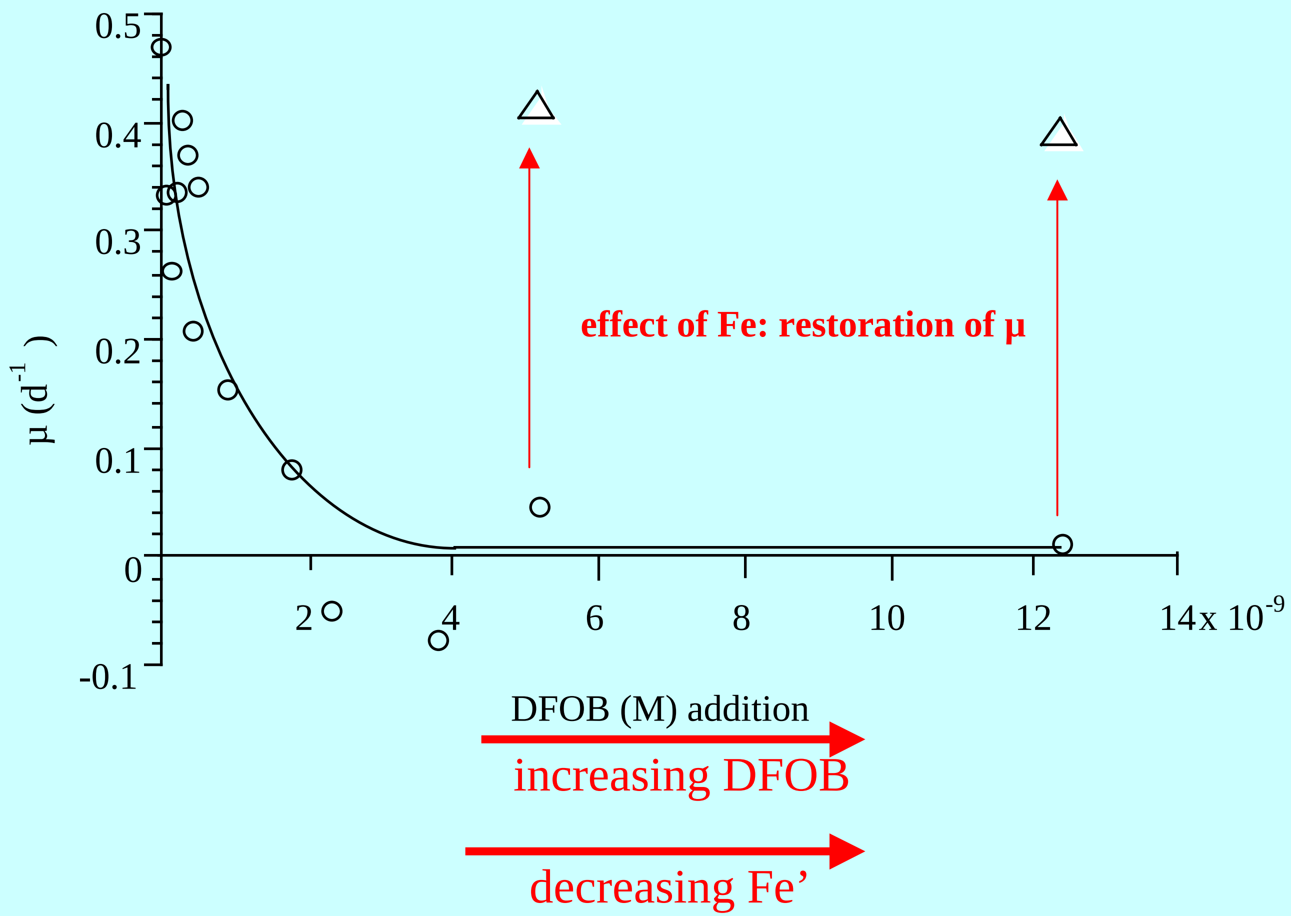


increasing DFOB

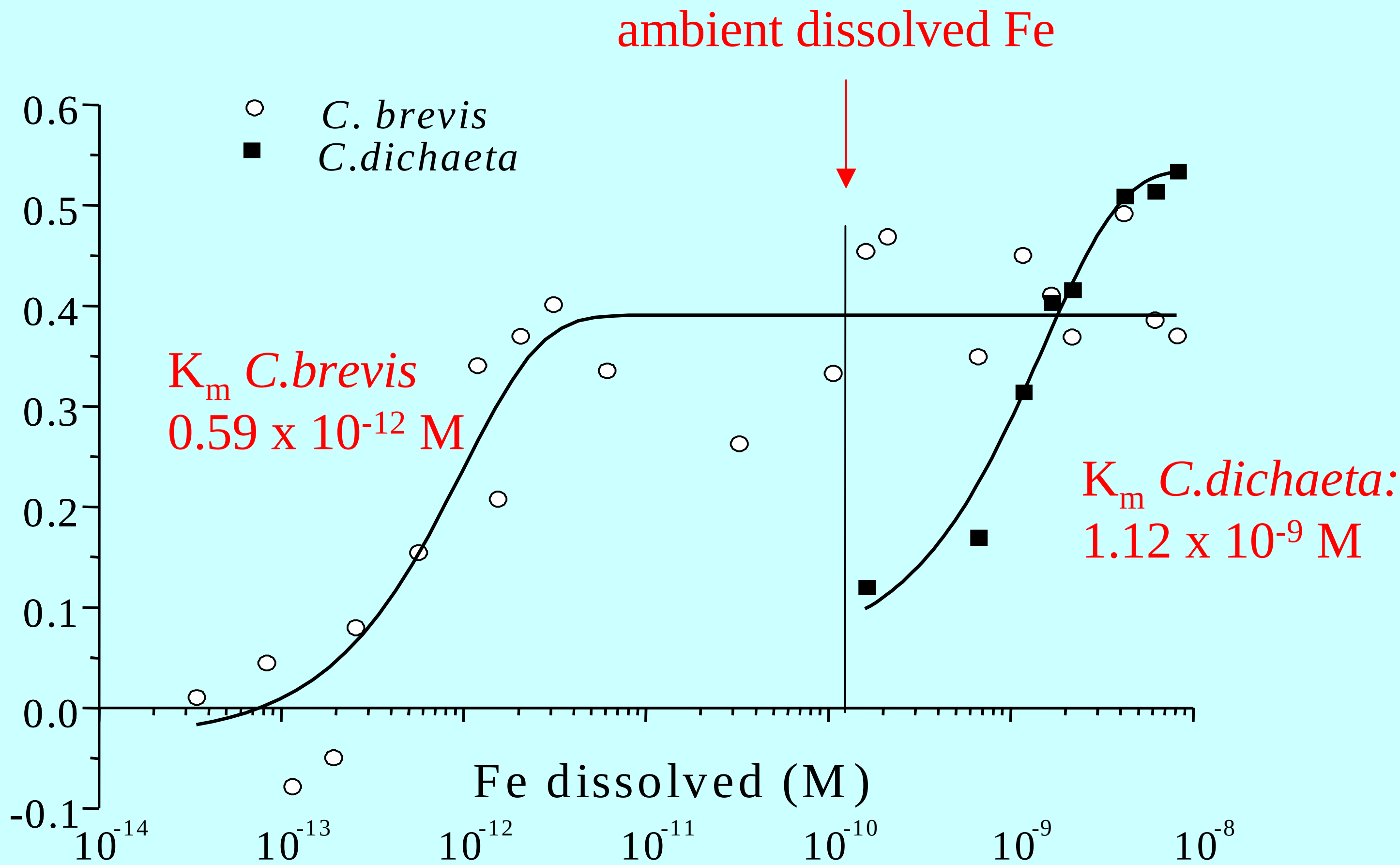
decreasing Fe’

Timmermans et al. Limnol & Oceanogr. 46: 699 - 703.

C. brevis, it works....
a limitation response



Timmermans et al. Limnol & Oceanogr. 46: 699 - 703.



In the Southern Ocean:

Large C. dichaeta is mostly Fe-limited except after Fe supply

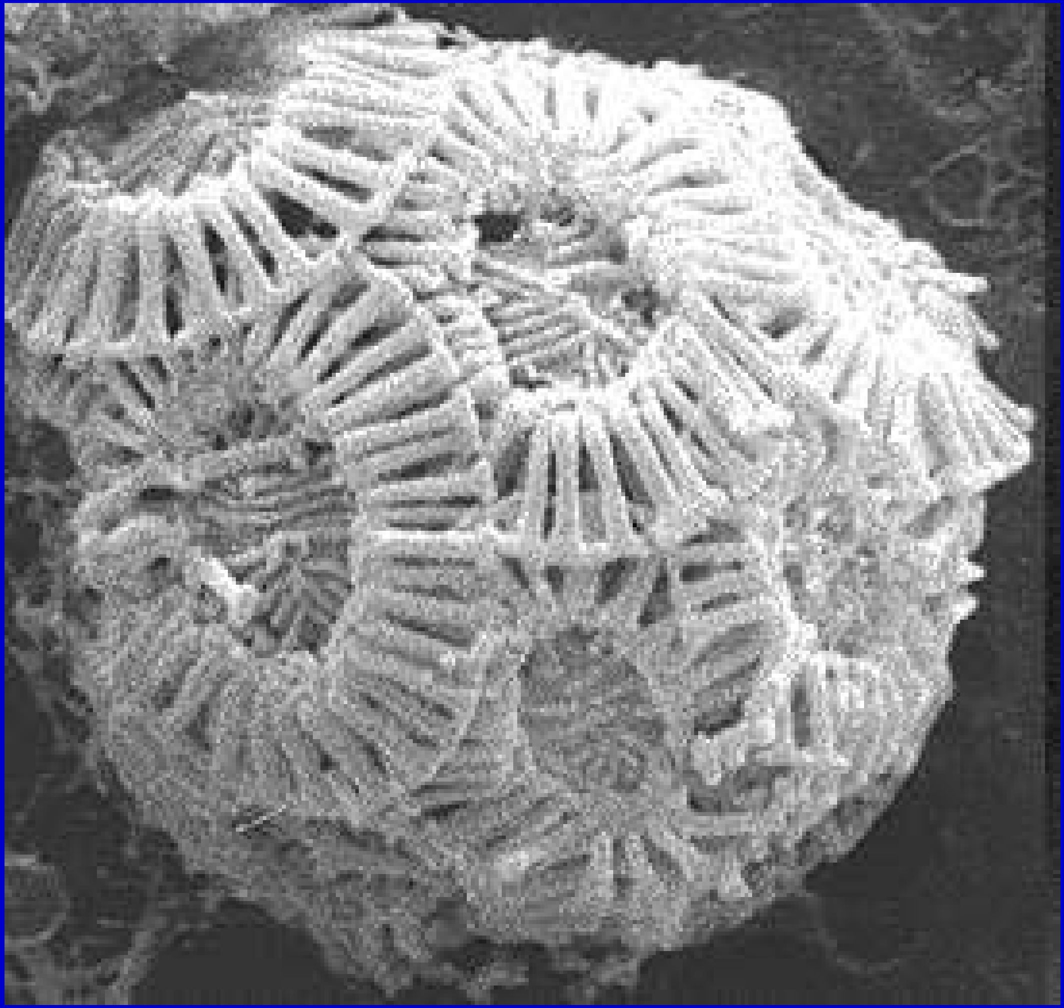
Small C. brevis is never Fe-limited but grazer-controlled

Paradigm Shift

- Old Paradigm (Sunda, Swift, Huntsman, 1991)
  - coastal diatom require more Fe than oceanic diatom
- New Paradigm
  - O.K. but third class of large oceanic diatoms having high Fe requirement
  - these large guys are driving export

Timmermans et al. Limnol & Oceanogr. 46: 699 - 703.

