

Cell death in lake phytoplankton communities

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SUMMARY

1. The fraction of living and dead phytoplankton cells in seven Florida lakes was assessed by using the cell digestion assay, a non-staining membrane permeability test. The cell digestion assay is an effective method to analyse cell viability in complex natural phytoplankton communities.
2. The lakes examined ranged widely in phytoplankton abundance and community composition. The variability in the percentage of living cells (% LC) was high among the taxonomic groups forming the different phytoplankton communities, ranging from 19.7% to 98% LC.
3. All cells within single cyanobacteria filaments were determined to be either dead or alive, suggesting physiological integration of the cells within colonies.
4. Within each lake, the dominant taxa generally exhibited the highest proportion of living cells. A high proportion of living cells was found to be a characteristic of the different taxa forming the communities of eutrophic lakes. The average value for the % LC for all groups comprising the phytoplankton communities in each of the lakes ranged from 29.9 ± 7.2 to 80.4 ± 4.0 (mean \pm SE) and varied strongly and positively with chlorophyll *a* concentration.
5. These results suggest phytoplankton cell death to be an important process structuring phytoplankton communities in lakes, particularly in oligotrophic ones.

Keywords: cell death, community structure, Florida lakes, membrane permeability, phytoplankton.

Introduction

Pioneering attempts at quantifying phytoplankton cell death in freshwater ecosystems (e.g. Jassby & Goldman, 1974; Knoechel & Kalff, 1978; Reynolds *et al.*, 1982) delivered a wide variety of results and subsequent interpretation. Although cell death was identified as an important phytoplankton loss factor in some systems, it was considered to be negligible in others (see Jassby & Goldman, 1974; Knoechel & Kalff, 1978; Reynolds *et al.*, 1982). A lack of reliable methods to discriminate dead from living cells hindered early progress in this area of research (e.g. Jassby & Goldman, 1974).

Relatively recent advances in the field of cell biology have provided new insights into the processes associated with cell death. These insights have, in turn, resulted in new concepts and also the development of new methods that might be applied to quantify phytoplankton cell death (e.g. Darzynkiewick, Li & Gong, 1994). In particular, the loss of the ability to maintain homeostasis, resulting in increased cell membrane permeability, has been identified as the event that characterises dead cells (Wyllie, Kerr & Currie, 1980; Ellis, Yuan & Horvitz, 1991; Darzynkiewicz, Li & Gong, 1994). Cell membrane permeability tests have, therefore, been developed for the discrimination between living and dead cells in cell biology (Wyllie *et al.*, 1980; Ellis *et al.*, 1991; Darzynkiewicz *et al.*, 1994). Recently, some membrane permeability tests have been successfully applied to quantify the proportion of living or viable cells in natural marine phytoplankton communities

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(Agustí & Sánchez, 2002). The results from these studies indicated that the proportion of dead cells could vary considerably among phytoplankton communities (Agustí & Sánchez, 2002; Agustí, 2004) and that the prevalence of dead cells may differ between the phytoplankton taxa comprising the communities (Agustí & Sánchez, 2002; Veldhuis, Kraak & Timmermans, 2002; Agustí, 2004). Whether the proportion of dead cells within natural freshwater phytoplankton communities are significant remains yet, untested, despite the fact that the new methods available might now render such a test possible.

The primary goal of this study was to quantify the proportion of living and dead cells in freshwater phytoplankton communities by applying a cell membrane permeability test – the cell digestion assay (see Agustí & Sánchez, 2002) – that allows for the discrimination between living and dead cells in natural phytoplankton communities. The cell digestion assay is an effective method to analyse cell viability in complex natural phytoplankton communities, because there is no staining of the cells with the morphology and optical properties of viable cells remaining unchanged after the assay, allowing the application of conventional methods, such as transmitted inverted or epifluorescence microscopy and flow cytometry, to identify and count phytoplankton (Agustí & Sánchez, 2002). The proportion of living phytoplankton cells was examined in freshwater communities of seven Florida lakes ranging widely in trophic status, phytoplankton abundance and communities composition. As a complement to the work described above, we compared results obtained for the cyanobacteria communities using the cell digestion assay (Agustí & Sánchez, 2002) to those obtained using an alternative membrane permeability test (Bac-light

Kit, Lee & Rhee, 1997) to further assess the adequacy of the cell digestion assay in discriminating living from dying phytoplankton cells collected from natural systems.

Methods

Surface water (c. 0.5 m depth) samples were collected with acid-cleaned Nalgene bottles from seven Florida lakes representing a broad range of trophic conditions (Table 1). Samples were collected between 6 and 17 March 2003, and only from lakes in close proximity to the laboratory to avoid delays between sampling and cell viability analyses (see below) which were conducted within 1 h of sample collection.

