RESEARCH ARTICLE

Ecological advantages from light adaptation and heterotrophic-like behavior in *Synechococcus* harvested from the Gulf of Trieste (Northern Adriatic Sea)

Alessandro Paoli[1], Mauro Celussi[1], Paola Del Negro[1], Serena Fonda Umani[2] & Laura Talarico[2]

[1]Department of Biological Oceanography, National Institute of Oceanography and Experimental Geophysics, Santa Croce, Trieste, Italy; and
[2]Department of Biology, University of Trieste, via Licio Giorgieri, Trieste, Italy

Correspondence: Alessandro Paoli, Department of Biological Oceanography, National Institute of Oceanography and Experimental Geophysics, via Auguste Piccard 54, Santa Croce, I-34010 Trieste, Italy. Tel.: +39 040 2249711; fax: +39 040 2249770; e-mail: apaoli@ogs.trieste.it

Received 15 October 2007; revised 14 January 2008; accepted 18 January 2008.
First published online 11 March 2008.

DOI:10.1111/j.1574-6941.2008.00459.x

Editor: Riks Laanbroek

Keywords
Synechococcus; light adaptation; phycobiliprotein; photoheterotrophy; exoenzymatic activity; carbon production.

Abstract

A preliminary study was carried out on a picocyanobacterial mixed culture harvested from the Gulf of Trieste (Northern Adriatic) and identified as *Synechococcus* spp. both by transmission electron microscopy observations, biliprotein composition and molecular analyses. Absorption and fluorescence spectra revealed phycourobilin and phycoerythrobilin chromophores, suggesting the presence of both CU- and C-phycoerythrin, besides phycocyanobilin chromophores typical for phycocyanins and allophycocyanins. Both biliprotein analyses and molecular identification indicated the presence of at least two *Synechococcus* subgroups presumably differing either in phycoerythrin type or in physiological traits. Among the exoenzymatic activities acting on different substrates, only aminopeptidase showed high hydrolysis rates and the uptake of organic molecules was positive for leucine but not for thymidine. The protein carbon mobilized was high compared with the leucine incorporation rates, resulting in low percentages of newly mobilized carbon utilized by cultures. The organic carbon incorporated as leucine was compared with the photosynthetically produced one, and the balance between the phototrophic- and heterotrophic-like processes was *c.* 3 : 1. Our findings suggest that the *Synechococcus* heterotrophy plays an important role in cell's metabolism, and that the photoheterotrophic behavior, together with their chromatic adaptation capability, might represent the key for the absolute dominance of this genus in the Adriatic Sea.

Introduction

The phycoerythrin-rich cyanobacteria of the *Synechococcus* type (Olson *et al.*, 1990; Sidler, 1994; Ting *et al.*, 2002; Six *et al.*, 2005), which inhabit the world's coastal and oligotrophic oceans at high concentrations (generally 10^6–10^7 cells L^{-1}), are also largely dominant within the autotrophic picoplankton (APP) community of the Northern Adriatic transitional, coastal and offshore waters (Bernardi Aubry *et al.*, 2006; Paoli & Del Negro, 2006; Paoli *et al.*, 2007). In this semi-enclosed basin, the APP, ranging between 10^5 and 10^9 cells L^{-1} depending mostly on seasons (see Paoli *et al.*, 2007 and references therein), contributes a percentage of 30–31% (with peaks up to 70%) to total phytoplankton carbon biomass (Bernardi Aubry *et al.*, 2006), and is responsible for a substantial, sometimes dominant, fraction of the primary production (see Magazzù & Decembrini, 1995 for a review). The now well-recognized importance of these organisms in marine carbon cycling has led to greatly increased interest in the physiology, ecology and molecular biology (Scanlan & West, 2002) of the genus. At least some *Synechococcus* strains, similar to several cyanobacterial genera, are known to acclimate to light quality through a process traditionally called complementary chromatic adaptation (Tandeau de Marsac, 1977; Talarico, 1996; Talarico & Maranzana, 2000; Grossman, 2003). These 'chromatically adapters' synthesize maximally phycocyanin in red light and phycoerythrin with high or low phycourobilin/phycoerythrobilin chromophore ratios in blue and green light respectively. According to Palenik (2001), the ability to adapt chromatically may help explain the global distribution of *Synechococcus*. Research has so far focused on nutrient

uptake, photosynthesis, motility and cell cycle behavior, but the *Synechococcus* photoheterotrophy has generally been considered to be of scarce ecological importance (see Zubkov *et al.*, 2003 and references therein). We believe that this physiological feature should not be underestimated when modeling marine ecosystems as this might lead to significant differences in balancing the carbon cycle, particularly in nearly oligotrophic areas like the Gulf of Trieste, where community respiration may exceed the generally low primary productivity (Fonda Umani *et al.*, 2007). Recently, Zubkov *et al.* (2003) stated that in oceanic oligotrophic waters the dominance of the genus *Prochlorococcus*, which consumes organic as well as inorganic nitrogen pools, may result in an overestimation of about 30% of bacterial secondary production and hence of computed organic matter decomposition and respiration. Moreover, similar to heterotrophic bacteria, different strains of the *Synechococcus* genus are also able to assimilate exogenous organic compounds like adenine, acetate, urea, leucine, uracil and thymidine (Martinez *et al.*, 1989) (see Martinez & Azam, 1993 and references therein). Another important ecological implication for *Synechococcus* lies in the protein turnover because they are able to hydrolyze oligo-peptides, especially in nitrogen-limited environments (Martinez & Azam, 1993).

In the present study, we investigated a picocyanobacterial mixed culture harvested from a widely monitored site in the Gulf of Trieste, which is one of the long-term research stations of the Northern Adriatic, included in the Italian Long-Term Ecological Research Network (LTER-Italy).

Cells were identified as *Synechococcus* sp. by means of an identification key (Bergey's Manual of Systematic Bacteriology) (Castenholz, 2001), transmission electron microscopy (TEM) observations, pigment and molecular analyses. We first analyzed the ultrastructural features and biliproteins of the cells and then focused on *Synechococcus* heterotrophic-like behavior by investigating the production of exoenzymes acting on different organic compounds (proteins, polysaccharides and lipids) as well as the uptake of organic molecules (leucine and thymidine incorporation) within the culture and two reference strains from the Provasoli–Guillard National Center for Culture of Marine Phytoplankton. We finally compared the organic carbon production via photosynthesis and via leucine incorporation, in order to evaluate the importance of the heterotrophic-like process within their carbon metabolism.