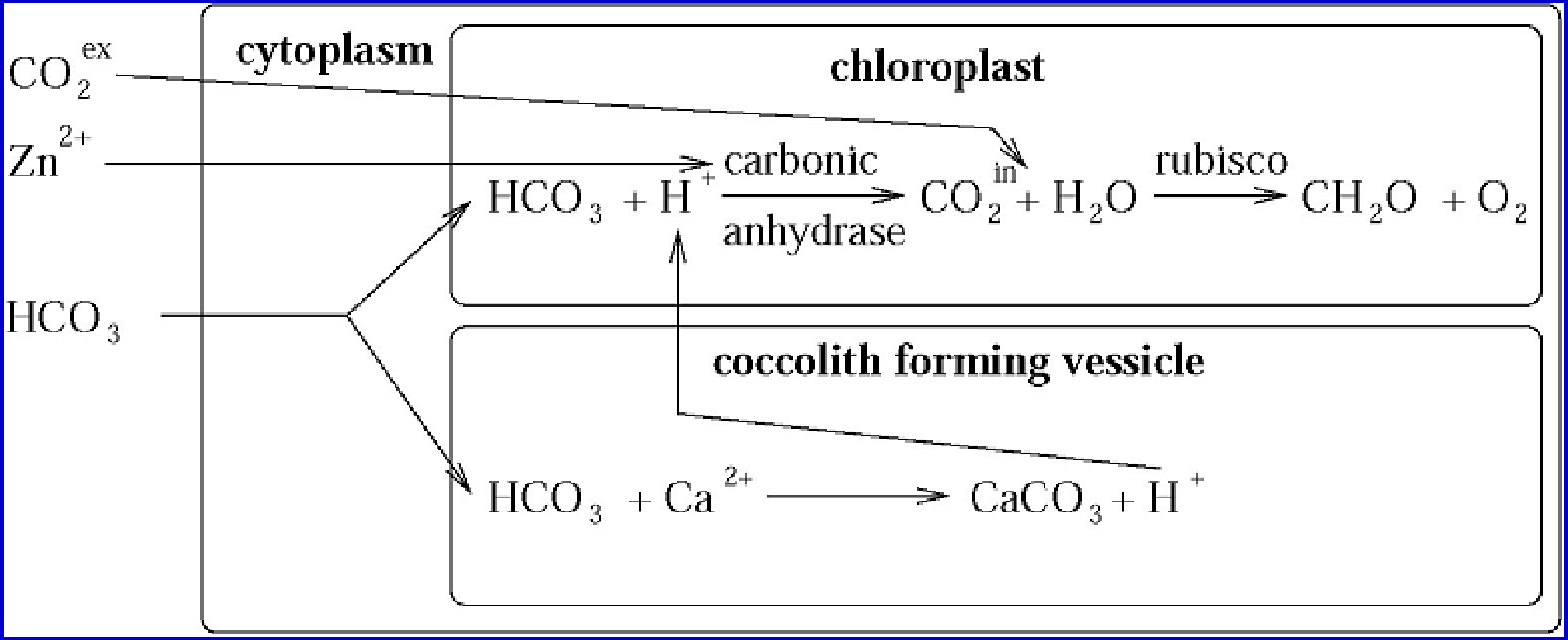
Emiliania huxleyi



excretes external $CaCO_3$ platelets

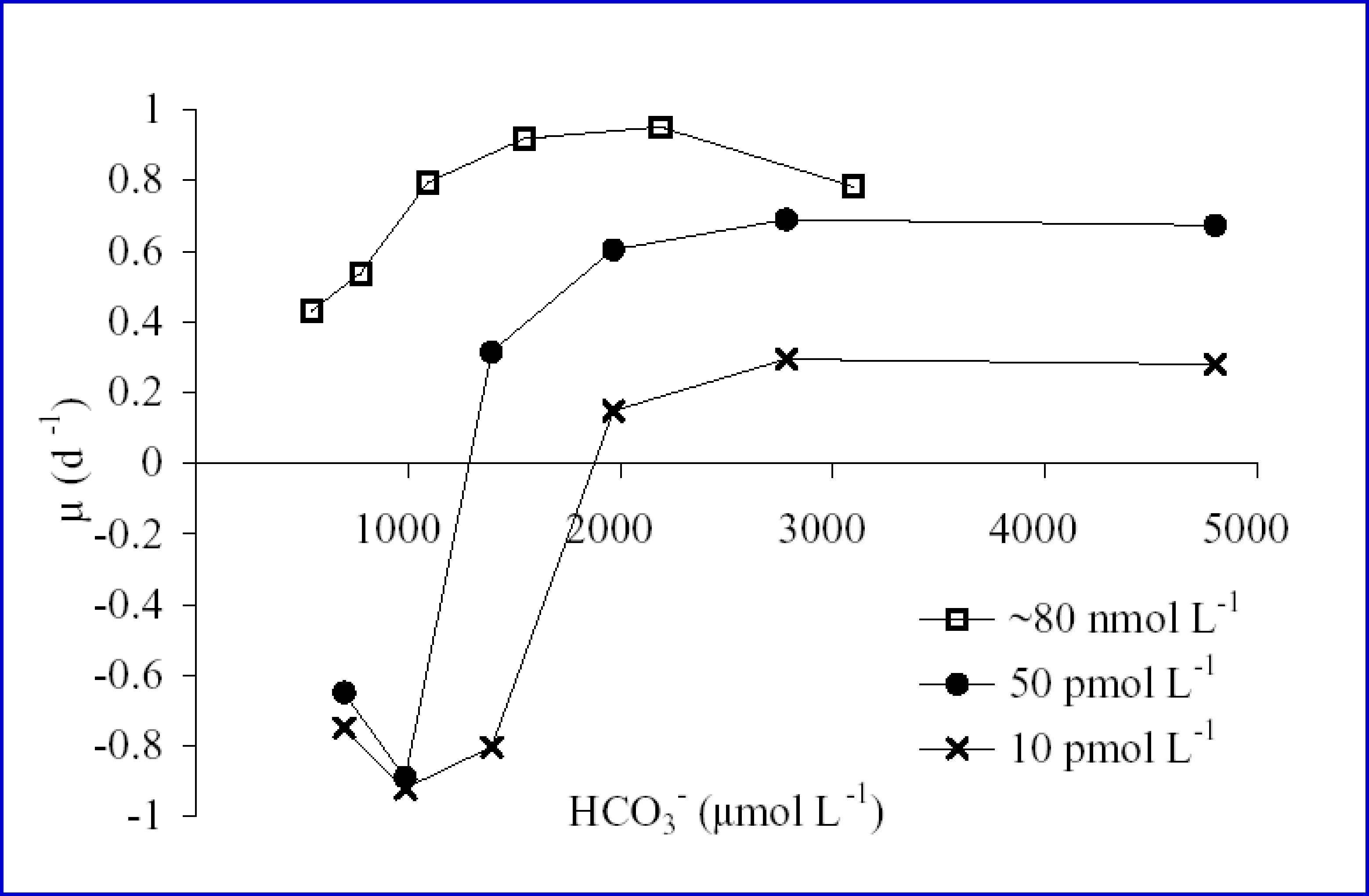
Concerted photosynthesis & calcification

- Zn-carbonic anhydrase permits fast use of [HCO_3^-]
- Calcification provides the necessary proton to make CO_2



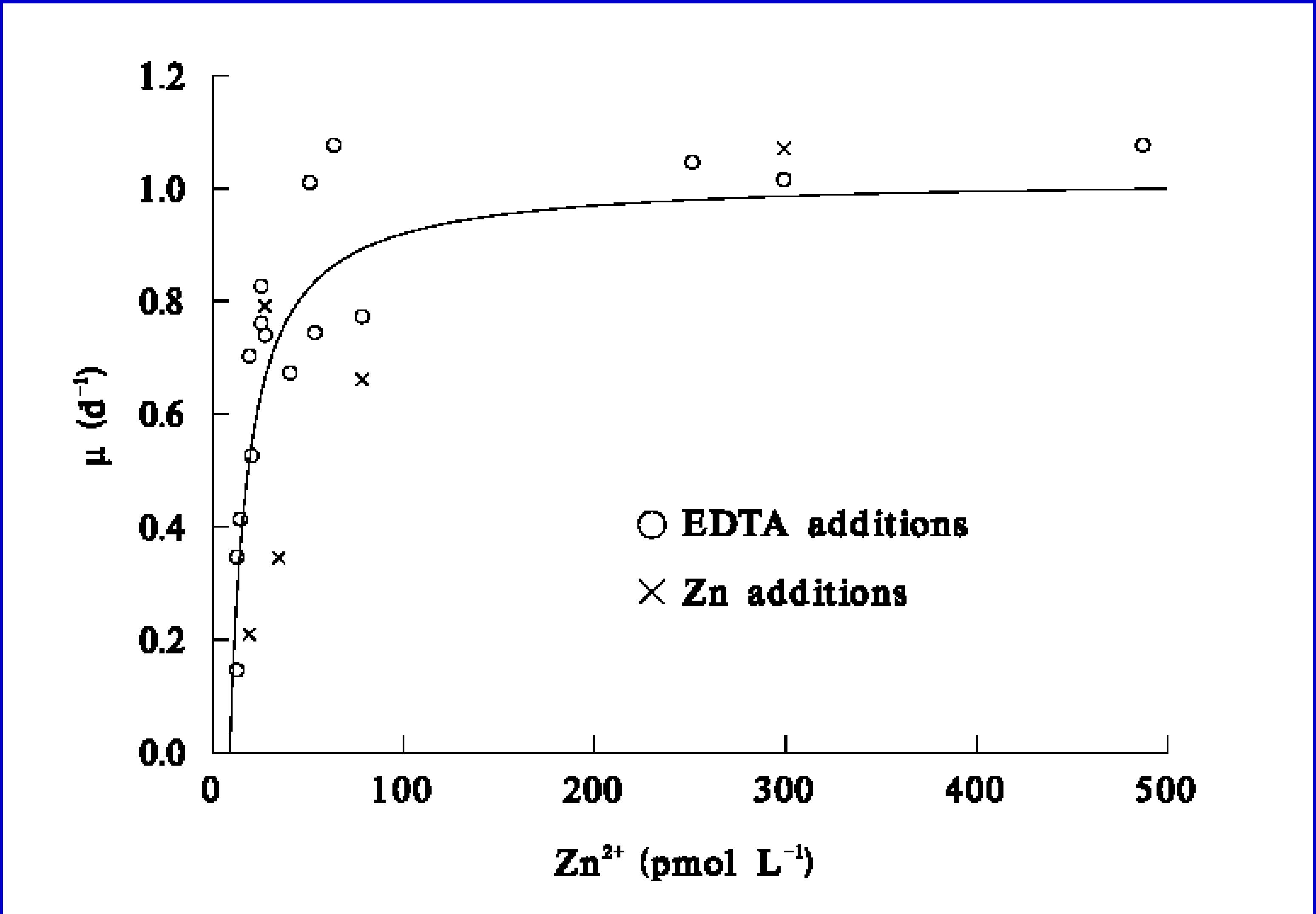
Buitenhuis, Timmermans and de Baar, Limnol.Oceanogr., in press