At the laboratory, total phosphorus concentrations were determined using the procedures of Murphy & Riley (1962) with a persulphate digestion (Menzel & Corwin, 1965). Total nitrogen concentrations were determined by oxidising water samples with persulphate and determining nitrate-nitrogen with second-derivative spectroscopy (D'Elia, Steudler & Corwin, 1977; Simal, Lage & Iglesias, 1985; Wollin, 1987). Specific conductance ($\mu\text{S cm}^{-1}$ at 25 °C) was measured using a Yellow Springs Instrument Model 35 conductance meter (YSI Inc., OH, U.S.A.). An Accumet model 10 pH meter (Fisher Scientific, GA, U.S.A.) calibrated with buffers of pH 4.0 and 7.0 was used to measure pH. Total alkalinity (mg L^{-1} as CaCO_3) was determined by titration with 0.02 N sulphuric acid (Method 2320 B; APHA, 1992).

Living and dead phytoplankton cells in natural communities were discriminated using the cell digestion assay, i.e. a membrane permeability test (Darzynkiewicz *et al.*, 1994) modified for phytoplankton cells by Agustí & Sánchez (2002). In the assay, cells are exposed briefly to the enzymes Trypsin and

Table 1 Chemical and biological characteristics of the Florida lakes examined at the time of sampling

	Cowpen	Alto	Alice	Lochloosa	Newnan	Wauberg	Bivans Arm
pH	5.9	5.8	7.5	7	6.9	8	9.7
Alkalinity (mg L^{-1})	0	2	85	40	21	13	64
Conductivity ($\mu\text{S cm}^{-1}$)	71	86	264	135	110	111	62
Total phosphorus ($\mu\text{g L}^{-1}$)	6	16*	18	36	118	123	177
Total nitrogen ($\mu\text{g L}^{-1}$)	140	670	610	3960	2370	2130	3800
Chlorophyll <i>a</i> ($\mu\text{g L}^{-1}$)	1	16	4.6	7.2	124.5	75	157.7

*Data from Florida LAKEWATCH (2005).

DNase I, which enter the cytoplasm of cells with damaged plasma membranes (i.e. necrotic or advanced apoptotic cells) resulting in the entire digestion of the cells, while having no measurable effect on the viability, morphology or function of live cells (Darzynkiewicz *et al.*, 1994; Agustí & Sánchez, 2002). The digestion of dead cells occurs as a consequence of fragmentation and hydrolysis of DNA by DNase I, and peptide hydrolysis by Trypsin, which penetrates the damaged cells. The digested dead cells are undetectable by microscopic observation and lose any fluorescence signals (both autofluorescence and staining), and are, therefore, effectively removed from the population (Darzynkiewicz *et al.*, 1994; Agustí & Sánchez, 2002).

Stock solutions of DNase I ($800 \mu\text{g mL}^{-1}$ in HBSS medium) and Trypsin (2% in HBSS medium), were prepared following Agustí & Sánchez (2002) and kept frozen at -25°C prior to use. The cell digestion assay was performed in duplicate using fresh samples from each of the seven lakes and initiated with the addition of $200 \mu\text{L}$ of DNase I solution for each mL of sample, followed by 15-min incubation period at 35°C . Then, $200 \mu\text{L}$ per mL of sample of Trypsin solution was added, followed by incubation for an additional 30 min at 35°C . Duplicate blank samples, i.e. fresh samples without the addition of digestion enzymes, were incubated for the same time and at the same temperatures. In samples where phytoplankton abundance was high (Bivans Arm, Newnan and Wauberg lakes, see Table 1), the viability of large phytoplankton cells ($>5 \mu\text{m}$) and colonies was analysed by using 2-mL fresh lake samples. In lakes where phytoplankton biomass was low, phytoplankton cells within a variable volume of water (0.25–0.5 L) were concentrated to a final volume of 10–20 mL with the aid of a Millipore cell concentrator fitted with a $2\text{-}\mu\text{m}$ polycarbonate filter. Duplicate 2-mL subsamples from the cell concentrate were used for both the blanks and cell digestion assay as described above. Immediately following the incubation periods, samples from the cell digestion assays were counted. Blank samples were fixed with glutaraldehyde (1% final concentration) and kept refrigerated for *c.* 2 h and then counted. In both cases, samples were placed in glass cuvettes and observed under a transmitted-light microscope to estimate the concentration of living and total phytoplankton cells respectively. The addition of fixatives to assay sam-

