Co-Limitation of Phytoplankton by Light and Multiple Nutrients

Hein de Baar,
Klaas Timmermans, Loes Gerrina, Erik Buitenhuis, Patrick Laan,
Christiane Lancelot, Olivier Aumont, Geraldine Sarthou, Andy Bowie,
Stephane Blain, Paul Worsfold
and many others in European research teams of
MERLIM, CARUSO, IRONAGES

Contents

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

![Graph showing the abundance of chemical elements versus atomic number.](image)

**Major Bio-Elements**
Abundances versus one million Si atoms

- Carbon: $10 \times 10^6$
- Nitrogen: $3 \times 10^6$
- Silicon: $1 \times 10^6$
- Phosphorus: $1 \times 10^4$
- Iron: $0.9 \times 10^6$
**Metals Abundance & Biological Evolution**

<table>
<thead>
<tr>
<th>Metal</th>
<th>Abundance (per million Si atoms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn</td>
<td>9550</td>
</tr>
<tr>
<td>Fe</td>
<td>900000</td>
</tr>
<tr>
<td>Co</td>
<td>2250</td>
</tr>
<tr>
<td>Ni</td>
<td>49300</td>
</tr>
<tr>
<td>Cu</td>
<td>522</td>
</tr>
<tr>
<td>Zn</td>
<td>1260</td>
</tr>
<tr>
<td>Ag</td>
<td>0.49</td>
</tr>
<tr>
<td>Cd</td>
<td>1.61</td>
</tr>
<tr>
<td>Hg</td>
<td>0.34</td>
</tr>
<tr>
<td>Pb</td>
<td>315</td>
</tr>
</tbody>
</table>

**Evolution used abundant metals: essential**

**Low abundant metals no bio-functions: toxic**

**Photosynthetic Oxygen Captured in Iron Formations**

\[ 4 \text{Fe(II)}_{\text{dissolved}} + 3 \text{O}_2 \rightarrow 2 \text{(Fe}_2\text{O}_3)_{\text{deposit}} \]

Dumb algae took away their own iron supply

Dumb algae took away their own iron supply

Dumb algae took away their own iron supply
2. Concepts of limitation

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


Emiliania huxleyi in pristine natural seawater

Driven into iron limitation by siderophore addition

Timmermans et al., in prep.

Multiple Limitations in Real Ocean

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

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


Biological function for Cd after all


join the Green Party

carbonic anhydrase

<table>
<thead>
<tr>
<th>Mn</th>
<th>Fe</th>
<th>Co</th>
<th>Ni</th>
<th>Cu</th>
<th>Zn</th>
</tr>
</thead>
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<tr>
<td>0.34</td>
<td>315</td>
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</tr>
</tbody>
</table>
Silver (Ag) resembles Copper (Cu)

North Pacific Ocean
(18°N, 108°W)

Martin et al. (1983)

Ag has better correlation with Si

Worldwide correlation Ag and Si

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


Fractionations Cu/Ag and Zn/Cd

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

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

(Also differences inorganic speciation)
Nutrient anomalies *Fragilariopsis kerguelensis* blooms


Fragilariopsis kerguelensis with heavily silicified armor ‘pantzer’
More Fe co-limitations major nutrients

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

Uptake by blooms in Ross Sea

<table>
<thead>
<tr>
<th>Arrigo et al. (1999)</th>
<th>Diatoms</th>
<th>Phaeocystis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N/P = 9.5</td>
<td>N/P = ~19</td>
</tr>
</tbody>
</table>

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

\[ y = 0.4756x + 16.394 \quad R^2 = 0.8868 \]

\[ y = 0.181x + 20.98 \quad R^2 = 0.7442 \]

\[ y = 0.0298x + 1.192 \quad R^2 = 0.6564 \]

\[ y = 0.0012x + 1.6159 \]

**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 *Emiliania huxleyi*

Different forms of Fe in seawater

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

EDTA dope would disturb all this
Fragilariopsis kerguelensis in natural Antarctic seawater

Nutrients Stoichiometry of Fragilariopsis kerguelensis

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Southern Ocean</th>
<th>Incubations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fe-deplete</td>
<td>Fe-deplete</td>
</tr>
<tr>
<td>Si : N</td>
<td>7.7</td>
<td>2.5</td>
</tr>
<tr>
<td>N : P</td>
<td>~ 5 ± 1</td>
<td>~ 5 ± 1</td>
</tr>
</tbody>
</table>

heavily silicified Frag.kerguelensis has higher Si/N ratio

Timmermans, van der Wagt, de Baar, in prep.
**Actinocyclus sp.**

- **Kₘ Feₐ₈₁₉**: 0.98 x 10⁻⁹ M
- **μₘₐₓ**: 0.34 d⁻¹

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**Elemental composition in relation to Feₐ₈₁₉**

