Environmental Microbiology (2007) **9**(6), 1415–1422 doi:10.1111/j.1462-2920.2007.01257.x

Coupling between autocatalytic cell death and transparent exopolymeric particle production in the marine cyanobacterium *Trichodesmium*

Ilana Berman-Frank,[1]* Gad Rosenberg,[1] Orly Levitan,[1] Liti Haramaty[2] and Xavier Mari[3]
[1]*Mina and Everard Goodman Faculty of Life Sciences, Bar Ilan University, Ramat Gan, 52900, Israel.*
[2]*Environmental Biophysics and Molecular Ecology, Institute of Marine and Coastal Sciences, Rutgers University, 71 Dudley Road, New Brunswick, NJ 08901, USA.*
[3]*IRD, UR 103, Noumea Center, BP A5, NC-98848 Noumea, New Caledonia.*

Summary

Extracellular polysaccharide aggregates, operationally defined as transparent exopolymeric particles (TEP), are recognized as an important conduit for carbon recycling and export in aquatic systems. Yet, the factors controlling the build-up of the TEP pool are not well characterized. Here we show that increased TEP production by *Trichodesmium*, an oceanic bloom-forming nitrogen-fixing (diazotrophic) cyanobacterium, is coupled with autocatalytic programmed cell death (PCD) process. We demonstrate that PCD induction, in both laboratory cultures and natural populations, is characterized by high caspase-like activity, correlates with enhanced TEP production, and occurs under iron and phosphorus starvation, as well as under high irradiance and oxidative stress. Enhanced TEP production was not observed in actively growing populations. We provide further evidence that iron is a key trigger for the induction of PCD. We demonstrate, for the first time, the concomitant enhanced build-up of the TEP pool when *Trichodesmium* is Fe-stressed. These results suggest a functional linkage between activation of caspases and PCD in *Trichodesmium* and regulation of vertical carbon and nitrogen fluxes. We hypothesize that modulation of TEP formation and its qualities by different mortality pathways could regulate the fate of phytoplankton blooms and particulate organic matter in aquatic ecosystems.

Received 21 December, 2006; accepted 9 January, 2007. *For correspondence. E-mail irfrank@mail.biu.ac.il; Tel. (+972) 3 5318214; Fax (+972) 4 6914842.

Transparent exopolymeric particles (TEP) (Alldredge *et al*., 1993) are increasingly recognized as an important component of carbon recycling and export in aquatic systems (Passow and Alldredge, 1994; Mari and Burd, 1998; Mari, 1999; Passow, 2000; 2002; Engel *et al*., 2004). The physicochemical properties, characteristics of distribution, and dynamics of TEP contribute significantly to trophic web structure and flux processes in the ocean (Passow, 2002). Transparent exopolymeric particle form from dissolved organic carbon released by phytoplankton (Passow, 2000) and, due to their high stickiness (Kiorboe and Hansen, 1993; Dam and Drapeau, 1995; Engel, 2000), can coagulate with other more conventional particles (e.g. bacteria, phytoplankton and detritus) to promote the formation of marine snow aggregates (Passow *et al*., 2001). Therefore, TEP formation and accumulation in the euphotic zone may directly enhance the downward vertical flux of major elements, such as carbon, and of adsorbed trace elements (Passow, 2002; Engel *et al*., 2004). Alternatively, when the TEP fraction is high, TEP-mediated aggregation can lead to a reduced settling velocity and even to an upward flux of substances due to the lower density of TEP compared with that of seawater (Passow, 2002; Engel *et al*., 2004; Mari *et al*., 2007).

While the role of TEP in regulating the fate of organic matter has been extensively addressed (see above references), the factors controlling the build-up of the TEP pool are not as clearly understood. Cyanobacterial blooms may serve as a significant source for the TEP pool via the production of large amounts of extracellular polymeric substances, consisting mainly of polysaccharides (Bertocchi *et al*., 1990; Gloaguen *et al*., 1995), which are known TEP precursors (Passow, 2000). Extracellular release and the production of TEP precursors may also increase under conditions of nutrient limitation coupled with sufficient light and carbon (Passow, 2002), leading to the accumulation of the TEP pool during bloom termination. While phytoplankton bloom formations are well studied, relatively few studies are available on the demise of blooms. Generally, grazers

and viruses are considered as typical agents of phytoplankton death.

Recently, an autocatalytic programmed cell death (PCD) has been documented in several phytoplankton species, including bloom-forming species (Berges and Falkowski, 1998; Vardi *et al*., 1999; Ning *et al*., 2002; Segovia *et al*., 2003; Berman-Frank *et al*., 2004; Franklin and Berges, 2004). Well documented in metazoans and higher plants, PCD refers to a genetically controlled cell suicide resulting from a cascade of interacting biochemical processes that include specific receptors, adapters, signal-kinases, proteases and nuclear factors (Leist and Nicotera, 1997; Aravind *et al*., 1999). Autocatalytic PCD in prokaryotic and eukaryotic phytoplankton displays morphological, physiological, biochemical and genetic characteristics reminiscent of apoptotic pathways in metazoans and higher plants and is induced by oxidative, dark, temperature, nutrient or salt stresses (Berges and Falkowski, 1998; Vardi *et al*., 1999; Ning *et al*., 2002; Segovia *et al*., 2003; Berman-Frank *et al*., 2004; Franklin and Berges, 2004).

