

## A non-diatom plankton bloom controlled by copepod grazing and amphipod predation: Preliminary results from the LOHAFEX iron-fertilisation experiment

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The most memorable LOHAFEX cruise came to an end on 17 March 2009 when RV *Polarstern* docked in the harbour of Punta Arenas (southern Chile) after successfully carrying out the longest iron fertilisation experiment so far (39 days). The two and a half month voyage, spent in the notorious Roaring Forties of the south-western Atlantic, was as close to an adventure as a research cruise can get these days. We first had to weather a totally unexpected political storm while we were selecting a suitable meso-scale eddy in which to conduct the experiment. Permission to go ahead with fertilisation came just as we had satisfied ourselves that we were indeed right in the centre of the selected eddy. After spreading 10 tonnes of dissolved ferrous sulphate over a patch of 150 km<sup>2</sup>, we followed, with excitement, the chemical and biological changes stimulated by iron fertilisation while chasing the patch circling within the eddy (Fig. 1). Some weeks later, to our chagrin, the eddy started collapsing and ejected our patch, which, nevertheless, miraculously “waited” for two weeks, squeezed between other eddies, until our day of departure.



Figure 2. Participants of the LOHAFEX cruise onboard the R/V *Polarstern* (photograph courtesy of T. Bresinsky).

We also witnessed the spectacular collapse of a stately iceberg (from a safe distance) and were rocked to near-panic by a series of freak waves during our farewell party. In short, it was an exceptionally stimulating and emotionally rewarding experience.

The joint Indo-German venture LOHAFEX (Loha is the Hindi word for iron), for which preparations started in 2005, was targeted for the productive south-western sector of the Antarctic Circumpolar Current (ACC) where we expected to fertilise a different type of plankton community to those of the severely iron-limited ACC studied by the previous five experiments. LOHAFEX was carried out by an interdisciplinary group of 48 scientists (29 Indian, 10 German and nine from five other countries; Fig. 2): the physicists selected the eddy, pin-pointed its centre (based on altimeter images, drift trajectories of two surface buoys, and an Acoustic Doppler Current Profiler survey) and kept track of the patch while the chemists and biologists followed the processes within it and compared them with outside waters.

In contrast to the rest of the ACC, silicic acid in the south-western Atlantic is depleted by summer also well south of the polar front, clearly due to the extensive spring diatom blooms that occur there and are conspicuous in satellite images of chlorophyll distribution. The sources of iron fuelling these blooms are varied:

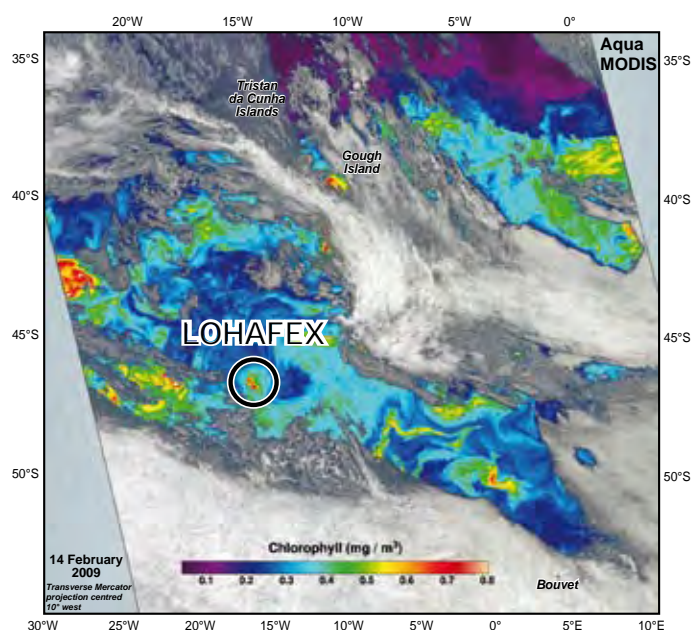


Figure 1. Chlorophyll a satellite picture of the LOHAFEX bloom from the NASA website ([http://oceancolor.gsfc.nasa.gov/cgi/image\\_archive.cgi](http://oceancolor.gsfc.nasa.gov/cgi/image_archive.cgi)).

