

Co-Limitation of Phytoplankton by Light and Multiple Nutrients

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Royal Netherlands Institute for Sea Research



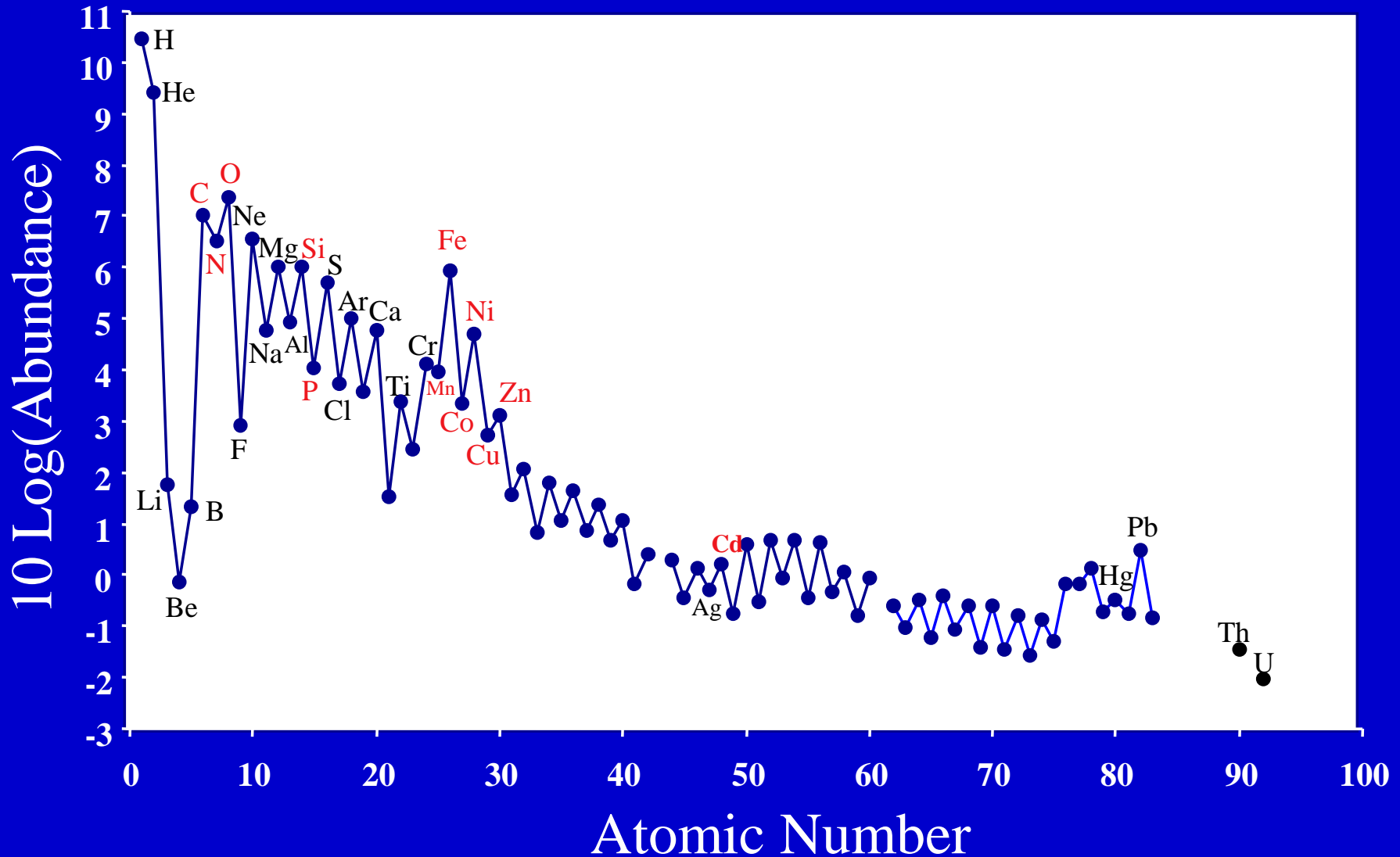
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European Union



Contents

- Building Blocks for Life
- Concepts of Limitation
- Observations in the Sea
- Growth Experiments
- Ironages
- GEOTRACES (GEOSECS II)
- Summary

Abundance of Chemical Elements



Major Bio-Elements

Abundances versus one million Si atoms

- Carbon • 10 x 10⁶
- Nitrogen • 3 x 10⁶
- Silicon • 1 x 10⁶
- Phosphorus • 1 x 10⁴
- Iron • 0.9 x 10⁶

Metals Abundance & Biological Evolution

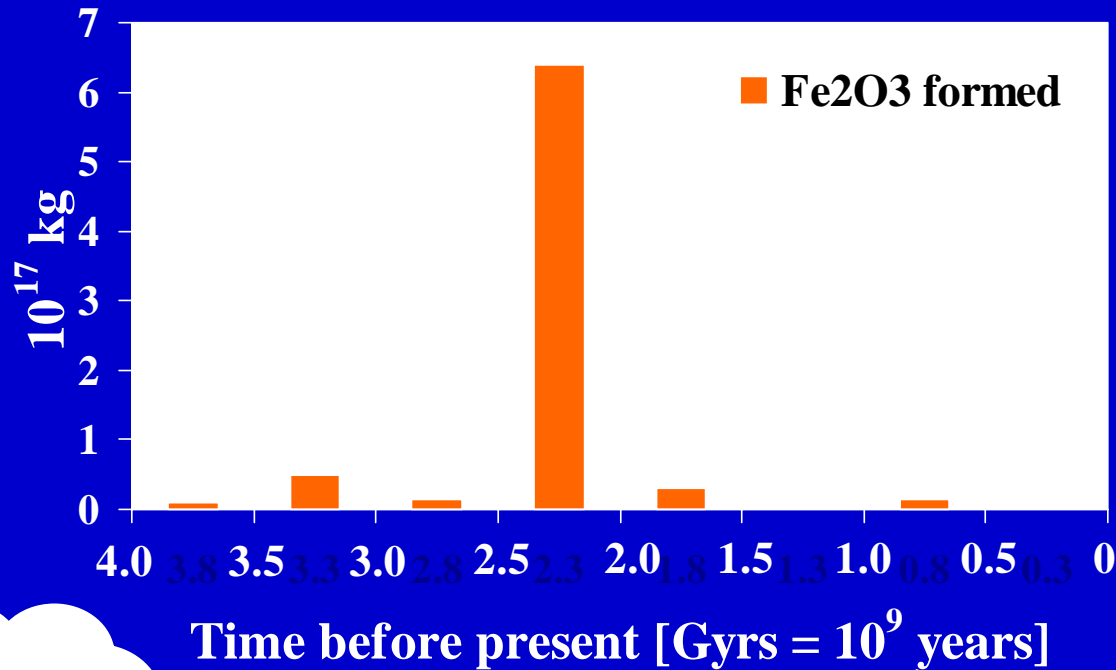
Mn 9550	Fe 900000	Co 2250	Ni 49300	Cu 522	Zn 1260	
				Ag 0.49	Cd ? 1.61	
					Hg 0.34	Pb 315

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



Dumb algae
took away their
own iron supply

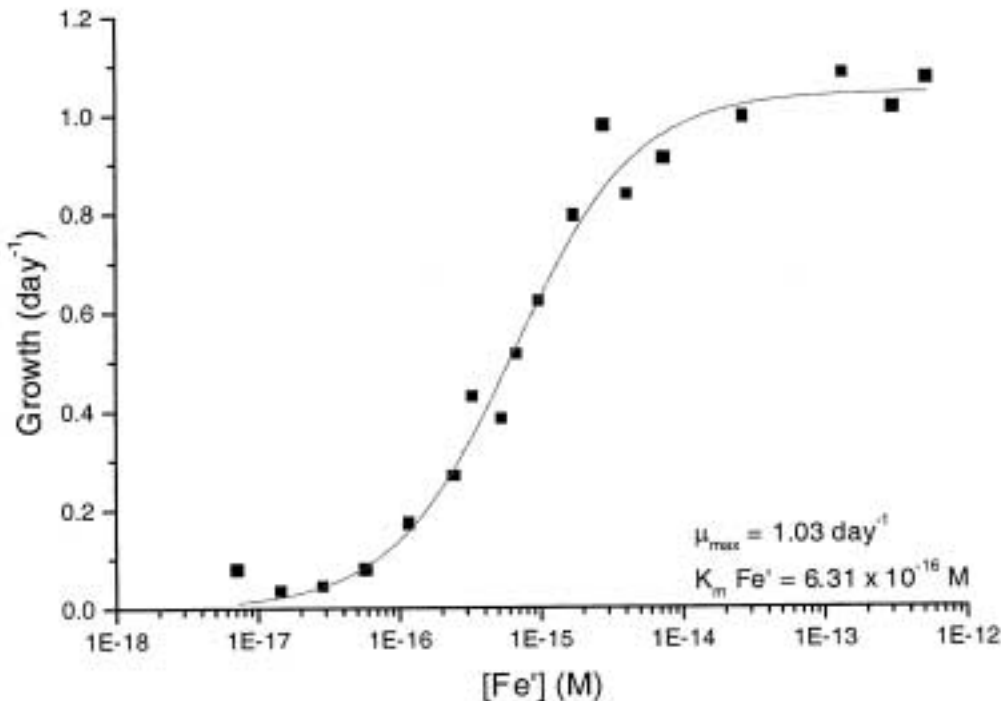
photosynthesis

oxygen in the air

2. Concepts of limitation

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

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, Hermann.



Emiliana huxleyi
in pristine natural seawater
driven into iron limitation by
siderophore addition

Timmermans et al., in prep.

Multiple Limitations in Real Ocean

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

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

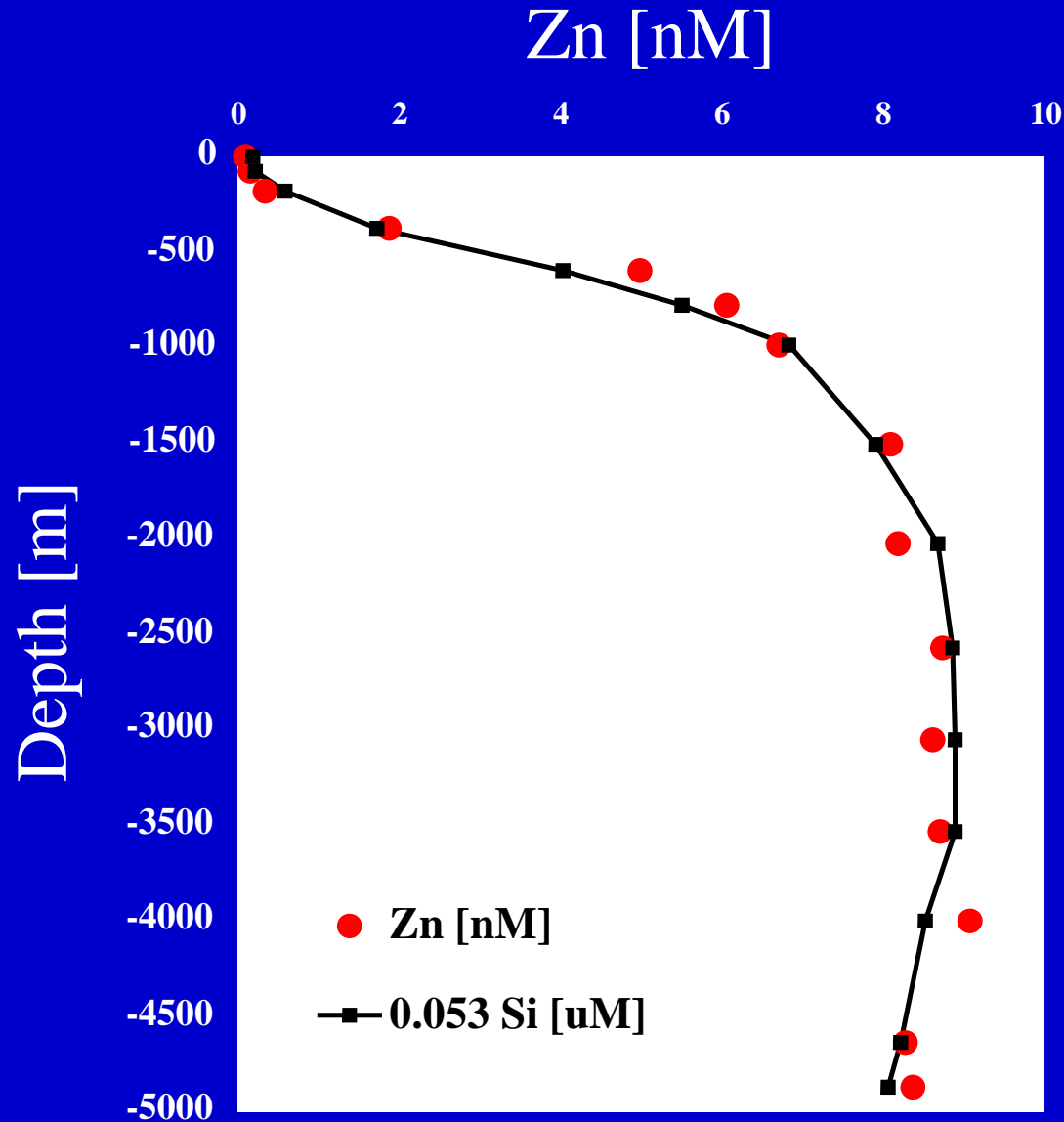
Some examples of interactions within the plant cell

- 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

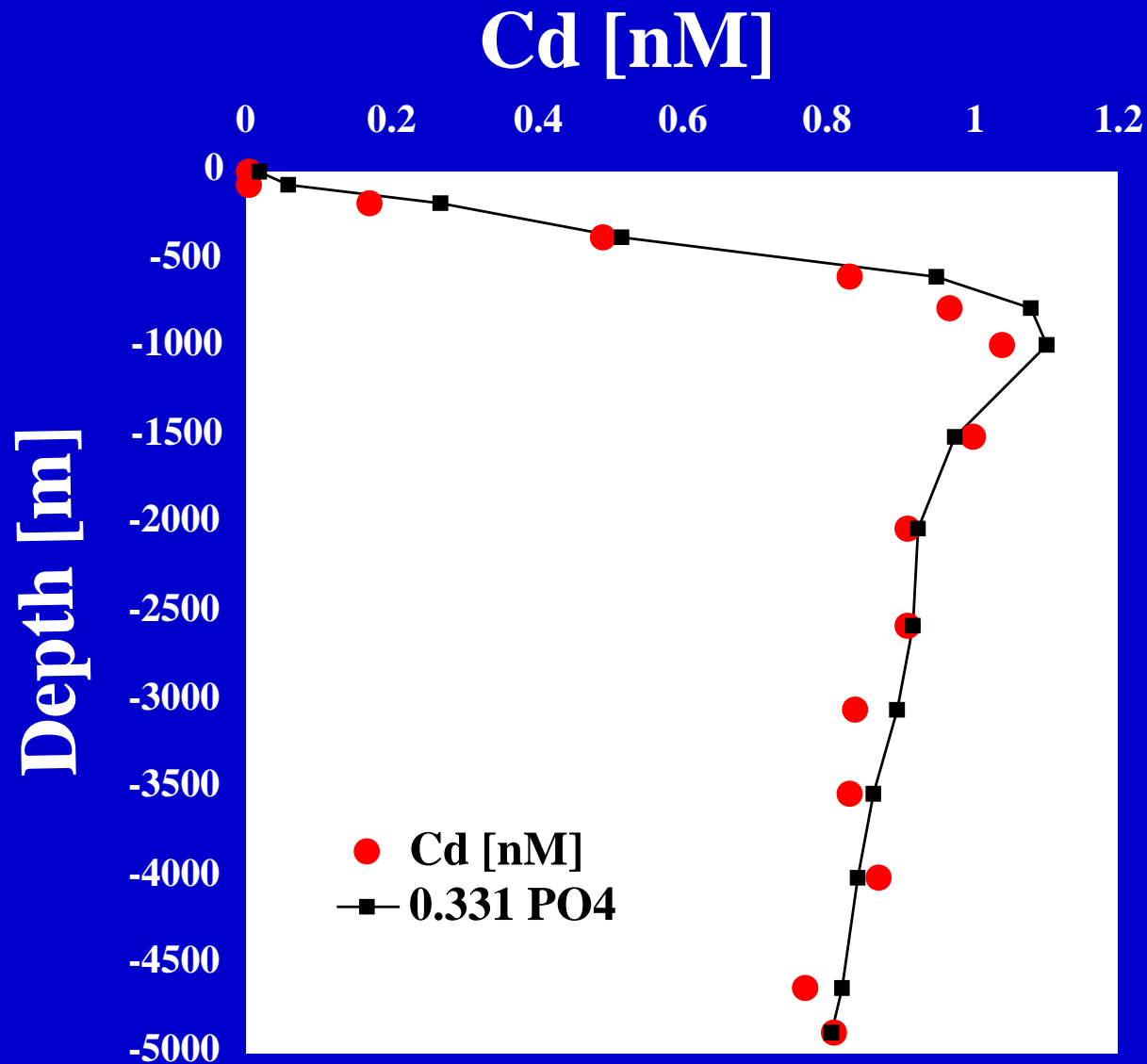
Zinc resembles Silicate



**North Pacific
Ocean
(33°N, 145°W)**

Bruland (1980)

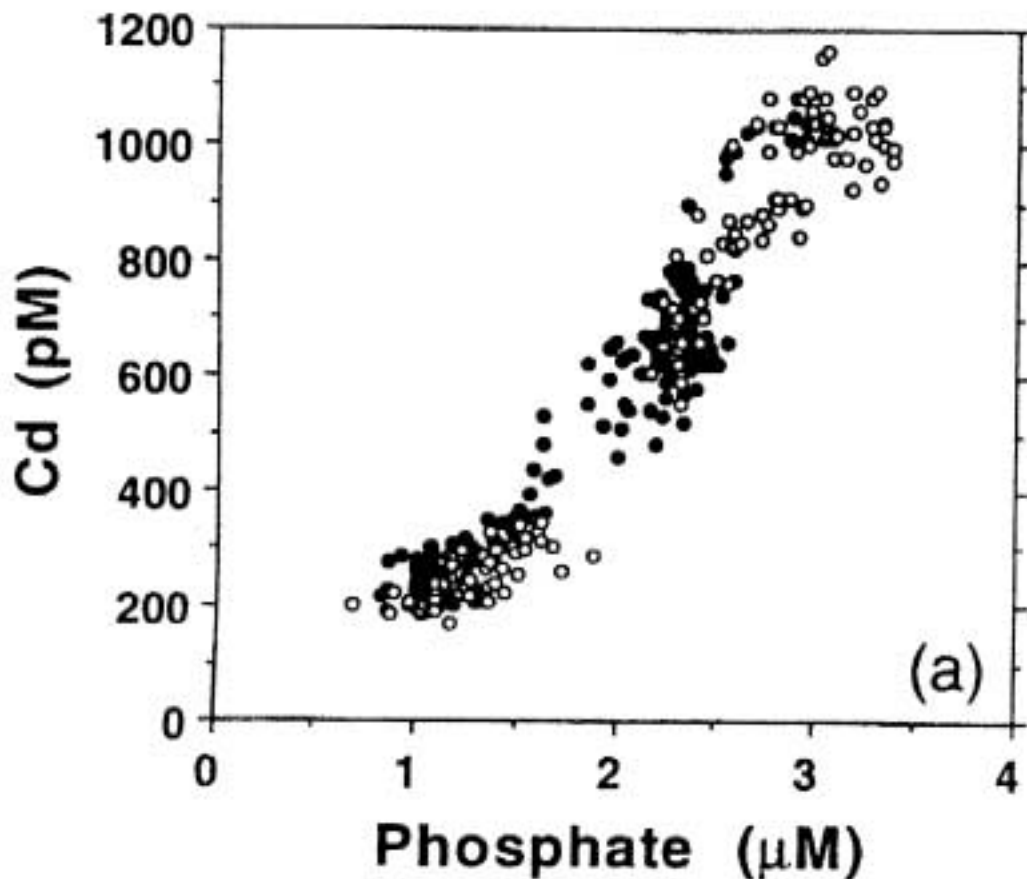
Cadmium resembles Phosphate



**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 $>1000\text{m}$ depth.

Open circles deBaar et al. (1994);

Filled circles new data Loscher et al (1998).