Materials and methods

Preparation of culture and culture conditions

Picocyanobacterial culture was harvested from the Gulf of Trieste (45°42′03″N–13°42′36″E) during a survey carried out in fall 2004 when *Synechococcus* generally reaches maximum abundances. Seawater collected at four depths from the surface to the bottom of the water column (max depth of 16 m) was prefiltered through a 10 μm mesh and 5 and 2 μm polycarbonate filters (Nuclepore) and then collected into an autoclaved glass flask. The prefiltrate was then filtered through 1 μm filters (Nuclepore) in order to minimize the heterotrophic bacteria content. The 1 μm filter content was resuspended into B medium (Agatha *et al.*, 2004) prepared with autoclaved artificial seawater and then divided into six replicate tubes and incubated under environmental conditions: 15 °C with a 12 : 12 light : dark cycle under 33 μmol m^{-2} s^{-1} PAR (OSRAM L18W21-Hellweiss LUMILUX Cool White). After *c.* 4 weeks under the above described conditions enrichment cultures were maintained by periodical transfers (2 weeks) into fresh medium. The culture obtained has been named SynTS. Filtrations allowed the complete removal of all organisms from the culture except for picocyanobacteria and an exiguous amount of heterotrophic bacteria, leading to a change in the phototrophic : heterotrophic bacteria ratio from *c.* 1 : 100 in seawater to $< 100 : 1$ within the culture. For comparison of exoenzymatic activities and leucine incorporation, two axenic *Synechococcus* strains, CCMP 1631 (*Synechococcus elongatus*, 248-01) and CCMP 1334 (DC2, WH7803, NEPCC549) from the Provasoli–Guillard National Center for Culture of Marine Phytoplankton (CCMP), were grown in B medium at 20 °C with a 12 : 12 light : dark cycle under 33 μmol m^{-2} s^{-1} PAR.

Systematic identification, fine structure and phycobiliproteins of the cells

The cultured cells were identified as *Synechococcus* according to the *Bergey's Manual of Systematic Bacteriology* (Castenholz, 2001). For TEM (Philips EM201) observations, aliquots of the culture were either centrifuged at 5900 ***g*** for 5 min or filtered on 0.2 μm polycarbonate filters (Nuclepore). Both preparations were fixed in 3% glutharaldehyde in 0.1 M phosphate buffer, pH 7.1, postfixed in 1% (w/v) osmium tetroxide in the same buffer and included in Spurr resin (Frisenda *et al.*, 2006). *In vivo* fluorescence excitation and emission spectra (Perkin Elmer LS 50B spectrofluorometer) were obtained using cultures in the logarithmic growth phase. Excitation and emission spectra were measured at fixed 652 nm λ_{Em} and 545 nm λ_{Ex} maxima, respectively. Both sterile B medium and 0.2 μm filtered cultures were used as the reference, and indicated fluorescence lower than 1% with respect to the culture. Spectra were smoothed with a 5 nm moving average. Biliprotein contents were also analyzed on crude extracts (Talarico & Kosovel, 1982; Honsell *et al.*, 1984) by spectrophotometry (Perkin-Elmer UV-VIS 554).

Cyanobacterial community structure

Culture aliquots (2 mL) were centrifuged at 20 800 ***g*** at room temperature for 10 min and then added with 175 μL of lysis buffer (400 mM NaCl, 750 mM sucrose, 20 mM EDTA, 50 mM Tris-HCl pH 9.0). DNA was extracted according to Boström *et al.* (2004). Briefly, cell lysis was preformed by adding lysozyme (final concentration 1 mg mL^{-1}), sodium dodecyl sulfate (final concentration 1%) and proteinase K (final concentration 100 mg L^{-1}). After the addition of 50 μg tRNA (from *Saccharomyces cerevisiae*, stock solution 10 mg mL^{-1}, Sigma) and 0.1 vol 3 M NaAc, DNA was precipitated with 0.6 vol isopropanol. Samples were then centrifuged and washed with 70% ethanol. The pellets were resuspended with 50 μL MQ. PCR and denaturing gradient gel electrophoresis (DGGE) were performed with cyanobacterial primers as described previously (Celussi *et al.*, 2008). Polyacrylamide gel slices containing the cyanobacterial DNA band of interest were excised using a sterile scalpel and eluted in 100 μL of MilliQ water overnight at $-20\,^{\circ}C$, followed by a freeze–thaw cycle. One microliter of thawed elution was reamplified using the CYA 781R-CYA 349F (Nübel *et al.*, 1997) primer without the GC clamp, and PCR product purified using the QIAquick PCR purification kit (Qiagen) according to the supplier's instructions.

Samples were sequenced using ABI Prism Big Dye dye-terminator chemistry (Applied Biosystems) using M13R primer and an ABI Prism 310 Genetic Analyzer (Applied Biosystems), according to the supplier's instruction. Sequences were aligned to known sequences in the GenBank database using BLAST (Altschul *et al.*, 1997). Multiple sequence alignment was performed using the CLUSTALX 1.81 program (Thompson *et al.*, 1997); the phylogenetic relationships were inferred by the neighbor-joining method and a phylogenetic tree was constructed with the program NJPLOT and finally edited for better graphics with CLC Free Workbench package version 4.0.1.

Synechococcus cell abundances

Culture aliquots (1 mL) were diluted 1 : 50 with artificial seawater and then fixed with 2% final concentration borate-buffered formalin (prefiltered through a 0.2 μm Acrodisc filter). Subsamples (5 mL) were filtered in triplicate onto 0.2-μm black-stained polycarbonate filters (Nuclepore). Filters were mounted on microscope slides, between layers of nonfluorescent immersion oil (Olympus), and counted within a few hours. Counts were made under a green (BP 480–550 nm, BA 590 nm) and a blue (BP 420–480 nm, BA 515 nm) filter set by epifluorescence microscopy (magnification, × 1000). Cells that auto-fluoresced orange were counted in at least 20 random fields and a minimum of 300 cells for each filter. Each value represents the mean of triplicate samples. Samples were processed until the coefficient of variation (CV = SD/Mean × 100) among three replicates was lower than 5%.

Heterotrophic bacteria

The presence of heterotrophic bacteria was monitored in the SynTS mixed cultures and in the CCMP 1631 and 1334 cultures during the whole experiment. Culture aliquots treated previously as described in the above section were stained for 15 min with 4′6-diamidino-2-phenylindole (DAPI, Sigma) at 1 μg mL^{-1} final concentration (Porter & Feig, 1980) and then filtered in triplicate onto 0.2-μm black-stained polycarbonate filters (Nuclepore). Heterotrophic bacteria were counted by epifluorescence microscopy under a UV (BP 330–385 nm, BA 420 nm) filter set. In each filter, at least 20 random fields and a minimum of 300 cells were counted.