Autocatalytic PCD also exists and operates in the diazotrophic cyanobacterium *Trichodesmium* spp. (Berman-Frank *et al*., 2004). *Trichodesmium* spp. form extensive blooms in the tropical and subtropical oceans and provide a significant source of 'new nitrogen' to these oligotrophic environments (Capone *et al*., 1997; Capone, 2001). Bloom termination may be due to copepod grazing (O'Neil, 1998), viral lysis (Ohki, 1999), or via an autocatalytic PCD that is induced in response to environmentally relevant stresses such as high irradiance, P and Fe starvation, and oxidative stress (Berman-Frank *et al*., 2004).

Currently the nature and mechanistic controls of PCD in phytoplankton remain virtually unexplored even though it may have important consequences to ecosystem and biogeochemical dynamics. The objective of this study was to examine potential coupling between autocatalytic PCD in *Trichodesmium* and the production of TEP that may regulate the fate of organic matter.

Results and discussion

Autocatalytic PCD in Trichodesmium *– a physiological response to nutrient and oxidative stress*

Ageing, nutrient (P and Fe starvation) or high light (oxidative) stress induce a genetically controlled PCD in *Trichodesmium* (Berman-Frank *et al*., 2004). Reduced photosynthetic capacity, measured as a decline in the quantum yield of photosystem II (F_v/F_m), characterizes the early physiological response of *Trichodesmium* to nutrient stress (P and Fe starvation) with values falling from 0.48 ± 0.14 in the control cultures to 0.27 ± 0.12 and 0.17 ± 0.02 in the [–Fe–P]-depleted and in the Fe-depleted cultures respectively. The photophysiological response is eventually mirrored in the chlorophyll a concentrations which dramatically decline after 2 days in the [–Fe] and in the [–Fe–P]-depleted cultures (Fig. 1A). The stressed cultures activate an autocatalytic PCD which is characterized by morphological and biochemical changes such as degradation and increased vacuolization of internal cellular components, with no evidence of plasma membrane rupture as observed under accidental necrotic death (Berman-Frank *et al*., 2004).

A unique family of cysteine aspartate-specific proteases, caspases, is involved in the initiation and activation of PCD in metazoans ranging from *Hydra* to humans (Thornberry and Lazebnik, 1998). Caspase orthologues (metacaspases) with similar catalytic properties have been reported in higher plants, yeast, eukaryotic algae, various bacterial species (reviewed in Bidle and Falkowski, 2004), and recently, in *Trichodesmium* undergoing PCD (Berman-Frank *et al*., 2004). Although caspase activity may sometimes occur without causing death (Wright *et al*., 1999), caspase activity, measured by cleavage of the caspase-specific substrates, is routinely used as a hallmark feature of cells undergoing autocatalytic PCD (Kohler *et al*., 2002). Our previous work showed that in *Trichodesmium* a caspase-like protein is indeed involved during PCD. This was evidenced by cross-hybridization to caspase-3, -8 and -9 antibodies, increased caspase-like activity with PCD induction, and the lack of activity when applying caspase-specific inhibitors (Berman-Frank *et al*., 2004). Here, we utilized this caspase-like activity, measured by cleavage of the caspase-specific substrate DEVD, as the cellular indicator of autocatalytic PCD (Figs 1–3). Healthy actively growing cultures show very little DEVD cleavage (Fig. 1B). Depletion of either Fe or both Fe and P in the medium caused a substantial increase in DEVD cleavage (Fig. 1B).

High DEVD cleavage was correlated with a large increase in TEP production again when either Fe or Fe and P were depleted (Fig. 1C). Transparent exopolymeric particle production was low in, nutrient-replete, healthy growing cells (Fig. 1C). One-way ANOVA and Tamahane Post Hoc analyses showed significant differences between treatments (d.f. = 3,9; $F = 25.4$, $P < 0.001$) with significant differences found between the controls and [–Fe] and [–Fe–P] ($P < 0.0001$). Compilation of independent experiments showed a significant positive correlation between TEP volume and DEVD cleavage representing caspase-like activity in PCD-induced cells ($r^2 = 0.66$, $n = 22$, $P < 0.05$) (Fig. 2).

