

Sampling techniques for quantitative analyses of calcareous marine phytoplankton

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Introduction

Phytoplankton is constituted by minute organisms but it is a key component of the oceanic ecosystem. It plays a key role in global biogeochemical cycles, particularly in the carbon-carbonate cycle (Honjo, 1976; Westbroek, 1991; Westbroek et al., 1994), but also in the sulphur cycle since it produces dimethylsulphoniopropionate (DMSP), the precursor of dimethyl sulphide (DMS) (Keller et al., 1989; Malin and Kirst, 1997) which may influence climate through stimulating cloud formation and influencing the Earth's radiative balance (Charlson et al., 1987; Simó and Pedrós-Alió, 1999). Some algae are known to produce stable lipid compounds which can be used as a tool to evaluate paleoclimatic changes (Volkmen et al., 1980; Brassell et al., 1986). These properties, together with the fact that the ubiquitous species *Emiliana huxleyi* is a recognized bloom forming alga (Holligan et al., 1993), confirm that the phytoplankton have an important role as active biogeochemical and climatic agents.

In order to assess their contribution to the global carbon cycle, absolute abundance determinations (cells/l) are needed. There are two basic methods for quantifying the abundance of calcareous marine phytoplankton: the Utermöhl settling method and the filtration method. Samples prepared using the Utermöhl method can only be analysed by light microscopy, whereas filter

preparations are suitable for both LM and scanning electron microscopy (SEM). The application of these two methods depends on the purpose of the study. The Utermöhl method has been widely used to quantify total phytoplankton composition in water samples, whereas filter preparations are mainly used to analyse calcareous marine phytoplankton. The use of different methods, filter funnels, microscopes or filter membranes may result in different cell density estimates.

Sampling method

Utermöhl method

Thirty milliliters of 20% hexamethyl tetramine buffered formalin (pH 7.5) are mixed in dark plastic bottles with 250 ml of seawater, transferred from Niskin bottles. These bottles are stored in the dark prior to analysis. For analysis, 100 ml sub samples are introduced into a settling chamber and the phytoplankton is allowed to settle for 72 h (Margalef, 1969). Phytoplankton cells are then identified and counted by the Utermöhl method using an inverted microscope equipped with phase contrast and bright field illumination.



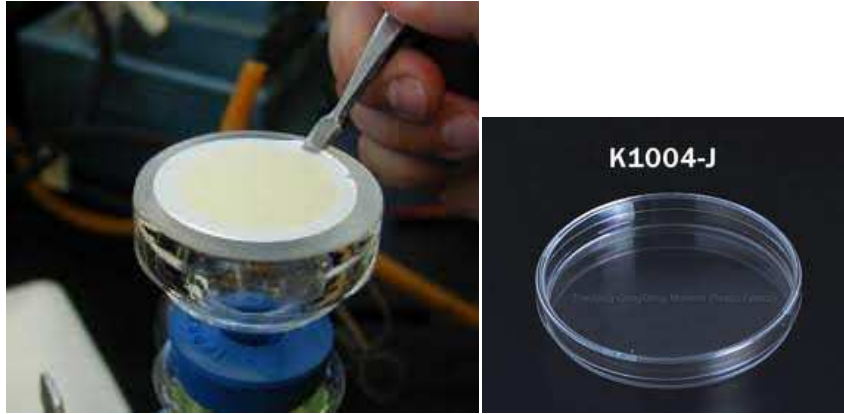
Relatively large Coccolithophores cells are identified and counted in alternate fields of the settling chamber, at a magnification of 160X. This corresponds to 50 ml of seawater analysed with a detection limit of 60 cells/l at a 95% confidence level. Where necessary, species identification can be confirmed at a magnification of 400X or 1000X. Relatively small species, such as *Emiliana huxleyi*, are counted and identified at a magnification of 400X in 64 squares. This corresponds to 1 ml of seawater analysed with a detection limit of 3000 cells/l at a 95% confidence level.

Filtration method

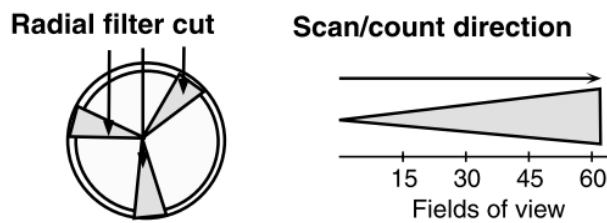
Up to 10 l of seawater are filtered on a 47 mm diameter cellulose mixed ester membrane filter or polycarbonate membrane filter using Millipore inline filter gaskets.



Each filter membrane are rinsed with distilled water (pH 8.5) immediately after filtration in order to remove all traces of sea salt. All membranes were stored in plastic Petri dishes and dried in an oven at 40-60°C for several hours. The seawater is filtered with an accuracy of 0.1 l.



For light microscope analyses a piece of filter membrane, cut along its radius, it's mounted onto a glass slide using Canada Balsam and fixed beneath a cover slip.



A similar sized piece of filter membrane can be mounted onto an aluminium stub using carbon tape and coated with 15 nm of gold for subsequent analysis in the SEM.





Light Microscope analyses

Cell counts can be carried out with a polarising light microscope using 63X or 100X objectives and a 10X eyepiece lens. The area represented by one field of view is 0.090 mm² and 0.037 mm², respectively, and the total observed area (52 fields of view along the radial cut) is 4.68 mm² and 1.92 mm² (see also Haidar and Thierstein, 2001).



SEM analyses

SEM equipped with a computer controlled stage at a magnification of 1500X and 3000X. Calculation of the observed filtration area relies on the positioning accuracy of the stage and the accuracy with which the area of one field of view can be calculated. The area of observation is the sum of the area of all single fields of view. This is easily estimated and controlled with LM, but it is more difficult to calculate and control in the SEM. In both cases, it is important

that single fields of view do not overlap to prevent double counting of specimens. Using a computer-driven stage makes it possible to control the size of the total observed area, to prevent overlapping and, therefore, avoiding the double counting of an area. A remotely controlled scanning electron microscope in combination with a imaging software analysis can be used to capture and store all images collected along a predefined transect.