Exoenzymatic activities (EEA)

Hydrolytic exoenzyme activities were measured with fluorogenic analogs of natural substrates (Hoppe, 1993): L-leucine-4-methylcoumarinile-7-amide (MCA, aminopeptidase), methyl umbelliferyl-β-D-glucoside (MUF, β-D-glucosidase), methyl umbelliferyl-β-D-galactoside (MUF, β-D-galactosidase) and methyl umbelliferyl oleate (MUF, oleate). Hydrolysis by bacteria was measured by incubating 2.5-mL subsamples with 200 μM leucine-MCA, MUF-β-glucoside, MUF-β-galactoside and MUF-oleate substrates for 1 h at 15 °C in the dark. The fluorescence released by enzymatic cleavage of the artificial substrates was measured fluorometrically, in quadruplicate, at 380/365 nm excitation and 440/455 nm emission for MCA/MUF substrates using a Perkin Elmer LS 50B fluorometer. Aminopeptidase activity was converted to protein mobilized using the conversion factor 72 μg C μmol^{-1}.

Primary carbon production

Cells productivity was measured by the ^{14}C technique (Steeman-Nielsen, 1952). Six aliquots (80 mL) from the SynTS mixed culture in the exponential phase of the growth curve were maintained in the dark for 30 min in order to minimize the photosynthetic activity. Replicate sample aliquots were inoculated with 222 KBq of $NaH^{14}CO_3$ and incubated for 2 h under culture conditions. The analysis was performed in quadruplicate against two dark bottle replicates, incubated for background measurements. At the end of incubation, 1, 2, 5 and 10-mL subsamples were acidified with a few drops of HCl 5 N for total primary production (PP) measurements. Extracellular release (ER) was measured by filtering 1, 2, 5 and 10-mL subsamples with 0.22-μm Millipore filters and then acidifying both the filter and the filtered water. 24 h after acidification, scintillation cocktail

was added to subsamples: 5 mL of Filter Count for filters and 10 mL of Ultima Gold for PP and ER. Activity was determined by a β-counter Packard Tri-Carb 300. Assimilation of carbon was calculated as described by Gargas (1975), assuming 5% isotope discrimination. Activity of the added $NaH^{14}CO_3$ and inorganic carbon concentration (tCO_2) were determined on the basis of total alkalinity measured in the same culture.

Secondary carbon production

Secondary carbon production was measured by the incorporation of ^{3}H-leucine (Smith & Azam, 1992) and ^{3}H-thymidine (Tdr) (Fuhrman & Azam, 1982). Quadruplicate 1.7 mL culture aliquots and two blanks [added with 90 μL 100% trichloracetic acid (TCA)] were amended with a 20 nM radiotracer and incubated in the dark at 15 and 5 °C for leucine and Tdr, respectively. Incubations were stopped with 100% TCA after 1 h. The extraction with 5% TCA and 80% ethanol was carried out using the microcentrifugation method (Smith & Azam, 1992). The incorporated radioactivity was determined by a β-counter (Packard Tri-Carb 300) after the addition of 1 mL scintillation cocktail (Ultima Gold MV; Packard). Incorporation of ^{3}H-leucine was converted into carbon produced via cyanobacterial protein production according to Simon & Azam (1989), assuming a twofold isotope dilution for leucine.

Results and discussion

Fine structure, biliprotein and culture identification of cells

Within the SynTS mixed cultures harvested from the Gulf of Trieste, TEM electron microscopy highlighted the dominance of *Synechococcus*-like cells (Fig. 1) that divided in a single plane. Cells were coccoid (Ø 0.8 μm) and rod shaped (from 1.0 to 1.6 μm long) with similar ultrastructural features. The external sheath(s) appeared to be poorly structured, the peptidoglycan layer between the outer and plasma membranes (Gantt, 1994) was barely visible and no periplasmic spaces were apparent. Thylakoids (thy) showed orderly parallel and concentric arrangements with peripheral location. Similar to eukaryotic red algae, interthylakoidal electron density indicated high concentrations of 'polydisperse' phycoerythrin (Talarico, 1990) that may act as a constraint on phycobilisome assembly (Talarico, 1996), depending on the light (irradiance/spectral composition) applied to the cultures (Talarico & Maranzana, 2000). In fact, only a few cells exhibited phycobilisomes (pbs) that were poorly structured and their presence is most probably due to some 'shade effect' of cell density in the culture. As a response to light quantity and quality, more or less structured phycobilisomes have been described for several cyanobacteria (Grossman, 2003) and eukaryotic red algae (Talarico, 1996; Talarico *et al.*, 1998; Talarico & Maranzana, 2000), both in the field and under culture. Other features such as an electron-transparent centroplasm with DNA filaments (n), several cytoplasmic inclusions, i.e. cyanophycin granules, polyphosphate bodies (pp) and carboxysomes (c), commonly seen in cyanobacteria (Kromkamp, 1987; Lefort-Tran *et al.*, 1988; Harano *et al.*, 2003), were also apparent.

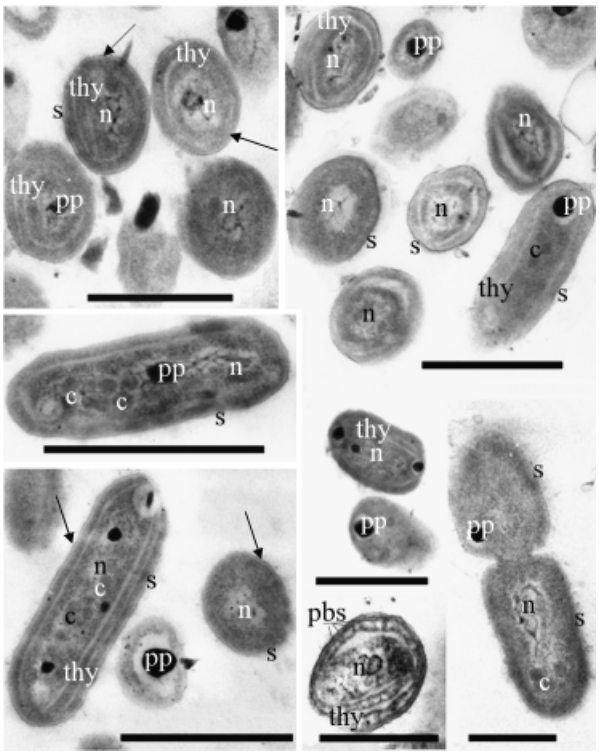

Fig. 1. TEM micrographs of *Synechococcus* mixed culture harvested from the Gulf of Trieste (SynTS). Ultrastructural features: poorly structured external sheath (s), no periplasmic spaces (arrows), peripheral thylakoids (thy) with phycobilisomes (pbs) rarely present in some cells, nucleoid with DNA filaments (n), numerous polyphosphate bodies (pp) and carboxisomes (c). The high electron density between thylakoids indicates 'polydisperse' biliproteins. Bars correspond to 1 μm.