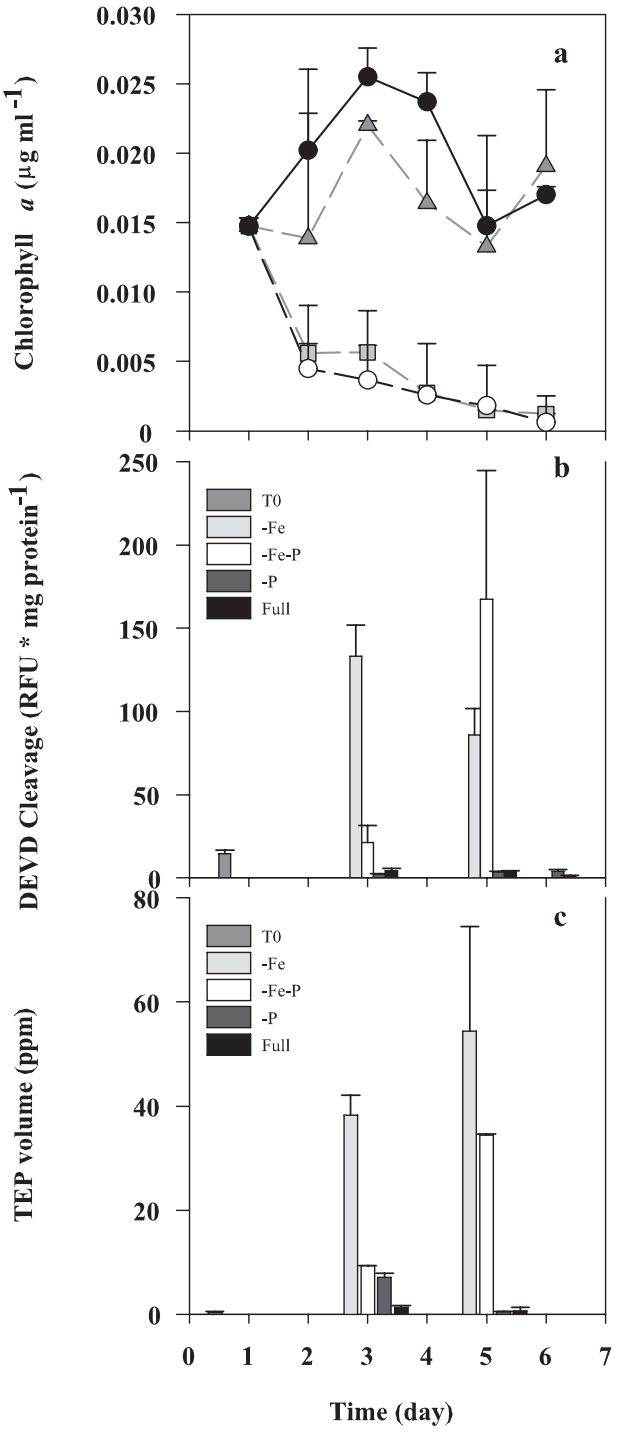

Fig. 1. Induction of autocatalytic PCD in cultures of *Trichodesmium* by nutrient depletion. (A) Changes in chlorophyll a concentrations over time of cultures that were divided into two to three replicate bottles of each of the following treatments: control (full medium – solid circles), P-depleted (triangles), Fe-depleted (empty circles) and [–P–Fe] (squares). Corresponding measurements of caspase-like activity obtained by DEVD cleavage (B) and calculated estimates of the volume of TEP aggregates (C). Values of TEP and DEVD cleavage correspond to the average from the replicate bottles (± standard deviations). Three measurements were taken from each bottle, respectively, for DEVD cleavage and for chlorophyll a.

Dynamics of PCD induction and TEP production under oxidative stress

The time scale for enhanced TEP production coincided with an increase in DEVD cleavage (Fig. 3). When natural populations were artificially exposed to high irradiance and, thus, to oxidative stress, both DEVD cleavage and TEP production were induced and increased from ~3 to 12 h after exposure (Fig. 3A), with instantaneous TEP and caspase-like production rates peaking at ~8 h after exposure (Fig. 3B).

Exposure to stress also resulted in modified characteristics of the TEP pool, as shown by the alteration of the spectral slope, δ. The observed increase of δ from ~4 to 17 h (Fig. 3A) derived from an increase of the large TEP fraction [i.e. > 20 µm equivalent spherical diameter (ESD)]. Microscopic observations revealed that most large TEP originated from the detachment of the polysaccharide capsule from *Trichodesmium* cells after PCD induction (Figs 3B and 4). This alternative mechanism of TEP production resulted in the massive release of long 'sock'-shaped TEP in the medium after instantaneous TEP production and caspase-like activity peaked (Fig. 4C). After release of these 'socks' TEP can be degraded and smaller TEP particles were observed when biomass mortality increased and caspase-like activity declined (Figs 3 and 4D). As TEP are usually produced by coagulation of TEP precursors of colloidal size (Passow, 2000), the TEP pool is thought to take its roots in the dissolved phase. Our

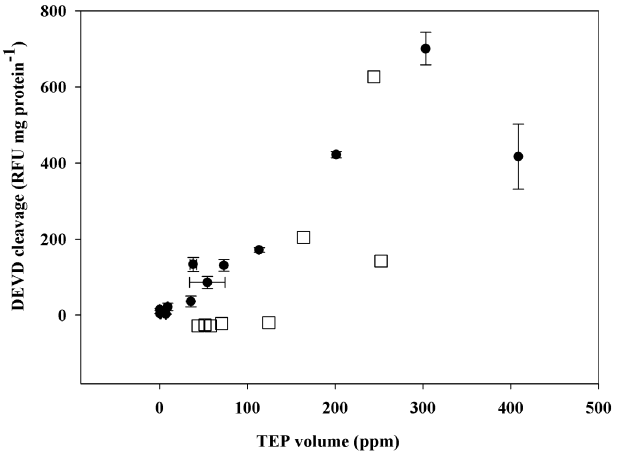

Fig. 2. Correlation between caspase-like activity (DEVD cleavage) and production of TEP (ppm) from three independent experiments examining the effects of nutrient (e.g. Fig. 1 and another similar experiment) (squares) and oxidative stress (Fig. 3) (circles) on laboratory and natural *Trichodesmium* populations. $R^2 = 0.66$, $n = 22$, $P < 0.05$. Error bars indicate standard errors within each individual experiment.