Biological function for Cd after all

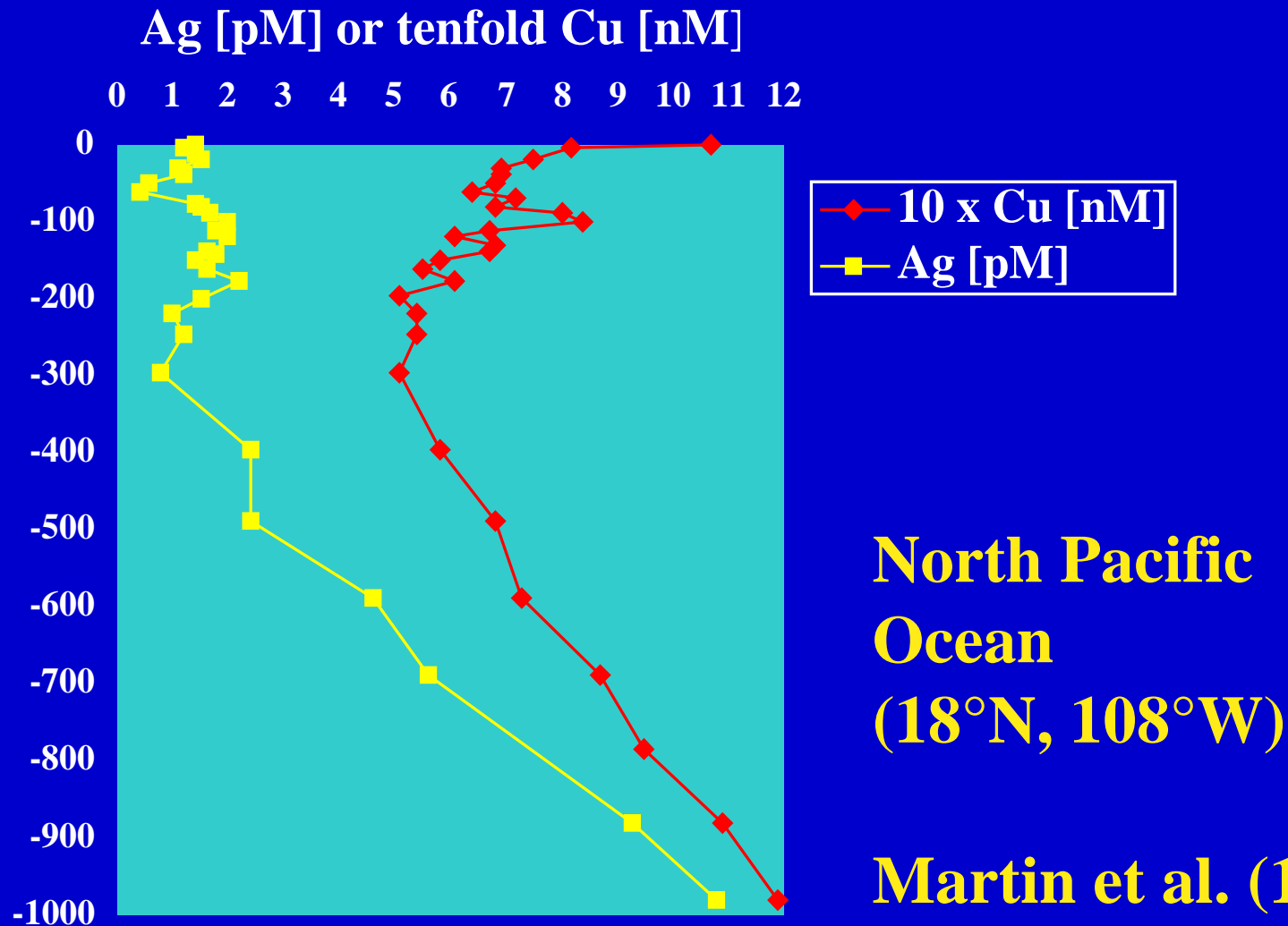
- Replacement of Zn by Cd in marine phytoplankton. Lee and Morel, Mar.Ecol.Prog.Ser., 127, 305-309, 1995
- A biological function for Cd in marine diatoms. Lane and Morel, Proc. Nat.Acad.Sci., 97, 4627-463, 2000

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

join the
Green
Party

Silver (Ag) resembles Copper (Cu)



Ag has better correlation with Si

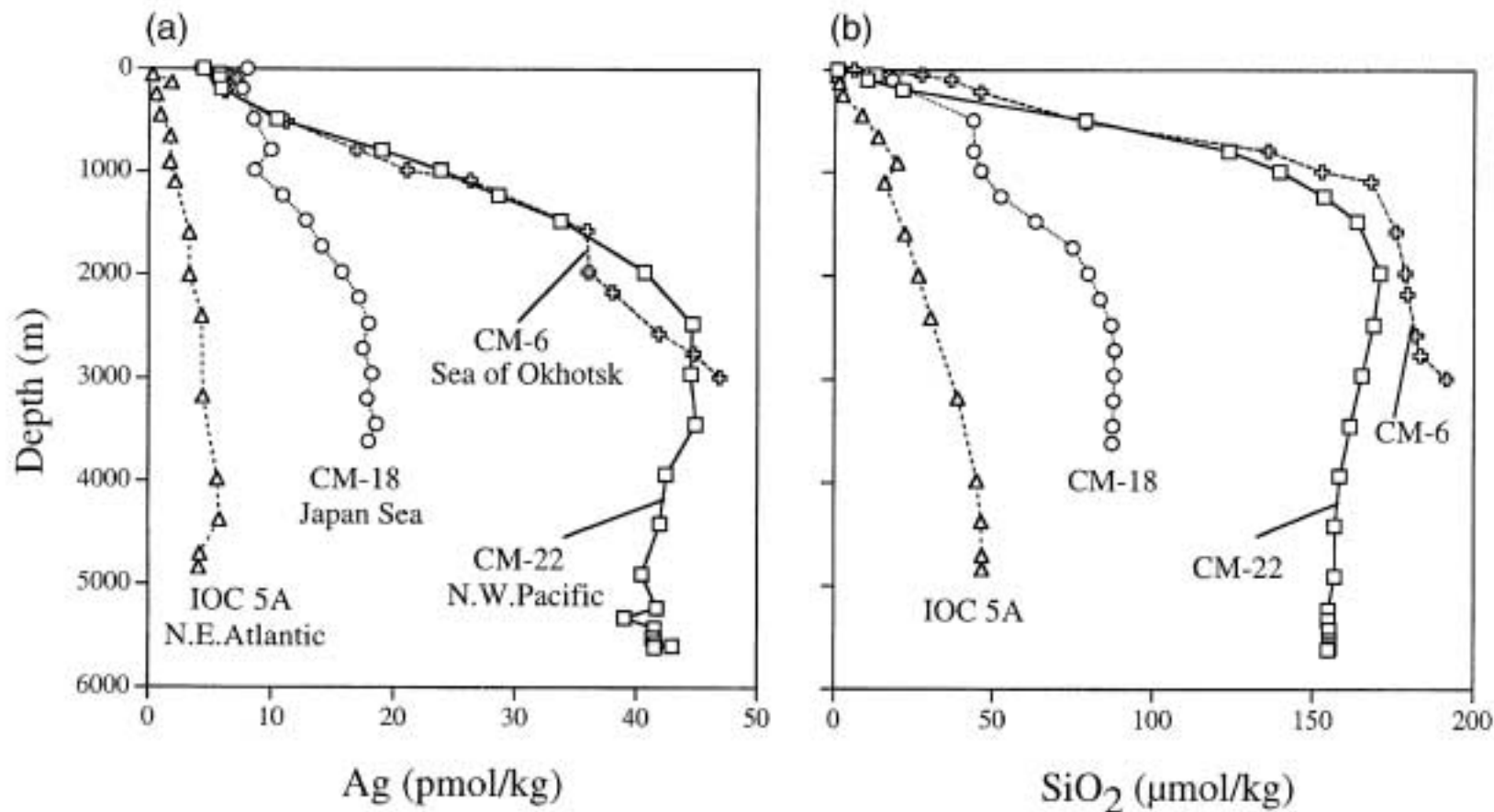


Fig. 5. The vertical profiles of dissolved Ag (a) and reactive Si (b) for different oceanic basins (the squares for the Northwest Pacific; circles for the Japan Sea; pluses for Sea of Okhotsk). The North Atlantic data indicated by triangles are based on the IOC station 5A (24°N, 23°W), after Fleaall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

Worldwide correlation Ag and Si

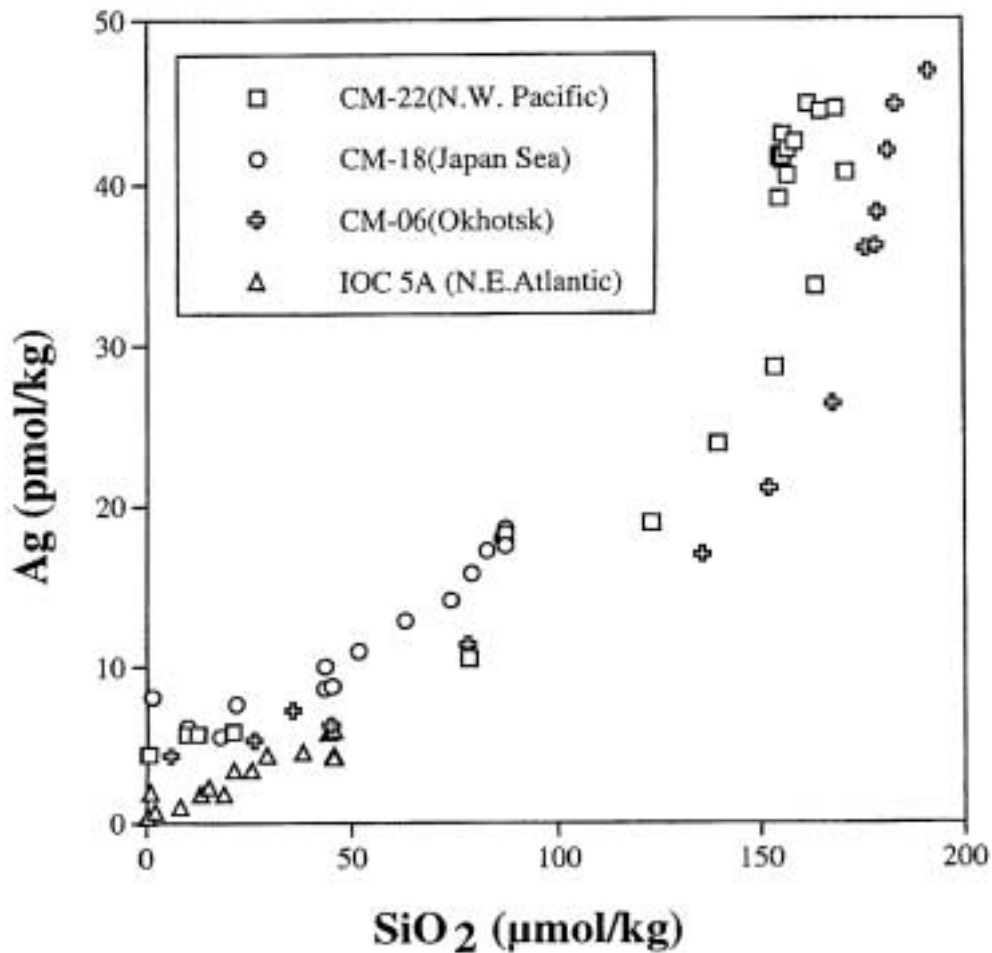


Fig. 6. Correlation between dissolved Si and Ag.

Ag/Si ratio
increases from
 $\sim 1.2 \cdot 10^{-6}$ in Atlantic
to
 $\sim 2.7 \cdot 10^{-6}$ in Pacific

Fractionations Cu/Ag and Zn/Cd

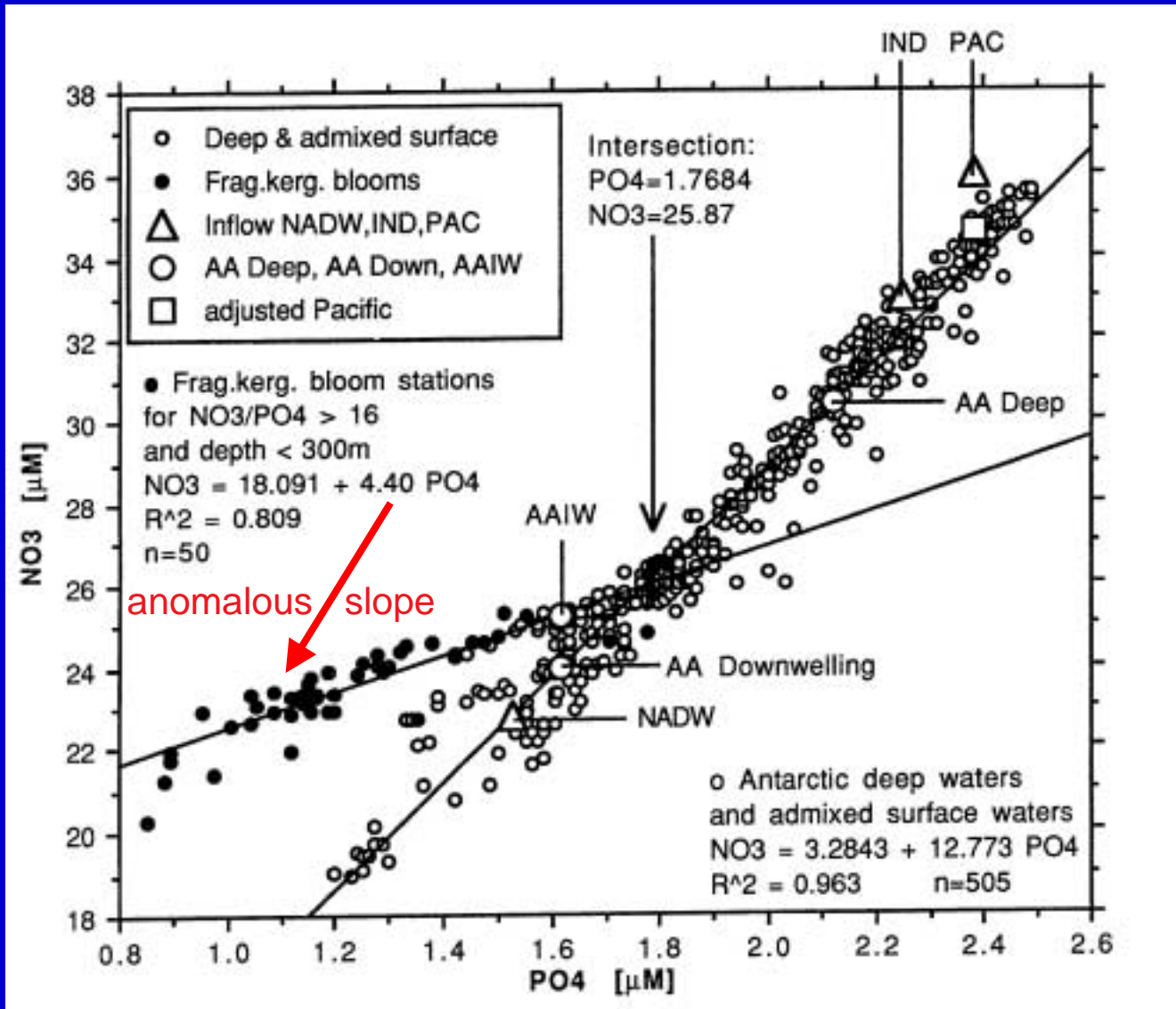
Periodic Table	Group 1b Cu /Ag	Group 2b Zn / Cd
Crustal abundance ratio	~1060	~780
Oceanic waters ratio	~8 <u>±</u> 3	~91
Fractionation factor	~130	~8.6

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





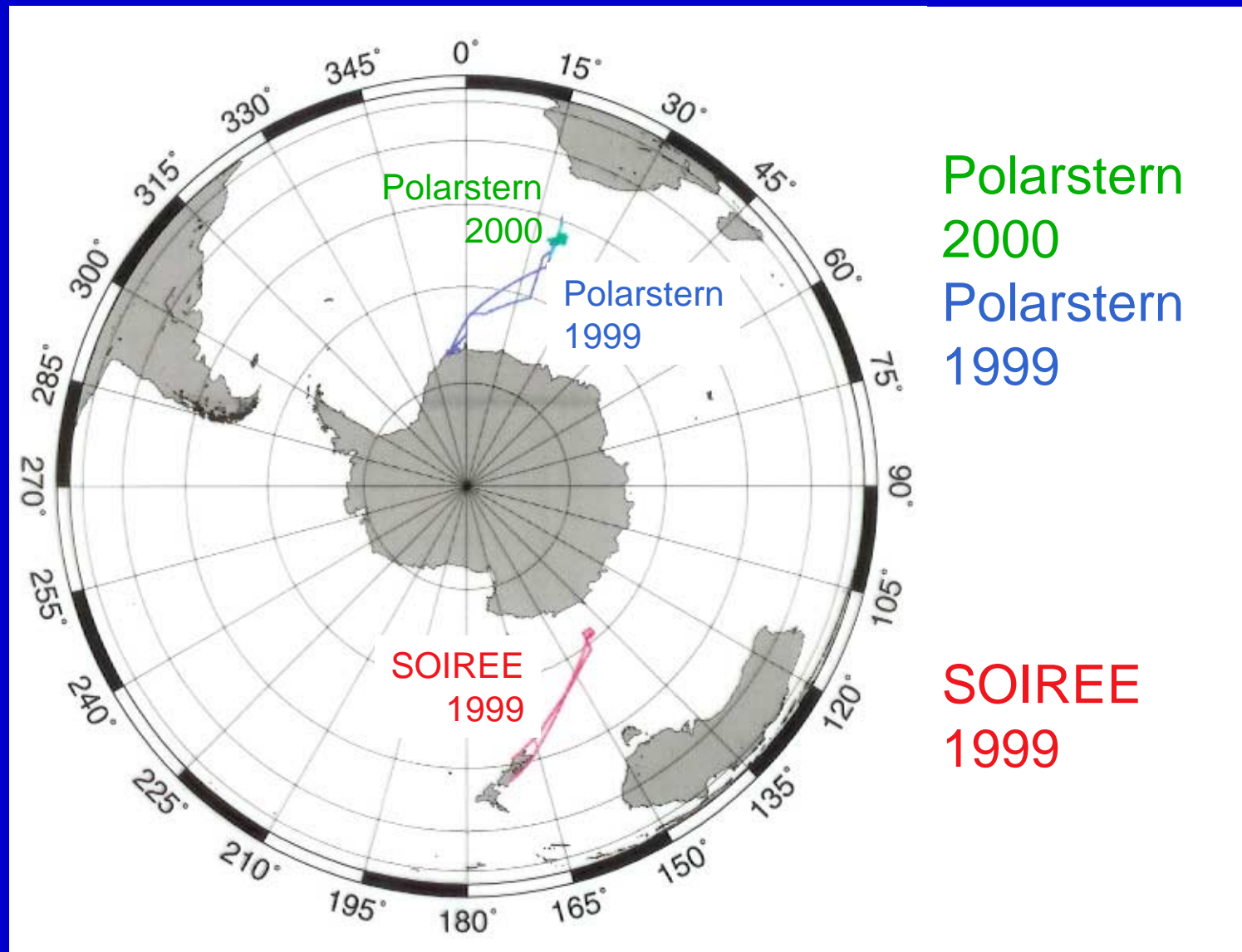
Frigilariopsis kerguelensis with heavily silicified armor 'pantzer'

More Fe co-limitations major nutrients

<u>Study</u>	<u>Fe-deplete</u>	<u>Fe-replete</u>
Southern Ocean (Takeda, 1998) plankton community	Si/N=2.3 N/P = 12	Si/N = 0.95 N/P = 14
Chaetoceros dicaeta	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 = 2.7 Si/N = 3.0	Si/N = 0.8 Si/N = 1.0 Si/N = 1.0