The *in vivo* fluorescence emission (Em) spectrum (Fig. 2) highlighted the presence of phycocyanobilin chromophores carried by both C-phycocyanin (652 nm Em λ_{max}) and by inner allophycocyanins (APC, APC-B and APC_{680} of the chromophorylated core-membrane linker L_{CM}) with the final emission at 680 nm λ_{max} (Talarico & Maranzana, 2000). The *in vivo* fluorescence excitation spectrum showed the presence of both phycourobilin and phycoerythrobilin chromophores (Fig. 2), in agreement with previous results on crude extracts from the same culture (Frisenda *et al.*, 2006). In fact, both absorption (Abs) and excitation (Ex) spectra revealed the presence of CU-phycoerythrin with

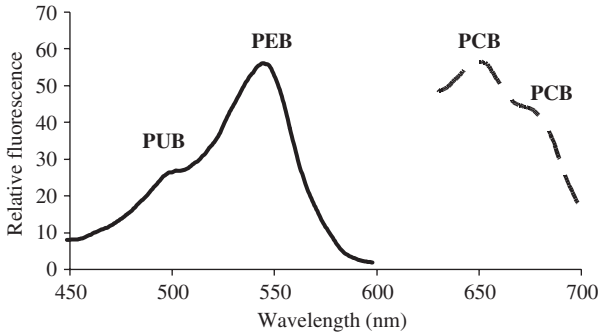

Fig. 2. *In vivo* fluorescence excitation (solid line) and emission (dashed line) spectra, measured at fixed 652 nm λ_{Em} and 545 nm λ_{Ex}, respectively. The peaks corresponding to phycourobilin, phycoerythrobilin and phycocyanobilin chromophores carried by γ, α and β subunits are indicated.

phycourobilin chromophores (Abs λ_{max} 494–498 nm) (MacColl, 1998), formerly named phycoerythrin II (Wilbanks & Glazer, 1993), as identified previously in the benthic *Oscillatoria* cf. *corallinae* (Abs λ_{max} 498 and 540 nm) (Hoffman *et al.*, 1990). Furthermore, given the amplitude and height of both Abs and Ex maxima at 540 nm, also a C-phycoerythrin (Abs λ_{max} 540 and 555 nm) carrying mostly phycoerythrobilin chromophores (MacColl, 1998) may be deducted.

Our findings would indicate the presence of at least two *Synechococcus* subgroups, presumably differing either in phycoerythrin type or in physiological traits. CU-phycoerythrin, similar to R-phycoerythrins of eukaryotic red algae (Talarico & Maranzana, 2000 and references therein), is particularly rich in phycourobilin (λ_{max} 494–498 nm) chromophores carried by its γ sub-units (Hoffman *et al.*, 1990; Wilbanks & Glazer, 1993). All C-phycoerythrins [including phycoerythrin I type, as classified by Ong & Glazer (1991)], but potentially also phycocyanins [a phycocyanin named R-phycoerythrin II by Ong & Glazer (1991) or CE-phycocyanin by MacColl (1998)], have prevailing phycoerythrobilin (λ_{max} 540–555 nm) chromophores on their α and β subunits (Sidler, 1994), although phycoerythrin-associated linkers (γ-L_R) chromophorylated with phycourobilin have been detected (Wilbanks & Glazer, 1993; Six *et al.*, 2005). It is worth noting that, unlike phycoerythrobilin, the phycourobilin chromophores have so far been identified in relatively few taxa, most of them belonging to genus *Synechococcus* (Olson *et al.*, 1990; Sidler, 1994; Toledo *et al.*, 1999; Palenik, 2001; Ting *et al.*, 2002; Six *et al.*, 2005; Ahlgren & Rocap, 2006). The presence of CU-phycoerythrin represents a competitive ecological advantage widening the absorption cross section in the blue-part of the light spectrum (Hoffman *et al.*, 1990; Wood *et al.*, 1998). These cells, besides being well adapted to green radiations for possessing phycoerythrobilin, also exhibit an efficient adaptation to the deepest layers of the water column for the presence (and/or major proportion) of phycourobilin. It has been thought that in marine strains the presence of two phycoerythrins, which are encoded by different genes (Sidler, 1994), reflects evolutionary chromatic adaptation to blue radiation dominating the light fields of the open oceans (Toledo *et al.*, 1999). Nevertheless, acclimation to light quality on physiological time scales (hours to days) may exist in some strains (Palenik, 2001; Ahlgren & Rocap, 2006), in contrast with the constancy of the phycourobilin to phycoerythrobilin ratio supposed previously for marine *Synechococcus*. In fact, this photoacclimation, largely demonstrated for other *Cyanobacteria* (Moore *et al.*, 1995; Grossman, 2003) and eukaryotic red algae (Talarico, 1996; Talarico & Maranzana, 2000), may result in low-high temporary phycourobilin or phycoerythrobilin contents with, thus, wide phycourobilin/phycoerythrobilin variations within the same strain.

In the shallow waters of the Gulf of Trieste (Northern Adriatic Sea), green light, which predominates in coastal waters, penetrates to a similar extent as blue light and reaches the maximum depth (Celussi *et al.*, 2006), thus resembling the light fields of the upper water column (0–30 m) of the open ocean (Ting *et al.*, 2002). This would explain the co-occurrence of populations with phycourobilin with those enriched in phycoerythrobilin, the former previously found only in coastal waters (Olson *et al.*, 1990) and the latter in an open ocean (Ting *et al.*, 2002).