contact with landmasses and islands, Patagonian dust and the many icebergs characteristic of this region. The core water of the eddy in which we carried out the experiment had evidently experienced such a bloom because silicon concentrations were at limiting levels down to 100 m depth, so further growth of diatoms was not possible. In a previous experiment (European Iron Fertilization Experiment: EIFEX) conducted at the same latitude and season but in silicon-rich water 1,000 km further east, a massive diatom bloom developed and reached 3 mg Chl m<sup>-3</sup> within three weeks. Despite consistently high levels of primary production (~1.5 g C m<sup>-2</sup> d<sup>-1</sup>), biomass accumulation stopped thereafter due to sequential fall-out of some early-response diatom species which was compensated by population growth of others that responded later. But the situation in the LOHAFEX bloom was different.

The phytoplankton community of mixed flagellates responded rapidly to the iron addition by increasing, as expected, Fv/Fm ratios (a measure of photosynthetic efficiency) above 0.4 and chlorophyll concentrations in the 60 m mixed layer doubled to 1.5 mg Chl m<sup>-3</sup> within two weeks but stopped increasing thereafter, although rates of primary production remained high (>1.0 g C m<sup>-2</sup> d<sup>-1</sup>). A second fertilisation three weeks later had no apparent effect indicating that the community was arrested in steady state despite abundant nutrients and sufficient light. However, there were changes in the composition of the phytoplankton that were not as easy to follow on board as in the case of large diatoms. The bulk of the biomass was contributed by small flagellates <5 µm with the smallest size fraction (~2 µm) increasing towards the end of the experiment to densities measured by flow cytometer and corroborated with counts made with an inverted microscope as high as >15 x 10<sup>6</sup> cells L<sup>-1</sup>. A significant fraction of the larger flagellates (~5 µm) were *Phaeocystis* solitary cells that started making numerous colonies in the second week, so we were expecting a *Phaeocystis* bloom to develop (Fig. 3). Dense blooms of balloon-like colonies of this alga are commonplace around the continent, however, the colonies disappeared by the third week. Although the community was dominated by pico- and nanophytoplankton it was functioning differently to the bacteria-based microbial "loop" which is the paradigm for planktonic recycling systems.

There was no evidence, as in the case of the EIFEX diatom bloom, that the lack of biomass accumulation was due to a balance between new growth and sinking out of older cells. Low to moderate sinking fluxes with no difference between inside and outside the patch were indicated by transmissometer profiles, thorium measurements, catches of neutrally buoyant sediment traps and vertical particle profiles recorded with the Underwater Video Profiler, version 5 (UVP5; a camera system mounted on the CTD which records particles down to 0.06 mm diameter from the surface to 3,000 m depth). So the excess biomass due to iron fertilisation must have been retained in the surface layer but not as phytoplankton cells.

The relatively high ammonia levels in the surface layer compared to outside waters were indicative of higher remineralisation rates but these could not have been due to bacteria because their numbers were well below the average reported from the region and their growth rates were moderate. Besides, there was no significant difference between the bacterial communities inside and outside the patch. Why they were suppressed in the entire region and not revived, as in other experiments, by iron addition is a mystery in itself begging an explanation. The only processes of bacterial and phytoplankton cell removal left are grazing by proto- and metazooplankton respectively, or death and disintegration by viral attack as has been demonstrated for bacteria and flagellates. At this stage we can only speculate on the possible role of viruses, but there is a strong case for copepod grazing being the structuring factor in the LOHAFEX bloom.

Luckily for us, the structure of the grazer community was unusually simple: the stocks of flagellate-grazing protozooplankton (ciliates and dinoflagellates) were much lower than during EIFEX, apparently controlled by heavy copepod grazing pressure, as there was an abundant food supply. Copepods were dominated by one representative each of the large (*Calanus simillimus*), medium (*Ctenocalanus citer*) and small (*Oithona similis*) size groups. The other copepods common in the ACC (*Rhincalanus*, *Pleuromamma*) were also present but in low numbers. In the integrated 0-100 m water column, the highest abundances estimated, from preliminary counts conducted onboard, ranged from 199 x 10<sup>3</sup> ind. m<sup>-2</sup> for *Oithona* spp. to 90 x 10<sup>3</sup> ind. m<sup>-2</sup> for *C. simillimus*, and 31 x 10<sup>3</sup> ind. m<sup>-2</sup> for *C. citer*, all recorded at in-patch stations within the 20th day after fertilisation.



