

BIOLOGICAL CONTROL OF HARMFUL ALGAL BLOOMS; THE PHAEOCYSTIS CASE STUDY.

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Massive blooms of *Phaeocystis* are annual events in European coastal waters and belong to the so-called non-toxic Harmful Algal Blooms (HAB). The species is polymorphic, including single cells (4 to 7 μm in diameter) and larger colonies, with numerous cells embedded in mucus. The single cells belong to the 'normal' phytoplankton community and are as such subject to biomass controlling factors like grazing by micro- and mesozooplankton. In contrast the size of the colonies, which can be up to several mm in the case of *P. globosa*, colonies of *P. pouchetii* are much smaller, do not 'fit' in the grazer controlled size range of the phytoplankton community. In particular the colony form can produce massive blooms in spring and early summer in just a few days. The foam on the beaches of the southern North Sea and its implications for tourism-related activities is the most visible aspect of the fate of the colonial matrix of *Phaeocystis*. Slime on fishing gear is another reported negative aspect of this HAB. Occasionally, during the degradation of the organic matter local anoxia in the water column occurs, killing massively shellfish like mussels and oysters.

The species not only blooms in just a few days but also disappears completely in a few day's. Biocontrollers (natural enemies), and in particular viruses, are thought to be responsible for the sudden termination of *Phaeocystis* blooms.

In order to have more insight in the bloom controlling factors and the role of colony formation in this process large-scale indoor mesocosm experiments were conducted as part of a large EU-sponsored program studying biological control of HABs. The aim of this experiment was to study the dynamics of the *Phaeocystis* blooms in relation to different environmentally realistic N/P ratios and the potential role of viruses and zooplankton grazing as bloom controlling factors. In two mesocosms nutrients were added in a balanced nutrient requirement for phytoplankton (N:P=16). In the other two mesocosms either a surplus of orthophosphate (N:P=4) or of nitrate (N:P=44) was added.

Before realising such a detailed study new tools and techniques were developed. Firstly, the detection and enumeration of viruses was improved using flow cytometry. Secondly, cell specific assays were adopted to detect the general viability of the different cell types of *Phaeocystis* (single cells and colonies). It was shown that virus infected algal cells rapidly lose their viability and thus can be distinguished easily from healthy cells. Finally, we succeeded in obtaining independent estimates of the gross growth rate of single and colony cells when co-occurring using the DNA replication rate as a tool. Because of the larger size of the colonies, up to mm's, this has for long been considered impossible. This last aspect is crucial in determining the whole set of growth and loss parameters of both cell types.

During the large-scale mesocosm experiments it was for the first time shown that viruses have an effect on the mitigation of a HAB-species but could not prevent the bloom formation of

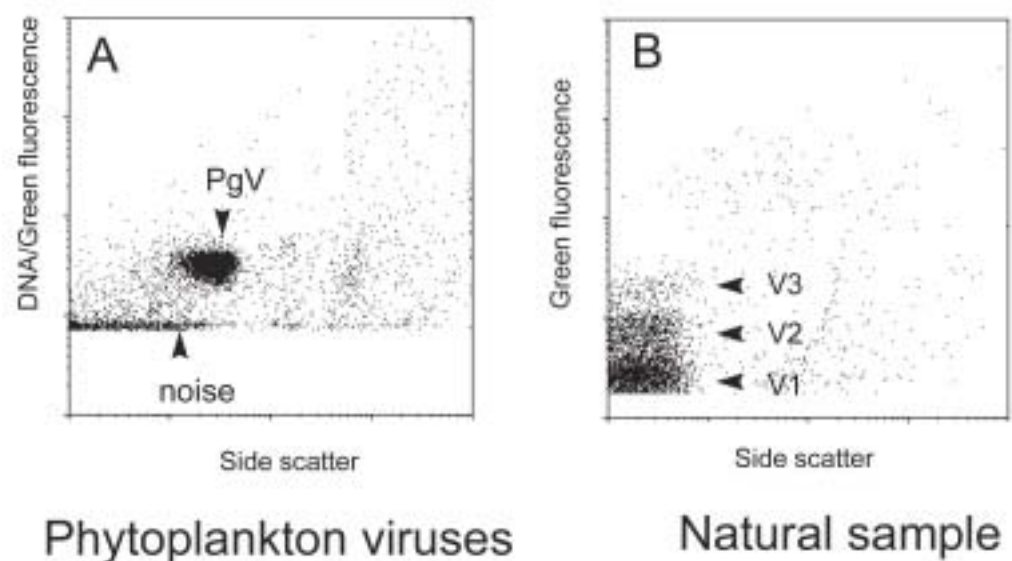
it. Blooms that developed in one week were terminated in the subsequent week with little or no differences between the different nutrient regimes. Enhanced rates of total phytoplankton cell lysis were recorded during the decline of the *P. globosa* blooms resulting in massive cell mortality. Only single cells were susceptible to viral induced mortality or zooplankton grazing, whereas colonies escaped from this process as long as they remained intact.

The colonial derived mucus formation resulted in large aggregates in the nutrient limited mesocosms. The colony structure not only prevented the cells inside the colony from being infected by viruses, as long as the colony was intact, but the large aggregates could trap free viruses as well. This last process will therefore also reduce viral infection of single cells.

The different experiments conducted to estimate the grazing upon the two morphotypes of *Phaeocystis* showed also some interesting results. Grazing by large mesozooplankton like copepods was low and when present other food sources were preferred above *Phaeocystis*. Moreover, the development of copepod nauplii (young copepods) was hindered in the presence of *Phaeocystis*. In contrast single cells were a major food source for the smaller sized zooplankton (microzooplankton). At time of massive production of the mucus also a succession in the microzooplankton species composition was observed.

The whole set of available data was used to construct an ecosystem model where a differentiation was made between the growth and mortality dynamics of single cells and colonies of *Phaeocystis*. The model included totally new scenarios so far unknown. Of crucial importance was the polymorphic appearance of *Phaeocystis* (as colonies and single cells). This did not only affect the mortality caused by grazing and viruses but could also explain the differences in gross growth rates of both cell types. Measurements indicated that the gross growth rate of colony cells was on average 2 times higher than that of the single cells. Combined with the reduced mortality rates this explained the massive blooming of in particular colonies. Furthermore, trapping of viruses by the mucus remnants of the colonies (Transparent Exopolymeric Particles) reduced the chances of infection through a general reduction of the viral abundance. The role of different nutrient ratios appeared of minor importance, except for the fact that the colonies tended to disintegrate when nutrients were depleted.

Despite some remaining uncertainties with respect to the parameterisation of the virus module the exercise to include the virus dynamics in the model was successful and should be considered as a major improvement of complex ecosystem models.



Flow cytometric signatures of particle size and DNA content. Picture A shows a typical virus found in cultures of *P. globosa*, picture B shows three different groups of viruses in a natural sample.