Growth on [HCO_3^-] at 3 different [Zn^{2+}]



Buitenhuis, Timmermans and de Baar, Limnol.Oceanogr., in press

Growth on $[Zn^{2+}]$ at constant $[HCO_3^-]$



Buitenhuis, Timmermans and de Baar, Limnol.Oceanogr., in press

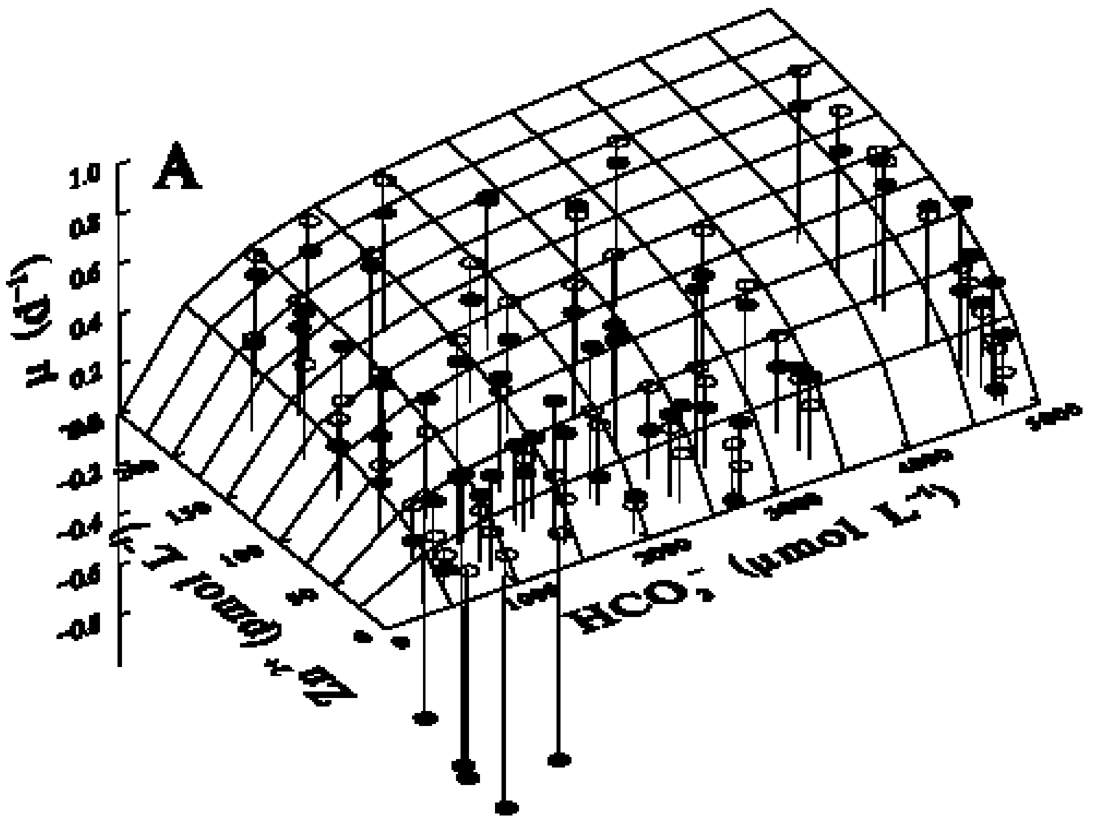
Suitable Equation for co-limitation ?

- A) Multiply two Monod equations
  - two nutrients act independently on growth rate
- B) Minimum nutrient governs growth rate
  - compare [N] with K_N to select one of two Monod
  - most suitable for independent nutrients
- C) Affinity for [HCO_3^-] depends on [Zn^{2+}]
  - most suitable concept for Zn-carbonic anhydrase

Which would provide the best fit ??