Figure 3. Small *Phaeocystis* colonies on *Corethron pennatum* spines (photograph courtesy of M. Montresor).

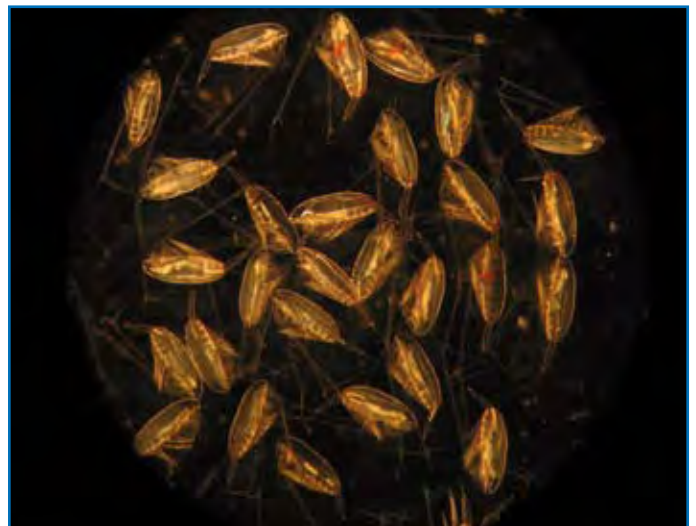


Figure 4. *Calanus simillimus* copepodites (photograph courtesy of M.G. Mazzocchi).

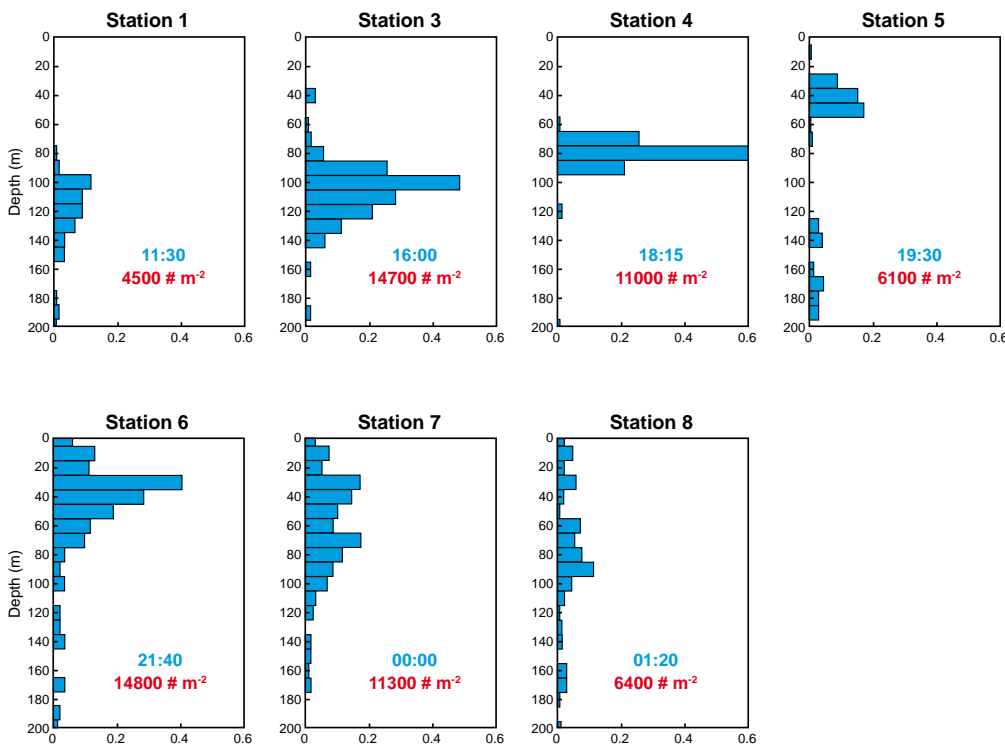


Figure 5. Copepod vertical distribution ( $N \text{ ind. L}^{-1}$ ) measured at 10 m vertical resolution step with the Underwater Video Profiler 5 along a south-north transect through the patch. The first (Station 1) and last two stations (Stations 7 and 8) were outside the fertilised patch. Data are unavailable for Station 2. The ship time (UTC-1) is given in blue and the integrated number of copepods per  $\text{m}^2$  for the first 200m is shown in red.