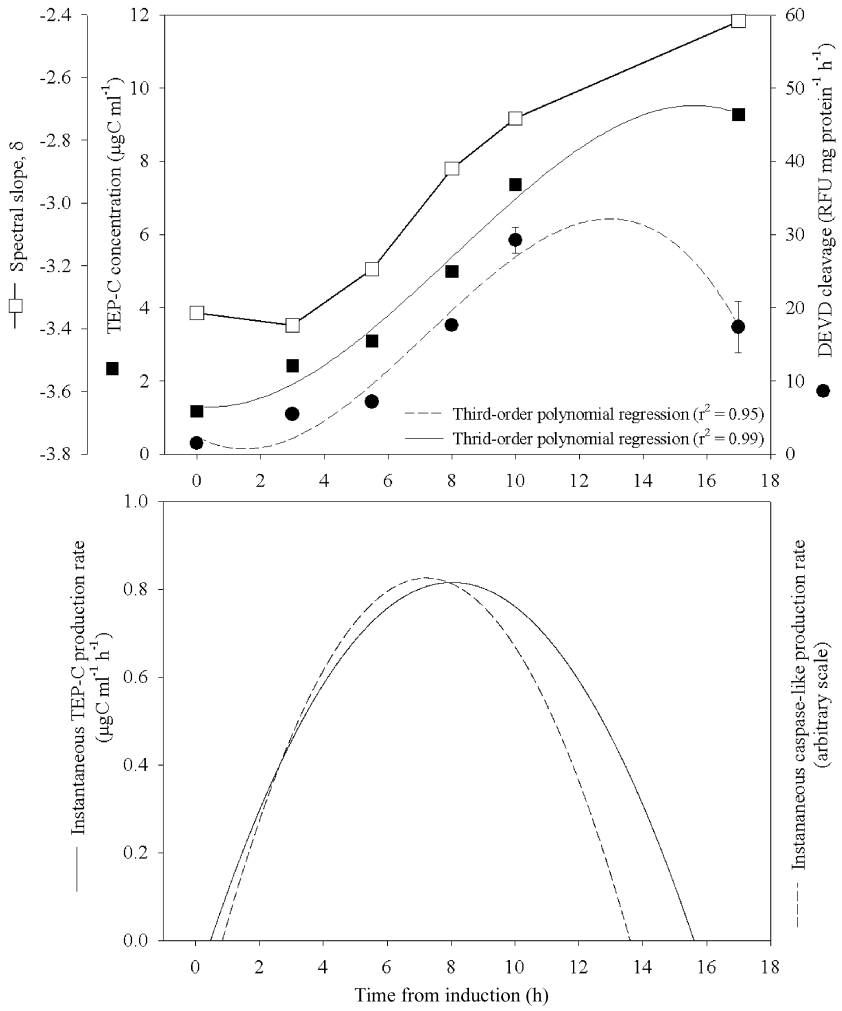

Fig. 3. Effect of high-irradiance and oxidative stress on induction of caspase-like activity (DEVD cleavage) and enhanced TEP production. (A) Temporal variations of the spectral slope, of the TEP-C concentration and of DEVD cleavage and (B) temporal variations of instantaneous TEP-C production rates and DEVD cleavage, estimated as the derivatives of the third-order polynomial fittings of TEP-C concentration and DEVD cleavage versus time. Calculation of TEP-C concentrations and of the spectral slopes from the TEP size spectra does not permit calculation of error estimates, as they are individually derived from a single size spectrum.

study showed that under specific circumstances TEP may also be produced directly as large particles (Fig. 4) and corresponds to a study from Lake Kinneret (Israel) showing that much of the TEP originates from bits and pieces of algae and from mucous-like excretions around bacteria and algae (Berman and Viner-Mozzini, 2001).

Contribution of the TEP pool to total particulate organic carbon during bloom demise induced by nutrient stress

In *Trichodesmium*, the contribution of the TEP pool to total particulate organic carbon (POC) varies as a function of nutrient stress (Table 1). When cells were P-limited, TEP comprised ~10% of the POC pool. Under Fe starvation TEP production increased drastically; eventually comprising almost the total pool of POC. A dual P and Fe starvation led to an intermediate state in which TEP constituted ~45% of the POC pool. Despite the uncertainties associated with the TEP size versus carbon content relationship, our experiments demonstrate that, on a similar time scale, varying nutrient stresses influence cellular growth and mortality responses differently. In the nutrient-depletion experiments, P depletion caused an increase in POC compared with the control but this was not mirrored in TEP carbon (TEP-C) or in DEVD cleavage as was the case when Fe was depleted. This may be due to the availability of intracellular stores of phosphorus in the form of phospholipid granules (Janson *et al*., 1995) reflected in flexible N : P stoichiometry (Krauk *et al*., 2006; White *et al*., 2006) or in *Trichodesmium*'s ability to utilize phosphonates (Dyhrman *et al*., 2006). In contrast, Fe is a crucial limiting nutrient for *Trichodesmium* with Fe starvation leading to both PCD induction and massive TEP production (Fig. 1). This is consistent with the high intracellular quotas of Fe required for both nitrogen-fixation and photosynthetic pathways in diazotrophs and especially for *Trichodesmium* (Reuter, 1988; Berman-Frank *et al*., 2001; Kustka *et al*., 2003; Tuit *et al*., 2004). Thus, we would expect that in oceanic areas with limited bioavailability of Fe, Fe would regulate not only N_2 fixation, primary production, and growth of *Trichodesmium* (Reuter, 1988; Falkowski, 1997; Berman-Frank *et al*.,