The DGGE band pattern of cyanobacterial amplicons showed the presence of seven ribotypes (Fig. 3), all affiliated to *Synechococcus,* and 16S rRNA gene sequences were deposited in GenBank (see Fig. 4 for accession numbers). Two different lineages were identified from the phylogenetic tree (Fig. 4), grouping together (1) SynTS3, SynTS4, SynTS2, SynTS1, and (2) SynTS6, SynTS7, SynTS5. These are the first data on the molecular identification of the phototrophic picoplankton from the Northern Adriatic Sea. The hypothesis that the two individual phylogenetic groups might be represented by strains with qualitatively different pigment contents (i.e. C-phycoerythrin and/or CU-phycoerythrin) would be in agreement with the evolutionary chromatic adaptation (Ting *et al.*, 2002; Qin *et al.*, 2005), and the different phycoerythrobilin and phycourobilin distributions between these groups would derive from selective expression either of genes encoding for C-phycoerythrin α and β subunits (carrying mostly phycoerythrobilin) or of other genes encoding for CU-phycoerythrin γ subunits (carrying phycourobilin) that are suitable for green and blue light fields, respectively. However, it has been demonstrated that high or low phycourobilin contents (when present) or diverse phycourobilin/phycoerythrobilin ratios displayed by the strains analyzed in the field and interpreted as genetically distinct populations are not useful characteristics for defining phylogenetic groups of *Synechococcus* (Toledo *et al.*, 1999). In the same study, several motile strains with widely

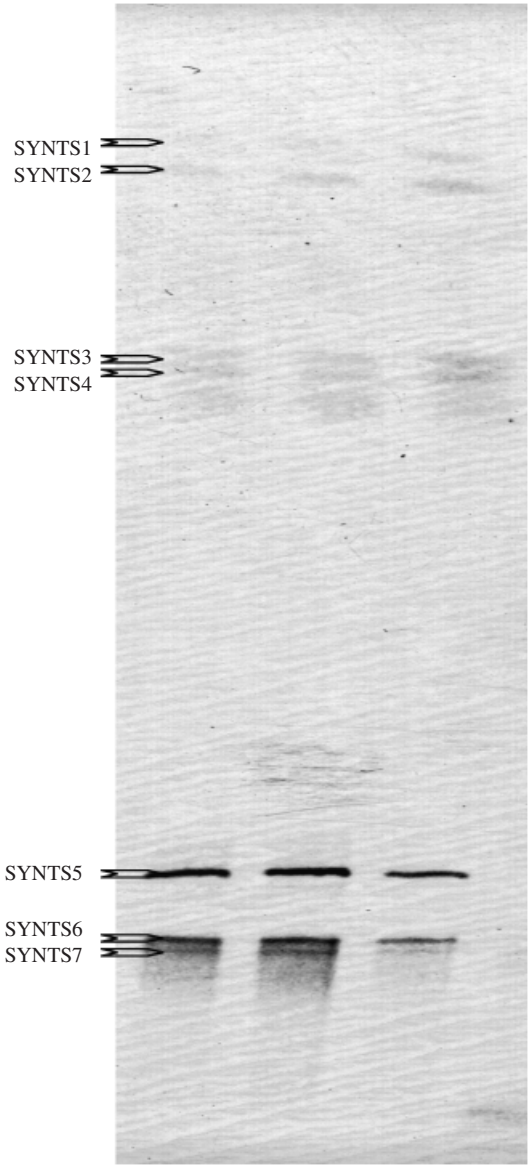

Fig. 3. DGGE profiles of PCR-amplified 16S rRNA gene fragments (c. 400 bp) from three replicates of template DNA subsamples from the SynTS mixed culture with a 30–70% denaturing gradient. Names next to the gel denote bands that were excised and used for sequence and phylogenetic analyses (Fig. 4).

different pigment (phycourobilin/phycoerythrobilin) ratios have been proven to form a monophyletic group, motility being a better marker of genetic affiliation than pigment complement. These results suggest that the genetic divergence we observed (Fig. 4) may rather reflect two different physiological responses to light, i.e. chromatic 'short term' acclimation (Talarico, 1996; Talarico & Maranzana, 2000). These two groups would possess quite different photoacclimation capabilities, in agreement with the findings of Palenik (2001), who clearly demonstrated that some marine strains of *Synechococcus* under culture were able to acclimate while others were not, despite similar phycourobilin and phycoerythrobilin spectral signatures. The hypothesis that these two lineages reflect two different physiological behaviors, possibly discriminating light (irradiance/spectral composition) 'adapters' and 'nonadapters', is further supported by the recent findings of Ahlgren & Rocap (2006), who isolated two new marine *Synechococcus* ecotypes with distinctive light (and nitrogen) physiologies under culture, thus proving that chromatic 'short term' adaptation is more widely distributed among *Synechococcus* strains than known previously. This underlines the need for further exploration of the physiological differences that allow for the coexistence of ecotypes fulfilling the same ecological niche. Additional insights into the adaptive mechanisms to light and other environmental factors would contribute to a new understanding of ecotype differentiation in the marine *Synechococcus* lineage (Ahlgren & Rocap, 2006), both on evolutionary and on physiological time scales. It is foreseeable that an increased number of genome sequences of cultured isolates, when available, will allow the comparison of genomic and physiological data that is crucial for understanding the ecological role (Hess, 2004) of these so widely distributed picocyanobacteria.

Synechococcus heterotrophic-like behavior

In Table 1, the range of variation of cells' abundance, aminopeptidase activity (AMA), protein carbon mobilization (C mob), Leucine incorporation and percentage of mobilized carbon utilization (C mob utilization) measured during the growth curve of the SynTS mixed culture and of the two axenic strains (CCMP 1334 and CCMP 1631) are reported. In Fig. 5, the trends of the above-mentioned parameters for the SynTS mixed culture are reported. During the whole study, the CCMP 1631 and 1334 cultures remained axenic while the phototrophic : heterotrophic bacteria ratio within the SynTS mixed culture ranged between 40 : 1 and 8 : 1 (Fig. 5), which means more than three orders of magnitude higher than the seawater one. The EEA were measured in order to characterize the carbon-containing substrates degradable by *Synechococcus* cultured strains: proteins (AMA), polysaccharides (β-glucosidase and β-galactosidase activities) and lipids (lipase activity). Among the four EEA investigated, only aminopeptidase showed high hydrolytic activity both for SynTS culture and for CCMP strains (Table 1). As described previously (Paoli *et al.*, 2005), high activities of the enzyme alkaline phosphatase were also found within the same cultures. The incorporation of macromolecules within cultures was positive for leucine but not for Tdr. Martinez & Azam (1993) also found high AMA within different *Synechococcus* strains, and thymidine incorporation was found for *Synechococcus curtus*