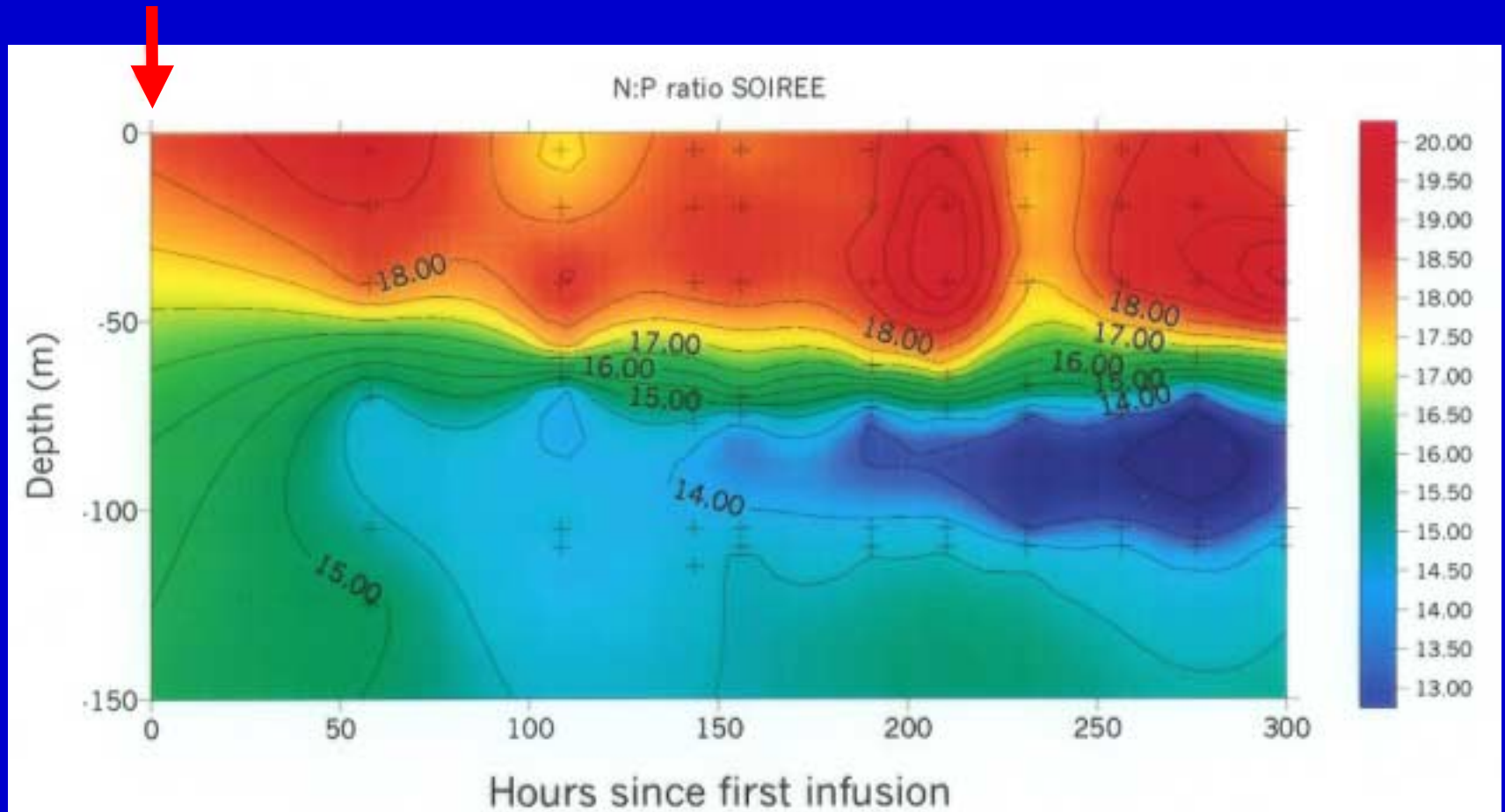
<u>Uptake by blooms in Ross Sea</u>	<u>Diatoms</u>	<u>Phaeocystis</u>
Arrigo et al. (1999)	N/P = 9.5	N/P = ~19

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



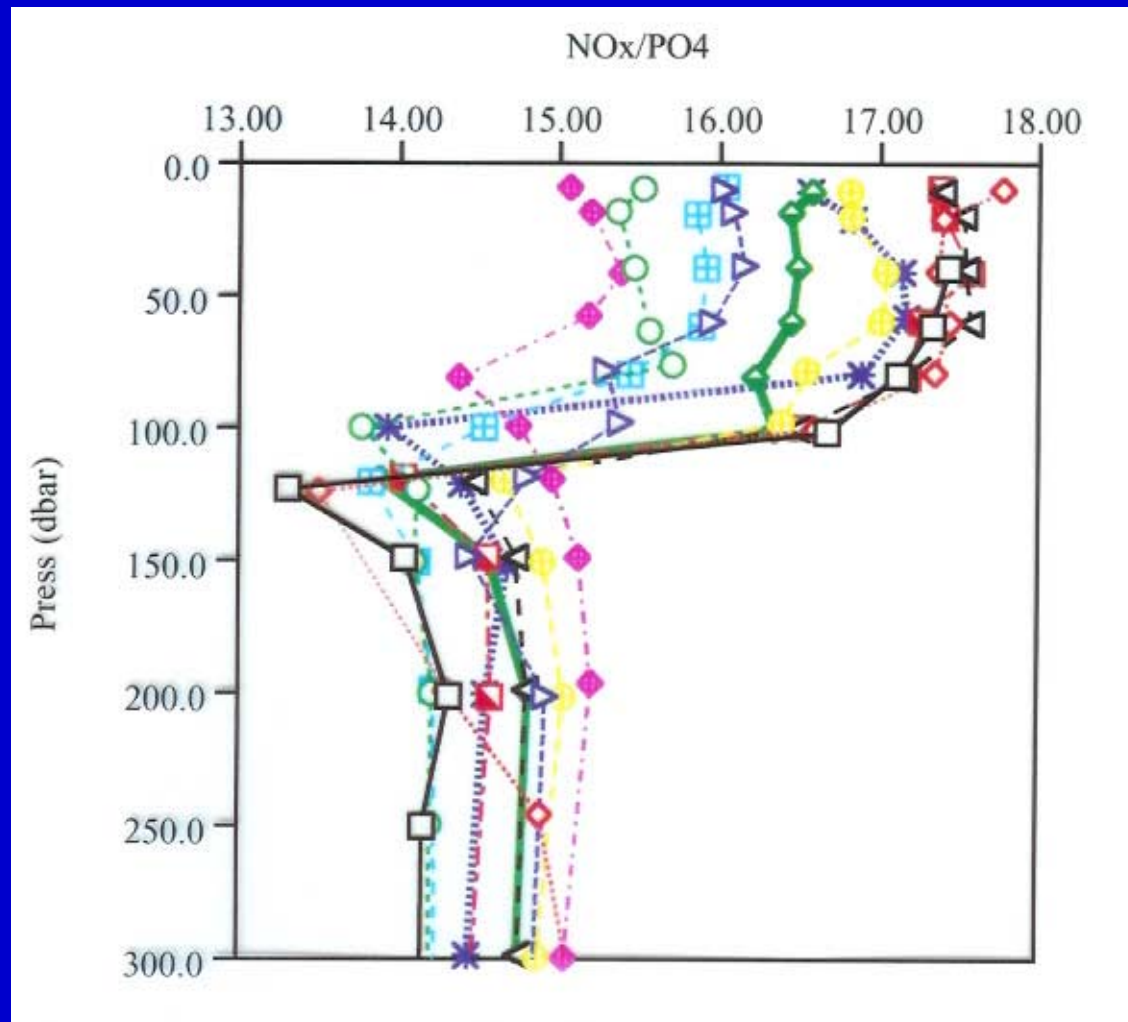
February 1999 SOI REE Nutrient Anomalies: *Fragilariopsis kerguelensis* strikes again

end of bloom season

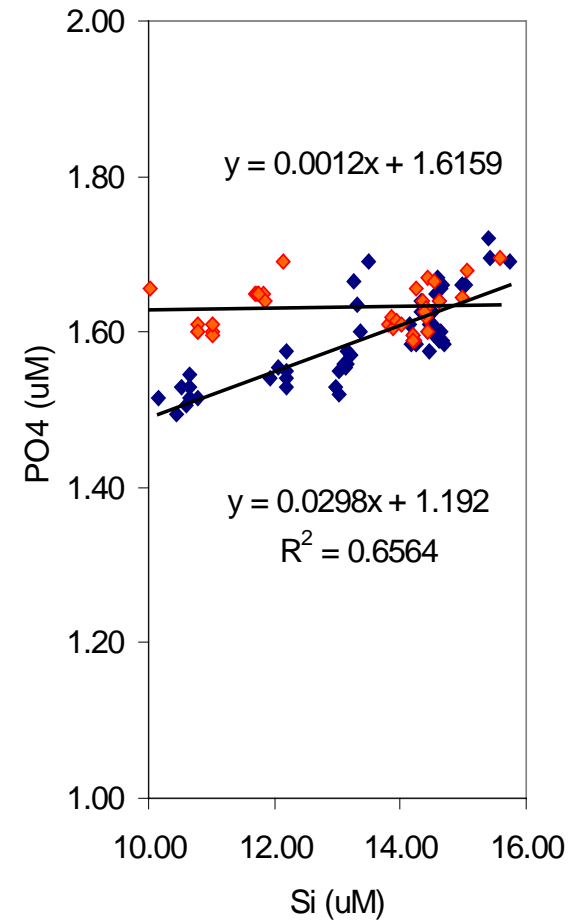
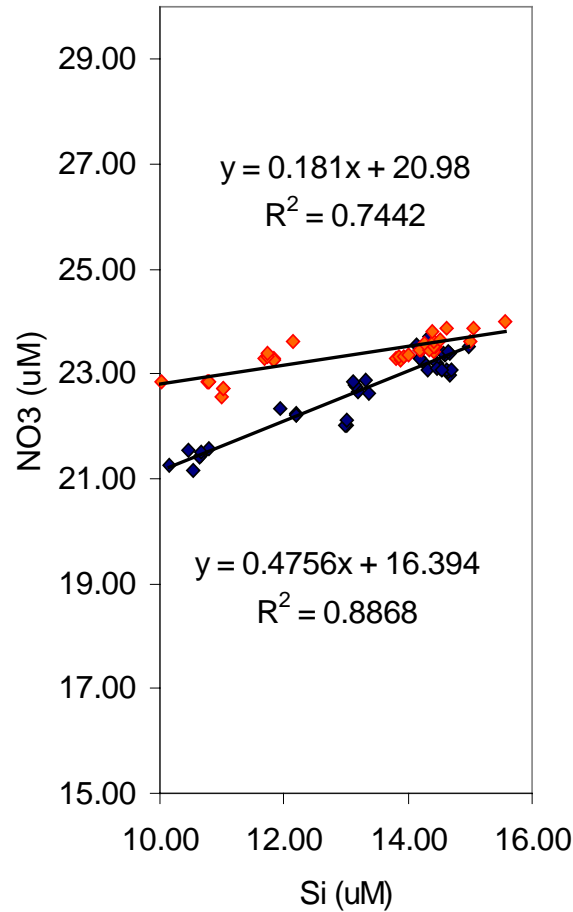
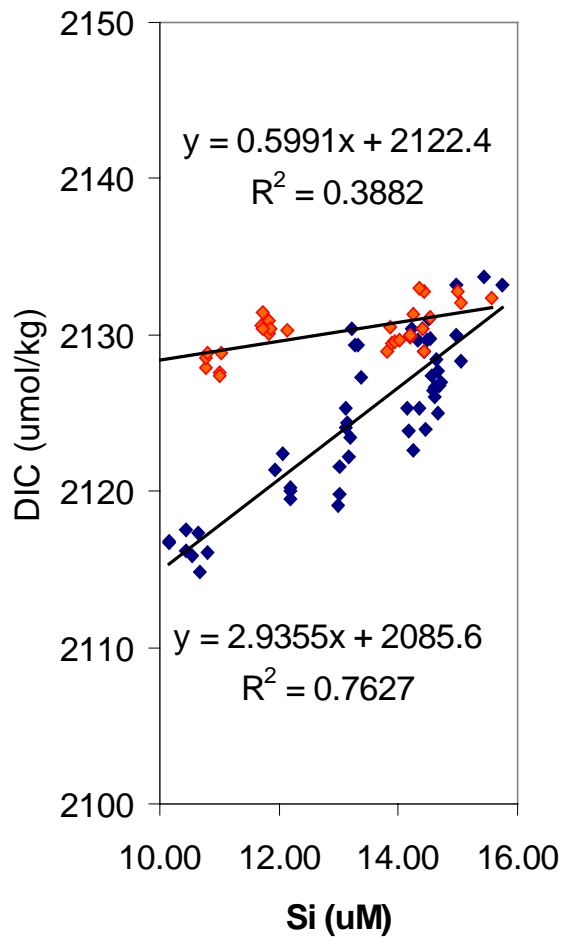


Nutrients data courtesy Stuart Pickmere, NIWA, New Zealand

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



Polarstern (2000) *in situ* Fe enrichment



◆ DIC/Si-inside ◆ DIC/Si-outside

◆ NO₃/Si inside ◆ NO₃/Si-outside

◆ PO₄/Si-inside ◆ PO₄/S-outside

Polarstern (2000) *in situ* Fe enrichment

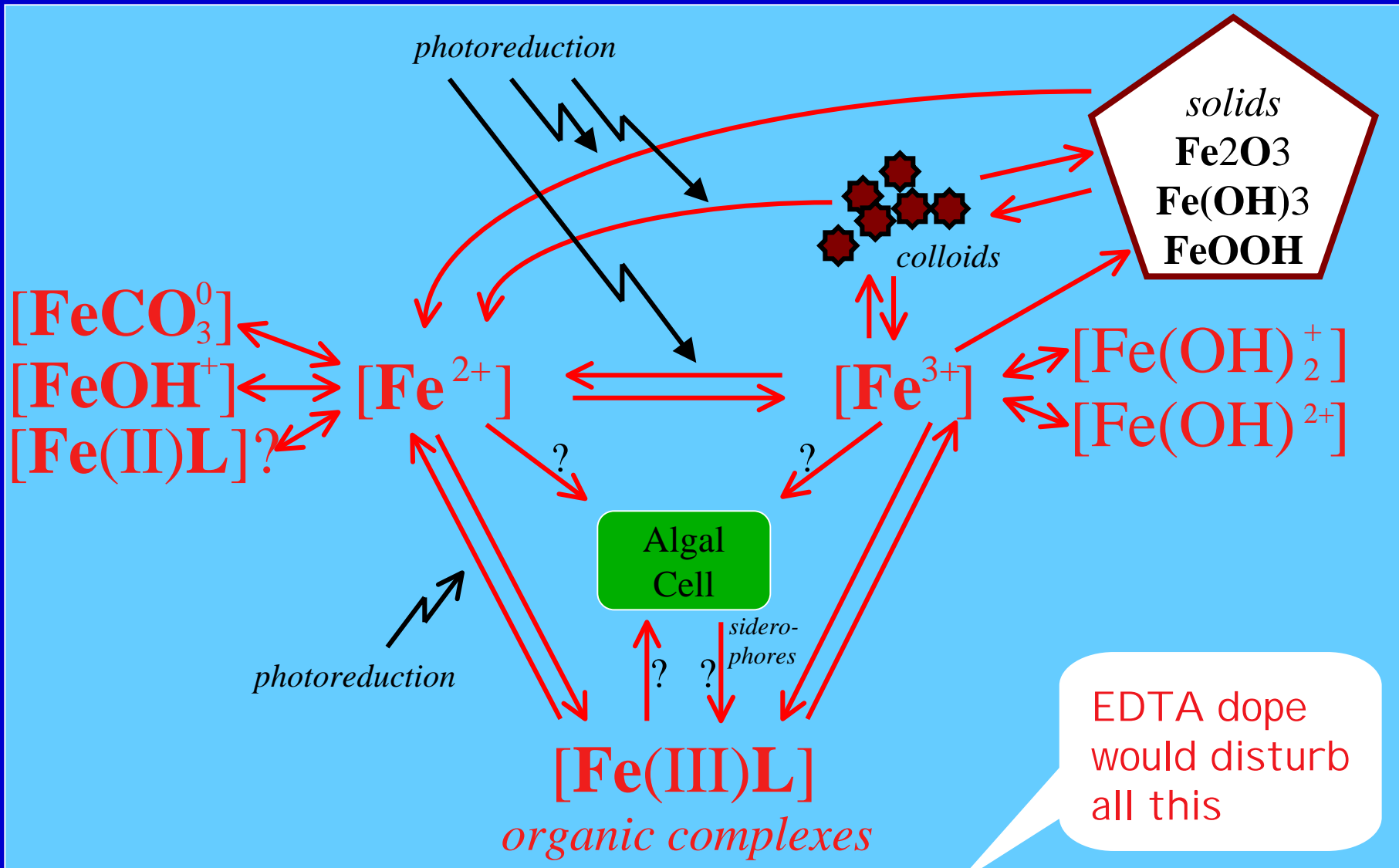
	Polarstern (2000)	Ironex II (1994)	Redfield (1934)	Takeda (1998)	
				-Fe	+Fe
$\Delta C/\Delta P$	82	90 + 5	106		
$\Delta C/\Delta N$	5.9	6.2 + 0.2	6.6		
$\Delta N/\Delta P$	12	14.3 + 0.2	16	12	14
$\Delta C/\Delta Si$	2.9	5.1 + 0.3			
$\Delta Si/\Delta N$	2.1			2.3	0.9
	in the patch plankton community	in the patch plankton community (Steinberg & Millero, 1998)		in bottles plankton community	

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

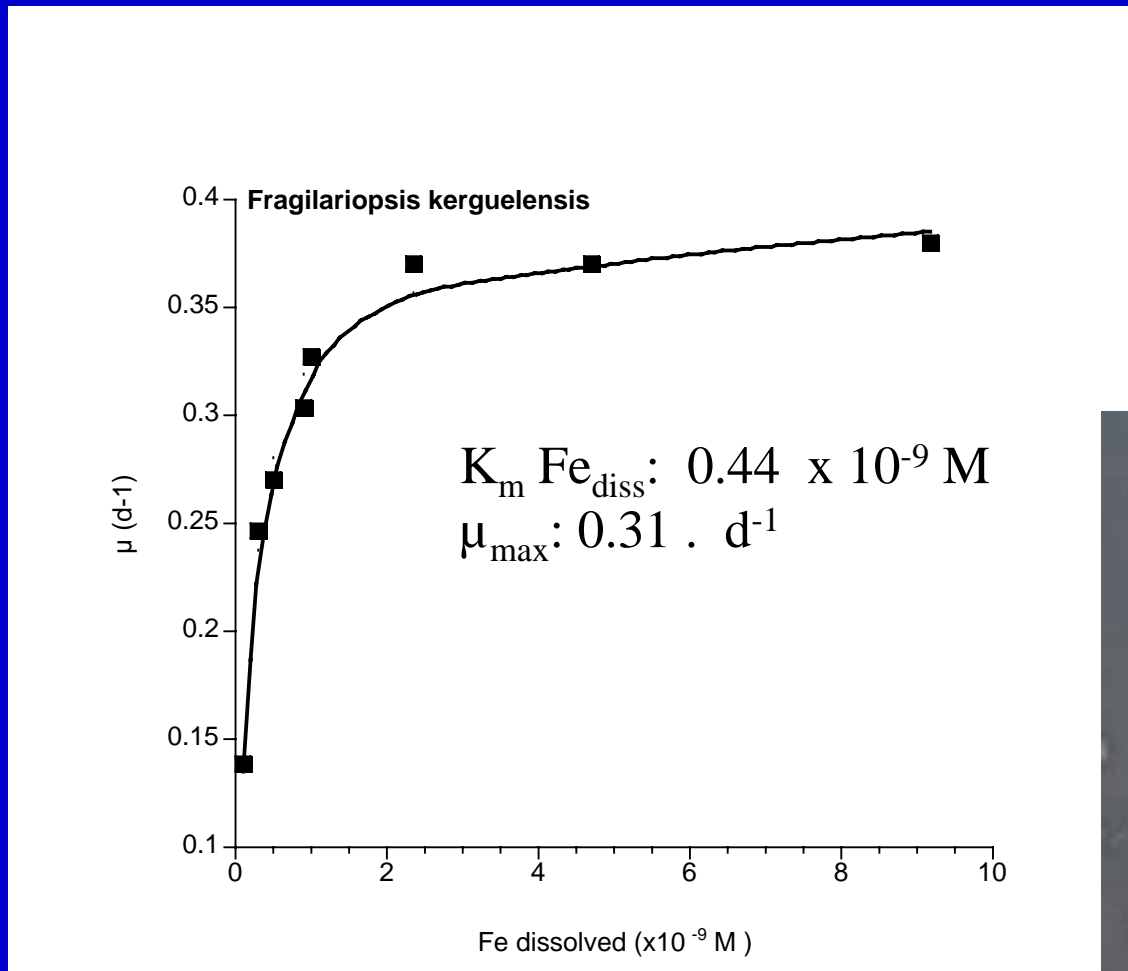
4. Growth Experiments

- Pristine natural seawater medium
- *Fragilariopsis kerguelensis*
- Diatoms are Forever
 - light & Fe co-limitation
 - small versus large *Chaetoceros sp.*
- Zn-HCO₃ co-limitation *Emiliana huxleyi*