ples containing the enzymatic cocktail is not recommended as enzymes and fixatives react forming a dense, opaque solution, avoiding microscopic examination under transmitted light (Agustí & Sánchez, 2002). Thus living cells were counted in, duplicated, fresh samples after incubation. As a consequence, the time devoted to taxonomic identification was limited. Phytoplankton were identified to genera; populations for which genera identification was not attained during the time of counting were characterised categorically, e.g. 'undetermined' large or small flagellates, or 'pennate diatom', indicating that those populations, despite the fact they were not taxonomically identified, were distinguishable from other populations present in the sample. To quantify the concentration of total and living cells in the small ($<5 \mu\text{m}$) fraction of the phytoplankton community, the cell digestion method was applied to fresh duplicate subsamples of variable volume (1–20 mL depending on cell abundance). Duplicate subsamples for blanks were incubated at the same temperature and time without enzymes as described above. After the incubation period, the samples were gently filtered onto 0.2–0.6 μm pore size black Nuclepore filters, washed several times with distilled water to remove the enzymes, fixed with glutaraldehyde (1% final concentration) and kept frozen until examined (*c.* 6 h) under an epifluorescence microscope fitted with a blue light filter (Nikon, NY, U.S.A.). Phytoplankton composition and cell viability of Cowpen Lake was only examined using epifluorescence microscopy due to the low abundance of large phytoplankton cells within the community in this system.

The effects of incubation conditions on the digestion of dead cells were assessed prior to the sampling programme described above using natural phytoplankton samples collected from Orange Lake (Gainesville, FL, U.S.A.). The effect of temperature on the digestion of dead cells was tested by running both the cell digestion assay and blanks at two temperatures, i.e. 25 and 35°C . The effect of enzyme concentration on the digestion of dead cells was tested by adding $200 \mu\text{L}$ per mL of sample of two different concentrations of DNase (400 and $800 \mu\text{g mL}^{-1}$ in HBSS medium) and Trypsin (1% and 2% in HBSS medium) and incubating replicated samples at 25 and 35°C as described above.

The results from the cell digestion assay regarding cell viability were compared with those obtained

using the BacLight™ Viability Kit (Molecular Probes, OR, U.S.A.; Lee & Rhee, 1997) for the natural cyanobacteria trichomes collected from Bivans Arm, Newnan and Wauberg lakes. The Bac-light™ Kit (Molecular Probes) is a cell membrane permeability test based on the simultaneous staining of living and dead cells with two DNA stains, i.e. Syto 9 and PI (propidium iodide) respectively. Living cells stained by Syto 9 yield green fluorescence, whereas dead cells stained by PI yield red fluorescence under blue light. Staining with the BacLight™ Viability Kit (Molecular Probes) was carried out in parallel with the cell digestion assays described above using the same fresh samples. A volume of 0.2 µL of the BacLight™ Viability Kit was added to duplicate 1-mL subsamples, stained for 10 min in the dark (Lee & Rhee, 1997) and gently filtered onto 0.6-µm pore size black nucleopore filters. After filtration, the samples were examined under an epifluorescence microscope fitted with a blue light filter (Nikon). In a separate effort, 0.5 L of water from Newnan Lake was maintained in darkness for 12 days at room temperature to induce death of cyanobacteria cells. After this time, subsamples were taken and cell viability in the cyanobacteria groups was analysed by applying both

the Bac-light™ Kit and the cell digestion assay, following the methods described above.

Results

The seven Florida lakes sampled varied widely with respect to phytoplankton abundance (as indicated by Chlorophyll *a* concentration), nutrient concentrations and other chemical properties (Table 1). Cowpen and Alto lakes, fairly oligotrophic systems, exhibited the lowest pH, alkalinity and conductivity (Table 1). Cowpen Lake had the lowest Chl *a* concentration ($1.0 \mu\text{g L}^{-1}$, Table 1) with a phytoplankton community dominated by picocyanobacteria and small flagellates (Fig. 1). Bivans Arm, Newnan and Wauberg lakes were the most eutrophic of the seven study systems (Table 1), with phytoplankton communities dominated by cyanobacteria (Fig. 1) of the genera *Anabaena*, *Spirulina*, *Oscillatoria* and *Microcystis*; although other groups, i.e. diatoms, chlorophyceae and undetermined flagellates, were also abundant (Fig. 1). These latter groups were the major components of the phytoplankton communities in lakes Alice, Alto and Lochloosa (Fig. 1).