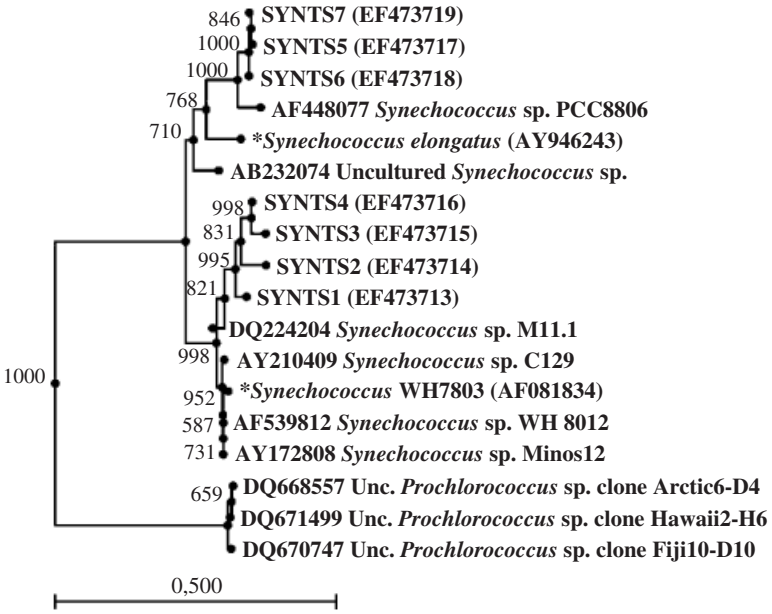

Fig. 4. Phylogenetic tree showing the relationships among all 16S rRNA gene cyanobacterial ribotypes considered in this study. The tree is unrooted with three *Prochlorococcus* strains as the outgroup and bootstrap values were attached to each branch as a measure of its confidence. The scale bar indicates 0.5 changes per nucleotide. The sequenced amplicons (c. 400 bp) are all affiliated to the genus *Synechococcus* and are indicated with the prefix SYN TS (GenBank accession numbers are provided in parenthesis). An asterisk indicates the two axenic reference strains used for the physiological studies.

Table 1. Maxima and minima of cell abundance, aminopeptidase activity (AMA), protein carbon mobilization (C mob), Leu incorporation (Leu), and percentage of mobilized carbon utilization (C mob utilization) measured during the growth curve of a *Synechococcus* mixed culture harvested from the Gulf of Trieste (SynTS) and two axenic cultures (CCMP 1334 and CCMP 1631) from the Provasoli–Guillard National Center for Culture of Marine Phytoplankton (CCMP)

		SynTS		CCMP 1334		CCMP 1631	
		Min	Max	Min	Max	Min	Max
Abundance	10^8 cells L^{-1}	39.4 ± 2.9	1827 ± 76	26.3 ± 1.6	208 ± 5.3	1.2 ± 0.1	53.0 ± 3.9
AMA	$\mu M\ h^{-1}$	0.2 ± 0.02	15.7 ± 0.9	0.1 ± 0.00	0.3 ± 0.02	2.3 ± 0.2	30.1 ± 1.3
C mob	$\mu g\ C\ L^{-1}\ h^{-1}$	14.8 ± 2.2	1127 ± 64	4.6 ± 0.09	19.4 ± 1.3	166 ± 16	2166 ± 95
Leu	$\mu g\ C\ L^{-1}\ h^{-1}$	1.7 ± 0.06	18.5 ± 1.3	0.03 ± 0.00	0.2 ± 0.01	0.2 ± 0.00	8.6 ± 0.4
C mob utilization	%	1.6 ± 0.01	11.6 ± 0.1	0.4 ± 0.00	2.3 ± 0.02	0.1 ± 0.00	0.4 ± 0.0

Each value represents the mean of triplicate (for cells abundance) and quadruplicate (for others) sample analysis ± SD. The standard propagated error is indicated for the C mob utilization.

(Martinez *et al.*, 1989) but not for the WH7803 strain (Cuhel & Waterbury, 1984). AMA paralleled the *Synechococcus* growth curves, and leucine incorporation rate increased strongly during the lag phase and then weakly and progressively during the whole exponential growth until the beginning of the senescent state of the cultures (see Fig. 5 for SynTS). AMA and leucine incorporation values are extremely high when compared with the seawater of the North Adriatic as well as the activities detected in mucilage aggregates (see Del Negro *et al.*, 2005 and references therein). AMA and leucine incorporation rates within SynTS culture were in the range of those found for the CCMP 1631 and CCMP 1334 strains (Table 1). The protein C potentially mobilized by AMA was particularly high when compared with the leucine incorporation (Table 1), resulting in low percentages of newly mobilized C utilization. Nevertheless, the SynTS culture showed the highest percentage of C utilization (up to 11.6%) among the investigated cultures, with the highest values in the lag phase decreasing toward the end of the growth curve (Fig. 5). We hypothesize that the higher percentage of C taken up with respect to the C mobilized in the lag phase would sustain the cell division processes characterizing the upcoming exponential phase. Because the aminopeptidase activity and leucine incorporation rates within the SynTS mixed culture were in the range of those found for CCMP 1631 and CCMP 1334, and by considering that the phototrophic : heterotrophic bacteria ratio within SynTS culture was always more than three orders of magnitude higher than the seawater one, we concluded that in the latter culture both activities could be primarily ascribed to *Synechococcus* cells and the heterotrophic bacterial contribution is negligible. The presence of high aminopeptidase activity and secondary carbon production within the mixed cultures confirms the role of