Buitenhuis, Timmermans and de Baar, Limnol.Oceanogr., in press

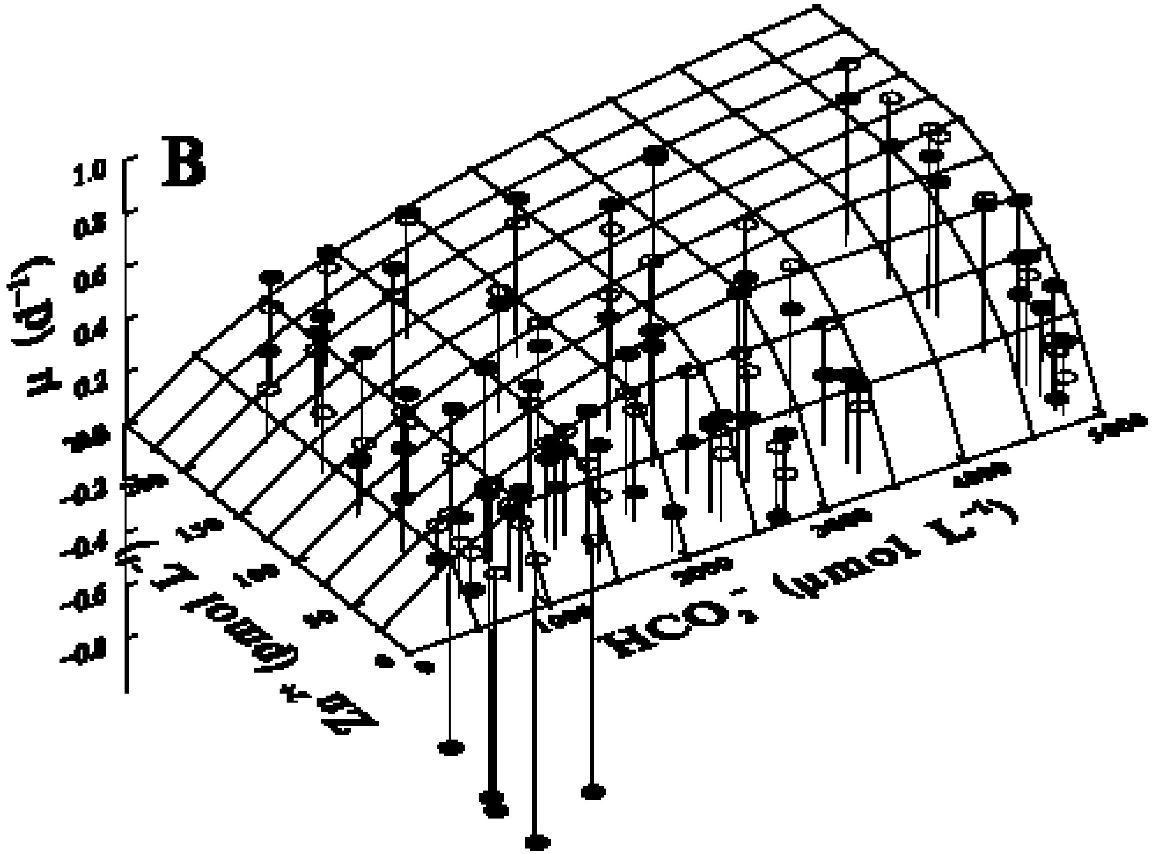
Multiply two Monod equations



filled circles are data;
open circles are intersect with 3-D model plane

best fit: mean residual on μ = 0.018 day^{-1}

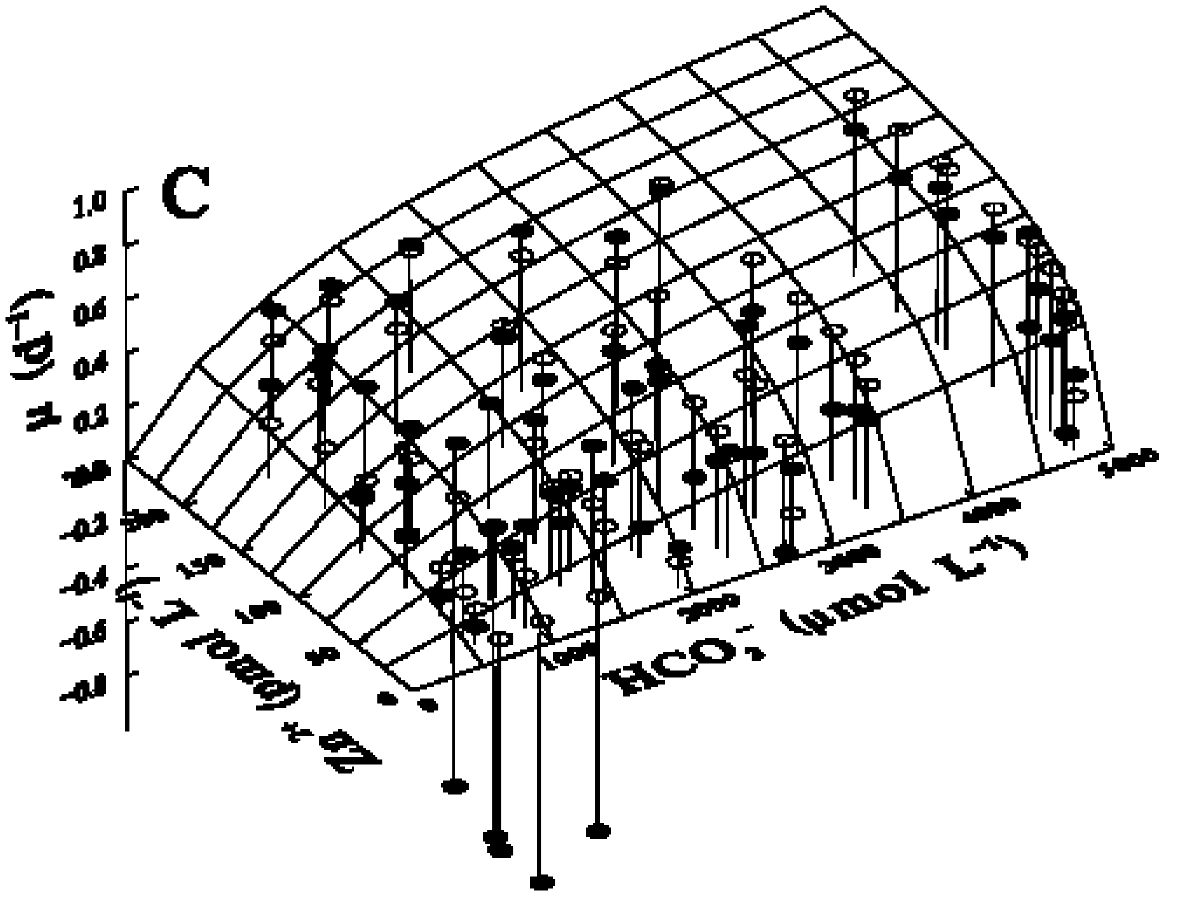
Minimum nutrient governs growth rate



filled circles are data;
open circles are intersect with 3-D model plane

best fit: mean residual on $\mu = 0.02$ day^{-1}

Affinity for $[HCO_3^-]$ depends on $[Zn^{2+}]$



filled circles are data;
open circles are intersect with 3-D model plane

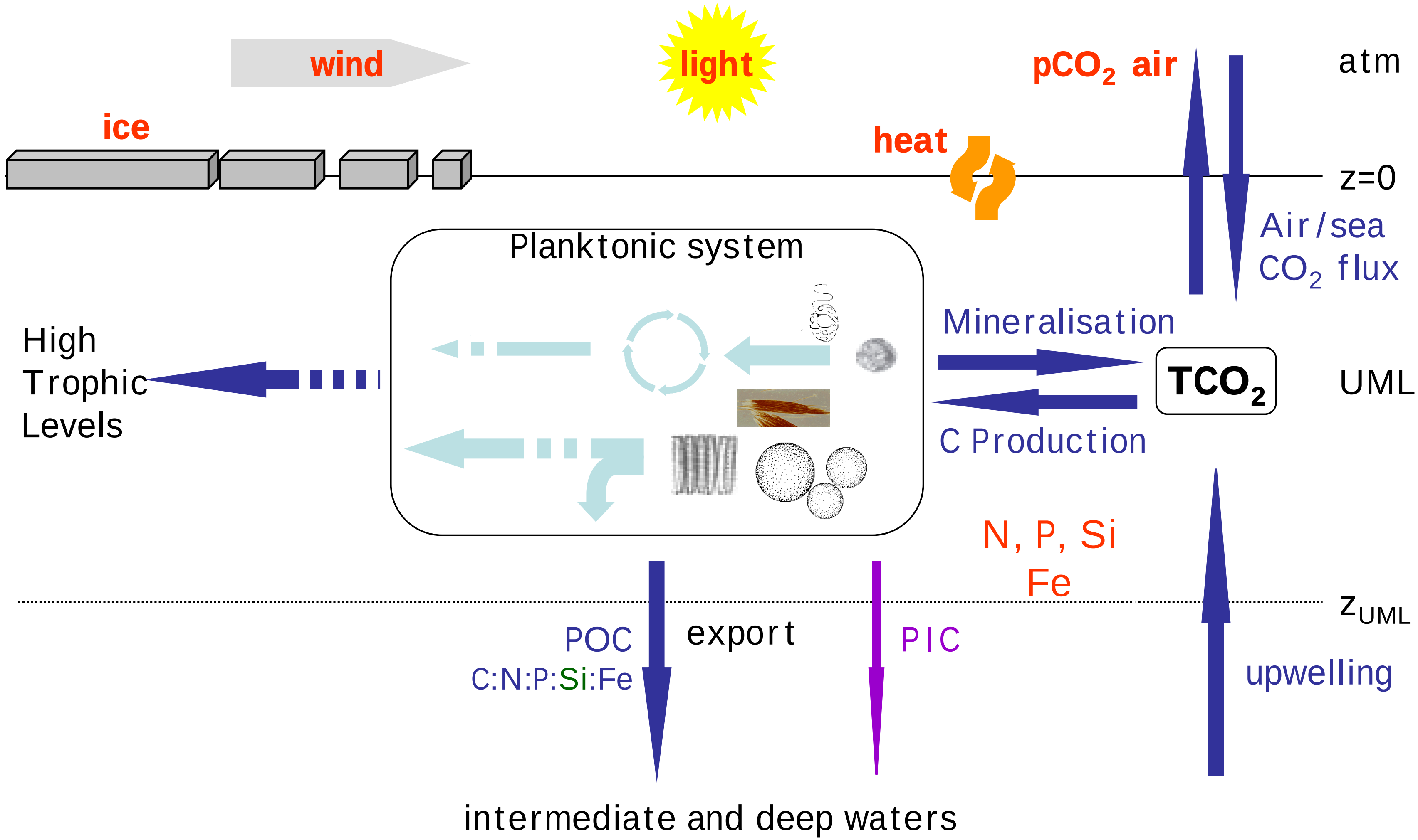
Best concept but fit not any better

best fit: mean residual on $\mu = 0.02$ day^{-1}

5. Iron Resources and Oceanic Nutrients; Advancement of Global Environment Simulations

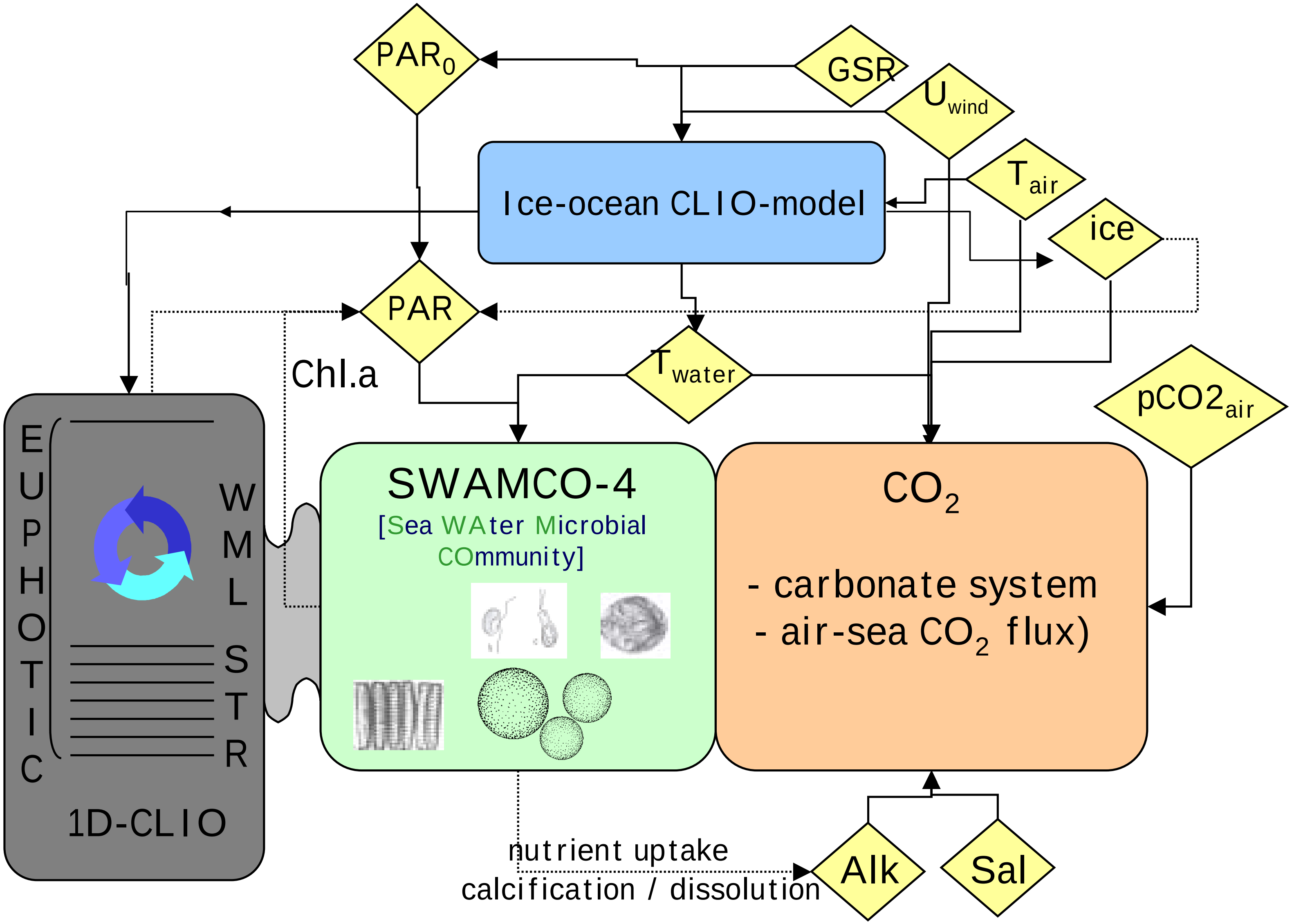
- Existing ecosystem model Southern Ocean
  - two plankton groups diatoms and nanoplankton
  - limitation by light and four nutrients N, P, Fe, Si
  - successful for Polar Front and for SOIREE
  - (Lancelot et al 2000; Hannon et al 2001)
- Advance to generic global model
  - five bloom-forming groups: diatoms, calcifiers, *Phaeocystis*, N2-fixers, pico-nano-plankton
  - limitation by light and four nutrients N, P, Fe, Si
  - embedding in Ocean Biogeochemical Climate Models