Different forms of Fe in seawater



Fragilariopsis kerguelensis in natural Antarctic seawater



80 μm



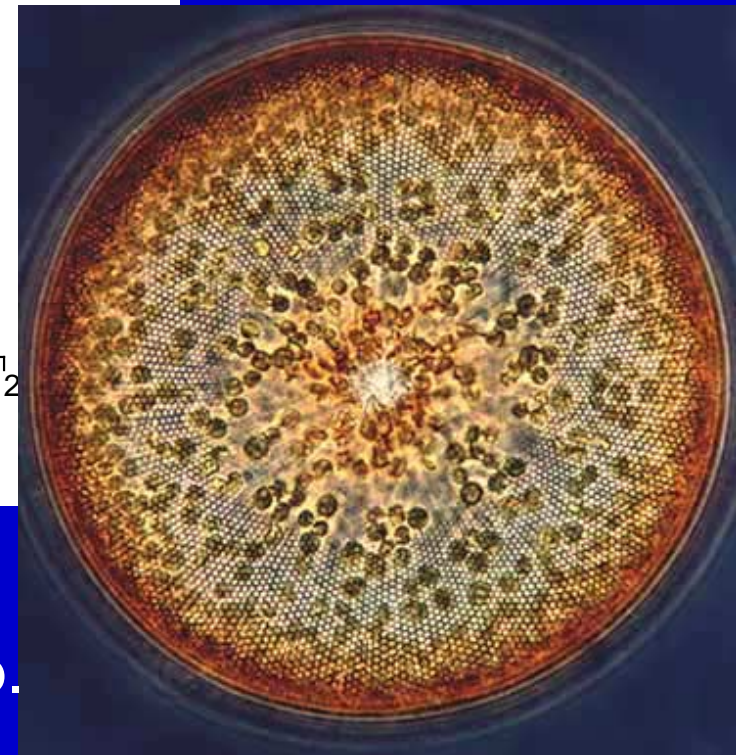
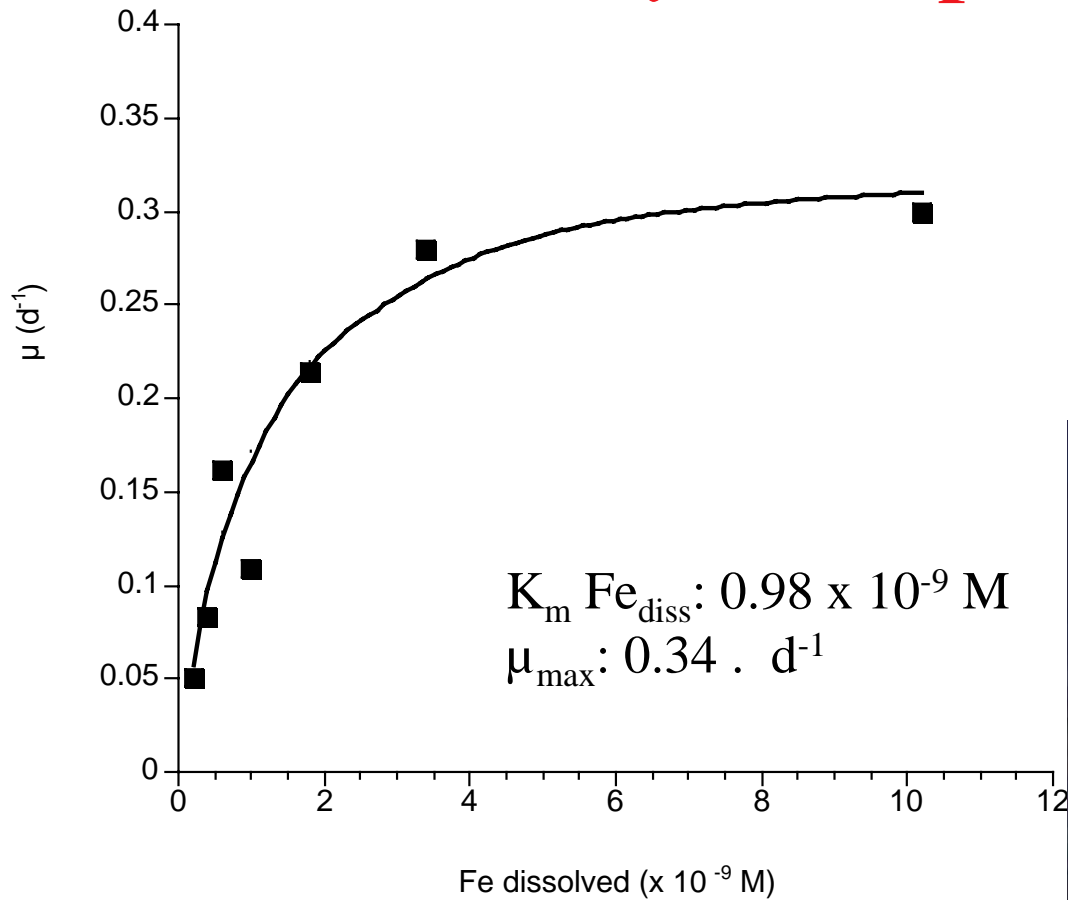
Timmermans, van der Wagt, de Baar, in prep.

Nutrients Stoichiometry of *Fragilariopsis kerguelensis*

<u>Ratio</u>	<u>Southern Ocean</u>		<u>Incubations</u>	
	<u>Fe-deplete</u>	<u>Fe-deplete</u>	<u>Fe-deplete</u>	<u>Fe-replete</u>
Si : N			7.7	2.5
N : P	$\sim 5 \pm 1$		$\sim 5 \pm 1$	$\sim 12 \pm 2$

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

Actinocyclus sp.





Elemental composition in relation to Fe_{diss}

mol per liter cell volume

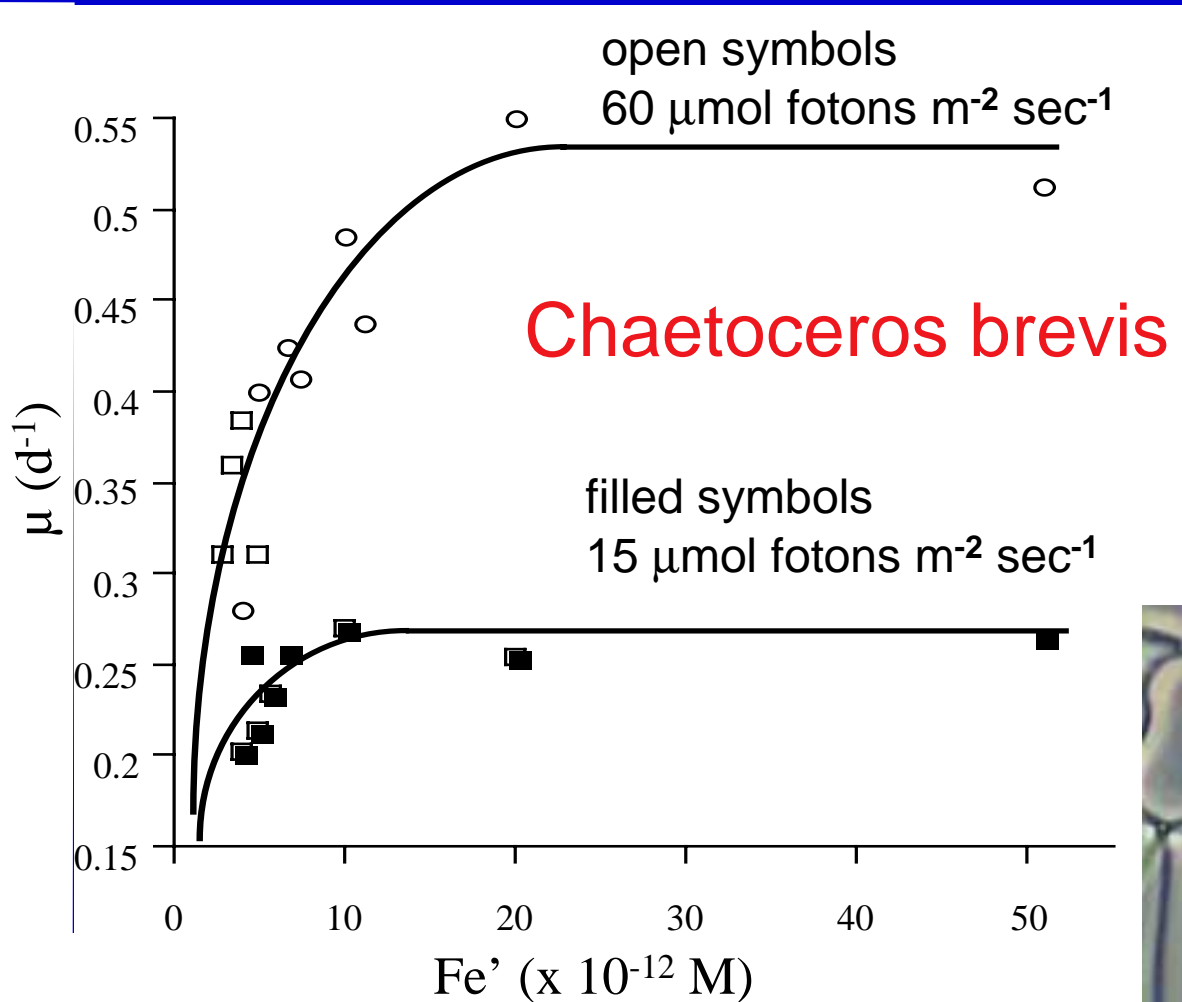
Actinocyclus sp.

Fe_{diss} ($\times 10^{-9}$ M)	Si	N	P	<u>Si : N</u>	<u>N:P</u>
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

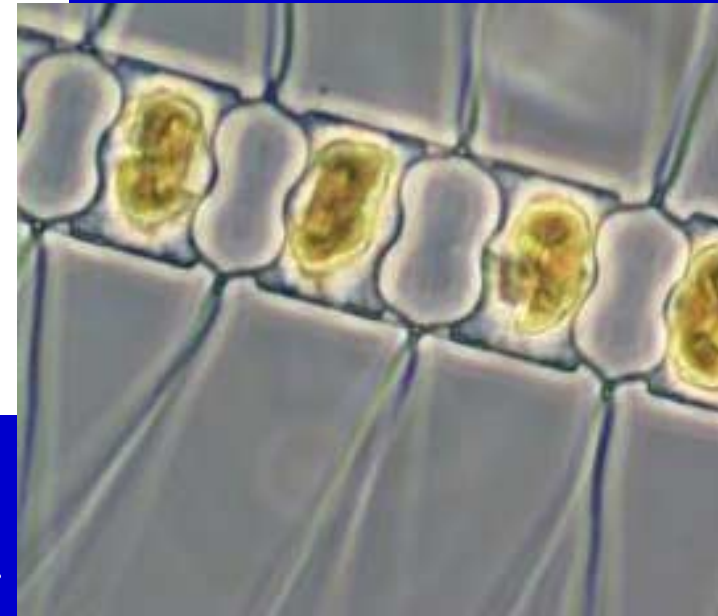


Klaas Timmermans et al., in prep.

Light and Fe co-limitation



single cells
4 - 6 μm diameter (small)

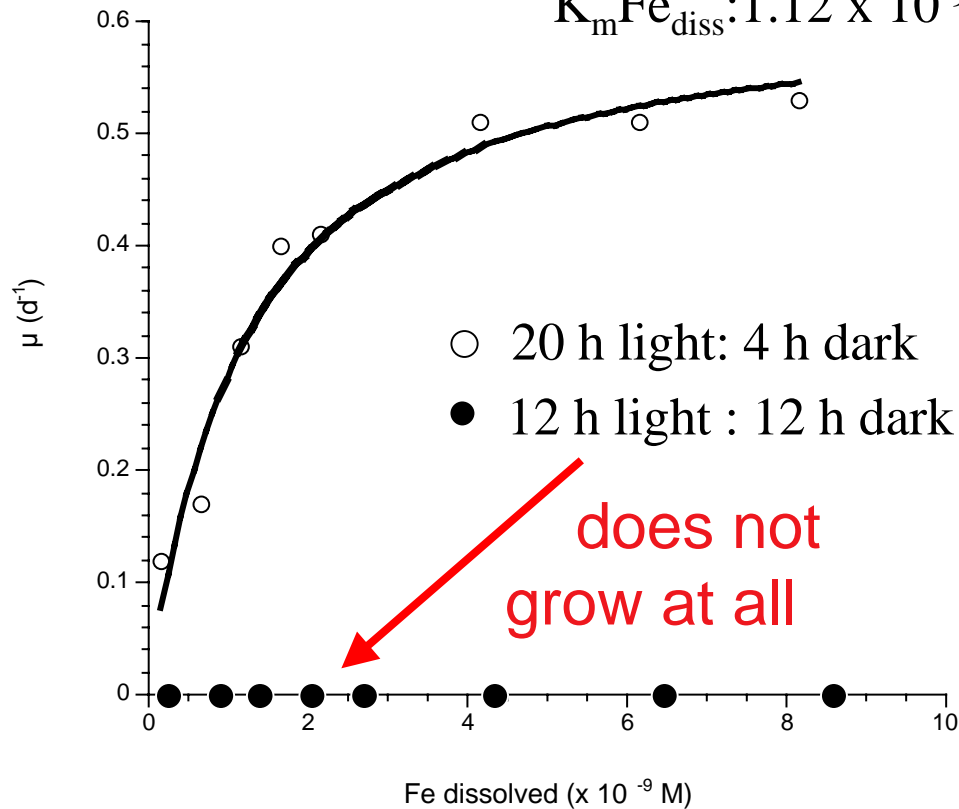


Timmermans et al. (2001), MEPS 287 - 297.

Chaetoceros dichchaeta

C. dichchaeta

$K_m \text{Fe}_{\text{diss}} : 1.12 \times 10^{-9} \text{ M}$



Chain-forming
large cells



Open Southern Ocean HNLC species

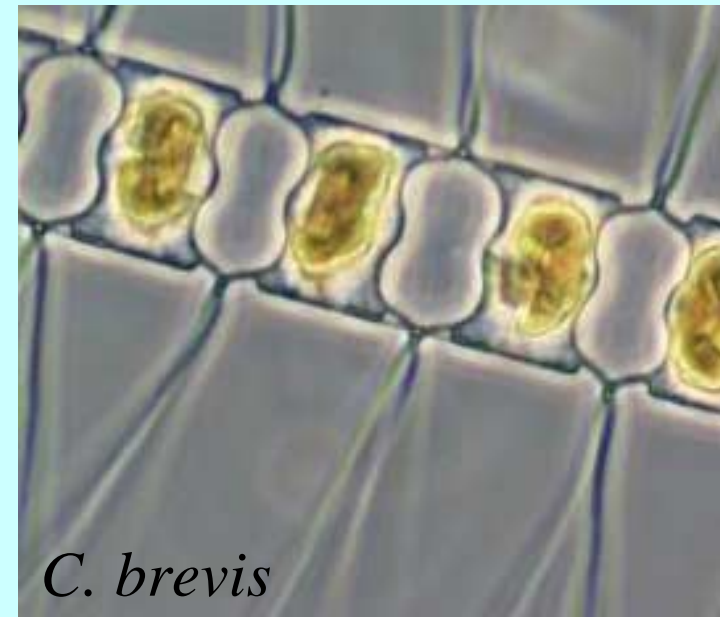
Large versus small
at optimal light levels

single cells 4 - 6 μm diameter (small)



C. dichaeeta

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

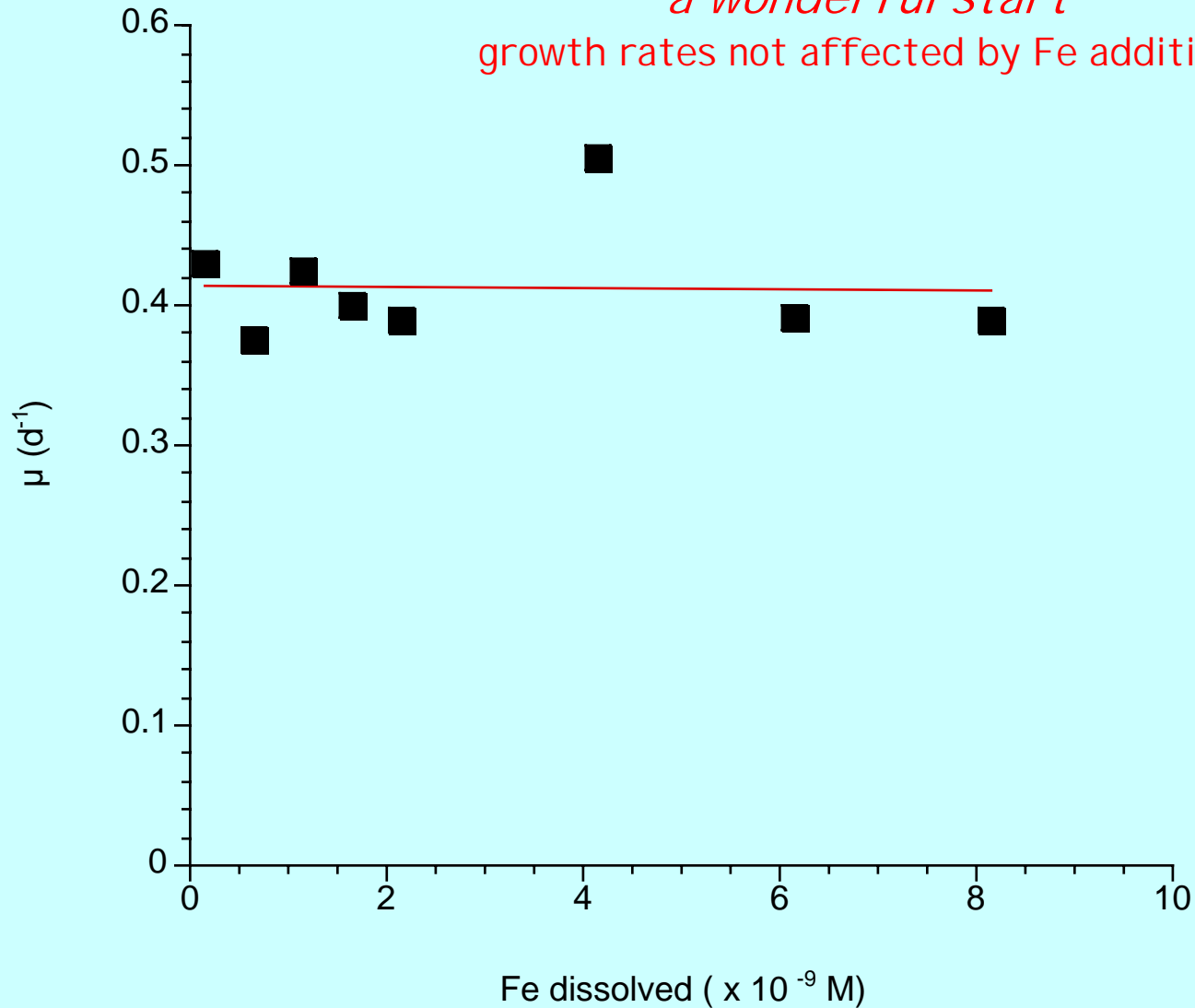


C. brevis

C. brevis in its pristine Antarctic seawater

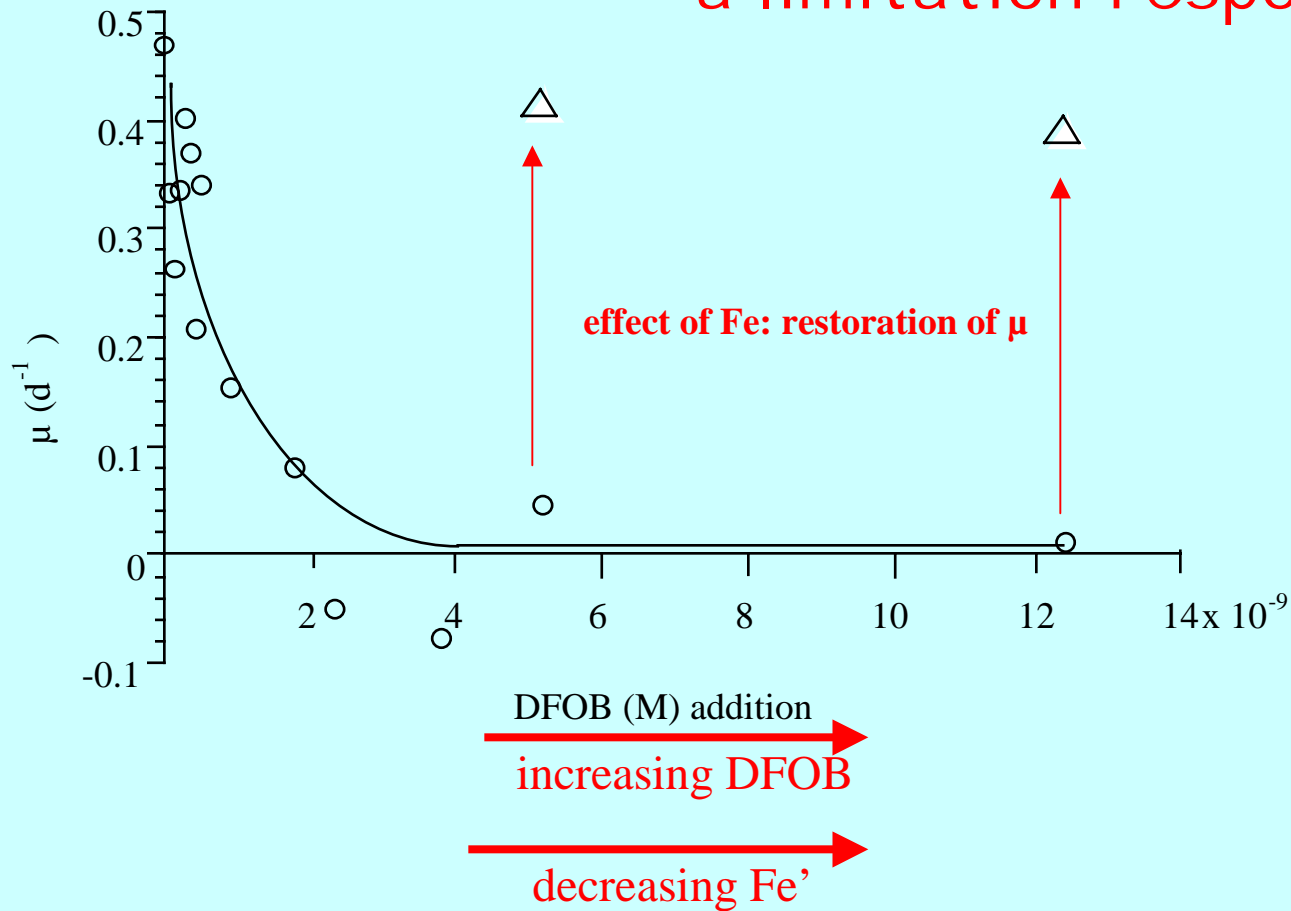
"a wonderful start"