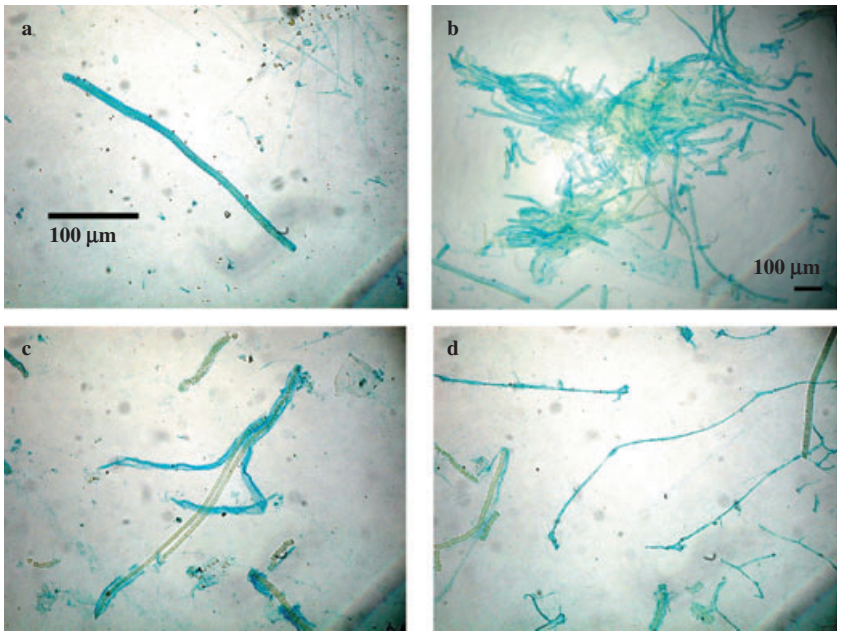

Fig. 4. Micrographs illustrating different stages of TEP production in *Trichodesmium* during the experiment depicted in Fig. 3. The pictures show the initial formation of TEP along the *Trichodesmium* trichomes 2–3 h after the start of PCD induction (A); a tuft-shaped colony of *Trichodesmium* with enhanced TEP production (B) and long 'sock'-shaped TEP peeling off the trichomes when PCD features were evident and caspase-like activity peaked ~8 h after induction (C); commonly observed smaller aggregates after cellular lysis ~17 h after induction (D).

2001; Kustka *et al*., 2003; Mills *et al*., 2004) but also the mortality pathway and TEP production.

Implications for Trichodesmium *and for the fate of organic matter produced during* Trichodesmium *blooms*

In the oceans, *Trichodesmium* blooms are frequently observed on the surface layer and regulate their position in the water column by modification of their gas vesicles (Walsby, 1978). One of the morphological characteristics associated with autocatalytic PCD in *Trichodesmium* is loss of gas vesicles required for flotation, with the population dividing into a large sinking fraction of cells and a smaller fraction of trichomes that remain suspended in the water column (Berman-Frank *et al*., 2004). These natural populations from New Caledonia are described here in the sphere experiment (Figs 2 and 3). In these populations, sinking trichomes had elevated (by a factor of 24–400) DEVD cleavage (17.1 RFU µg protein^{-1}) compared with buoyant trichomes (0.30 ± 0.22 RFU µg protein^{-1}; range 0.04–0.70) and lysed after 24 h. Transparent exopolymeric particle production was associated with high DEVD cleavage (i.e. cells undergoing PCD) and thus sinking cells (Figs 2 and 3).

Positive coupling between PCD and TEP production may have important implications for the fate of organic matter produced during *Trichodesmium* blooms. The build-up of the TEP pool enhances particle aggregation by increasing both the apparent stickiness of particles and the collision frequency, thus, modifying the vertical flux of substances (Passow *et al*., 2001; Engel *et al*., 2004). The TEP-mediated formation of large aggregates may either enhance or retard the downward flux of matter depending on the density of aggregates compared with seawater. Whereas TEP-mediated aggregates are assumed to increase rates of sedimentation from the surface, a relative increase of TEP concentration within aggregates may prolong their residence time in the euphotic layer (Alldredge and Crocker, 1995; Mari *et al*., 2007), and even create an upward flux of substances (Azetsu-Scott and Niven, 2005). In our sphere experiments, using natural populations, the positive coupling between cells undergo-

Table 1. Contribution of the TEP-C pool to total POC during nutrient starvation experiment, in the mother culture (T0) and in the four treatments after 1 week incubation (T1).

		T1			
Treatments	T0	Control	[–P]	[–Fe]	[–P–Fe]
Measured POC (µg ml^{-1})	9.4	6.43 ± 1.19	13.83 ± 3.45	4.03 ± 1.54	8.21 ± 1.31
Estimated TEP-C (µg ml^{-1})	0.44	2.54	1.64	4.88	3.74
Contribution of TEP to total POC (%)	4.7	39.5	11.9	121.1	45.6

F_v/F_m and POC concentrations are average values (± standard deviations, $n = 3$). Calculation of TEP-C concentrations from the TEP size spectra does not permit calculation of error estimates, as they are individually derived from a single size spectrum.

ing PCD and TEP production (Fig. 3) was observed predominantly in the sinking fraction of cells (Berman-Frank *et al*., 2004) suggesting higher sedimentation rates for cells producing high TEP.