Control of the carbon cycling in the upper ocean



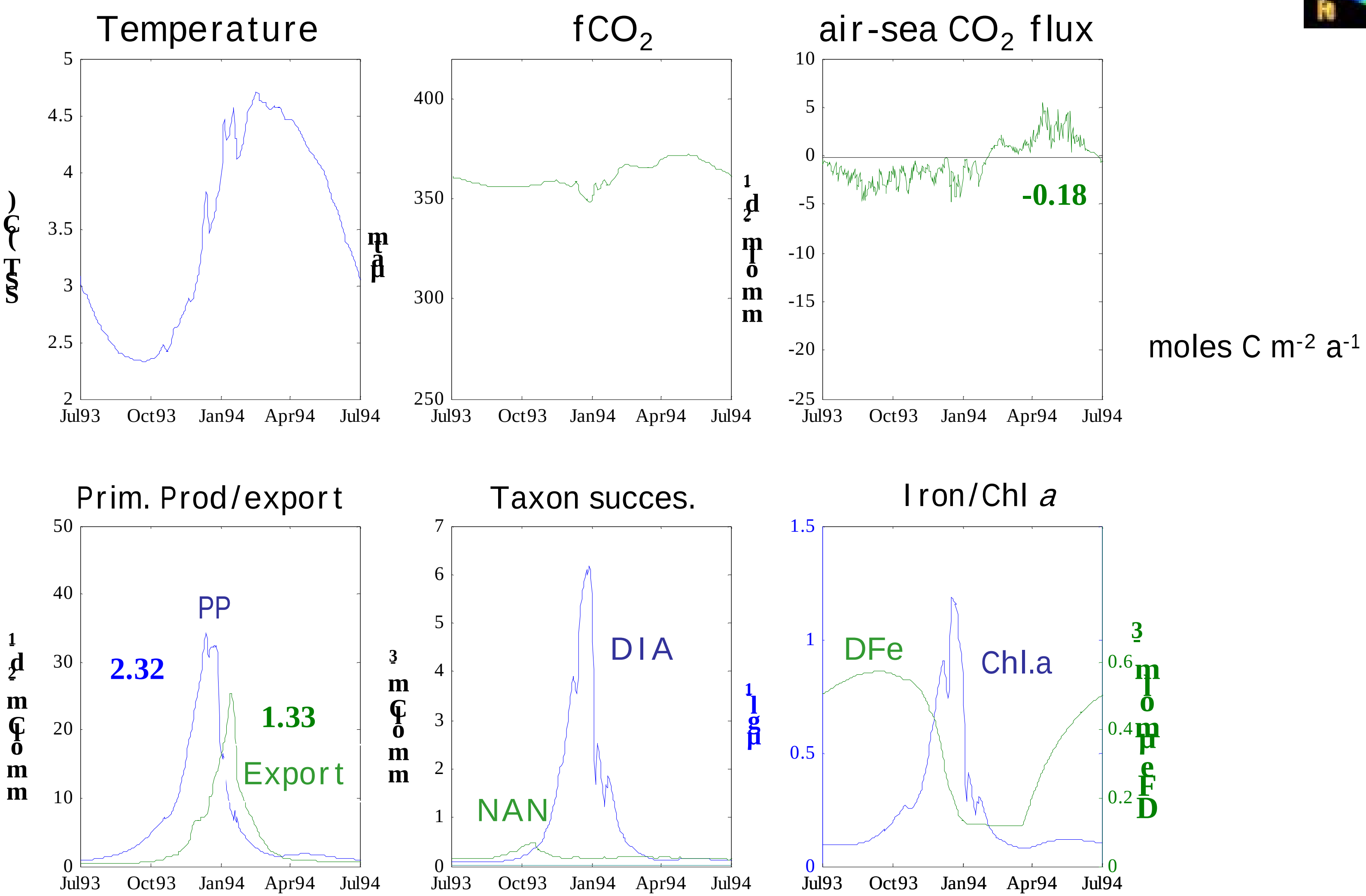
Christiane Lancelot, Nice 2003 lecture

Structure of the coupled biological-chemical-physical 1D model



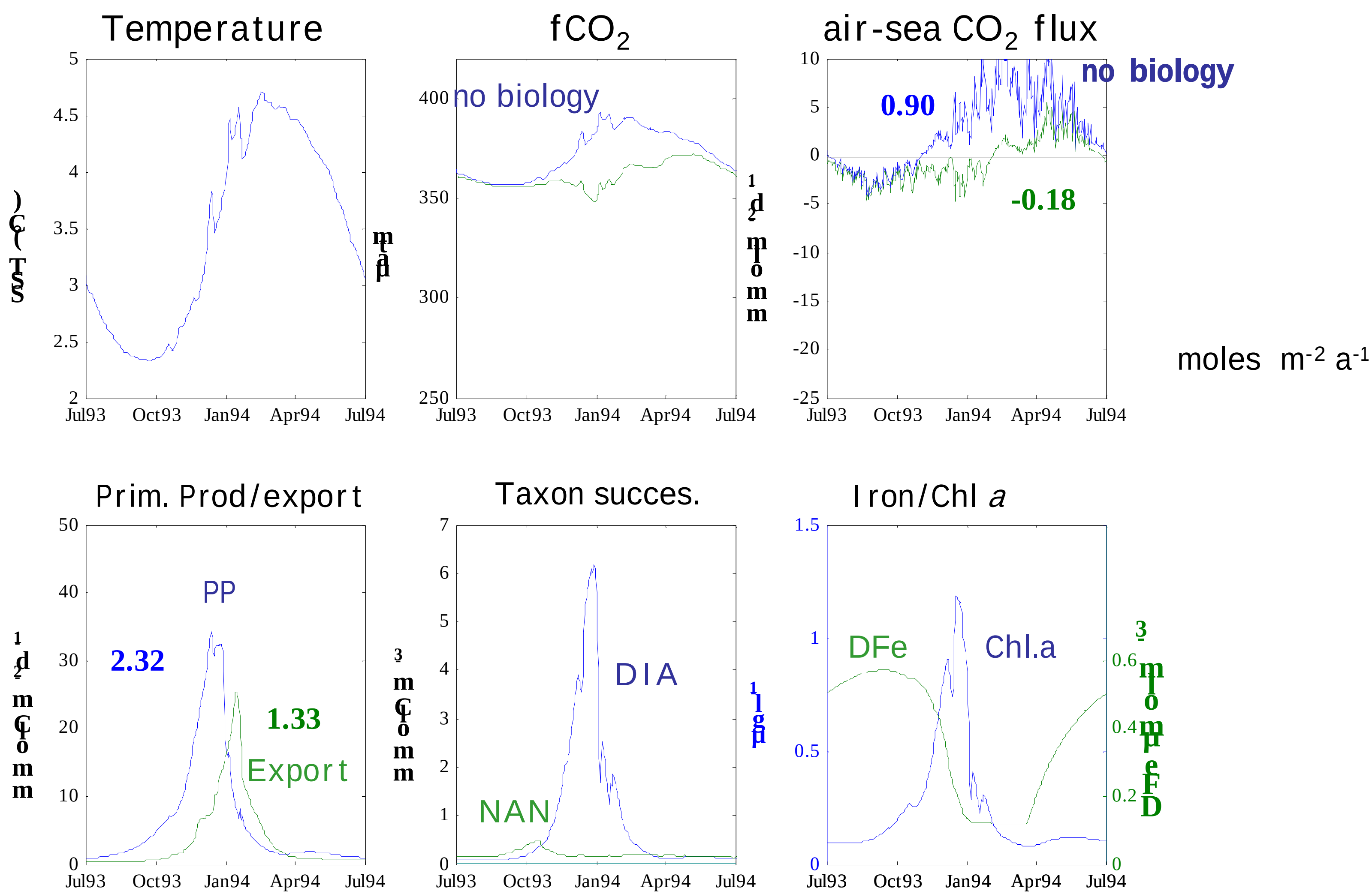
Christiane Lancelot, Nice 2003 lecture

1D SWAMCO-4 results at KERFIX [1993] :
moderate diatom bloom and low CO_2 sink



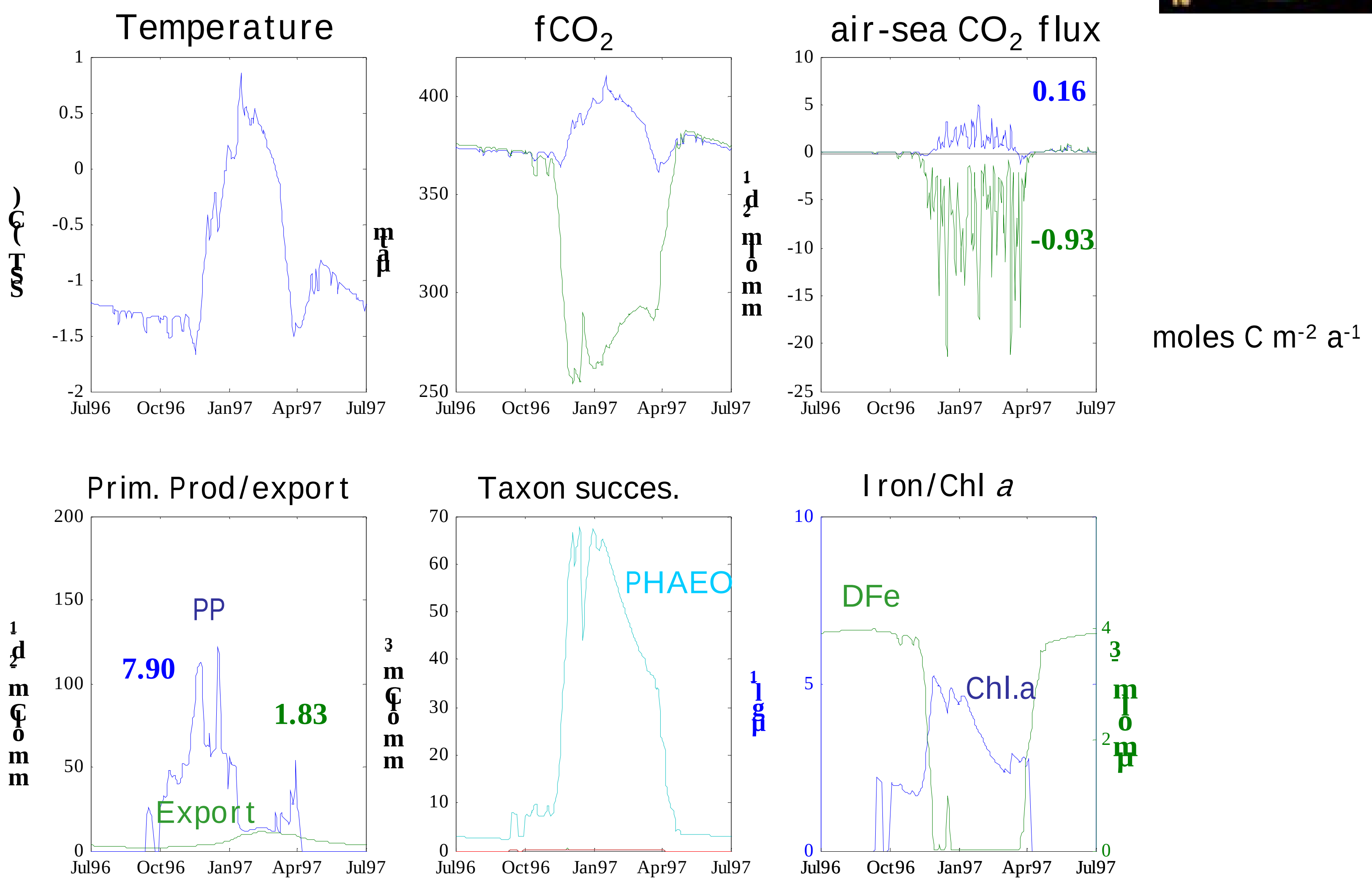
Christiane Lancelot, Nice 2003 lecture

1D SWAMCO-4 results at KERFIX [1993] :
Thermodynamic & biological control of air-sea CO2 fluxes



