Eukaryotic Picoplankton Composition and Succession during the Iron Fertilization Experiment LOHAFEX in the Southern Ocean

C. Wolf, E. Kilias, I. Schulz, K. Metfies

Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany

IPY 2012 Conference Montréal, Canada
23.04.2012
LOHAFEX

- RV *Polarstern* cruise ANT XXV/3 (austral summer 2009)
- joint Indo-German experiment
- Atlantic sector of the Antarctic Circumpolar Current (ACC)

LOHA = iron (Hindi)
FEX = fertilization experiment
overall intention of the experiment:
→ investigate the fate of iron fertilized bloom biomass

general outcome:
• experiment was carried out in a silicic acid depleted mesoscale eddy:
  → prevented diatoms from accumulating biomass
  → domination of nano- and picoplankton (<10 µm)
• fertilization had little effect on vertical flux
  → heavy grazing of large copepod population
Objectives

- influence of iron fertilization on eukaryotic pico- and nanoplankton (<6 µm)
- composition and succession during the experiment

microscopy: cell counts
molecular approach: 454-pyrosequencing
### Sampling

#### microscopy

<table>
<thead>
<tr>
<th>days after fertilization</th>
<th>location</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>(in patch)</td>
</tr>
<tr>
<td>2</td>
<td>in patch</td>
</tr>
<tr>
<td>4</td>
<td>out patch</td>
</tr>
<tr>
<td>5</td>
<td>in patch</td>
</tr>
<tr>
<td>10</td>
<td>in patch</td>
</tr>
<tr>
<td>14</td>
<td>in patch</td>
</tr>
<tr>
<td>16</td>
<td>out patch</td>
</tr>
<tr>
<td>23</td>
<td>in patch</td>
</tr>
<tr>
<td>25</td>
<td>in patch</td>
</tr>
<tr>
<td>30</td>
<td>out patch</td>
</tr>
<tr>
<td>33</td>
<td>in patch</td>
</tr>
<tr>
<td>35</td>
<td>out patch</td>
</tr>
<tr>
<td>37</td>
<td>in patch</td>
</tr>
<tr>
<td>38</td>
<td>out patch</td>
</tr>
</tbody>
</table>

#### 454-pyrosequencing

<table>
<thead>
<tr>
<th>days after fertilization</th>
<th>sample name</th>
<th>location</th>
<th>iron [nmol/l]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>L2</td>
<td>(in patch)</td>
<td>0.19</td>
</tr>
<tr>
<td>10</td>
<td>L3</td>
<td>in patch</td>
<td>0.24*</td>
</tr>
<tr>
<td>16</td>
<td>L4</td>
<td>out patch</td>
<td>0.19</td>
</tr>
<tr>
<td>18</td>
<td>L5</td>
<td>in patch</td>
<td>1.10</td>
</tr>
</tbody>
</table>

* not measured in the center of the patch

microscopy: CTD 0-80 m

454-pyrosequencing: CTD 20 m
**Methods**

**microscopy:**
- cells were identified and counted in transects using an inverted light microscope
- biovolume and biomass was determined

**molecular approach (454-pyrosequencing):**
- amplification of highly variable V4-region of 18S rRNA gene (app. 670 bp)
- quality check, chimera check and assembling of reads (97% identity)
- placement in a reference tree (Phyloassigner)

454-pyrosequencing process (Rothberg and Leamon 2008)
Results

Microscopy

Integrated biomass
(0-80 m, all cells)
Results

integrated cell counts
(0-80 m, cells <6 µm)
Results

454-pyrosequencing 0.2-5 μm

- No iron addition
  - +10 days
  - +18 days
  - +16 days

- Iron addition

<table>
<thead>
<tr>
<th>Treatment</th>
<th>L2</th>
<th>L3</th>
<th>L4</th>
</tr>
</thead>
<tbody>
<tr>
<td>No iron addition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron addition</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- N = 11517
- N = 28586
- N = 17968
- N = 20762
Results

454-pyrosequencing
0.2-5 µm

N = 28586
Phaeocystis sp.
unc. Prymnesiophyte
unc. Micromonas sp. 1
unc. Micromonas sp. 2
unc. Syndiniales 6
unc. Syndiniales 5
unc. Syndiniales 3
unc. Syntina
Monomastix sp.
unc. Bathycoccus sp.
Pseudochattonella sp.
Pirsonia sp.

N = 17968
Phaeocystis sp.
rare biosphere
unc. Micromonas sp. 1
unc. Micromonas sp. 2
unc. Syndiniales 6
unc. Syndiniales 5
unc. Syndiniales 3
unc. Stramenopila
unc. Stramenopila
unc. Pelagophyte
unc. Labyrinthulid

N = 20762
Phaeocystis sp.
rare biosphere
unc. Micromonas sp. 1
unc. Micromonas sp. 2
unc. Monomastix sp.
unc. Bathycoccus sp.
unc. Pelagophyte
unc. Labyrinthulid

N = 11517
Phaeocystis sp.
rare biosphere
unc. Micromonas sp. 1
unc. Micromonas sp. 2
unc. Monomastix sp.
unc. Bathycoccus sp.
unc. Pelagophyte

+18 days
+10 days
iron addition
no iron addition
+16 days
Results

MDS plot (Bray Curtis) integrated cell counts (0-80 m, cells <6 µm)

MDS plot (Jaccard) 454-pyrosequencing (0.2-5 µm)
Conclusions

- iron fertilization slightly enhanced biomass production during the first three weeks
- but composition of eukaryotic <6 μm fraction did not change significantly (no winner or looser)
- rather natural/temporal succession than iron induced succession
- <6 μm assemblage was dominated by *Phaeocystis* sp., prasinophytes (*Micromonas* sp., *Monomastix* sp.) and small dinoflagellates (*Syndiniales*)
Thank you for your attention!

Acknowledgements:
- E. Kilias and K. Metfies
  (molecular support)
- I. Schulz
  (microscopy analysis)
- S. Frickenhaus and F. Kilpert
  (bioinformatical support)
- P. Assmy
  (advisory support)

Questions?