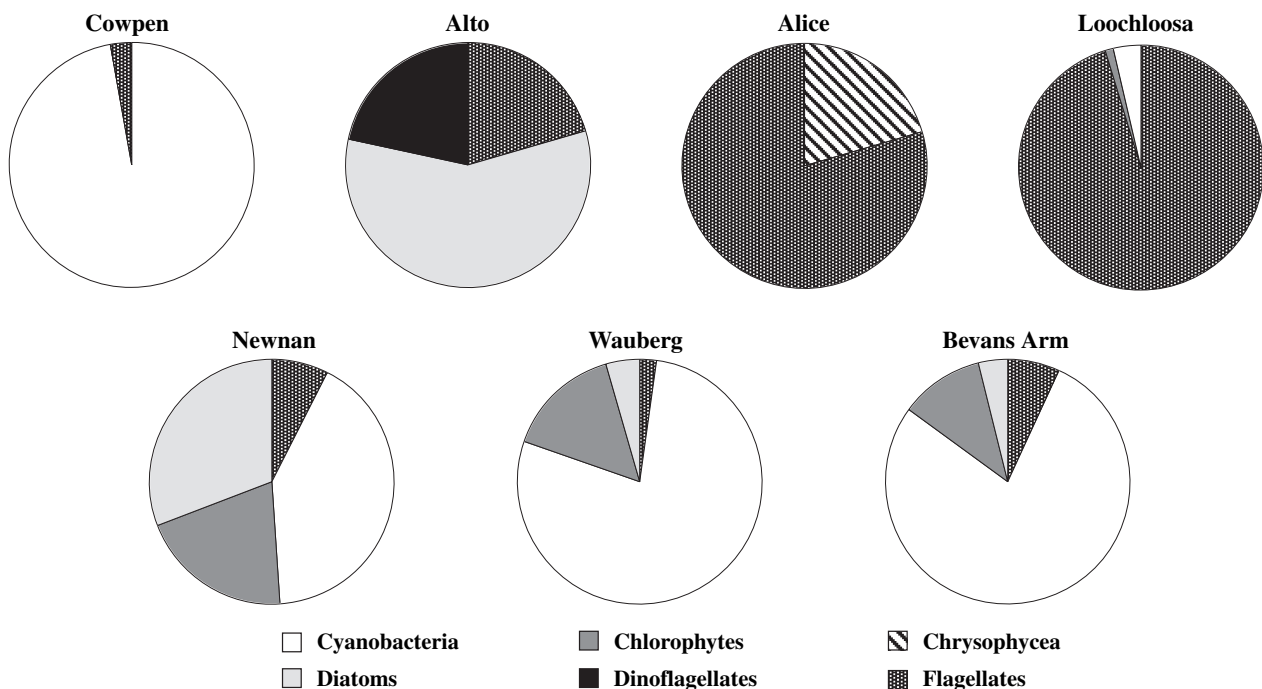


Fig. 1 Pie charts showing the phytoplankton community composition, in terms of cell abundance, and relative dominance of different taxa found in the seven Florida lakes examined.

	Enzyme solution (1/2 stock)		Enzyme solution (stock)	
	Assay temperature		Assay temperature	
	35 °C	25 °C	35 °C	25 °C
Pennate diatom	58.4 ± 7.2%	56.6 ± 11.3%	39.7 ± 4.4%	42.0 ± 3.2%
<i>Scenedesmus</i> sp.	75.0 ± 14.3%	69.6 ± 13.8%	65.2 ± 14.6%	62.9 ± 18.1%
Flagellates	45.0 ± 2.9%	50.0 ± 9.6%	42.9 ± 5.5%	53.4 ± 11.2%

Stock solutions = 800 µg ml⁻¹ DNase and 2% Trypsin; both in HBSS medium.

The effects of varying incubation conditions on results of the cell digestion assay were assessed using data for a pennate diatom, *Scenedesmus* sp., and undetermined flagellates, as these were the most abundant phytoplankton in the samples collected from Orange Lake. There was no statistical difference in the efficiency of the cell digestion assay when conducted at 25 or 35 °C (Table 2; Wilcoxon's signed-ranks test, $P = 0.157$). Although there was a tendency for the assay to be more efficient at the stock concentrations, especially in the case of diatoms (Table 2), differences were not statistically significant (Wilcoxon's signed ranks test, $P = 0.068$). We note here that initial incubations with the enzymatic cocktail using samples from the more acidic lakes, Cowpen and Alto lakes specifically (Table 1), resulted in a precipitate that could be observed in the sample under transmitted light. To avoid interference with the identification and counting of cells, the samples from these lakes were incubated using less-concentrated enzyme solutions, corresponding to half of the concentration of stock solutions, which greatly reduced the formation of precipitate.

We observed that entire trichomes of *Anabaena* sp., *Spirulina* sp. and *Oscillatoria* sp., and not single cells within the trichomes, were digested during the assay, suggesting that cells within colonial trichomes are in similar physiological state. The same result was observed when applying the BacLight™ Viability Kit to natural cyanobacteria samples. Entire trichomes appeared to fluoresce green (live cells) or red (dead cells). In no case did we observe both green and red fluorescent cells within the same trichome. The percentages of living cyanobacteria cells obtained using the cell digestion assay were tightly correlated with the percentages obtained using the BacLight™ Viability Kit (Fig. 2) and the slope of the relationship obtained when comparing the results from both methods did not differ significantly ($P > 0.05$) from

Table 2 Variability in the percentage of living cells from natural phytoplankton samples from Orange Lake achieved after the cell digestion assay as a consequence of the effect of varying the enzyme (Trypsin and DNase I) concentrations and temperature of the assay