<table>
<thead>
<tr>
<th>Feₐ₈₁₉ (x10⁻⁹ M)</th>
<th>Si</th>
<th>N</th>
<th>P</th>
<th>Si : N</th>
<th>N-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>18.25</td>
<td>0.69</td>
<td>1.48</td>
<td>27</td>
<td>0.47</td>
</tr>
<tr>
<td>0.45</td>
<td>17.25</td>
<td>0.75</td>
<td>1.50</td>
<td>23</td>
<td>0.50</td>
</tr>
<tr>
<td>0.65</td>
<td>9.88</td>
<td>0.56</td>
<td>1.38</td>
<td>18</td>
<td>0.41</td>
</tr>
<tr>
<td>1.05</td>
<td>5.69</td>
<td>0.59</td>
<td>0.78</td>
<td>10</td>
<td>0.76</td>
</tr>
<tr>
<td>1.85</td>
<td>4.02</td>
<td>0.52</td>
<td>0.52</td>
<td>8</td>
<td>1.00</td>
</tr>
<tr>
<td>3.45</td>
<td>3.66</td>
<td>0.53</td>
<td>0.63</td>
<td>7</td>
<td>0.85</td>
</tr>
<tr>
<td>10.45</td>
<td>2.36</td>
<td>0.61</td>
<td>0.33</td>
<td>4</td>
<td>1.86</td>
</tr>
</tbody>
</table>

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Klaas Timmermans et al., in prep.
Light and Fe co-limitation

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

Chaetoceros brevis

open symbols
60 µmol fotons m⁻² sec⁻¹

filled symbols
15 µmol fotons m⁻² sec⁻¹

Fe⁺ (x 10⁻¹² M)

µ (d⁻¹)

4 - 6 µm diameter (small)

Chaetoceros dichaeta

C. dichaeta
K_mFe⁺₁.12 x 10⁻⁹ M

20 h light: 4 h dark
12 h light : 12 h dark

KmFe_diss:1.12 x 10⁻⁹ M

does not grow at all

Chain-forming large cells

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

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

C. brevis

single cells 4 - 6 µm diameter (small)

Open Southern Ocean
HNLC species

Large versus small
at optimal light levels


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


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

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

Emiliania huxleyi

excretes external CaCO3 platelets

Concerted photosynthesis & calcification

• Zn-carbonic anhydrase permits fast use of \([\text{HCO}_3^-]\)
• Calcification provides the necessary proton to make \(\text{CO}_2\)

Buitenhuis, Timmermans and de Baar, Limnol.Oceanogr., in press
Growth on \([\text{HCO}_3^-]\) at 3 different \([\text{Zn}^{2+}]\)

![Graph 1](image1)

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

Growth on \([\text{Zn}^{2+}]\) at constant \([\text{HCO}_3^-]\)

![Graph 2](image2)

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 $\text{[HCO}_3^\text{-}]$ depends on $\text{[Zn}^{2+}]$
  - most suitable concept for Zn-carbonic anhydrase

Which would provide the best fit??


---

Multiply two Monod equations

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

best fit: mean residual on $\mu = 0.018 \text{ day}^{-1}$
Minimum nutrient governs growth rate

Affinity for $[\text{HCO}_3^-]$ depends on $[\text{Zn}^{2+}]$
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 CO₂ sink

Christiane Lancelot, Nice 2003 lecture
1D SWAMCO-4 results at KERFIX [1993]:
Thermodynamic & biological control of air-sea CO2 fluxes

Christianne Lancelot, Nice 2003 lecture

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

Christianne Lancelot, Nice 2003 lecture
PISCES 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.
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 (Boye, 2000) show good agreement. Also shown is dissolved iron at stations 6 (37°28.5'S, 22°36.7'W) and 8 (42°34.1'N, 23°02.3'W) of Boye (2000). For comparison dissolved Fe at 47°N, 26°W (Martin et al., 1995a) and 54°N, 13°W (Landing et al., 1995) are also shown. Drafted after Boye (2000) and Boye et al. (submitted (a)).

True and accurate dissolved Fe values still are puzzling.

Certified standard is urgently needed.
Atlantic Fe distribution in Hamburg model

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

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IRONAGES standard: collection

---

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

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

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
Scientific Committee for Oceanic Research SCOR
European Union Programs MERLIM, CARUSO, IRONAGES
National Science Agencies (NWO, NERC, DFG, CNRS)
Our universities and institutes

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

European Union