Clearly, *C. simillimus*, consisting almost entirely of late juvenile stages (CIV–CV copepodites) with large lipid sacs, overwhelmingly dominated copepod biomass (Fig. 4). Apparently, this population had developed during the previous bloom as there were very few adult females still present. The UVP5 images (Fig. 5) indicated that the bulk of the population was concentrated around 100 m depth during the day and dispersed within the 60 m mixed surface layer at night where they left behind large numbers of faecal pellets. There was a distinct decline over the course of the experiment in numbers and diversity of plankton collected by a 20  $\mu\text{m}$  mesh net routinely examined on board. As many of the missing species were found in copepod pellets, particularly tintinnid loricae, diatom frustules, cell walls of a common, large dinoflagellate (*Ceratium pentagonum*), foraminifera shells, it is most likely that heavy grazing pressure exerted by the copepods was responsible. The shift to the smallest phytoplankton size class, in the veritable absence of ciliates, could also be attributed to “size escape” from copepod grazing pressure.

Since the *C. simillimus* population was not growing in numbers, the food was presumably being converted into lipid reserves by this deep diapausing species. Because lipids are hydrocarbons,

the essential elements, including iron, were being recycled back to the phytoplankton. The necessary calculations will only be possible after all the samples from the various depth ranges sampled with the two multineets deployed at all major stations as well as the samples from grazing experiments have been processed. Due to marked spatial patchiness, reflected in wide variation in integrated standing stocks at both in- and out-stations, we cannot yet say whether copepod numbers were higher inside the patch due to congregation from the surroundings. However, the copepods inside the patch were eating more as indicated by the faecal pellet production rates expressed as volume of faecal pellets egested, which were about double inside as compared to outside the fertilised patch (67 and 39  $\times 10^6 \mu\text{m}^3 \text{ ind.}^{-1} \text{ d}^{-1}$ , respectively) (Fig. 6). This could well have accounted for the fate of the extra biomass inside the patch because the community structures inside and outside, in contrast to diatom-dominated blooms, were essentially similar in all respects.

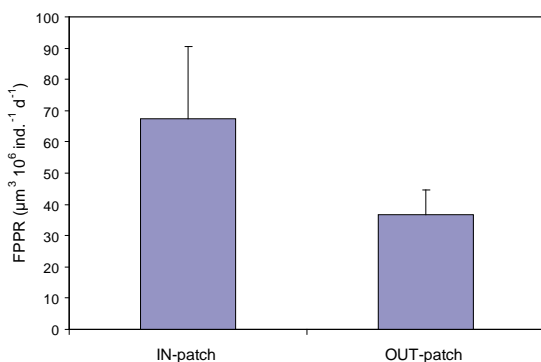


Figure 6. Faecal pellet production rate (as volume,  $\mu\text{m}^3 \times 10^6 \text{ ind.}^{-1} \text{ d}^{-1}$ ) in *Calanus simillimus* averaged at the stations inside the fertilised patch (days 4–36 after fertilisation) and outside the patch (days 16–35).



Figure 7. *Themisto gaudichaudii* (photograph courtesy of H.E. González).

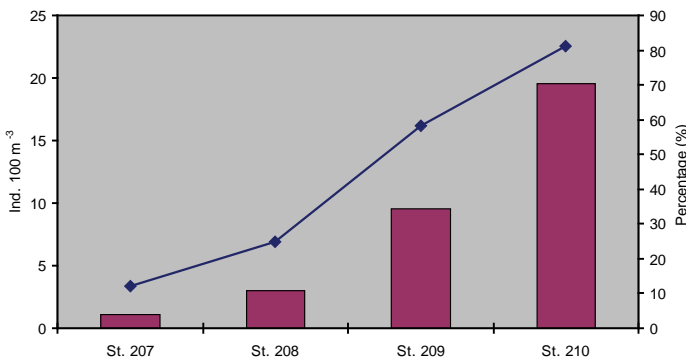


Figure 8. Abundance of *Themisto gaudichaudii* (columns) and its percentage contribution (line) to macrozooplankton abundance in rectangular mid-water trawl samples along a transect.