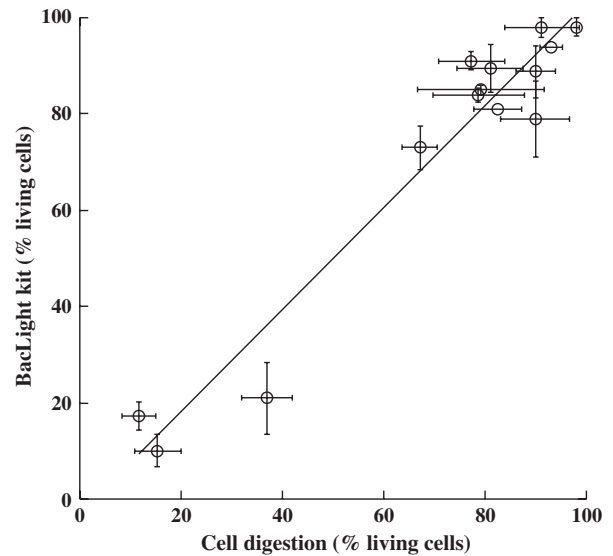


Fig. 2 The relationship between the percentage of living cyanobacteria cells (% LC) obtained using the BacLight™ Viability Kit and the % LC obtained using the cell digestion assay, examined in natural populations of *Anabaena* sp., *Spirulina* sp. and *Oscillatoria* sp. from Bivans Arm, Newnan and Wauberg lakes. The solid line represents the linear regression equation % LC (BacLight™ Viability Kit) = -3.17 + 1.06 % LC (cell digestion), $R^2 = 0.94$. The lowest viability values (% LC < 40%) corresponded to cyanobacteria populations of Newnan Lake maintained in darkness for 12 days.

1.0. The lowest percentage of living cyanobacteria cells shown (less than 40%, Fig. 2) corresponded to the Newnan Lake water kept for 12 days in the dark.

The variability in the percentage of living cells (% LC) was high among the groups forming the different phytoplankton communities in the seven lakes examined, ranging from 19.7% to 98% LC (Figs 3 & 4). The two groups representing the phytoplankton community in Cowpen Lake both exhibited a low % LC (Fig. 3). In other lakes, however, we found high variability in the percentages of living cells between the groups comprising the phytoplankton community.

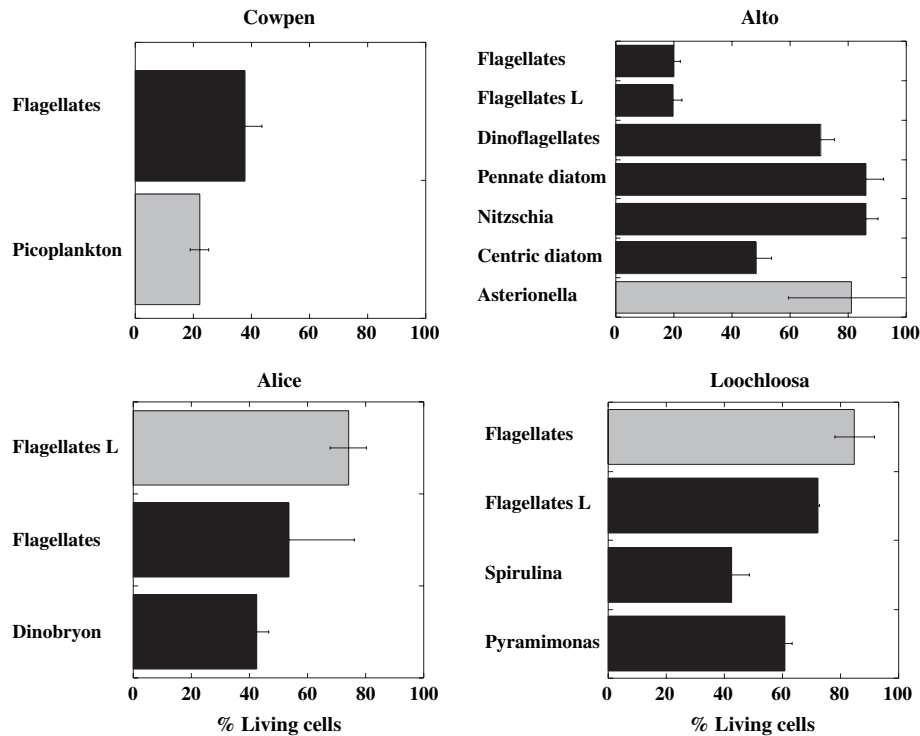


Fig. 3 The percentage of living cells (average \pm SE) of the different phytoplankton populations forming the communities in the oligotrophic and mesotrophic lakes. L Flagellates = large flagellates. Grey bars represent the % LC of the most abundant group in the community.