growth rates not affected by Fe additions



Add DFOB siderophore to tie down the iron

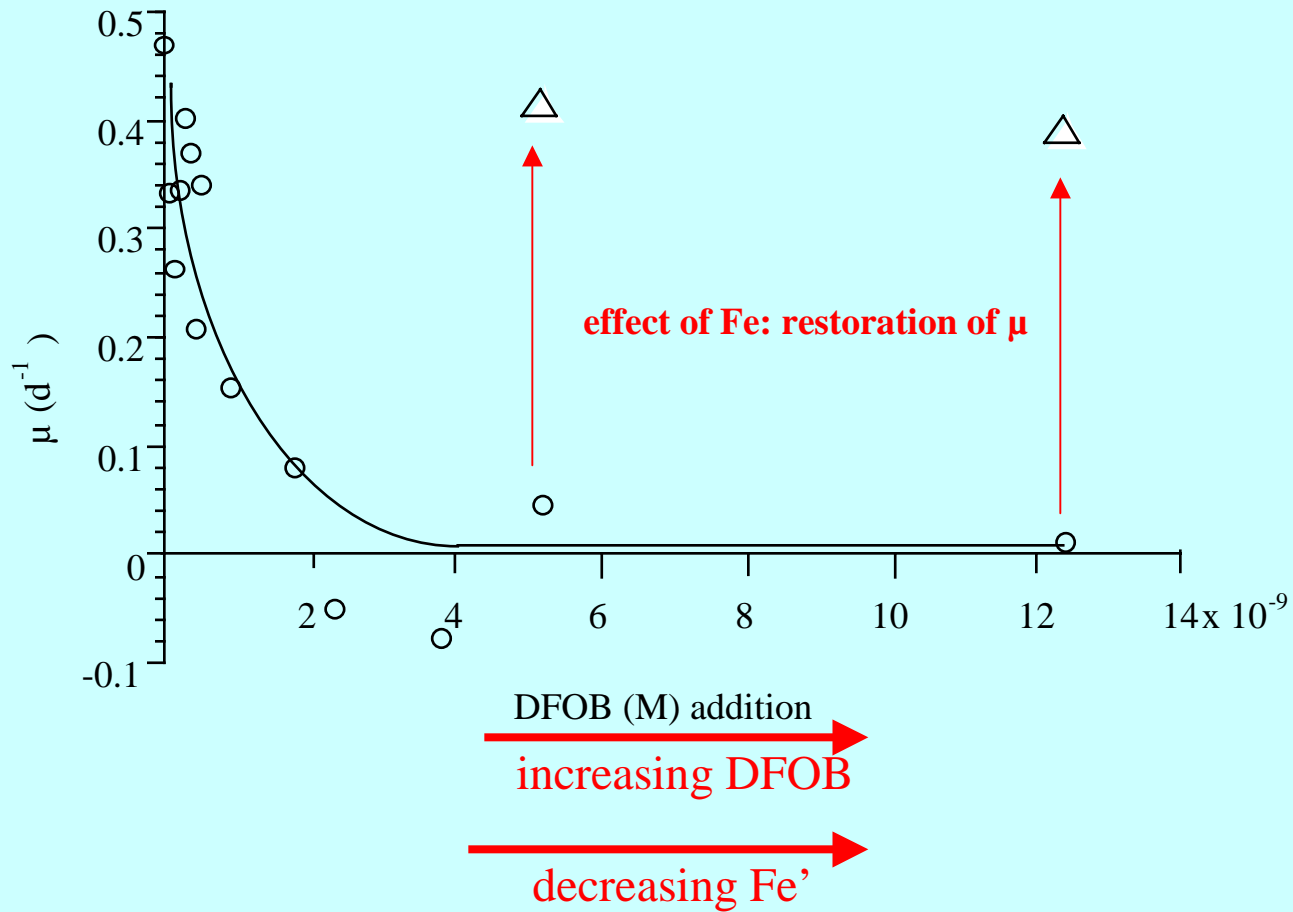
C. brevis, it works...
a limitation response



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

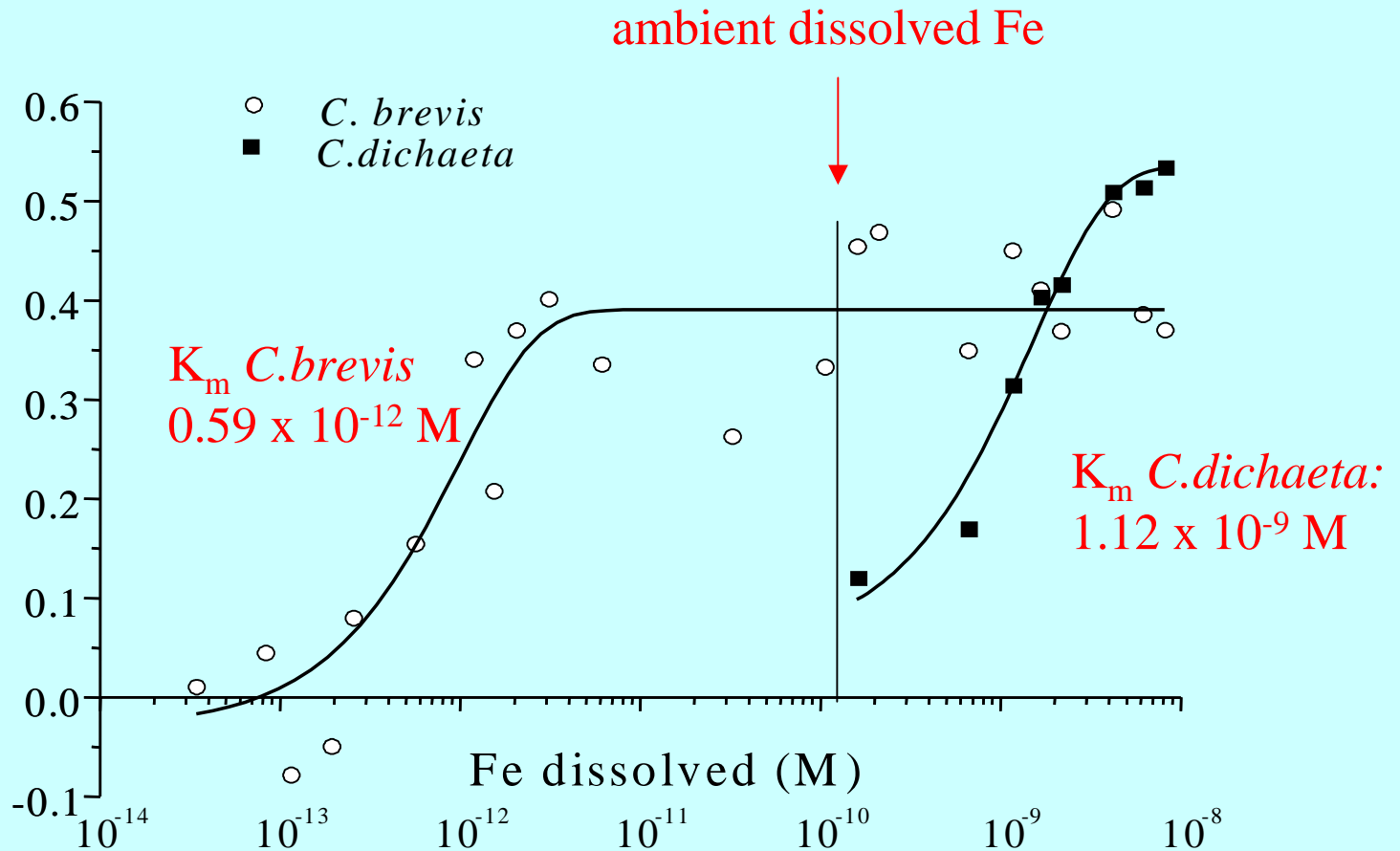


C. brevis, it works.... a limitation response



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





In the Southern Ocean:

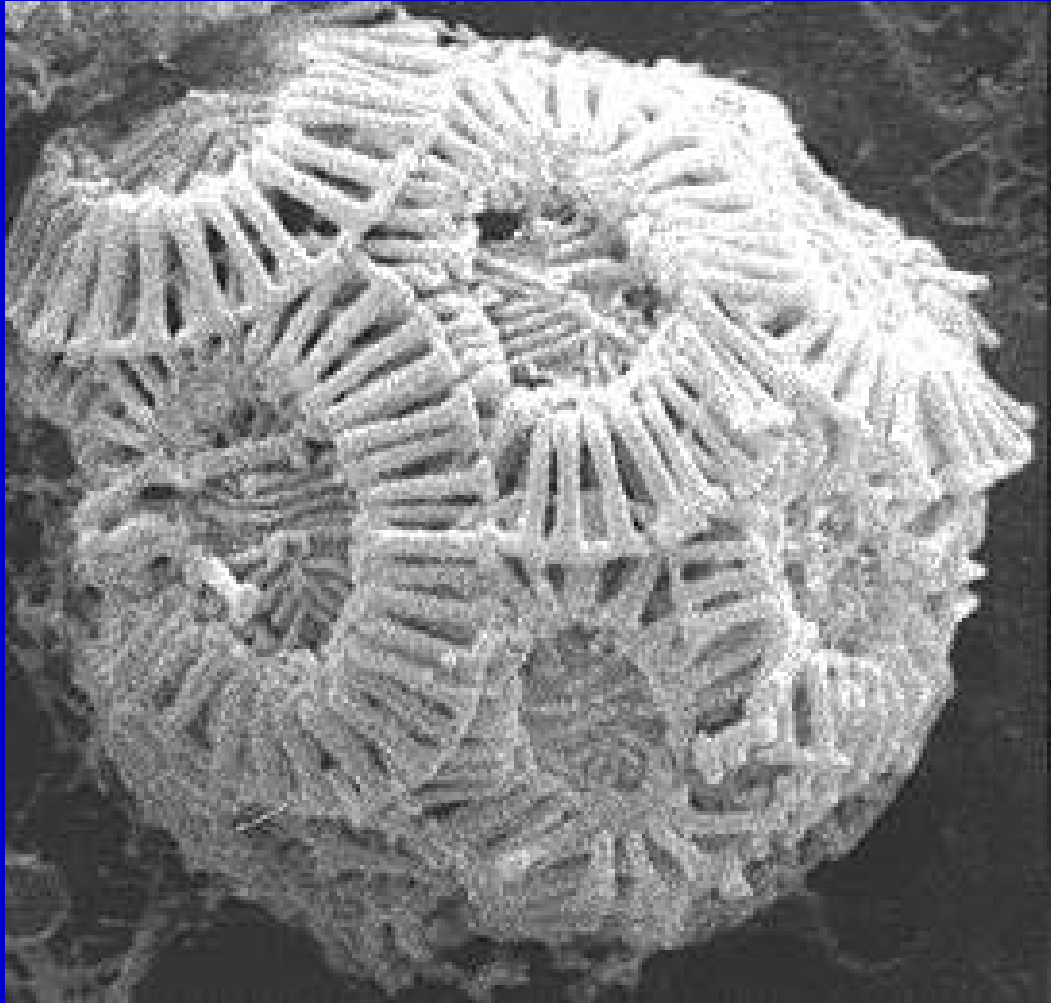
Large *C. dicaeta* is mostly Fe-limited except after Fe supply
Small *C. brevis* is never Fe-limited but grazer-controlled

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



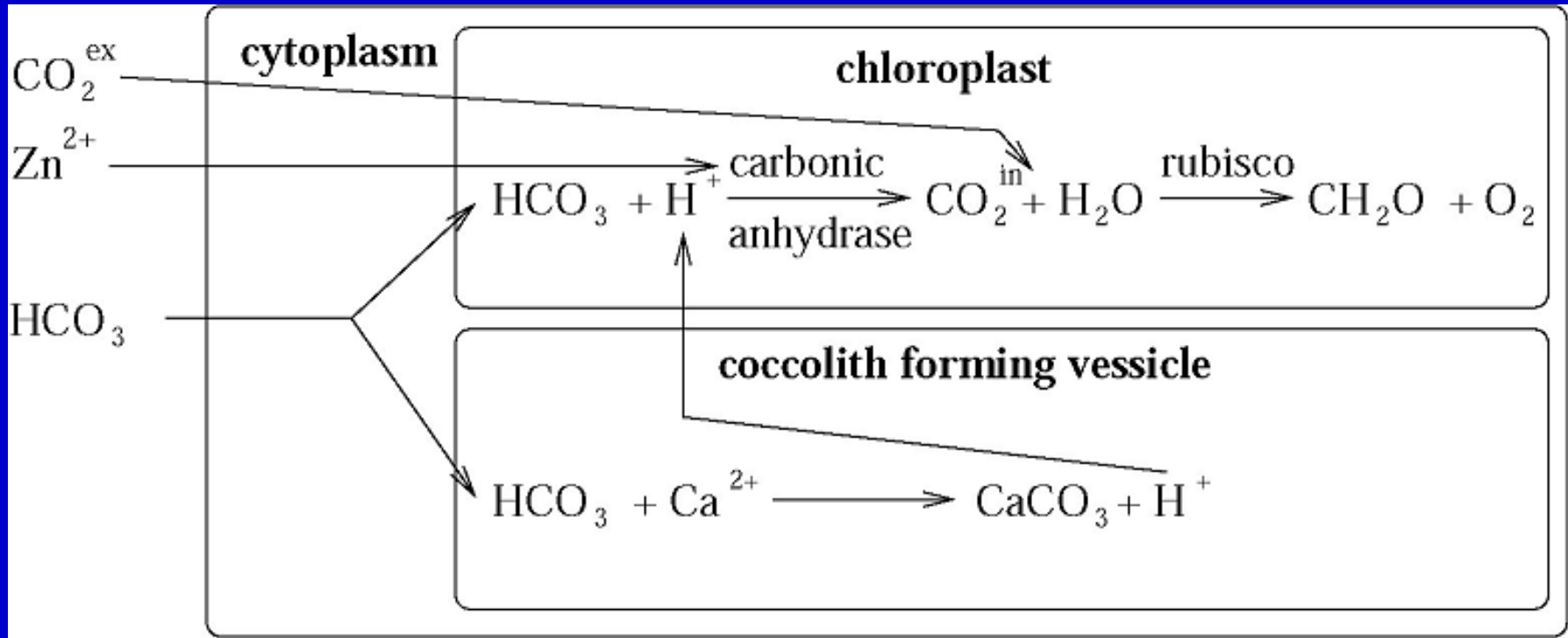
Emiliana huxleyi



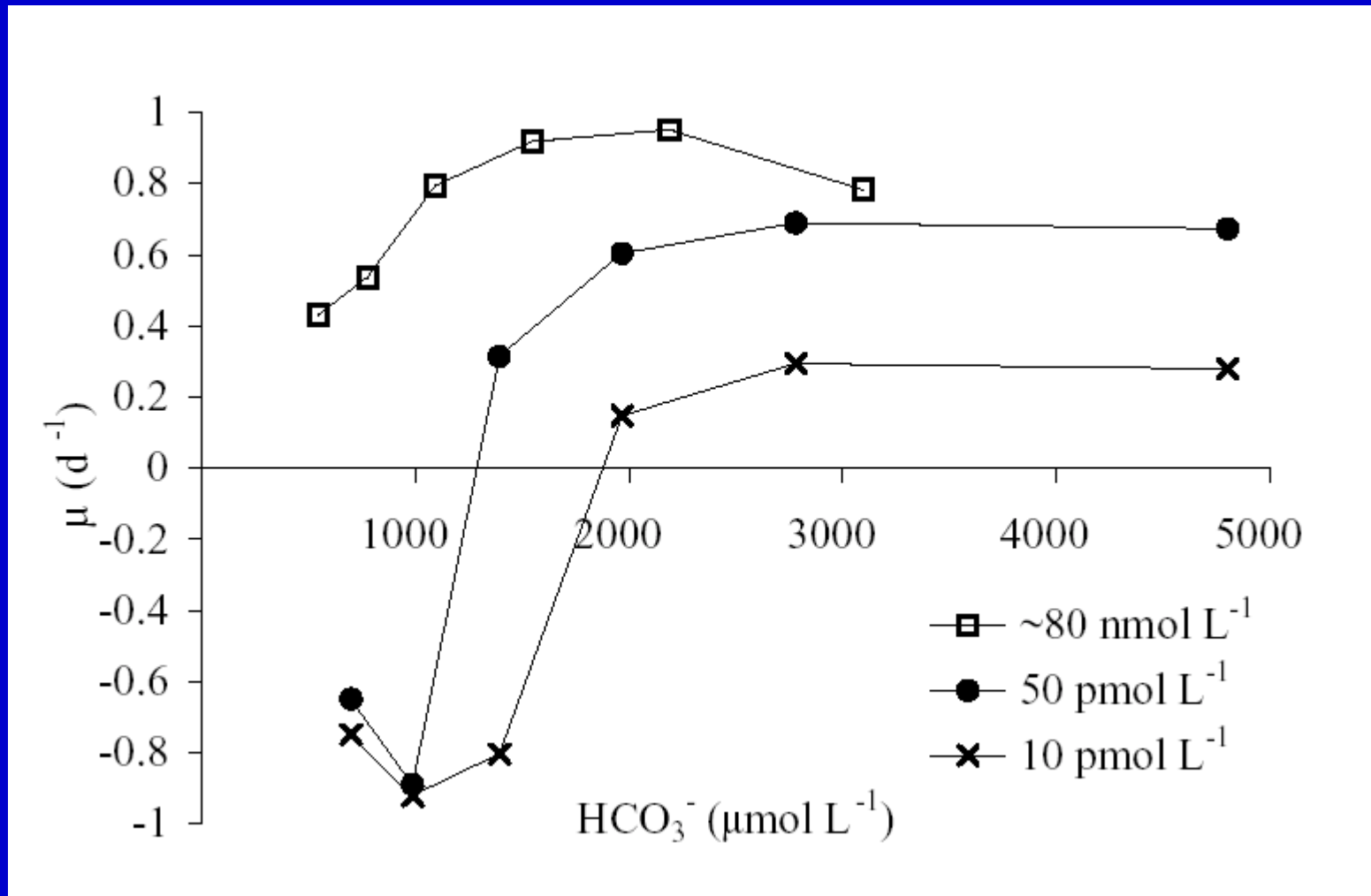
excretes external CaCO_3 platelets

Concerted photosynthesis & calcification

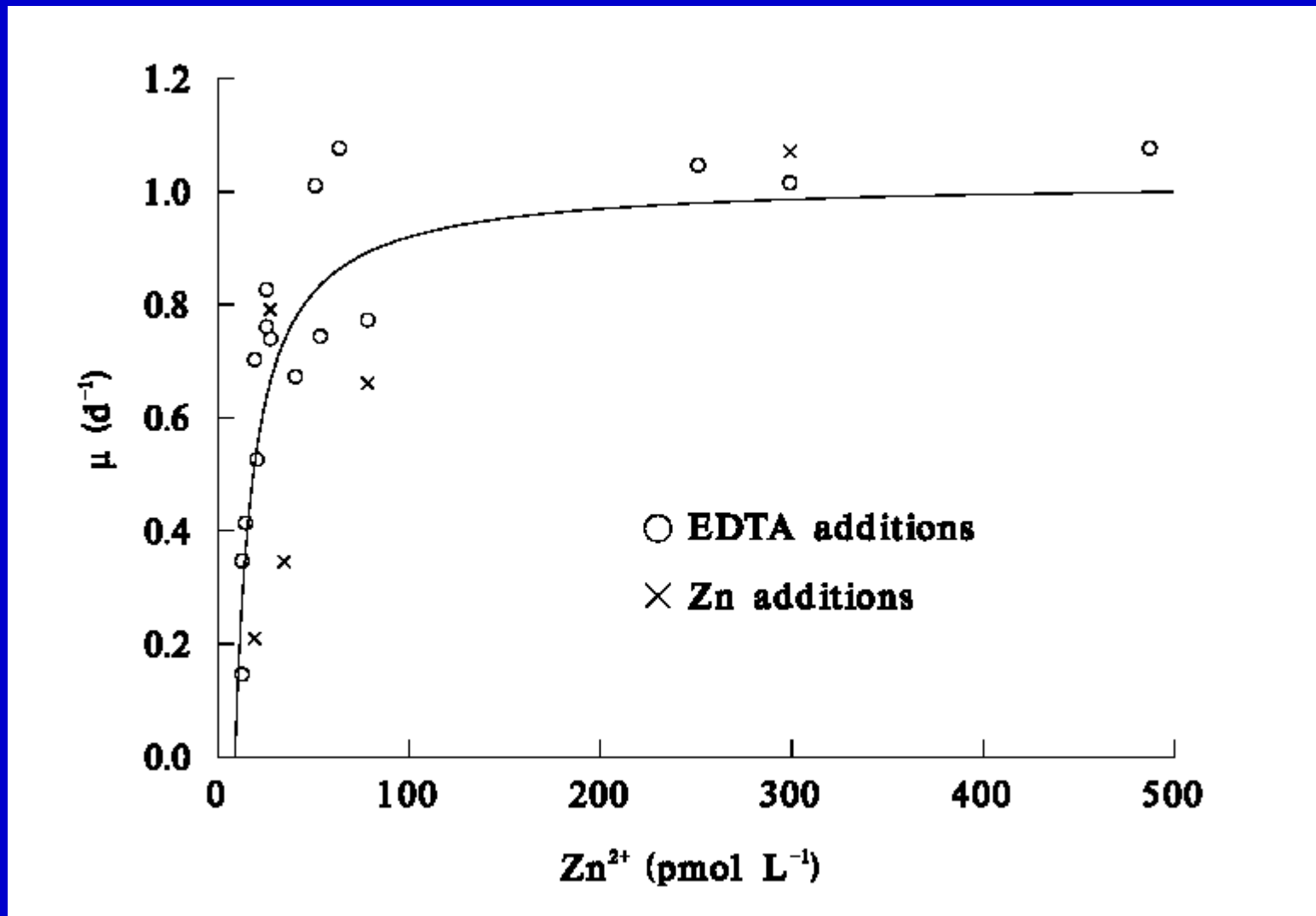
- Zn-carbonic anhydrase permits fast use of $[\text{HCO}_3^-]$
- Calcification provides the necessary proton to make CO_2