Our study provides further evidence that iron is a key trigger for the induction of PCD and, demonstrates for the first time, the concomitant enhanced build-up of the TEP pool when *Trichodesmium* is starved for Fe. We suggest that bloom demise catalysed by Fe depletion would be characterized by a TEP-dominated standing stock of POC, resulting in an enhanced downward flux of organic matter. Therefore, the global contribution of *Trichodesmium* blooms to vertical export of material may be to increase the downward flux when Fe flux to the oceans, and subsequent Fe availability to *Trichodesmium*, is low (via the PCD-high TEP production pathway). Alternatively, high Fe flux to the oceans may promote the formation of high-biomass, low-TEP forming *Trichodesmium* blooms (more likely terminating via necrotic low-TEP pathways such as grazing or viral lysis). These may promote recycling in the surface oceans. We do not currently know the chemical and buoyancy characteristics of *Trichodesmium*-produced TEP and whether these features change in populations undergoing PCD or necrotic death. Moreover, we do not know how the myriad associated populations found within *Trichodesmium* colonies and blooms (Nausch, 1996; Sheridan *et al*., 2002) utilize and/or influence the formed TEP or the process of PCD. Extensive research is required to test these hypotheses on *Trichodesmium* especially for natural populations under bloom conditions.

Experimental procedures

Trichodesmium *source and stress conditions*

Trichodesmium IMS101 cultures in YBC II medium (Chen *et al*., 1996) were grown for three independent experiments (different mother cultures) in two laboratories at 25°C to 26°C with a 12:12 light/dark cycle at ~85 μmol quanta m^{-2} s^{-1} and constant aeration. Experimental treatments were initial stock inoculates (identified as T0); control (5×10^{-5} M KH_2PO_4; 4×10^{-7} M $FeCl_3$); P-depleted conditions (YBC II with no addition of phosphate); Fe-depleted conditions (YBC II with no addition of iron) and in P and Fe-depleted conditions (YBC II with no addition of either phosphate or iron). Phosphorus, iron and dual starvations are identified as [–P], [–Fe] and [–P–Fe]. Stock cultures were transferred to two replicate bottles of each treatment by gravity filtration and several washes in the sterilized medium of the respective treatments. Biomass was followed in the respective treatments until it crashed (several days and up to a week in each treatment).

Natural *Trichodesmium* populations, predominantly *T. erythraeum*, were collected using 35 μm net tows from surface waters off Noumea (New Caledonia) during December 2002. To isolate *Trichodesmium*, tows were size fractionated and zooplankton was separated from *Trichodesmium* by phototaxis. *Trichodesmium* were hand-picked, placed in a separating flask in filtered seawater (< 0.2 μm), irradiated with ~450 μmol quanta m^{-2} s^{-1} and populations were followed with time until the biomass crashed. Experimental data presented here augment the experiments and populations described in Berman-Frank and colleagues (2004) with TEP production measured on the same populations.

Measurement of caspase-like activity

Cells filtered on 5 μm Nucleopore filters were frozen in a buffer containing 100 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 10% sucrose, 500 μM ethylenediaminetetraacetic acid and 10 mM dithiothreitol. After sonication, the extract was incubated with 50 μM Z-DEVD-AFC (Calbiochem) for 24 h at 26°C. Fluorescence was read in a Spectra Max Gemini XS plate reader (excitation 400 nm, emission 505 nm).

Photosynthetic efficiency

Fast repetition rate fluorometer measurements of fluorescence kinetics were used to derive the maximum photochemical quantum yield of photosystem II (F_v/F_m), a measure of photosynthetic efficiency and physiological status of the cells (Kolber *et al*., 1998).

Determination of TEP

Transparent exopolymeric particles were stained with Alcian Blue (Alldredge *et al*., 1993) and TEP size spectra were determined from 1 to 5 ml samples filtered onto 0.2 μm polycarbonate filters, after transfer of the particles retained on a microscope slide (Passow and Alldredge, 1994). For each slide, TEP size spectra were determined by counting and sizing TEP at successive magnifications using a compound light microscope (Mari and Burd, 1998). Ten images were taken per slide and for each magnification and the TEP size spectra were compiled by combining the size distributions obtained at each magnification. The ESD of each TEP was calculated by measuring of its cross-sectional area with a semiautomatic image-analysis system (ImagePro Plus, MediaCybernetics). Counts were combined and classified according to their ESD. Transparent exopolymeric particle size distributions were described using a power relation of the type $dN/d(d_p) = kd_p^{\delta}$, where d_p is the ESD and dN is the number of particles per unit volume in the size range d_p to $[d_p + d(d_p)]$. The spectral slope, δ, describes the size distribution and was estimated from regressions of $\log[dN/d(d_p)]$ versus $\log[d_p]$.

Transparent exopolymeric particle carbon concentration

Transparent exopolymeric particle size spectra were used along with the well-defined carbon–size relationship $TEP\text{-}C = 0.25\ r^{2.55}$ (pg C TEP^{-1}), where *TEP-C* (pg C) is the carbon content of a given TEP particle with a radius r (μm) (Mari, 1999), to estimate the size of the TEP-C pool. This

approach provides only rough estimates of the size of the TEP-C pool because this relationship was determined for TEP produced from exudates of organisms other than *Trichodesmium* (i.e. diatoms). Different sources of TEP are likely to result in TEP with varying carbon contents. As the above relationship is the only tool so far available in the literature allowing the estimation of TEP-C concentration from the size spectra, it was used in the present study.

Particulate organic carbon concentration

Samples for POC measurements were immediately filtered onto 25 mm Whatman GF/F filters (nominal pore size = 0.7 μm) pre-combusted at 550°C for 2 h. After filtration the filters were dried at 60°C for 24 h and then frozen for later analysis on a CHN-analyser.

Comparison of TEP production and caspase-like enzymatic activity

In order to compare enzymatic activity detected from DEVD cleavage, with TEP-C concentration at a given time we compare production rates. During the time-series oxidative stress experiment, such estimates of TEP-C and caspase-like production rates can be directly obtained by calculating the derivatives of the TEP-C and DEVD cleavage versus time plots (Fig. 3). The derivatives obtained have an informative value and provide a conceptual representation of the correlation between TEP-C and caspase-like production rates.