For instance, in Lake Alto, diatoms showed percentages of living cells greater than 80%, but flagellates had values around 20% LC (Fig. 3). In other lakes, all the represented groups showed a high % LC, e.g. phytoplankton from Bivans Arm (Fig. 4). In general, the most abundant group in the phytoplankton community showed the highest cell viability, e.g. small flagellates in Lake Loochloosa (74.5% LC, Fig. 3), *Asterionella* sp. in Lake Alto (81.1% LC, Fig. 3), *Spirulina* sp. in Newnan Lake (93% LC, Fig. 4), *Oscillatoria* sp. in Lake Wauberg (81.2%, Fig. 4) and *Oscillatoria* sp. and *Spirulina* sp. in Bivans Arm (98% and 91% LC, respectively, Fig. 4). The average value for the % LC for all groups comprising the phytoplankton communities in each of the seven study lakes ranged from 29.9 ± 7.2 to 80.4 ± 4.0 (mean \pm SE, Fig. 5). The mean % LC of the phytoplankton within a specific system increased with increasing Chl *a* concentration; lower values of % LC were observed at lakes with low Chl *a* concentration and higher % LC in communities from lakes with high Chl *a* concentration (Fig. 5).

Discussion

Our results demonstrate that the methods recently applied to quantify cell death in marine phytoplankton communities (Agustí & Sánchez, 2002; Agustí, 2004) can be successfully applied to those in freshwater. Having established this, we note that dead cells were prevalent within some of the freshwater phytoplankton communities we sampled and suggest that cell death is likely to be an important process affecting the structure and function of freshwater phytoplankton communities. The proportion of living phytoplankton cells varied widely, both between the lake communities examined and also within the groups comprising any single community. Nevertheless, the numerically dominant taxa in each lake, regardless of trophic status, tended to have a higher proportion of living, healthy cells, than other taxa present in the community. These observations lend credence to our suggestion above that phytoplankton cell death is likely to be an important process structuring communities in freshwater systems, par-

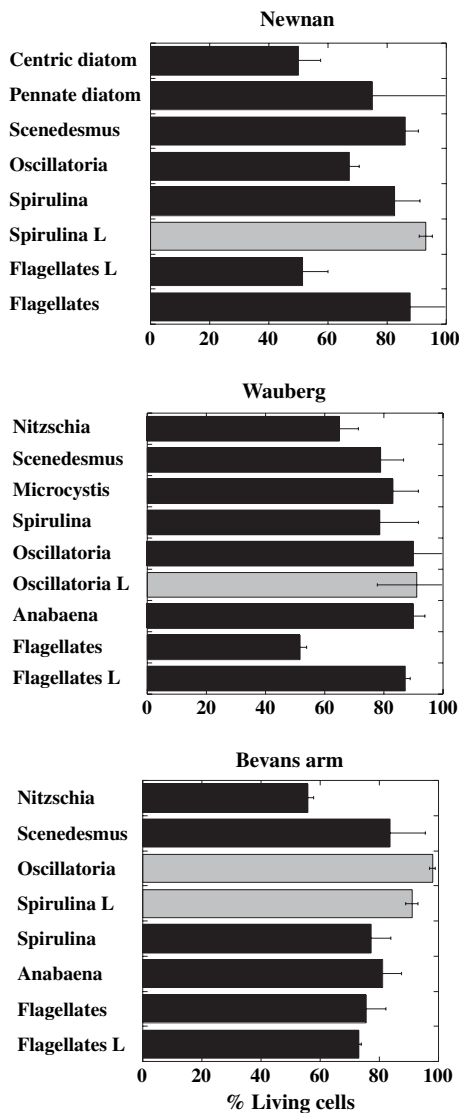


Fig. 4 The percentage of living cells (average \pm SE) of the different phytoplankton populations forming the communities in the more eutrophic lakes sampled. L = large-celled population. Grey bars represent the % LC of the most abundant group in the community.

allel to recent findings for oceanic phytoplankton communities (Agustí & Sánchez, 2002; Veldhuis *et al.*, 2002; Agustí, 2004).

The applicability of the cell digestion assay used in marine systems (Agustí & Sánchez, 2002) to freshwater samples was successful. Minor problems, such as enzyme-induced precipitate in the samples from the more acidic lakes tested, could be easily solved by using a diluted enzyme solution (half of the stock concentration of Agustí & Sánchez, 2002), with no