Macrozooplankton was dominated by the swarm-forming, hyperiid amphipod *Themisto gaudichaudii*, an active, aggressive, indiscriminate carnivore which attacks organisms much larger than itself with its formidable clawed appendages (Fig. 7). Its high densities in the region were the most likely reason why other large zooplankton, including euphausiids but in particular chaetognaths and salps, which thrive on copepods and small flagellates respectively, were present in relatively small numbers. The integrated average abundance of *Themisto gaudichaudii* in the upper 100 m water column (17 night catches with the rectangular midwater trawl) was two-fold higher inside (24 ind. 100m<sup>-3</sup>) than outside the patch (12 ind. 100m<sup>-3</sup>). This was confirmed by a transect of four trawls conducted from outside to inside the patch at the end of the experiment (Fig. 8). What attracted swarms of this species to the patch is not clear at this stage. As it is practically the only visual predator in this region, the strong diurnal migrations and remarkable transparency of its potential prey, in particular salps, can be attributed to selection by this predator. The impact of predation by *Themisto* on the copepods will be evaluated from the results of feeding experiments.

Surprisingly little is known about the biology and quantitative distribution of this interesting predator which is known to be the dominant zooplanktivore in productive regions of the northern ACC, extending northward along the Patagonian shelf in the southwest Atlantic. It seems to at least partially fill the niche occupied by small swarming fish almost everywhere else further north and is the major conduit from plankton to vertebrates and cephalopods, analogous to the role played by the much better known Antarctic krill (*Euphausia superba*) to the south of its range. Indeed *Themisto* has been called “krill of the north” and is reported to be the main food of the squid *Illex argentinus* which is the target of the intense squid fishery along the Patagonian shelf break, prominent in satellite images of the world by night, because lights are used to attract squid. So why does only this region shine like an inhabited coastline in the night and why is there nothing similar in other parts of the ocean? Is it a bottom-up condition, driven by local hydrography mixing nutrient- with iron-rich waters along the shelf edge, or does the unique biology of *Themisto* hold the key to the explanation? Would long-term, larger-scale iron fertilisation in the ACC duplicate the processes along the Argentinean shelf edge?

A major conclusion from LOHAFEX is that, despite high growth rates, biomass of non-diatom phytoplankton can be kept in check by grazing pressure of oceanic copepods which can establish a recycling system analogous to, but different from, the classic microbial food web. The fact that copepods increased their feeding rates inside the patch indicates that they were food-limited in the surrounding waters. As a result, biomass accumulation was modest as was vertical flux of organic carbon, hence net uptake of atmospheric CO<sub>2</sub> by the fertilised phytoplankton was only marginally different to the unfertilised, surrounding water. This result conforms with observations that massive phytoplankton blooms in the ocean are almost exclusively due to diatoms (coccolithophorid blooms are prominent in satellite images but usually contain much less biomass than the spring and upwelling diatom blooms) and highlights a fundamental difference between oceanic and coastal waters where many non-diatom phytoplankton species (such as *Phaeocystis* and *Ceratium*) contribute to dense blooms. Presumably the larger zooplankton stocks on a square metre basis in oceanic as compared to coastal waters can exert top-down control on the structure of the ecosystem and, in the absence of silicon, prevent uptake of nutrients to limiting concentrations.

The main interest in iron fertilisation experiments has focused on the biological carbon pump because of its implications for sequestration of atmospheric CO<sub>2</sub>. LOHAFEX has shown that an adequate supply of silicic acid is a prerequisite for significant, deep carbon sequestration, thereby restricting the region of the Southern Ocean where significant amounts of carbon dioxide can be sequestered from the atmosphere. Of course, we cannot say what happened to the system in the surface layer after the *C. simillimus* population departed for diapausing, how much carbon they took down with them and how much was retained in the deep ocean by predation at depth. Certainly, the C:N ratio of diapausing copepods will differ considerably from sinking diatom aggregates. Further experiments will yield new insights on physical, chemical and biological processes that govern the functioning of planktonic ecosystems. These fundamental control mechanisms can only be studied under *in situ* conditions as it is impossible to simulate natural grazing pressure under laboratory or mesocosm conditions. More open ocean perturbation experiments, analogous to the whole-lake experiments carried out by limnologists, are called for to study the impact of zooplankton grazing pressure in different regions and seasons on phytoplankton biomass and composition, hence ultimately the global carbon cycle.

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