Growth on $[\text{HCO}_3^-]$ at 3 different $[\text{Zn}^{2+}]$



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

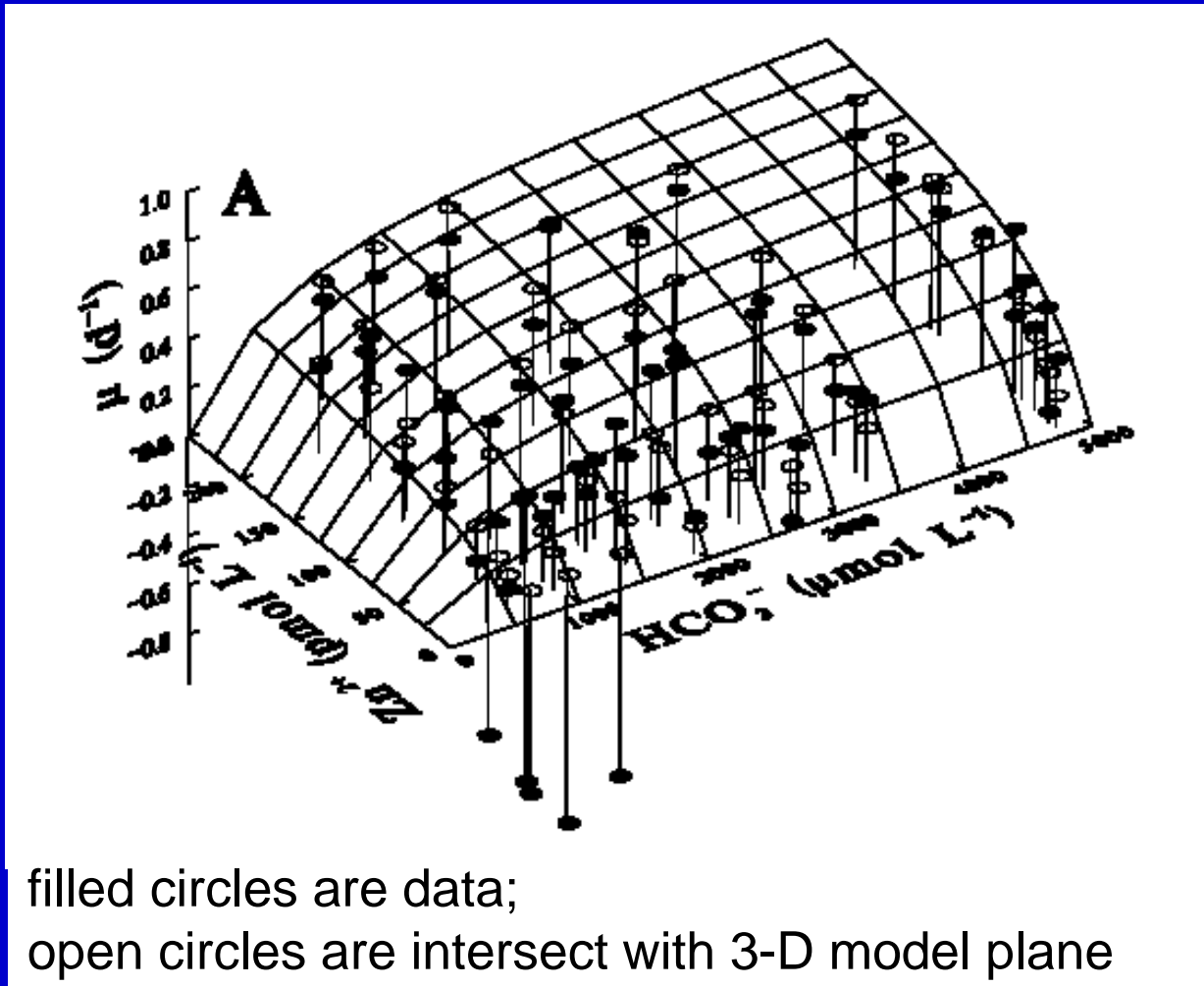


Suitable Equation for co-limitation ?

- 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

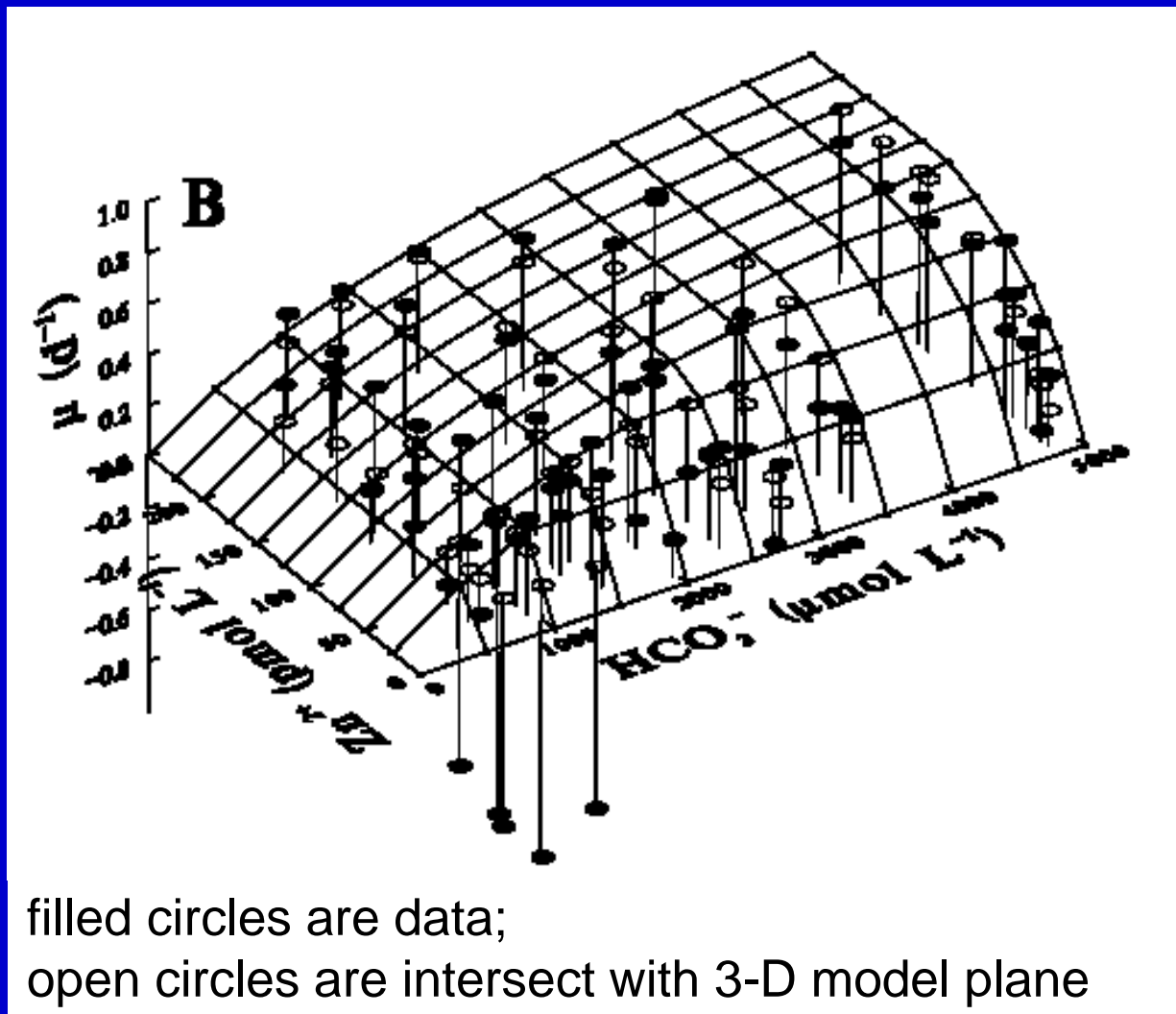
Which would provide the best fit ??

Multiply two Monod equations



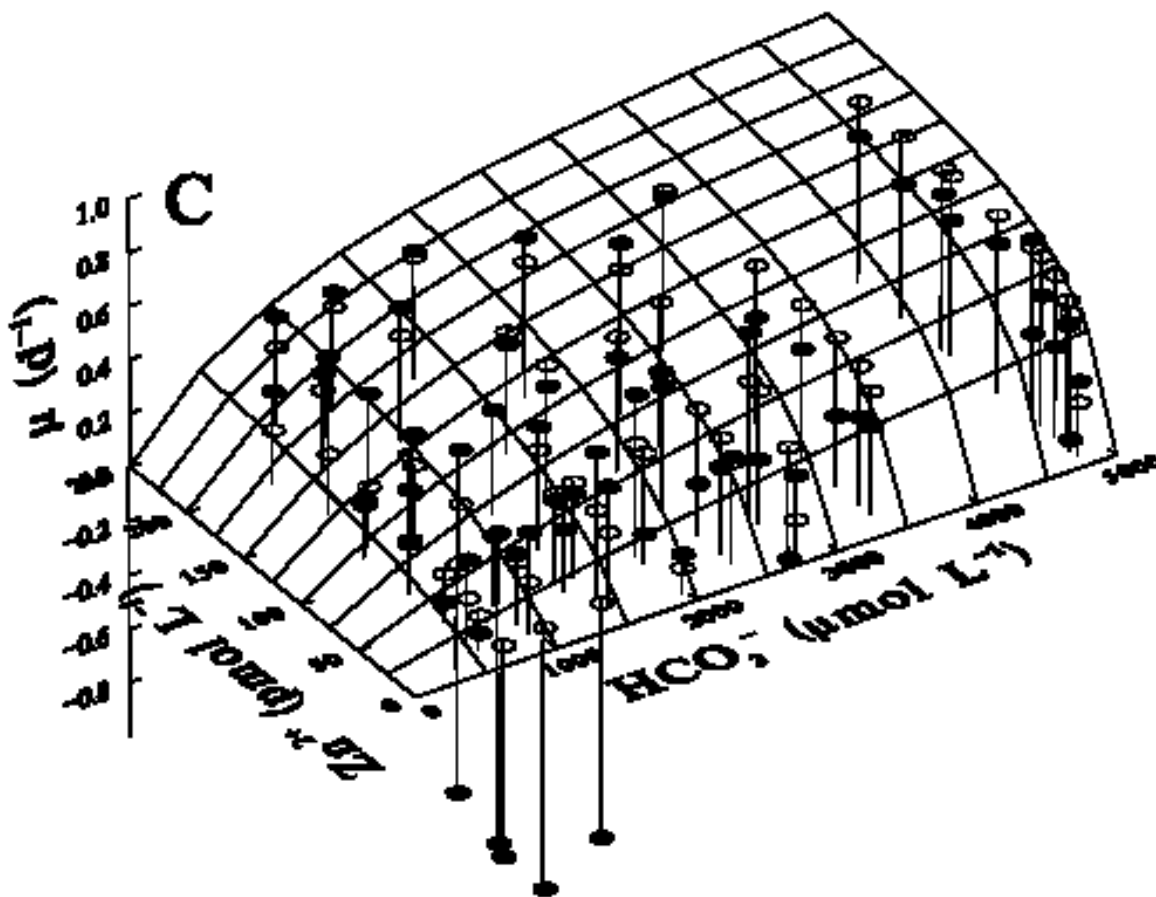
best fit: mean residual on $\mu = 0.018 \text{ day}^{-1}$

Minimum nutrient governs growth rate



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

Affinity for $[\text{HCO}_3^-]$ depends on $[\text{Zn}^{2+}]$



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

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

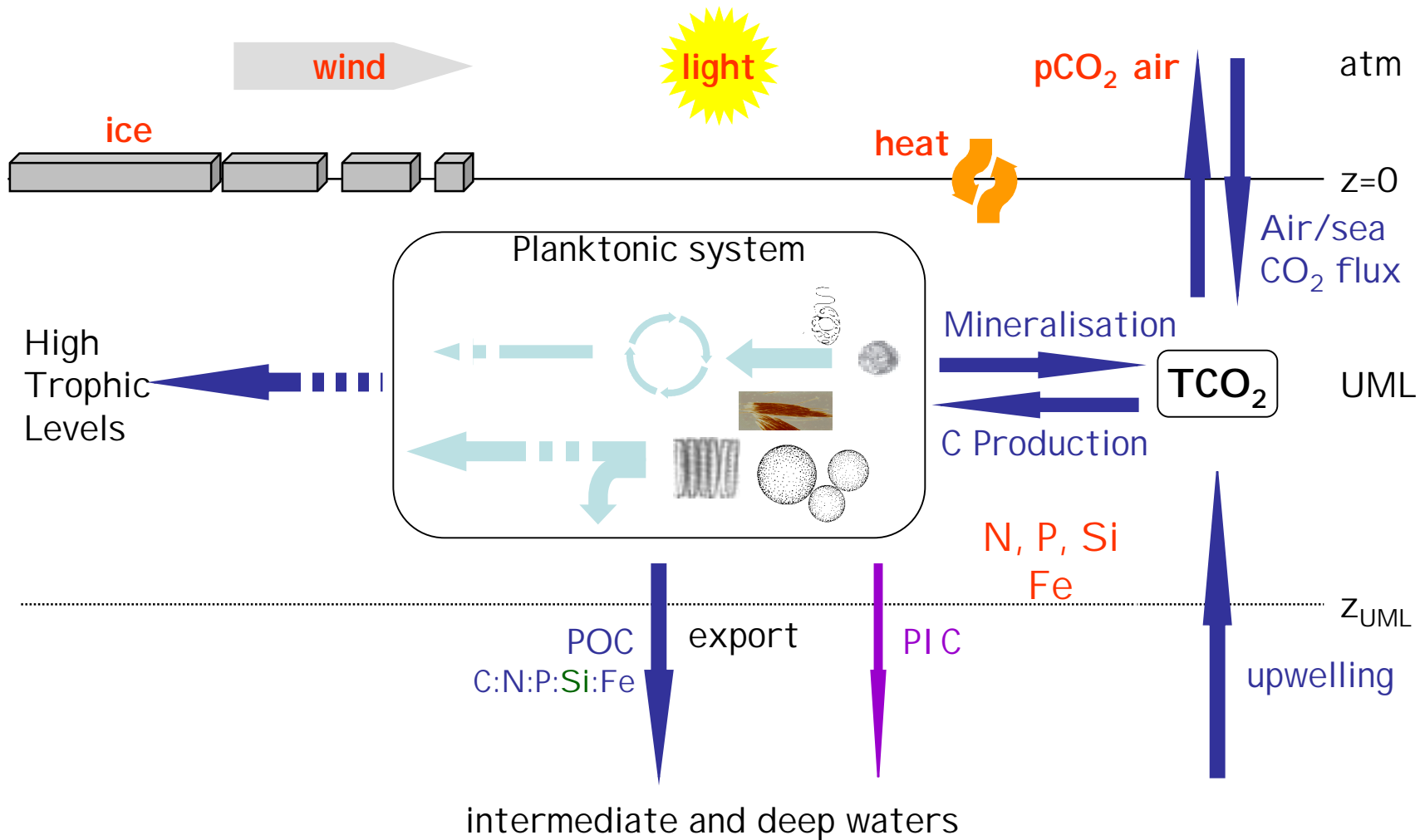
Best
concept
but fit
not any
better

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

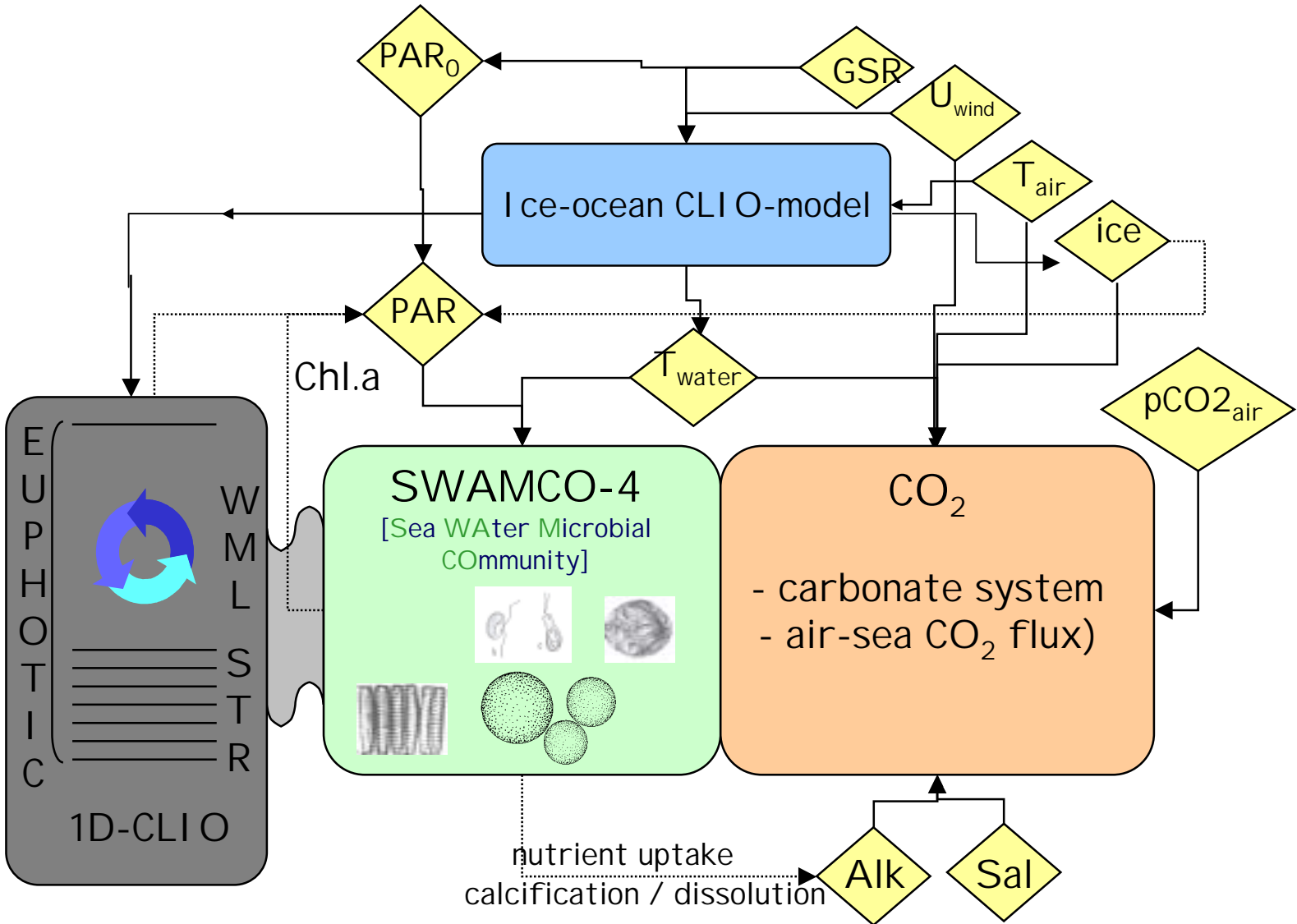


- Existing ecosystem model Southern Ocean
 - two plankton groups diatoms and nanoplankton
 - limitation by light and four nutrients N, P, Fe, Si
 - successful for Polar Front and for SOI REE
 - (Lancelot et al 2000; Hannon et al 2001)
- Advance to generic global model
 - five bloom-forming groups: diatoms,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