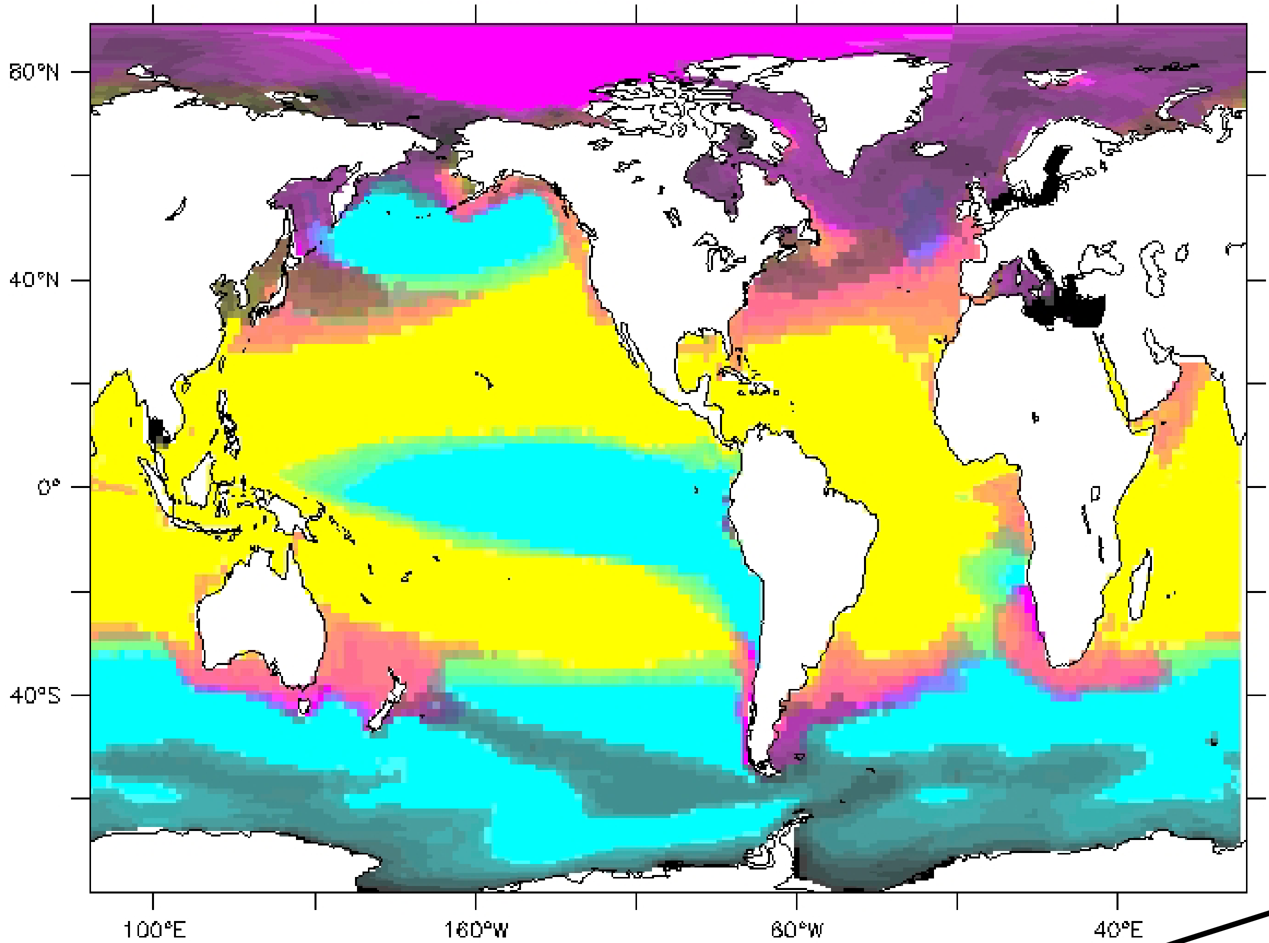
Christiane Lancelot, Nice 2003 lecture

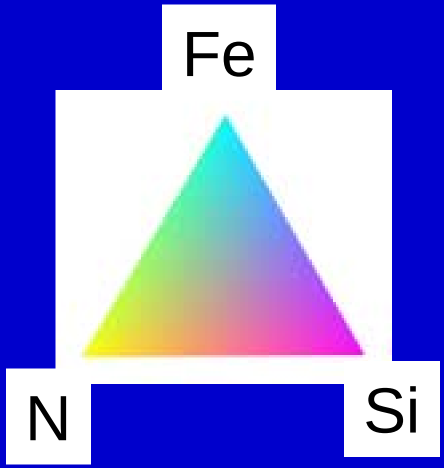
1D SWAMCO-4 results at AESOPS [1996]: Thermodynamic & biological control of air-sea CO_2 fluxes



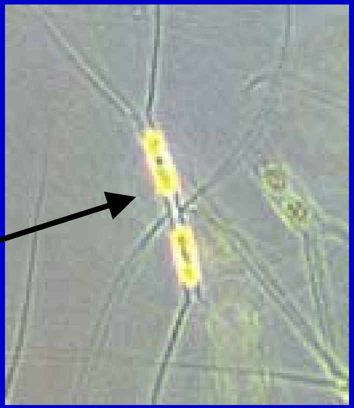
Christiane Lancelot, Nice 2003 lecture

PISCES Model by Olivier Aumont: Co-limiting of 4 taxa by 3 nutrients





Example: the Diatoms

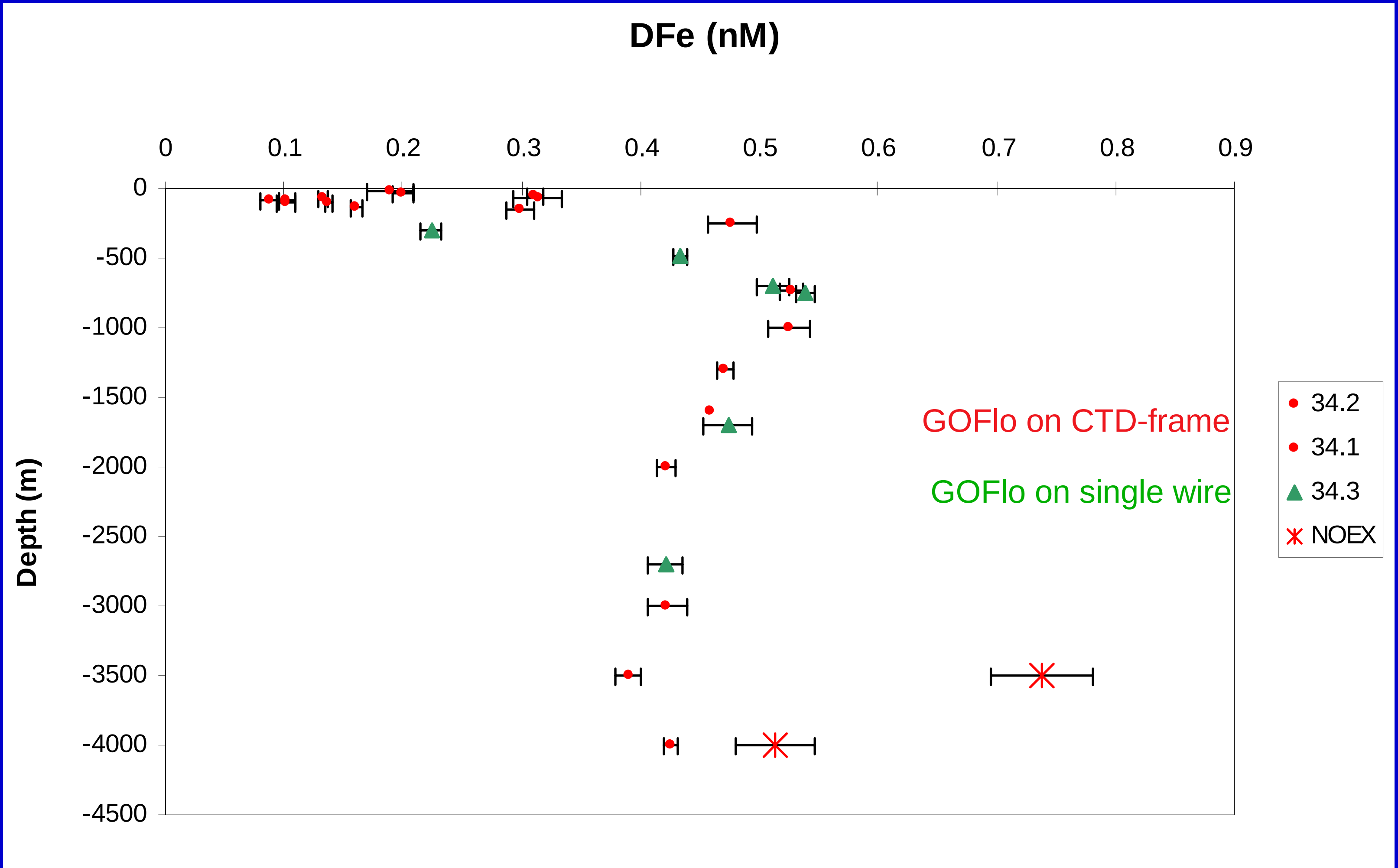


6. GEOTRACES (GEOSECS I I)

Epoxy-coated stainless steel prototype frame;
final type of titanium or carbon fibre, within own clean van


driver unit
pneumatics
Air tubes
link
GoFlo with
rotating
ball valves
NOEX
expanding
silicone closures

Routine deep profiling with ultraclean CTD frame and cable: allows GEOSECS II for trace elements

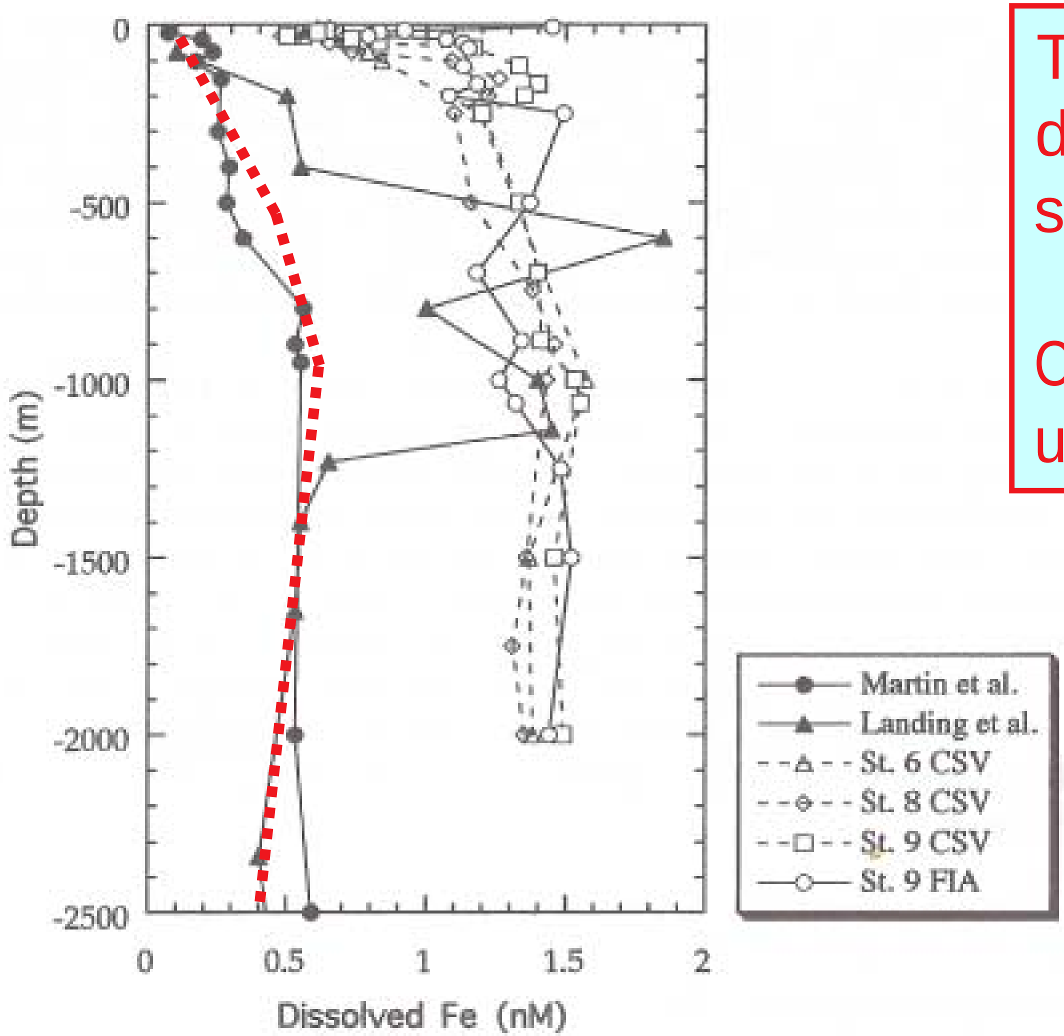


Geraldine Sarthou, Stephane Blain, Patrick Laan, Klaas Timmermans

October 2003 cruise IRONAGES-3 off West Africa

True and accurate dissolved Fe values still are puzzling.