Acknowledgements

We thank A. Le Bouteiller and C. Dupouy for providing assistance during the set-up of the experiments in New Caledonia. This research was supported by IRD, by the PROOF-DIAPAZON program (JGOFS-France). Support for L.H. was provided by the National Science Foundation in a Biocomplexity Grant to P.G. Falkowski.

References

Alldredge, A.L., and Crocker, K.M. (1995) Why do sinking mucilage aggregates accumulate in the water column. *Sci Total Environ* **165:** 15–22.

Alldredge, A.L., Passow, U., and Logan, B.E. (1993) The abundance and significance of a class of large, transparent organic particles in the ocean. *Deep-Sea Res* **40:** 1131–1140.

Aravind, L., Dixit, V.M., and Koonin, E.V. (1999) The domains of death: evolution of the apoptosis machinery. *Trends Biochem Sci* **24:** 47–53.

Azetsu-Scott, K., and Niven, S.E.H. (2005) The role of transparent exopolymer particles (TEP) in the transport of Th-234 in coastal water during a spring bloom. *Cont Shelf Res* **25:** 1133–1141.

Berges, J.A., and Falkowski, P.G. (1998) Physiological stress and cell death in marine phytoplankton: induction of proteases in response to nitrogen or light limitation. *Limnol Oceanogr* **43:** 129–135.

Berman, T., and Viner-Mozzini, Y. (2001) Abundance and characteristics of polysaccharide and proteinaceous particles in Lake Kinneret. *Aquat Microb Ecol* **24:** 255–264.

Berman-Frank, I., Cullen, J.T., Shaked, Y., Sherrell, R.M., and Falkowski, P.G. (2001) Iron availability, cellular iron quotas, and nitrogen fixation in *Trichodesmium*. *Limnol Oceanogr* **46:** 1249–1260.

Berman-Frank, I., Bidle, K., Haramaty, L., and Falkowski, P.G. (2004) The demise of the marine cyanobacterium, *Trichodesmium* spp., via an autocatalyzed cell death pathway. *Limnol Oceanogr* **49:** 997–1005.

Bertocchi, C., Navarini, L., Cesaro, A., and Anastasio, M. (1990) Polysaccharides from cyanobacteria. *Carbohyd Polym* **12:** 127–153.

Bidle, K.D., and Falkowski, P.G. (2004) Cell death in planktonic, photosynthetic microorganisms. *Nat Rev Microbiol* **2:** 643–655.

Capone, D.G. (2001) Marine nitrogen fixation: what's the fuss? *Curr Opin Microbiol* **4:** 341–348.

Capone, D.G., Zehr, J.P., Paerl, H.W., Bergman, B., and Carpenter, E.J. (1997) *Trichodesmium*, a globally significant marine cyanobacterium. *Science* **276:** 1221–1229.

Chen, Y.B., Zehr, J.P., and Mellon, M. (1996) Growth and nitrogen fixation of the diazotrophic filamentous nonheterocystous cyanobacterium *Trichodesmium* sp IMS 101 in defined media: evidence for a circadian rhythm. *J Phycol* **32:** 916–923.

Dam, H.G., and Drapeau, D.T. (1995) Coagulation efficiency, organic-matter glues and the dynamics of particles during a phytoplankton bloom in a mesocosm study. *Deep-Sea Res II Top Stud Oceanogr* **42:** 111–123.

Dyhrman, S.T., Chappell, P.D., Haley, S.T., Moffett, J.W., Orchard, E.D., Waterbury, J.B., and Webb, E.A. (2006) Phosphonate utilization by the globally important marine diazotroph *Trichodesmium*. *Nature* **439:** 68–71.

Engel, A. (2000) The role of transparent exopolymer particles (TEP) in the increase in apparent particle stickiness (alpha) during the decline of a diatom bloom. *J Plankton Res* **22:** 485–497.

Engel, A., Thoms, S., Riebesell, U., Rochelle-Newall, E., and Zondervan, I. (2004) Polysaccharide aggregation as a potential sink of marine dissolved organic carbon. *Nature* **428:** 929–932.

Falkowski, P.G. (1997) Evolution of the nitrogen cycle and its influence on the biological sequestration of CO2 in the ocean. *Nature* **387:** 272–275.

Franklin, D.J., and Berges, J.A. (2004) Mortality in cultures of the dinoflagellate *Amphidinium carterae* during culture senescence and darkness. *Proc R Soc Lond B Biol Sci* **271:** 2099–2107.

Gloaguen, V., Morvan, N., and Hoffmann, L. (1995) Released and capsular polysaccharides of Oscillatoriaceae (Cyanophyceae, Cyanobacteria). *Algol Stud* **78:** 53–69.

Janson, S., Siddiqui, P.J.A., Walsby, A.E., Romans, K.M., Carpenter, E.J., and Bergman, B. (1995) Cytomorphological characterization of the planktonic diazotrophic cyanobacteria *Trichodesmium* spp from the Indian Ocean and Caribbean and Sargasso Seas. *J Phycol* **31:** 463–477.