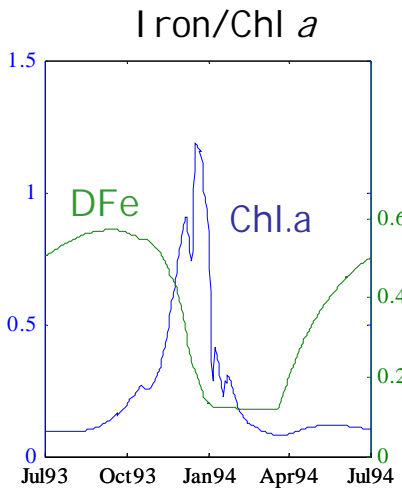
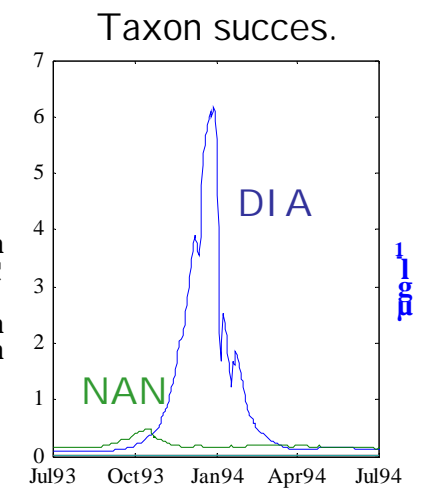
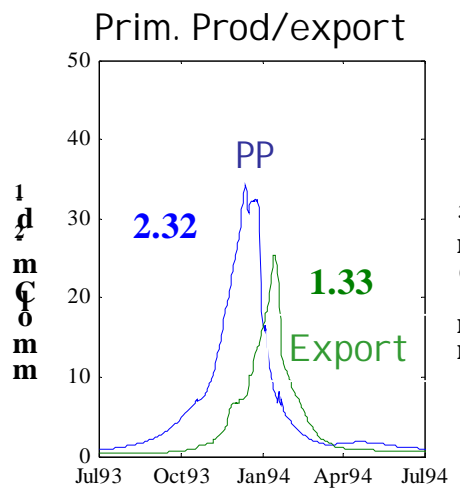
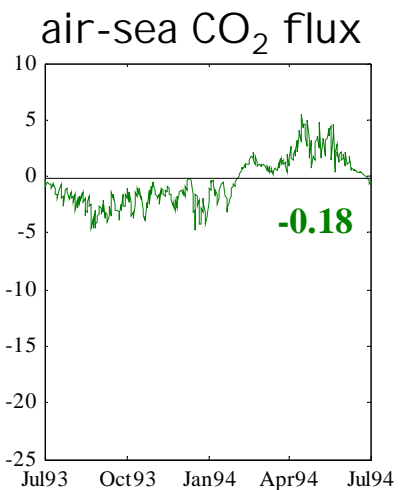
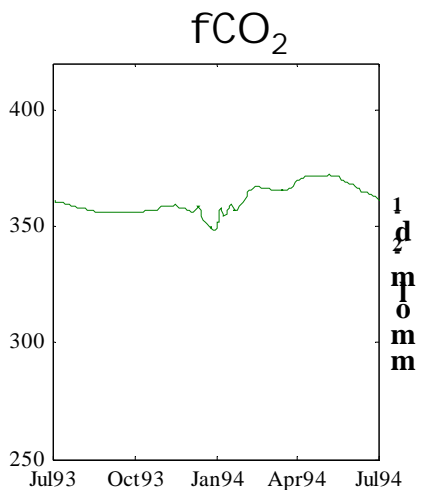
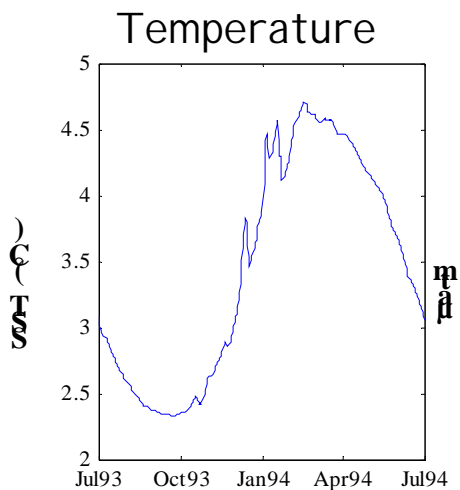
Control of the carbon cycling in the upper ocean



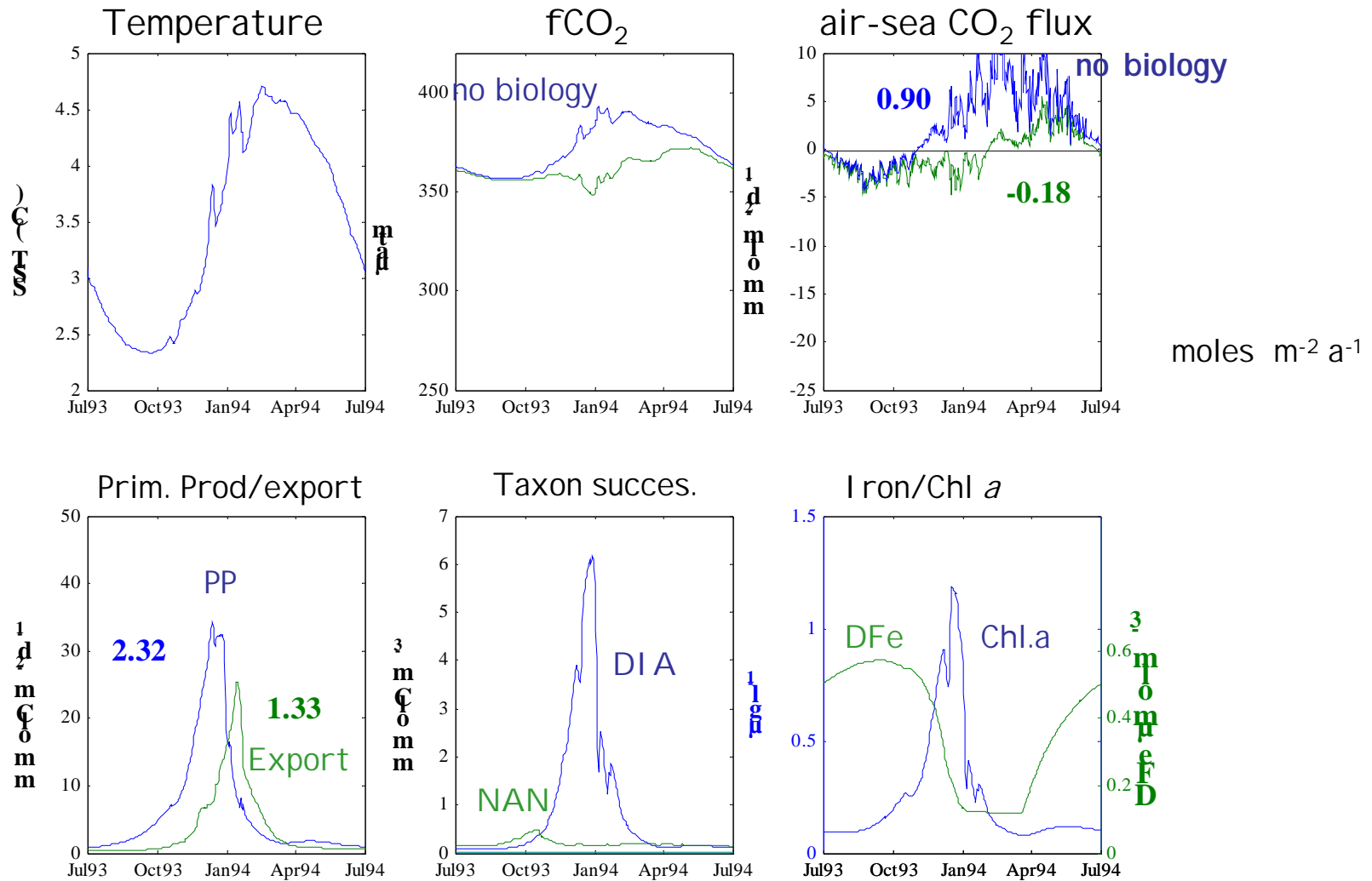
Structure of the coupled biological-chemical-physical 1D model



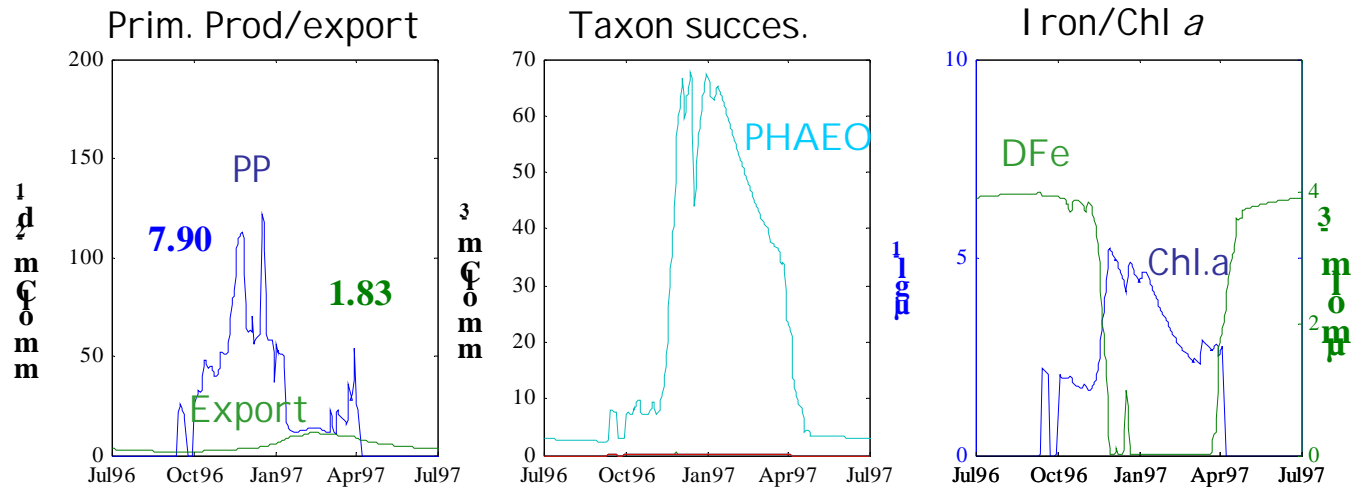
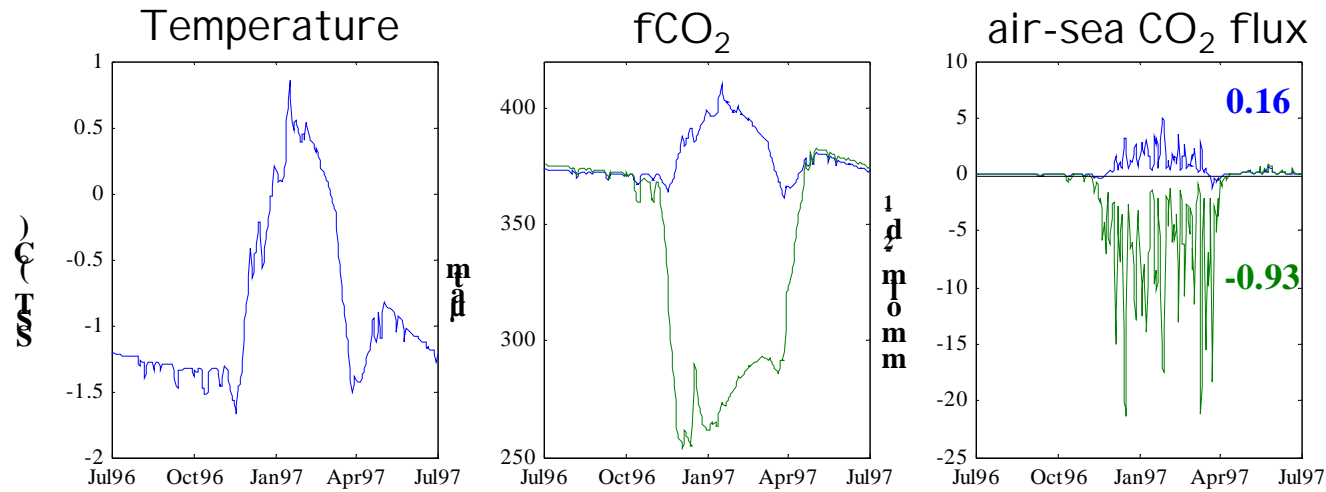
1D SWAMCO-4 results at KERFI X [1993] : moderate diatom bloom and low CO₂ sink



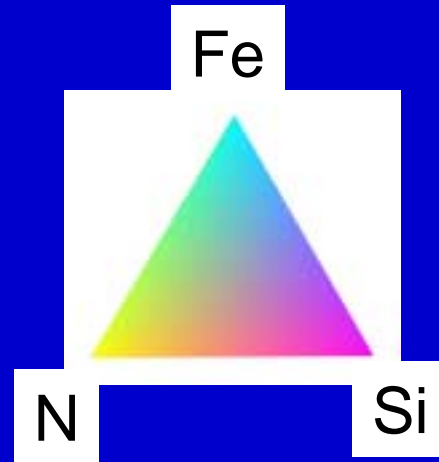
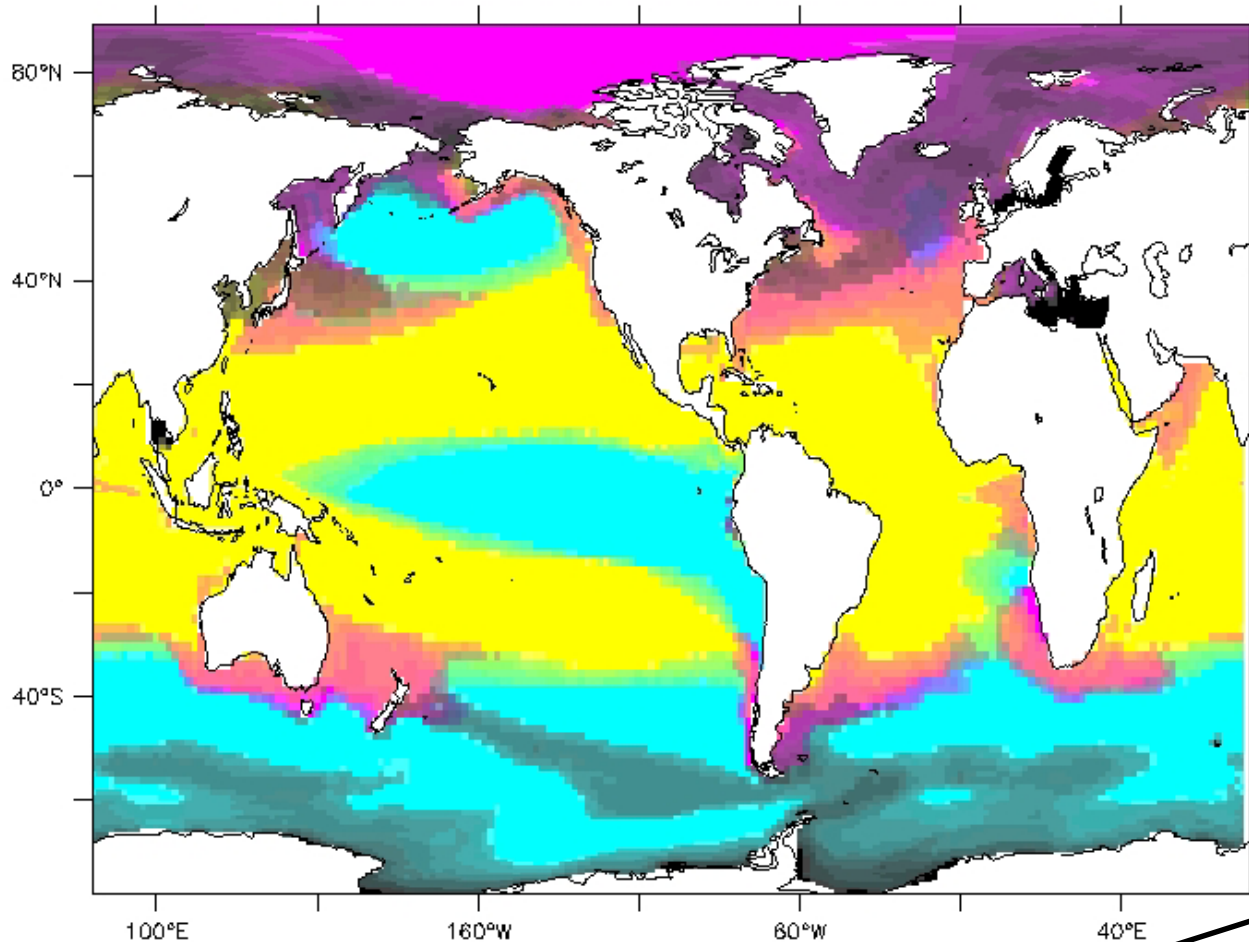
1D SWAMCO-4 results at KERFIX [1993] : Thermodynamic & biological control of air-sea CO₂ fluxes



1D SWAMCO-4 results at AESOPS [1996]: Thermodynamic & biological control of air-sea CO₂ fluxes



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



Example: the Diatoms



6. GEOTRACES (GEOSECS II)





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



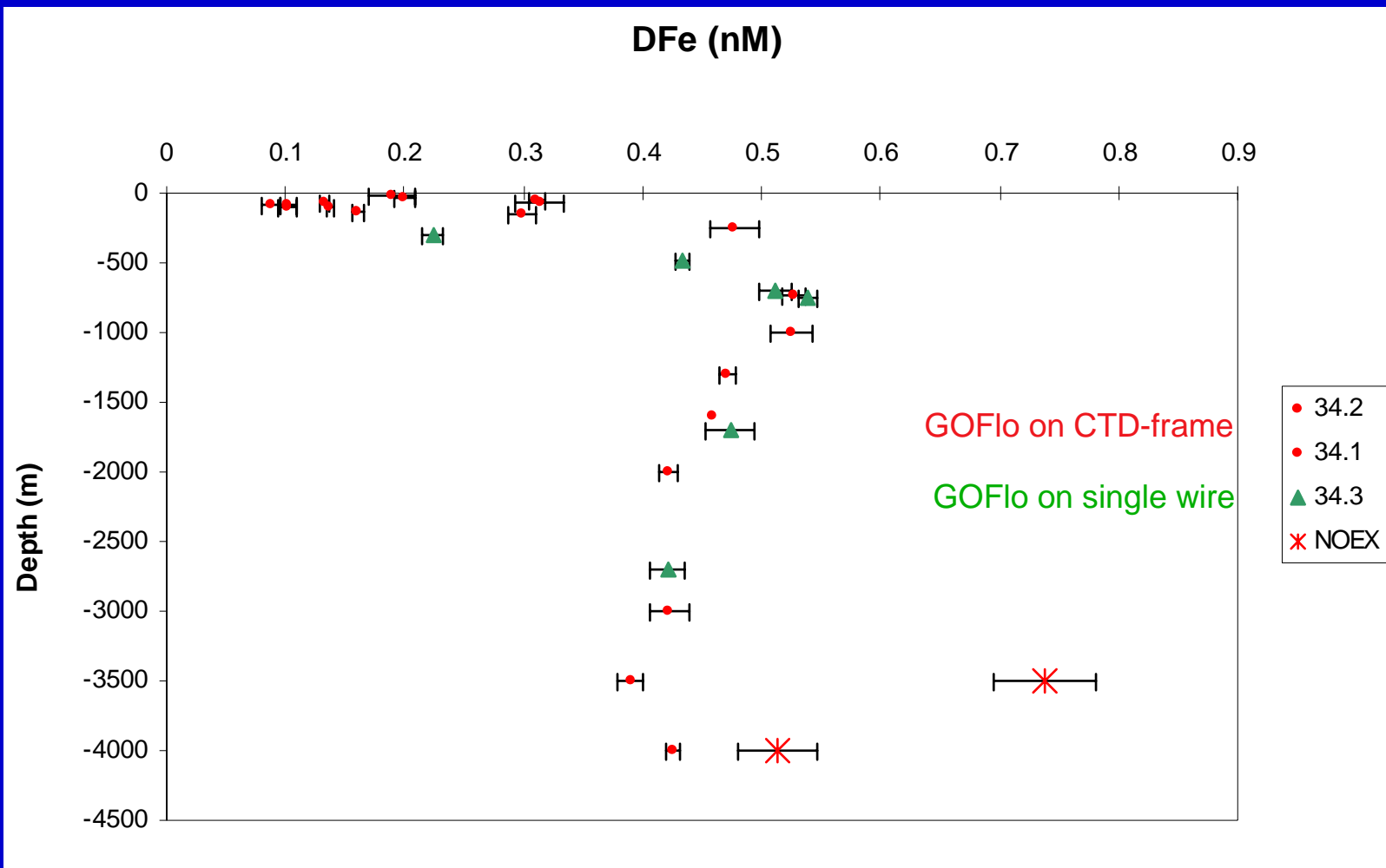
driver unit pneumatics

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

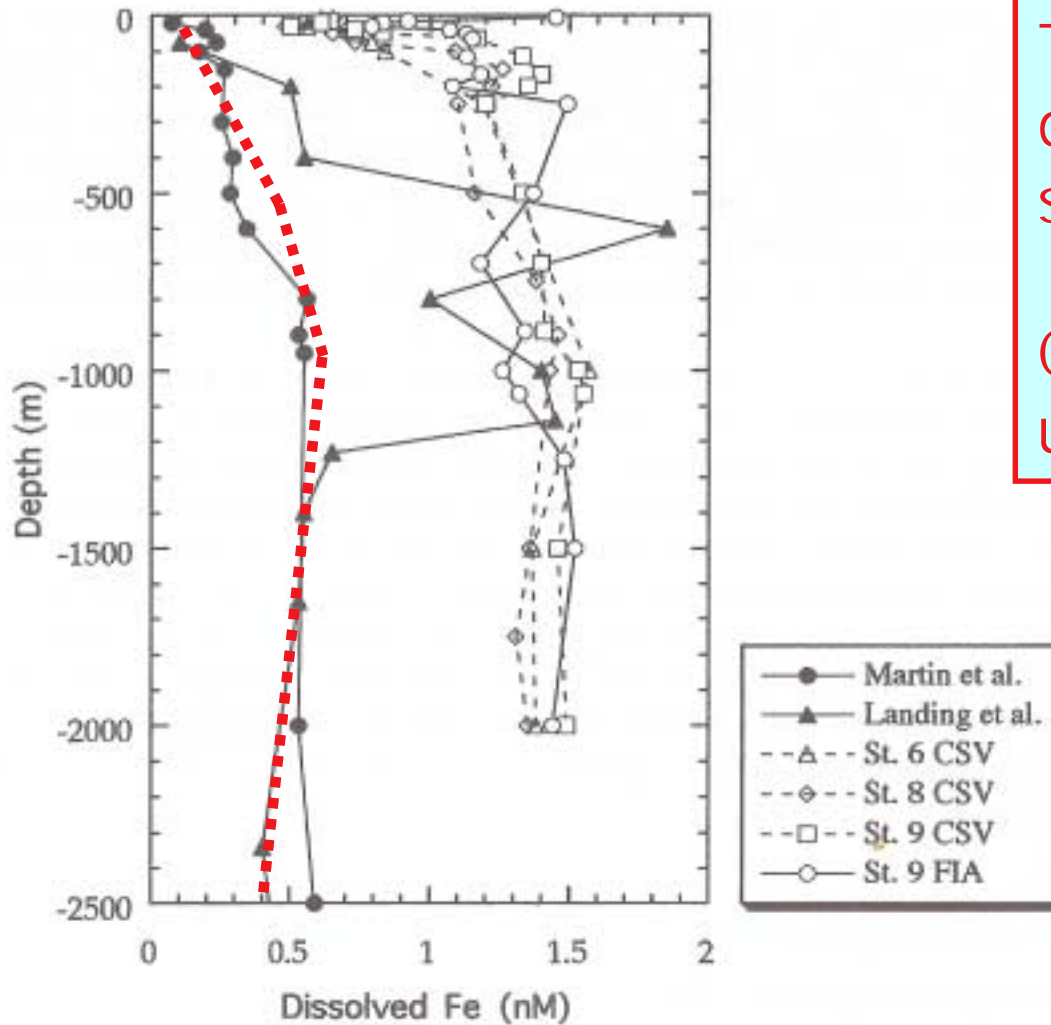


Figure 18. Vertical profiles of dissolved Fe at station 9 (40°N, 23°W) in the Northeast Atlantic Ocean. Duplicate analyses of total dissolved Fe by FIA-CL after acidifying to pH 2 (de Jong *et al.*, 2000) and dissolved Fe by CSV after UV digestion at pH 8 (Boyé, 2000) show good agreement. Also shown 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)).