Certified standard is urgently needed



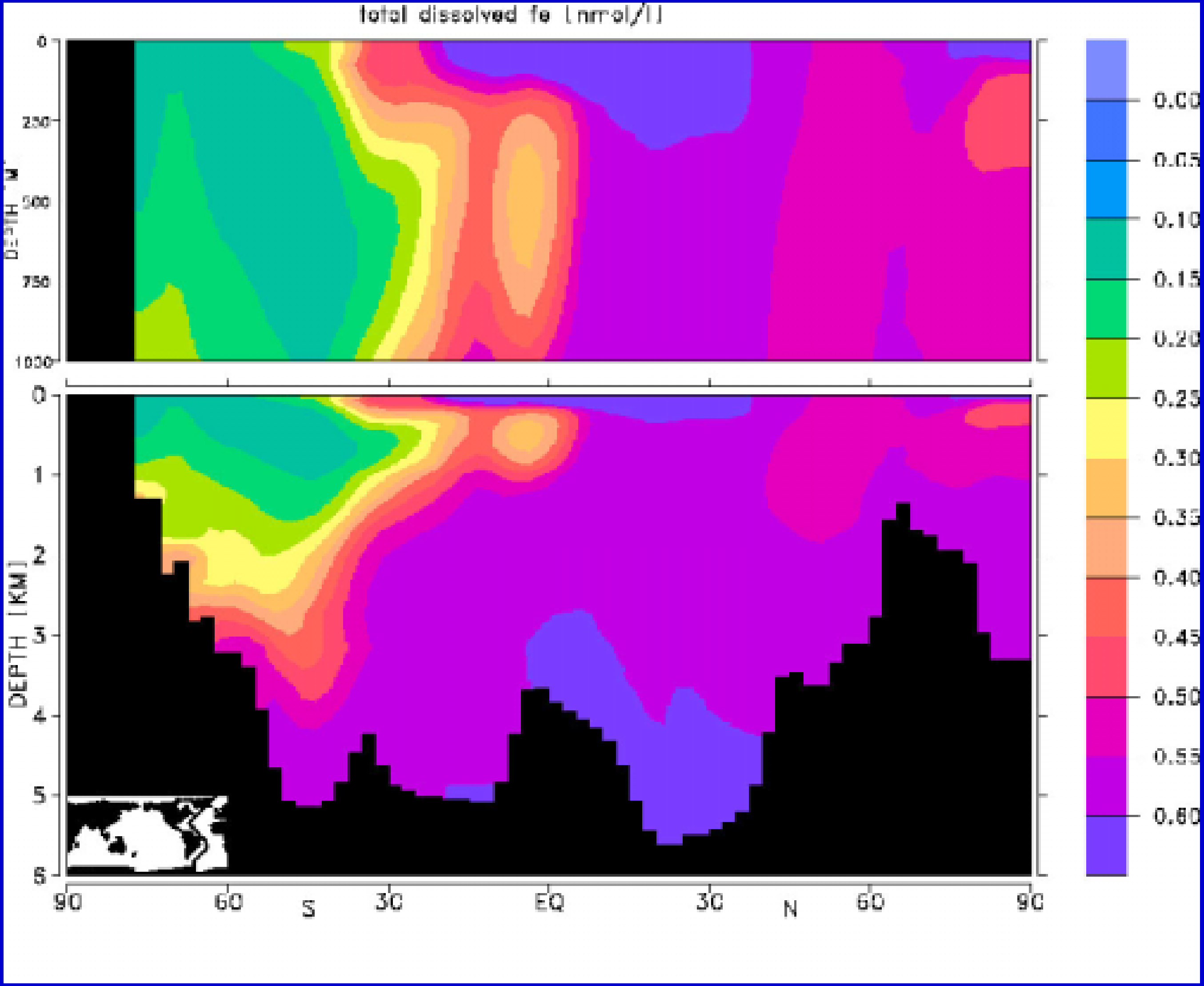
- outliers not shown
- IOC station

Figure 18. Vertical profiles of dissolved Fe at station 9 (40°N, 23°W) in the Northeast Atlantic Ocean. Duplicate analyses of total dissolved Fe by FIA-CL after acidifying to pH 2 (de Jong *et al.*, 2000) and dissolved Fe by CSV after UV digestion at pH 8 (Boyé, 2000) show good agreement. Also shown is dissolved iron at stations 6 (37°28.9′N, 22°58.7′W) and 8 (42°34.18′N, 23° 02.34′W) of Boyé (2000). For comparison dissolved Fe at 47°N, 20°W (Martin *et al.*, 1993a) and 34°N, 13°W (Landing *et al.*, 1995) are also shown. Drafted after Boyé (2000) and Boyé *et al.* (submitted (a)).

de Baar and de Jong (2001) Chapter in: Biogeochemistry of Iron in Seawater

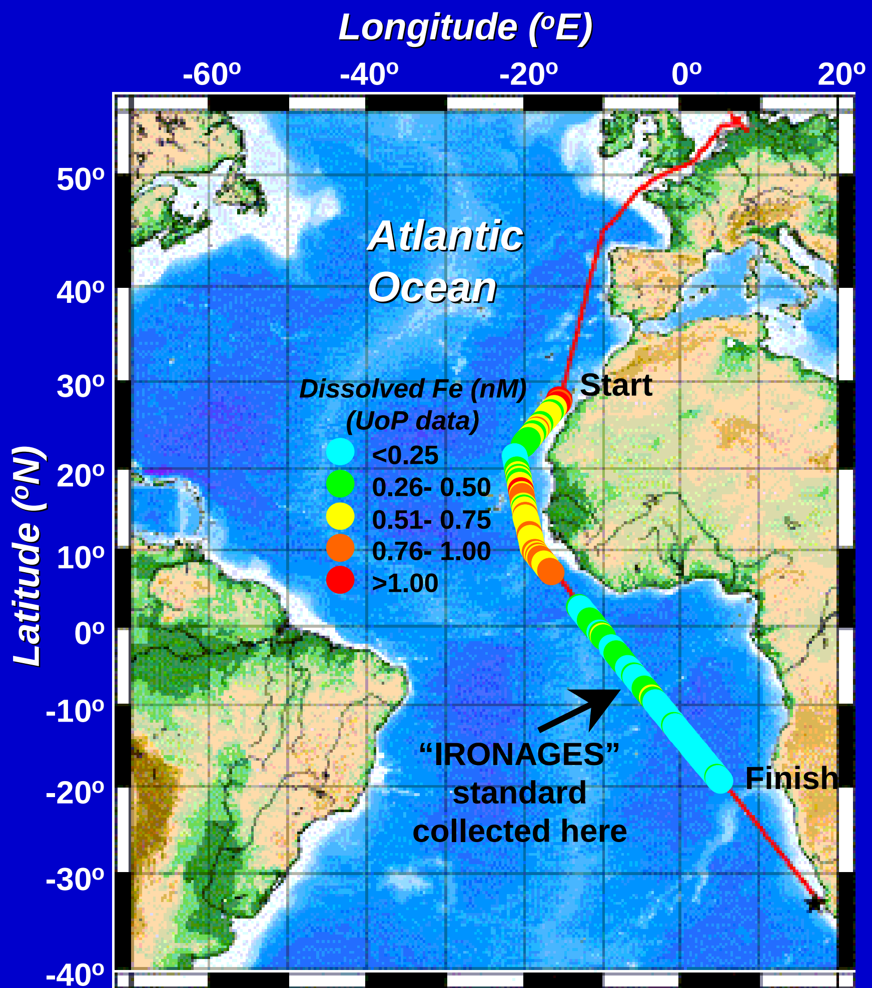
Atlantic Fe distribution in Hamburg model

Modelers are ready to go, but lack of good Fe data for validation

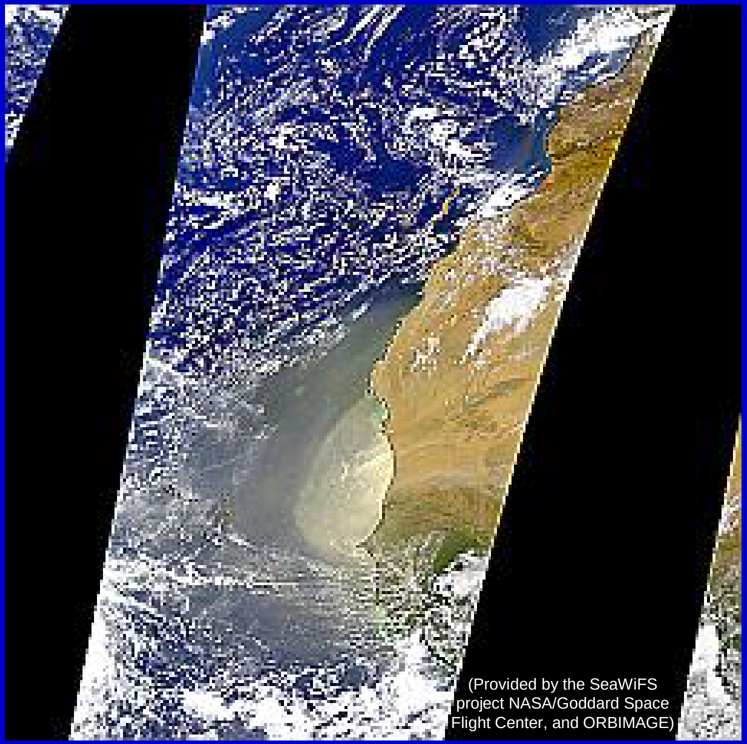


Six and Maier-Reimer, European Ironages project modeling

IRONAGES standard: collection



IRONAGES-1 Cruise, Sep 29th–Oct 23rd 2000



Dust storm on 25th Sept 2000 off Western Africa observed by SeaWiFS satellite

Analytical challenge - how to collect, preserve and distribute sea water samples for the preparation of a low level iron in sea water CRM?

