12 Harmful Algae and Cell Death

M.J.W. Veldhuis and C.P.D. Brussaard

12.1 Introduction

Mortality can be defined as the number of deaths in a given time, place or population, i.e. it is the loss term of population dynamics. For phytoplankton it is the opposite of cell population growth and is responsible with other loss factors for a reduction in the population size of a species (Reynolds 1984). In summary, this can be expressed as:

\[ \mu_{\text{gross}} = \mu_{\text{net}} + \text{grazing} + \text{lysis} + \text{sinking} + \text{physical transport} \]  

(Eq. 12.1),

where \( \mu_{\text{gross}} \) is the specific growth rate; \( \mu_{\text{net}} \) is the net increase in cell abundance; grazing is the loss of algal cells due to herbivore grazing; cell lysis is the rupture of the cell membrane and loss of cytoplasm caused by autocatalytic mortality, pathogenicity (viral, bacterial, fungal) or stress-induced death; sinking is the loss of cells due to sedimentation and burial; and physical transport is dilution by lateral/vertical dispersal.

For a long time, grazing and sedimentation have been considered as the main factors reducing the size of natural phytoplankton populations. In the last decade of the 20th century cell lysis became recognised as an additional and significant loss factor for phytoplankton cells, affecting not only algal population dynamics and species succession, but also the flux of energy and matter to other trophic levels (Van Boekel et al. 1992; Brussaard et al. 1996; Agustí et al. 1998).

The fact that phytoplankton cells die has been noticed by anyone who has ever cultured algal species (Fig. 12.1a). Directly upon transfer into fresh medium, a slight decline in total cell abundance is often noted. More obviously, the cell abundance in batch cultures typically declines after having reached a maximum due to limitation by nutrients or light (shelf-shading of dense cultures). Detailed laboratory studies clearly revealed significant specific cell lysis that was caused by the physiological stress of nutrient limitation (Brussaard et al. 1997; Lee and Rhee 1997) or light (Berges and Falkowski...
Interestingly, a form of intrinsic cell death under N-controlled conditions (using continuous cultures) was observed for *Ditylum brightwellii*, which was independent of the growth rate (Brussaard et al. 1997). In addition to environmentally relevant factors such as nutrients and light, infection by pathogens is an important factor inducing algal cell death (Imai et al. 1993; Brussaard 2004). Both growth and mortality are traditionally based on net changes in total population abundance, but this provides no direct information on the viability status of the individual cells (in microbiology, viability refers to an organism being capable of reproducing under appropriate conditions, see Singleton and Sainsbury 1994).

Moreover, the change in photochemical efficiency of the PSII reaction centre (Fv/Fm; e.g. Berges and Falkowski 1998), the reduction in $^{14}$C-primary productivity, and the loss of pigmentation (Veldhuis et al. 2001) that are typically associated with declines in cell abundances provide only information on the population as a whole. The recent development of sensitive fluorescent viability or live/dead stains has allowed distinction of the living and dead proportions within a population. Also, the use of dyes in combination with flow cytometry allows the tracing of functional properties at the level of the single cell (Fig. 12.1b and 12.2; Collier 2000; Veldhuis and Kraay 2000). Various vital staining cell assays were specifically developed for phytoplankton, including HABs, and application of these methods, both in the laboratory and the field, showed the co-occurrence of viable and non-viable phytoplankton cells (Crippen and Perrier 1974; Dorsey et al. 1989; Jochem 1999; Brussaard et al. 2001; Veldhuis et al. 2001). The measured viability as a proportion of the total population still needs to be converted into a specific loss rate. This is relatively easy under laboratory conditions using specific formulas (Brussaard et al. 1997), by monitoring batch cultures over time or using continuous cultures in steady state, but measuring such parameters in field populations is more difficult.

Fig. 12.1. Changes in the abundance of A total cells and B live cells (abundance and as percentage of total cells) of a standard culture of *Chaetoceros calcitrans* after staining with the live/dead stain SYTOX