Kiorboe, T., and Hansen, J.L.S. (1993) Phytoplankton aggregate formation – observations of patterns and mechanisms

of cell sticking and the significance of exopolymeric material. *J Plankton Res* **15:** 993–1018.
Kohler, C., Orrenius, S., and Zhivotovsky, B. (2002) Evaluation of caspase activity in apoptotic cells. *J Immunol Methods* **265:** 97–110.
Kolber, Z.S., Prasil, O., and Falkowski, P.G. (1998) Measurements of variable chlorophyll fluorescence using fast repetition rate techniques: defining methodology and experimental protocols. *Biochim Biophys Acta* **1367:** 88–106.
Krauk, J.M., Villareal, T.A., Sohm, J.A., Montoya, J.P., and Capone, D.G. (2006) Plasticity of N : P ratios in laboratory and field populations of *Trichodesmium* spp. *Aquat Microb Ecol* **42:** 243–253.
Kustka, A., Sanudo-Wilhelmy, S., Carpenter, E.J., Capone, D.G., and Raven, J.A. (2003) A revised estimate of the iron use efficiency of nitrogen fixation, with special reference to the marine cyanobacterium *Trichodesmium* spp. (Cyanophyta). *J Phycol* **39:** 12–25.
Leist, M., and Nicotera, P. (1997) The shape of cell death. *Biochem Biophys Res Commun* **236:** 1–9.
Mari, X. (1999) Carbon content and C : N ratio of transparent exopolymeric particles (TEP) produced by bubbling exudates of diatoms. *Mar Ecol-Prog Ser* **183:** 59–71.
Mari, X., and Burd, A. (1998) Seasonal size spectra of transparent exopolymeric particles (TEP) in a coastal sea and comparison with those predicted using coagulation theory. *Mar Ecol-Prog Ser* **163:** 63–76.
Mari, X., Rochelle-Newall, E., Torréton, J.-P., Pringault, O., and Jouon, A. (2007) Water residence time: a regulatory factor of the DOM to POM transfer efficiency. *Limnol Oceanogr* **52:** 808–819.
Mills, M.M., Ridame, C., Davey, M., La Roche, J., and Geider, R.J. (2004) Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic. *Nature* **429:** 292–294.
Nausch, M. (1996) Microbial activities on *Trichodesmium* colonies. *Mar Ecol-Prog Ser* **141:** 173–181.
Ning, S.B., Guo, H.L., Wang, L., and Song, Y.C. (2002) Salt stress induces programmed cell death in a prokaryotic organism *Anabaena*. *J Appl Microbiol* **93:** 15–28.
Ohki, K. (1999) A possible role of temperate phage in the regulation of *Trichodesmium* biomass. *Bull Inst Oceanogr (Monaco)* **19:** 287–291.
O'Neil, J.M. (1998) The colonial cyanobacterium *Trichodesmium* as a physical and nutritional substrate for the harpacticoid copepod *Macrosetella gracilis*. *J Plankton Res* **20:** 43–59.
Passow, U. (2000) Formation of transparent exopolymer particles, TEP, from dissolved precursor material. *Mar Ecol-Prog Ser* **192:** 1–11.
Passow, U. (2002) Transparent exopolymer particles (TEP) in aquatic environments. *Prog Oceanogr* **55:** 287–333.
Passow, U., and Alldredge, A.L. (1994) Distribution, size and bacterial-colonization of transparent exopolymer particles (TEP) in the ocean. *Mar Ecol-Prog Ser* **113:** 185–198.
Passow, U., Shipe, R.F., Murray, A., Pak, D.K., Brzezinski, M.A., and Alldredge, A.L. (2001) Origin of transparent exopolymer particles (TEP) and their role in the sedimentation of particulate matter. *Cont Shelf Res* **21:** 327–346.
Reuter, J.G. (1988) Iron stimulation of photosynthesis and nitrogen fixation in *Anabaena* 7120 and *Trichodesmium* (Cyanophyceae). *J Phycol* **24:** 249–254.
Segovia, M., Haramaty, L., Berges, J.A., and Falkowski, P.G. (2003) Cell death in the unicellular chlorophyte *Dunaliella tertiolecta*: an hypothesis on the evolution of apoptosis in higher plants and metazoans. *Plant Physiol* **132:** 99–105.
Sheridan, C.C., Steinberg, D.K., and Kling, G.W. (2002) The microbial and metazoan community associated with colonies of *Trichodesmium* spp, a quantitative survey. *J Plankton Res* **24:** 913–922.
Thornberry, N.A., and Lazebnik, Y. (1998) Caspases: enemies within. *Science* **281:** 1312–1316.
Tuit, C., Waterbury, J., and Ravizzaz, G. (2004) Diel variation of molybdenum and iron in marine diazotrophic cyanobacteria. *Limnol Oceanogr* **49:** 978–990.
Vardi, A., Berman-Frank, I., Rozenberg, T., Hadas, O., Kaplan, A., and Levine, A. (1999) Programmed cell death of the dinoflagellate *Peridinium gatunense* is mediated by CO(2) limitation and oxidative stress. *Curr Biol* **9:** 1061–1064.
Walsby, A.F. (1978) The properties and buoyancy providing role of gas vacuoles in *Trichodesmium Ehrenberg*. *Br Phycol J* **13:** 103–116.
White, A.E., Spitz, Y.H., Karl, D.M., and Letelier, R.M. (2006) Flexible elemental stoichiometry in *Trichodesmium* spp. and its ecological implications. *Limnol Oceanogr* **51:** 1777–1790.
Wright, M.E., Han, D.K., Carter, L., Fields, S., Schwartz, S.M., and Hockenbery, D.M. (1999) Caspase-3 inhibits growth in *Saccharomyces cerevisiae* without causing cell death. *FEBS Lett* **446:** 9–14.