Paul Worsfold, Nice 2003 lecture

IRONAGES standard: sampling

- 1000 l HDPE cubic tank
- Filled to 700 l over 8 h
- South Atlantic Ocean, 6.0°S 5.6°W
- Acidified to ~pH 2 using 700 ml Q-HCl
- Homogenised by gentle shaking of tank

Paul Worsfold, Nice 2003 lecture

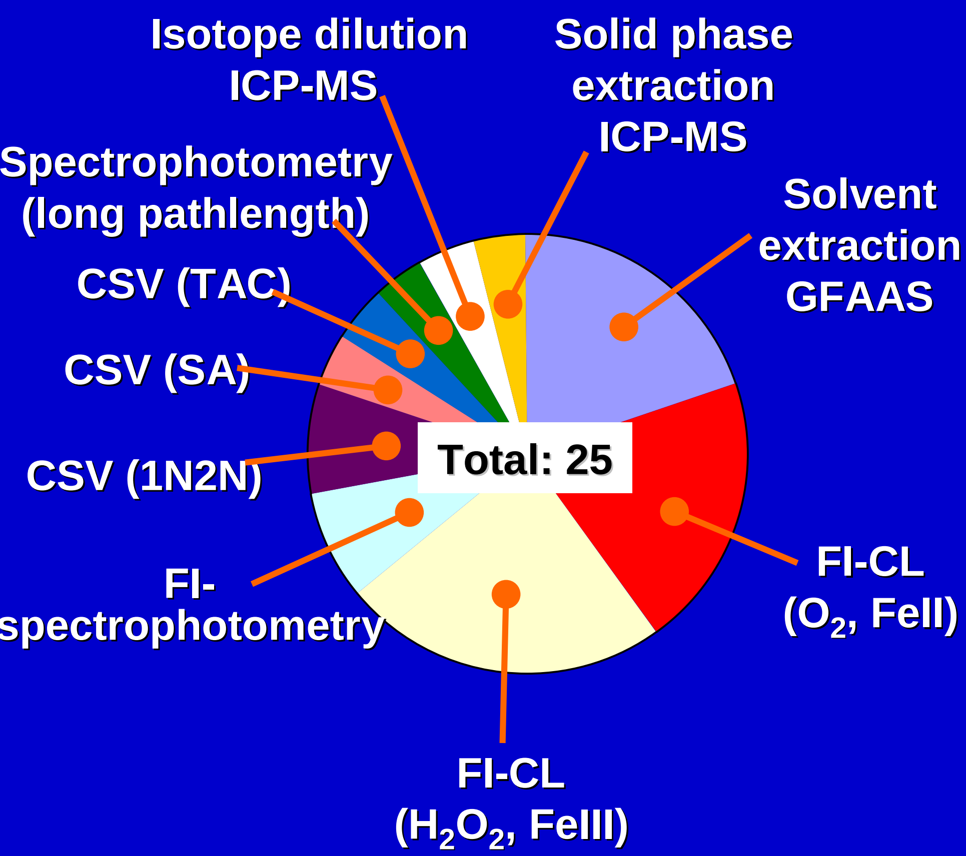
IRONAGES standard: bottling

- Transfer from tank to clean laboratory using Teflon FEP line and peristaltic pump
- 200 x 1 l LDPE bottles filled in two batches - 160 UoP & 40 NIOZ

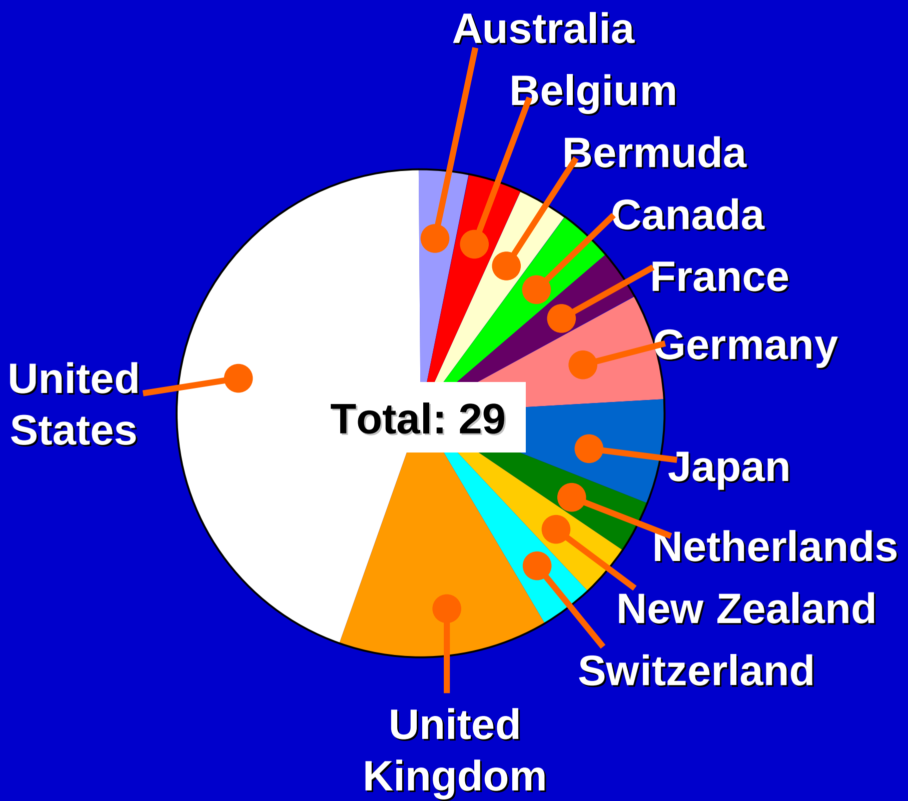
- Trials underway for:
  - homogeneity
  - time-series stability
  - sample storage
- Other bottles sent to 25 worldwide iron laboratories

Paul Worsfold, Nice 2003 lecture

IRONAGES standard: participants/methods



Analytical methods used during the IRONAGES exercise



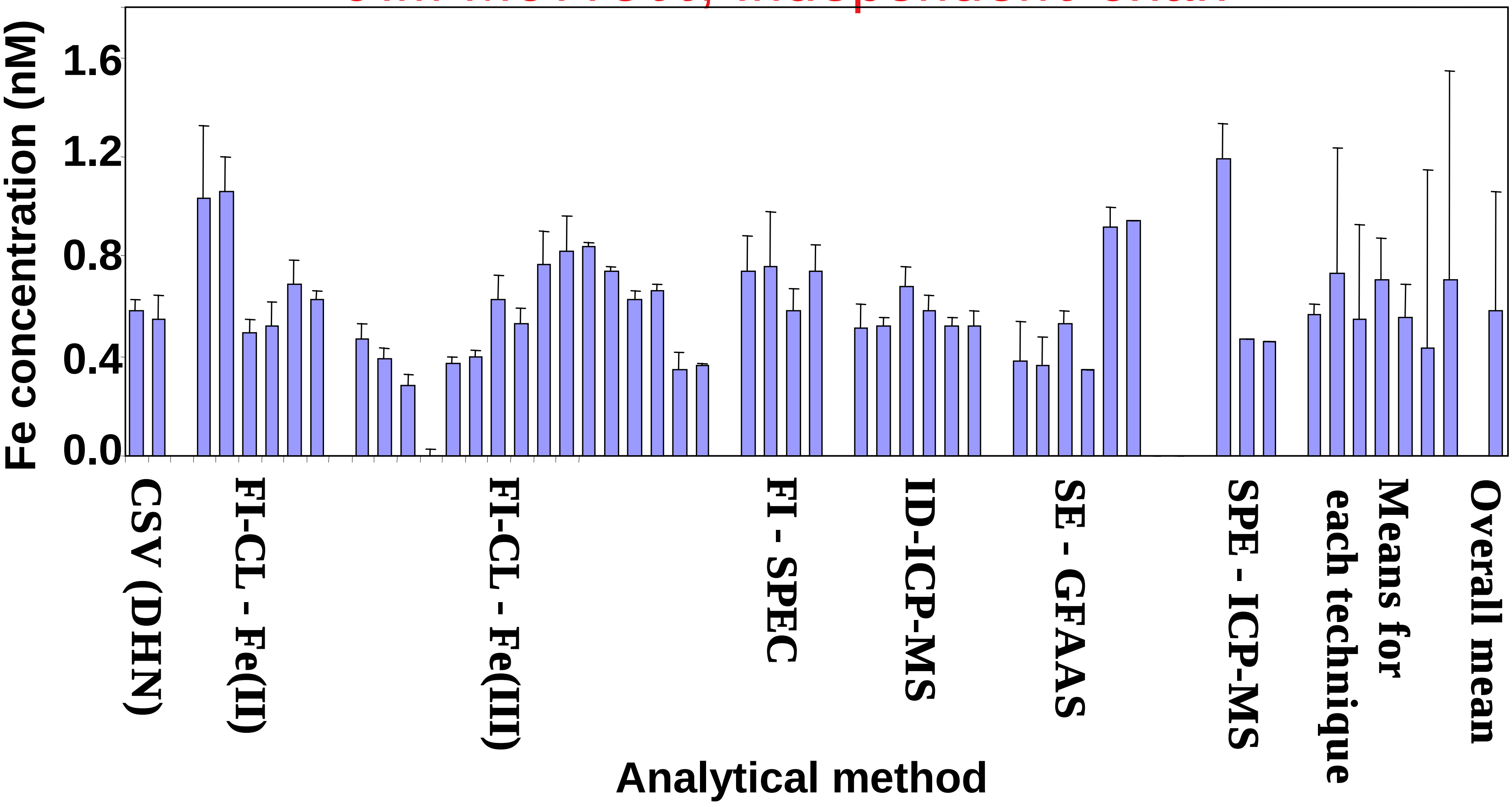
Laboratories participating in the "Ironcal" workshop, San Antonio, January 2000

Paul Worsfold, Nice 2003 lecture

Data courtesy of Andrew Bowie (University of Tasmania, Australia)

Laboratory data versus analytical method

Jim Moffett, independent chair



Paul Worsfold, Nice 2003 lecture

Towards GEOTRACES (GEOSECS II)

- Imbalance of ocean sciences
  - plenty modeling of the virtual ocean
    - armchair oceanography: cheap and easy
  - not much real data in real ocean
  - accuracy, certification, calibration is underfunded
- need for certified standards
    - **nitrate, phosphate, silicate**
    - **essential metals Fe, Mn, Zn, Co, Cd**

Summary

- Co-limitation is the rule
- Single limiting factor is exceptional
- Southern Ocean nice and simple
  - only light and Fe as two co-limitations
  - only two taxa: diatoms and Phaeocystis
- Oligotrophic central gyres
  - surface waters uncharted for all nutrients
  - NO3, PO4, SiO4 in nanomoles or picomoles ?
  - Fe, Mn, Zn, Co, Cu ?
  - seasonality of these nutrients ?
- New concepts beyond Liebig (1840 !) and M&M (1913 !) are needed
  - dynamics beyond steady state
  - co-limitations beyond single factor

The End

With many thanks for support by

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National Science Agencies (NWO, NERC, DFG, CNRS)

Our universities and institutes

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Royal Netherlands Institute for Sea Research

Europese Unie
European Union