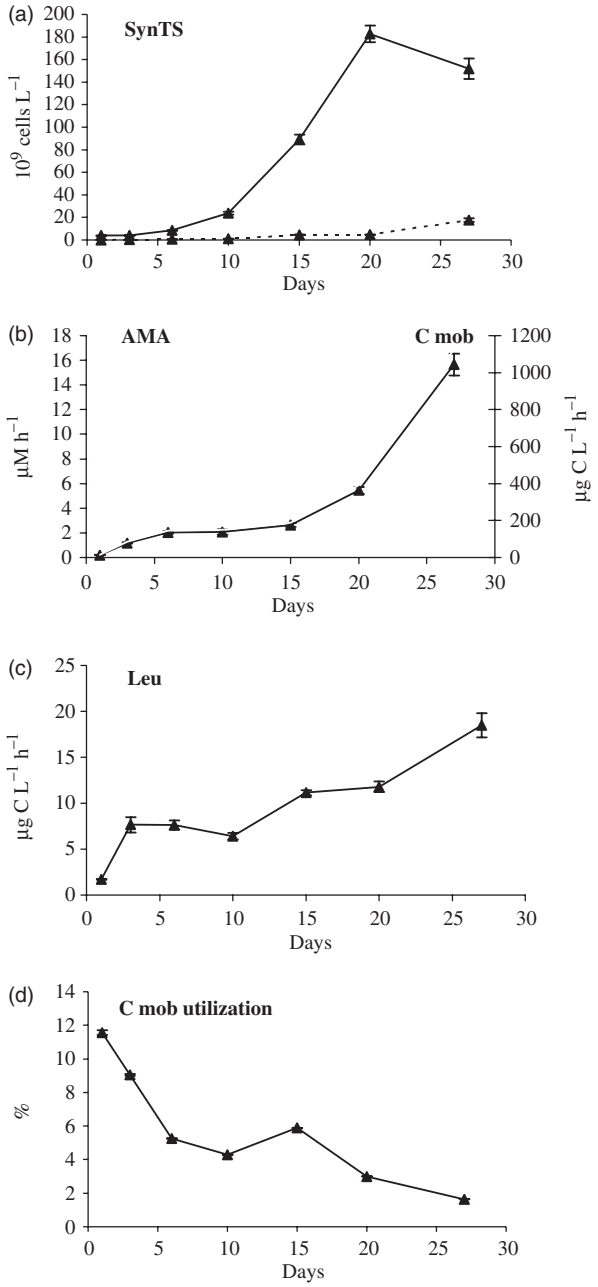

Fig. 5. (a) *Synechococcus* cells (continuous line) and heterotrophic bacterial (dashed line) abundance, (b) AMA–protein carbon mobilization (C mob) (both represented by the continuous line), (c) leucine incorporation and (d) percentage of mobilized carbon utilization (C mob utilization) measured during the growth curve of a *Synechococcus* mixed culture harvested from the Gulf of Trieste. Each value represents the mean of triplicate (for cells abundance) and quadruplicate (for others) sample analysis ± SD. The standard propagated error is indicated for the C mob utilization.

Synechococcus in C remineralization, particularly through the degradation/utilization of proteins and not of sugars or lipids. Notwithstanding leucine incorporation is generally considered to be an important mode of carbon utilization for marine organisms; this process could also represent an important strategy for nitrogen recovery for *Synechococcus*. As a result of their strong N dependence (Collier *et al.*, 1999), most of the marine cyanobacteria developed some strategies that are alternative to inorganic N absorption like N_2 fixation, exoenzymatic degradation and direct uptake of low-molecular-weight organic compounds rich in N. A study on *Synechococcus* revealed that its element assimilation ratio (C : N : P : S = 95 : 16 : 3 : 1) is quite high for N (Cuhel & Waterbury, 1984); thus, leucine and other amino acids would represent an important source of organic nitrogen. The high leucine incorporation rate may also represent an energetically less expensive way for 'constructing' biliproteins, given their massive presence, as discussed previously. It is reasonable to think that biliproteins would represent an important inner N source in cases of N limitation in the surrounding environment.

Measurements of the primary carbon production (PP) were performed within SynTS culture during the exponential phase of the growth curve. *Synechococcus* cells and heterotrophic bacterial abundances were *c.* 5.4×10^{10} and 1.1×10^9 cells L^{-1}, respectively. The PP analyses showed similar results by considering different aliquots of the same replicates (1, 2, 5 and 10 mL). A total production of 34.6 ± 0.9 μg C L^{-1} h^{-1} with no detectable extracellular release (Fig. 6) was observed. Thus, the inorganic carbon fixed by photosynthesis was completely transformed in biomass and not released by exudation processes or the exuded carbon, if any, was immediately consumed by the heterotrophic bacteria. The comparison of the organic carbon produced photosynthetically with the organic carbon incorporated as leucine (11.1 ± 0.3 μg C L^{-1} h^{-1}) yielded an *c.* 3 : 1 ratio (Fig. 6).

According to these results, a clear photoheterotrophic behavior characterizes the *Synechococcus* cells harvested

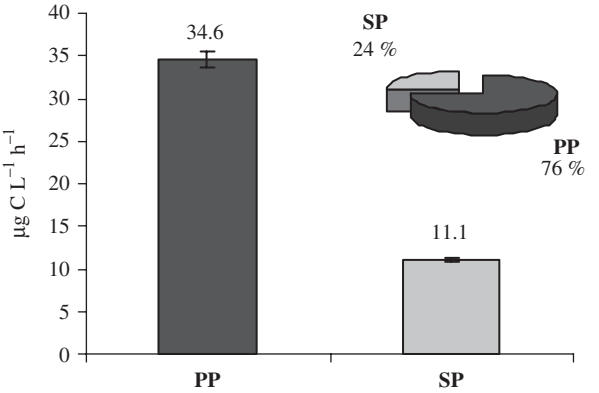

Fig. 6. Primary (PP) and secondary (SP) carbon production within the SynTS mixed culture during the exponential phase of the growth curve measured as a rate of carbon fixation via photosynthesis and leucine incorporation, respectively.

from the Gulf of Trieste and this might represent a key to the successful large-scale distribution of this genus within this area as well as over the entire Adriatic and Mediterranean sea. In addition to the important ecological advantages given by their small dimension (i.e. a reduced sedimentation rate, a more efficient light energy absorption, together with a more efficient inorganic nutrients' adsorption) (Fogg, 1995), their ability to adapt chromatically to underwater light fields represents an important mechanism for their competitive dominance over planktonic microalgae. Moreover, the capacity to regenerate N and P inorganic nutrients (Martinez & Azam, 1993; Paoli *et al*., 2005) and to utilize organic compounds taken up from the surrounding water (Cuhel & Waterbury, 1984; Martinez *et al*., 1989) allows *Synechococcus* to live in different marine environments, from oligotrophic to highly productive ones. In the Gulf of Trieste, which is characterized by a generally low trophic state (Fonda Umani *et al*., 2007) that could rapidly turn into a very high state due to the impulsive nature of the local rivers' outflow, *Synechococcus* cells are always abundant during the whole year (Paoli & Del Negro, 2006). They might be adapted to directing their metabolism to remineralization rather than production processes in relation to the temporary trophic state of the basin. The ability of *Synechococcus* to adapt to an annual (but also on a short time-scale of days) (Paoli *et al*., 2006) wide variation in hydrological and chemical-physical factors (Cushman-Roisin *et al*., 2001) may help to explain the significant increase in their abundances over the last decades in the Gulf of Trieste (Paoli & Del Negro, 2006), whereas a general decrease in microphytoplankton abundance (and particularly diatoms) was observed (Fonda Umani *et al*., 2004). The *Synechococcus* role in the degradation of the organic matter as well as in the regenerated production processes might place them in competition with the heterotrophic bacteria and this might justify their similar, both seasonal and multiyear, patterns of distribution (Paoli & Del Negro, 2006). Our findings support the idea of Zubkov *et al.* (2003), according to whom the classical clear distinction between auto- and heterotrophic microorganisms in the sea should be reconsidered, because heterotrophic bacteria can use photosynthesis (e.g. Gómez-Consarnau *et al*., 2007) and, conversely, photosynthetic cyanobacteria can utilize key nutrients and organic compounds heterotrophically.