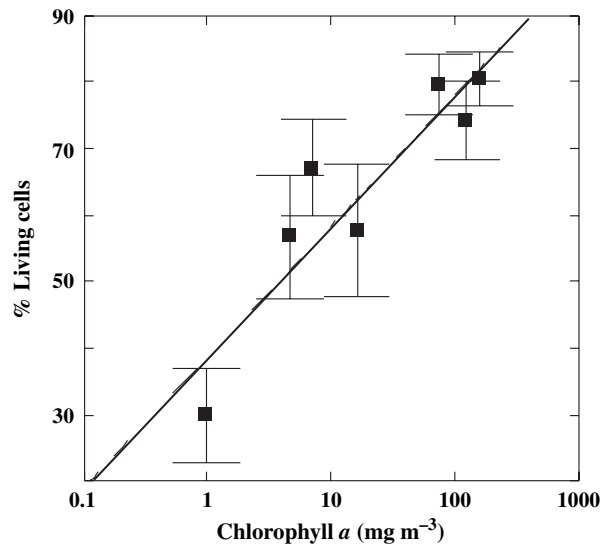


Fig. 5 The relationship between the percentage of living cells (% LC) of the phytoplankton communities (average \pm SE of the % LC of the different populations) and the chlorophyll *a* concentration in the Florida lakes. The solid line represents the fitted regression equation $\% \text{ LC} = 38.6 + 19.6 \log \text{Chl } a \text{ (}\mu\text{g L}^{-1}\text{)}$, $R^2 = 0.91$.

significant loss in digestion efficiency. The problem of precipitation described for acidic lakes ($\text{pH} < 6$) is most probably related to the differences in the pH between the HBSS medium (HBSS, Sigma, nominal $\text{pH} = 7.4 \pm 0.3$; Sigma Chemical Company, MO, U.S.A.) used to prepare the enzyme solutions and the low pH of some lakes. Thus, the precipitation issue may possibly be avoided by using another medium, with a lower pH, to prepare the enzymatic solutions.

Agustí & Sánchez (2002) recommended that the cell digestion assay used here be carried out at 35°C . However, we have demonstrated that the digestion efficiency of the enzymes was not significantly affected by using a lower temperature, i.e. 25°C , suggesting that the outcome of the assay is relatively robust with regard to small changes in the experimental conditions when using natural phytoplankton communities. The use of a lower incubation temperature during the assay will, by reducing the possibility of a thermal shock during the incubation, allow the application of the cell digestion assay in phytoplankton growing in colder waters than those examined (Agustí & Sánchez, 2002; Agustí, 2004).

Agustí & Sánchez (2002) have discussed previously the problems of vital stains, particularly when applied to natural phytoplankton communities, which render

the cell digestion method used here a superior probe of phytoplankton viability. Fluorescent vital stains used to differentiate living and dead cells often yield red fluorescence in dead cells [e.g. PI, propidium iodine, Darzynkiewicz *et al.* (1994) and BacLight staining kit, Lee & Rhee (1997)], which is confounded with the natural red fluorescence (from chl *a*) of phytoplankton cells, except when used with cyanobacteria (Lee & Rhee, 1997), which natural orange-yellow fluorescence does not interfere with the red colour of dead cells stained by the kit. Vital stains often yield a gradient of staining intensity within the cells (Gasol & del Giorgio, 2000), which introduces ambiguity in the separation between positively (living) and negatively (dead) stained cells (Gasol & del Giorgio, 2000). In contrast to the many problems of vital stains, the enzymatic cell digestion method is free of ambiguity, for cells are either totally digested or remain intact. In addition, the cell digestion method does not involve fluorescence signals that may interfere with natural fluorescence of phytoplankton, and the resulting cell suspensions can be examined under transmitted light microscopy, thereby readily allowing the taxonomic identification of living and dead cells in natural phytoplankton communities.

The percentage of living phytoplankton cells as determined using two different membrane permeability tests, i.e. the cell digestion assay and the BacLight™ Viability Kit, examined in natural phytoplankton communities were in excellent agreement indicating that the results from the cell digestion assay are comparable, as observed previously for cultured phytoplankton (Agustí & Sánchez, 2002; Agustí, 2004), with those obtained using other cell membrane permeability tests.

All cells within single cyanobacteria filaments of *Spirulina* sp., *Oscillatoria* sp. and *Anabaena* sp. were determined to be either dead or alive, which strongly suggests physiological integration of the cells forming colonial filaments. Physiological integration within filaments in cyanobacteria has been demonstrated previously by Pearl (2000) who reported physical separation of processes within the filament as indicated by the presence of specialised cells, heterocysts, used for N₂ fixation although other cells in the filament are responsible for photosynthesis. Such integration has been observed also in non-diazotrophic cyanobacteria (Pearl, 2000). Our findings, in com-

bination with those of Pearl (2000), lead us to suggest that cyanobacteria filaments should be considered as units when analysing cell death properties.

The strong, positive relationship between the average percentage of living cells within phytoplankton communities and chl *a* concentration showed that the proportion of phytoplankton living cells increased from low values in oligotrophic lakes to a dominance of living cells in the most eutrophic lakes sampled. This tendency indicates that the percentage of living cells in freshwater systems, and therefore, the importance of cell death, could be a function of the lake trophic status. These results are in agreement with those reported for oceanic systems, where the highest incidence of cell death, measured both as phytoplankton lysis rates and the % LC in the communities, occur in the most oligotrophic waters (Agustí *et al.*, 1998; Agustí *et al.*, 2001; Agustí, 2004). Moreover, higher proportion of viable bacteria cells has been reported to occur in eutrophic lakes than in oligotrophic lakes (del Giorgio & Scabrough, 1995). These observations suggest that cell death should be an important process structuring phytoplankton communities and carbon flow in oligotrophic lakes.