True and accurate dissolved Fe values still are puzzling.

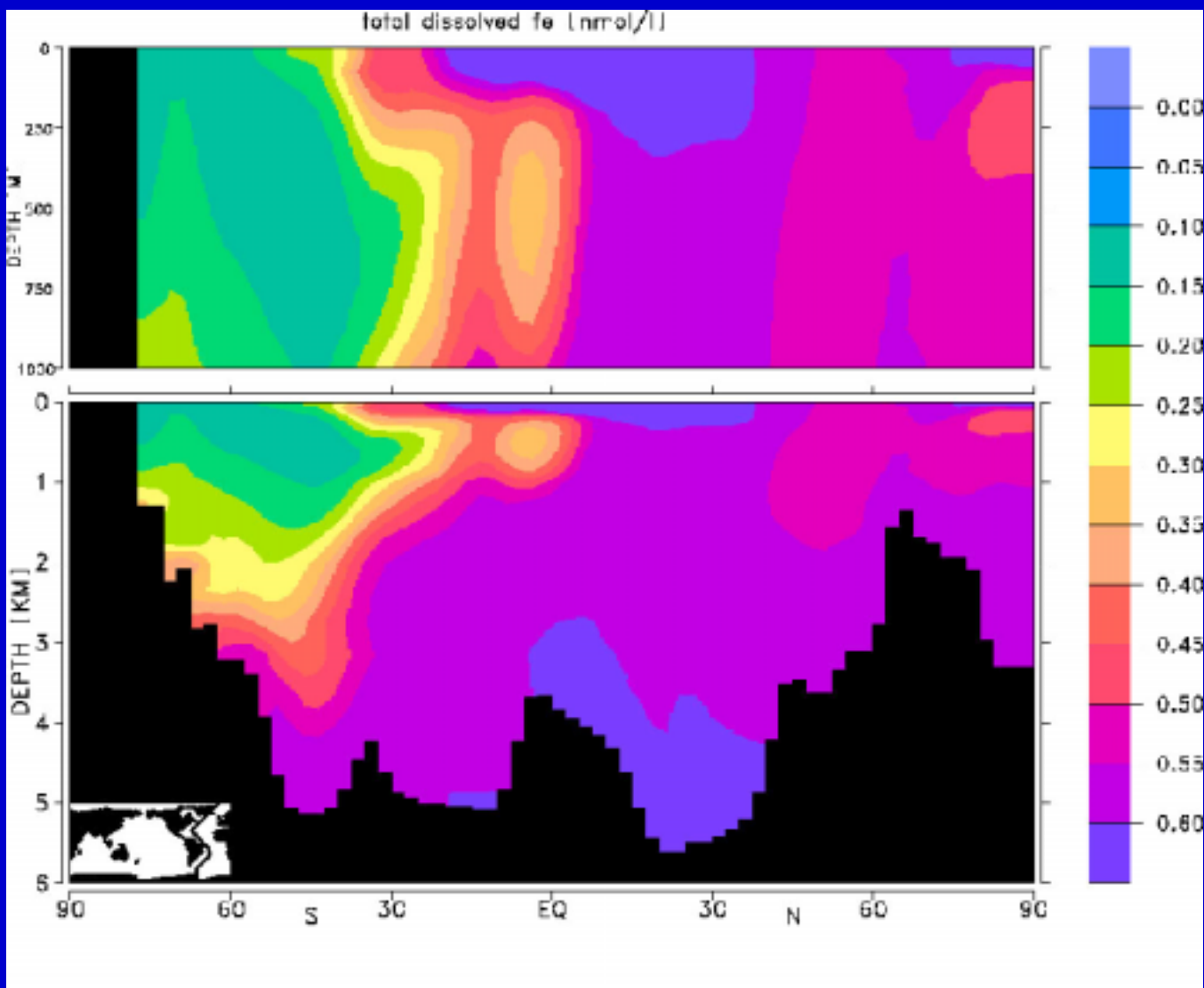
Certified standard is urgently needed

- outliers not shown
- IOC station

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

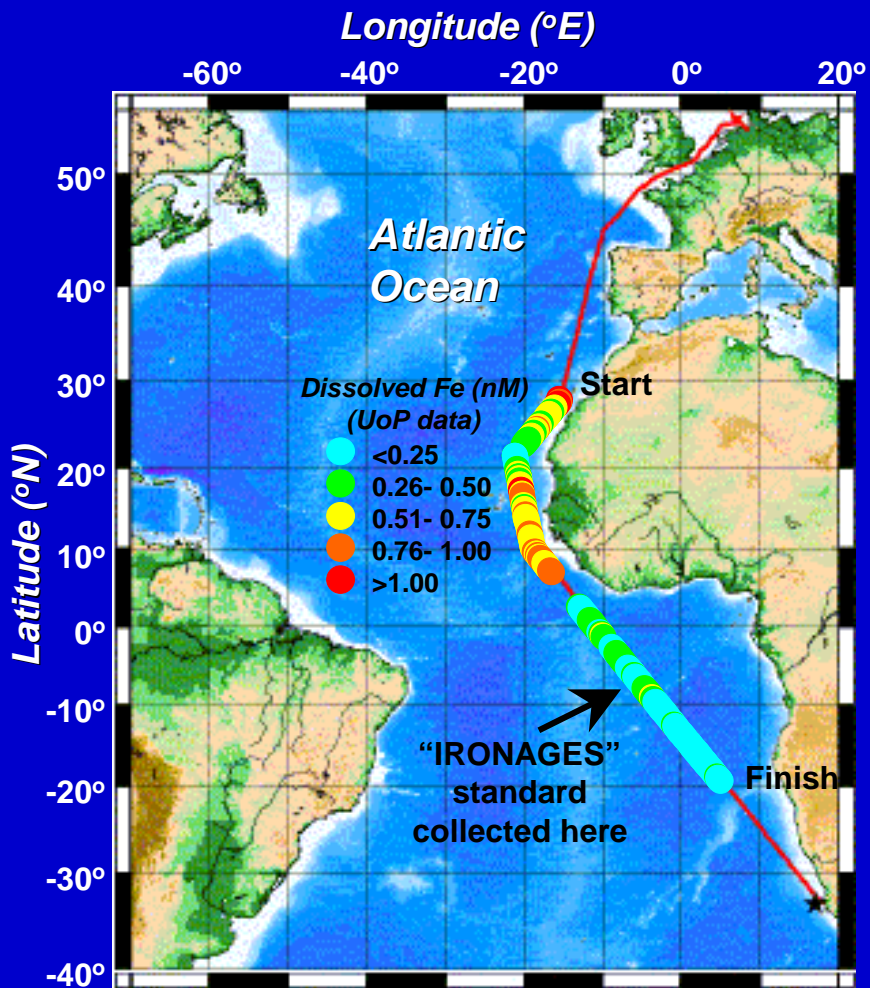
Atlantic Fe distribution in Hamburg model

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

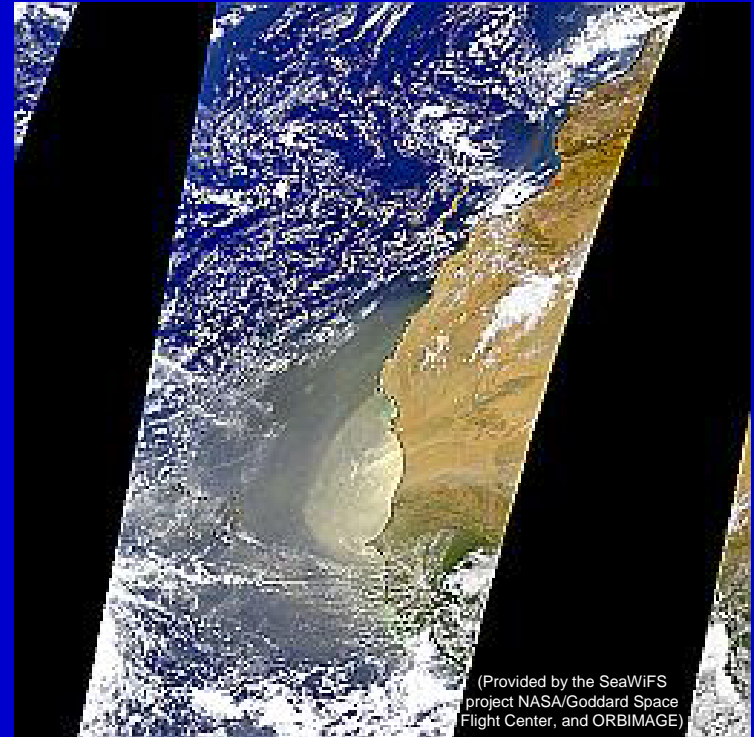


Six and Maier-Reimer, European Ironages project modeling

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?

IRONAGES standard: sampling

R. V. Polarstern



- 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



HDPE tank



Towed fish

Paul
Worsfold,
Nice 2003
lecture



Towed fish

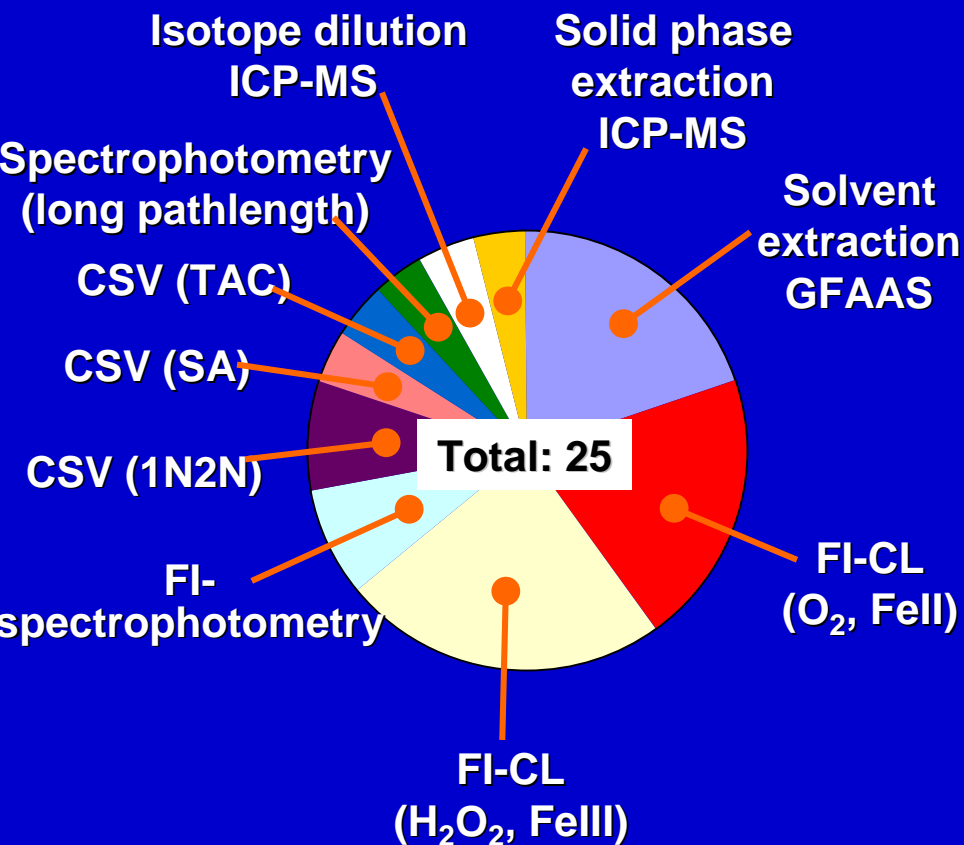
IRONAGES standard: bottling

- 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

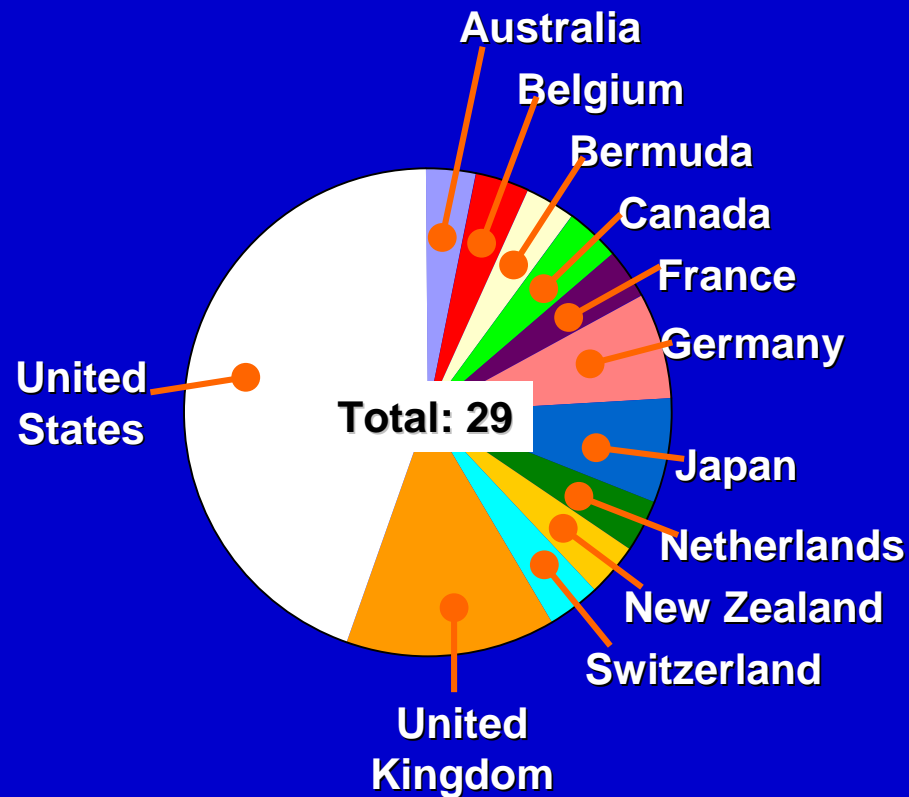


- Trials underway for:
 - homogeneity
 - time-series stability
 - sample storage
- Other bottles sent to 25 worldwide iron laboratories

IRONAGES standard: participants/methods



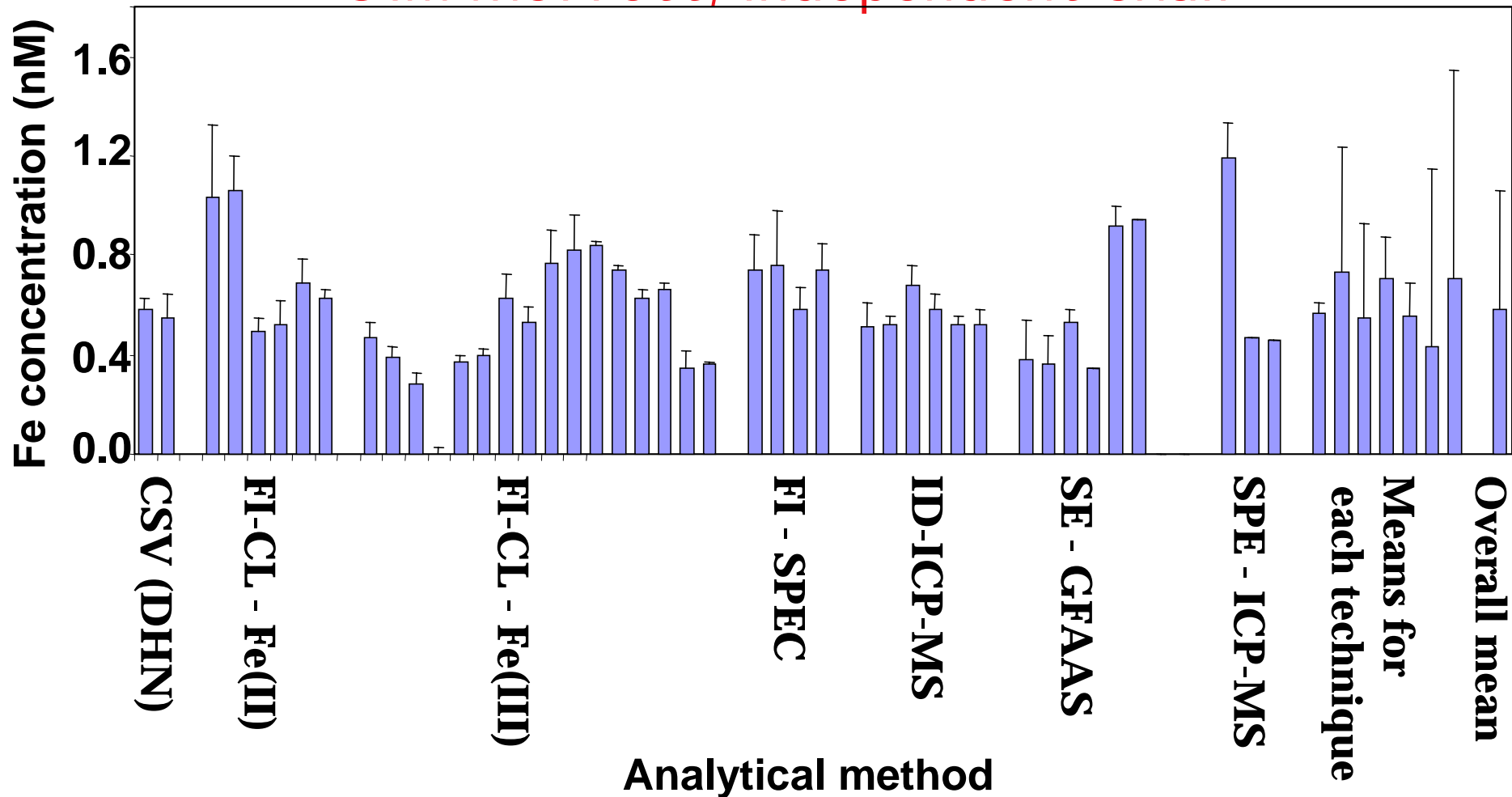
Analytical methods used during the IRONAGES exercise



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

Laboratory data versus analytical method

Jim Moffett, independent chair



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
 - NO_3 , PO_4 , SiO_4 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

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Our universities and institutes

Koninklijk Nederlands Instituut voor Onderzoek der Zee
Royal Netherlands Institute for Sea Research



Europese Unie
European Union