Although there are few published data pertaining to cell death and lysis of freshwater phytoplankton, some studies have indicated that this process could play a principal role in the population dynamics of phytoplankton. Jassby & Goldman (1974), for example, reported lysis rates of 0.8 day⁻¹ for Castle Lake, an oligotrophic system. More recently Berman & Wynne (2005) reported substantial phytoplankton lysis rates in Lake Kinneret, resulting in a yearly averaged rate of 0.91 day⁻¹, with more rapid rates corresponding to seasons of lower Chl *a* concentrations. Some data on the proportion of living and dead phytoplankton cells in natural freshwater communities have been published, but it is restricted to diatoms (Knoechel & Kalff, 1978). Knoechel & Kalff (1978) examined the population dynamics of five species of diatoms in mesotrophic Lake Hertel using track autoradiography and found low rates of phytoplankton lysis. However, the investigators found a close relationship between cell death and sinking, suggesting that cell death was considerably higher than indicated by the calculated rates. Others have quantified the decline of diatoms by quantifying the empty diatom valves (e.g. Lund, Mackereth & Mortimer, 1963). Although this approach provides a minimum estimate of the damaged cells in

the community, it does not consider dying cells that still conserve cellular contents. As a result, most reports of cell lysis in natural freshwater phytoplankton communities have not assessed directly the status of the cells and provide little or no information on the impact of cell death on the different populations present in the communities.

Although phytoplankton cell death has seldom been quantified, examination of the dynamics of dissolved and particulate organic matter in lakes has suggested the existence of a non-predatory death of phytoplankton (Wetzel, 1995). As cell viability is a static indicator of the state of the cells, the time elapsed between the physiological death of a cell and its subsequent lysis (i.e. the release of the cellular components) cannot be determined. The time dead cells remain in the water column is, therefore, also unknown. However, the presence of dying phytoplankton cells within the phytoplankton communities studied here were detected because they showed increased membrane permeability, which implies important losses of soluble cellular compounds (e.g. Myklestad, 2000). High release rates of extracellular products resulting from algal photosynthesis have been observed in lakes and attributed to increased phytoplankton cell membrane permeability (see Wetzel, 1975). Cell death results in the release of the cytoplasmic contents, which then become available to support bacterial metabolism. Thus, the observation that phytoplankton cell death is inversely related to lake trophic status in Florida lakes is consistent with the tendency for bacterial communities to use a larger share of phytoplankton production in unproductive compared to productive freshwaters (del Giorgio, Cole & Cimbleris, 1997).

The quantification of the proportion of living phytoplankton cells in natural communities by using the cell digestion assay revealed that phytoplankton cell death in the lakes examined was not uniform among the constituent phytoplankton groups in any of the lakes we examined. Differences in the proportion of dead cells within the groups comprising the phytoplankton communities indicate an important variability in the capacity to survive in the same environment for coexisting taxa. In the more eutrophic lakes examined, a high proportion of living cells were found to be a characteristic of the different taxa within the community, indicating that the environmental condi-

tions were favourable for most taxa. The high proportion of dying cells found within the communities of the oligotrophic Florida lakes sampled indicated, however, that most phytoplankton groups were stressed by environmental conditions in those lakes. This finding is, however, in agreement with the higher diversity found within eutrophic freshwater systems in Florida (e.g. Agustí, Duarte & Canfield, 1991) that should be the result of the survival of a large number of species within those systems. In fact, the variable distribution in the proportion of living cells found in the natural populations of picocyanobacteria in the Central Atlantic Ocean, helped identify the segregation of niches in the water column of *Synechococcus* and *Prochlorococcus*, which coexist in the oligotrophic ocean (Agustí, 2004). Hence, coexisting phytoplankton groups may differ greatly in their capacity to cope with different stressors.

The ability to identify dying and living cells within natural freshwater phytoplankton communities as demonstrated here by using the cell digestion assay, opens new avenues for the study of phytoplankton population dynamics in lakes by including population losses by cell death and their importance in the evolution of community structure, persistence of species, stability or perturbation and many other aspects that have been prevented in the past because reliable methodologies were not available to quantify cell death. The finding that high proportions of phytoplankton cells are often dying in natural waters helps to explain the observations of high release rates of organic compounds by phytoplankton in lakes (e.g. Wetzel, 1975) that could not be attributed to healthy, actively growing cells (Sharp, 1977). Finally, our finding that phytoplankton cell death appears to be higher in oligotrophic than in eutrophic lakes, has profound consequences for the coexistence of species, diversity and the fate and dynamics of organic matter in these freshwater ecosystems that need be addressed throughout.

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