Acknowledgements

The authors would like to thank B. Cataletto and D. Virgilio for their support in carrying out and discussing molecular and primary production analyses, P. Frisenda for TEM preparations, A. Colaviti and F. Bolzan for stimulating debates on biliproteins and 'light' and E. De Vos for language revision.

References

Agatha S, Strüder-Kypke MC & Beran A (2004) Morphological and genetic variability in marine planktonic ciliate *Laboea strobila* Lohmann, 1908 (Ciliophora, Oligotrichia), with notes on its ontogenesis. *J Eukaryot Microbiol* **51**: 267–282.

Ahlgren NA & Rocap G (2006) Culture isolation and culture-independent clone libraries reveal new marine *Synechococcus* ecotypes with distinctive light and N physiologies. *Appl Environ Microbiol* **72**: 7193–7204.

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W & Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**: 3389–3402.

Bernardi Aubry F, Acri F, Bastianini M & Pugnetti A (2006) Picophytoplankton contribution to phytoplankton community structure in the Gulf of Venice (NW Adriatic Sea). *Internat Rev Hydrobiol* **91**: 51–70.

Boström KH, Simu K, Hagström Å & Riemann L (2004) Optimization of DNA extraction for quantitative marine bacterioplankton community analysis. *Limnol Oceanogr: Methods* **2**: 365–373.

Castenholz RW (2001) Oxygenic photosynthetic cyanobacteria. *Bergey's Manual of Systematic Bacteriology, Vol. 1*, 2nd edn (Boone DR *et al*., eds), pp. 473–599. Springer.

Celussi M, Paoli A, Gerin R & Vinzi E (2006) Control on planktonic cyanobacteria development: the role of light quality. *Biol Mar Medit* **13**: 951–953.

Celussi M, Paoli A, Bernardi Aubry F, Bastianini M & Del Negro P (2008) Diel microbial variations at a coastal Northern Adriatic station affected by Po River outflows. *Estuar Coast Shelf Sci* **76**: 36–44.

Collier JL, Brahamsha B & Palenik B (1999) The marine cyanobacterium *Synechococcus* sp. WH 7805 requires urease (urea amidohydrolase, EC 3.5.1.5) to utilize urea as nitrogen source: molecular-genetic and biochemical analysis of the enzyme. *Microbiology* **145**: 447–459.

Cuhel RL & Waterbury JB (1984) Biochemical composition and short term nutrient incorporation patterns in a unicellular marine cyanobacterium, *Synechococcus* (WH 7803). *Limnol Oceanogr* **29**: 370–374.

Cushman-Roisin B, Gačić M, Poulain P-M & Artegiani A (2001) *Physical Oceanography of the Adriatic Sea*. Kluver Academic Publishers, Dordrecht.

Del Negro P, Crevatin E, Larato C, Ferrari C, Totti C, Pompei M, Giani M, Berto D & Fonda Umani S (2005) Mucilage microcosms. *Sci Total Environ* **353**: 258–269.

Fogg GE (1995) Some comments on picoplankton and its importance in the pelagic ecosystem. *Aquat Microb Ecol* **9**: 33–39.

Fonda Umani S, Beran A, Parlato S, Virgilio D, Zollet T, De Olazabal A, Lazzarini B & Cabrini M (2004) *Noctiluca scintillans* Macartney in the Northern Adriatic Sea: long-term dynamics, relationships with temperature and eutrophication, and role in the food web. *J Plankton Res* **26**: 1–17.

Fonda Umani S, Del Negro P, Larato C, De Vittor C, Cabrini M, Celio M, Falconi C, Tamberlich F & Azam F (2007) Major inter-annual variations in microbial dynamics in the Gulf of Trieste (northern Adriatic Sea) and their ecosystem implications. *Aquat Microb Ecol* **46**: 163–175.

Frisenda P, Celussi M, Paoli A & Talarico L (2006) Study of picocyanobacteria in the Gulf of Trieste (Northern Adriatic Sea): a methodological approach. *Biol Mar Medit* **13**: 971–974.

Fuhrman JA & Azam F (1982) Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica, and California. *Appl Environ Microbiol* **39**: 1085–1095.

Gantt E (1994) Supramolecular membrane organization. *The Molecular Biology of Cyanobacteria* (Bryant DA, ed), pp. 119–138. Kluwer Academic Publishers, Dordrecht.

Gargas E (1975) A manual for phytoplankton primary production studies in the Baltic. *The Baltic Marine Biologist*, *Vol. 2* (Gargas E, ed), pp. 1–18. Water Quality Institute, Hørsholm.

Gómez-Consarnau L, González JM, Coll-Lladó M, Gourdon P, Pascher T, Neutze R, Pedrós-Alió C & Pinhassi J (2007) Light stimulates growth of proteorhodopsin-containing marine Flavobacteria. *Nature* **445**: 210–213.

Grossman AR (2003) A molecular understanding of complementary chromatic adaptation. *Photosynthesis Res* **76**: 207–215.

Harano K, Ishida H, Kittaka R, Kojima K, Inouir N, Tsukamoto M, Satoh R, Himeno M, Iwaki T & Wadano A (2003) Regulation of the expression of ribulose-1,5-bisphosphate carboxylase/oxygenase (EC 4.1.1.39) in a cyanobacterium, *Synechococcus* PCC7942. *Photosynthesis Res* **78**: 59–65.

Hess WR (2004) Genome analysis of marine photosynthetic microbes and their global role. *Curr Opinion Biotechnol* **15**: 191–198.

Hoffman L, Talarico L & Willmotte A (1990) Presence of CU-phycoerythrin in the benthic blue-green alga *Oscillatoria* cf. *corallinae*. *Phycologia* **29**: 19–26.

Honsell E, Kosovel V & Talarico L (1984) Phycobiliprotein distribution in rhodophyta: studies and interpretations on the basis of their absorption spectra. *Bot Mar* **27**: 1–16.

Hoppe HG (1993) Use of fluorogenic model substrates for extracellular enzyme activity (EEA) measurement of bacteria. *Handbook of Methods in Aquatic Microbial Ecology* (Kemp PF *et al.*, eds), pp. 423–431. CRC Press, Boca Raton, FL.

Kromkamp J (1987) Formation and functional significance of storage products in cyanobacteria. *New Zeal J Mar Freshwater Res* **29**: 457–465.

Lefort-Tran M, Pouphile M, Spath S & Packer L (1988) Cytoplasmic membrane changes during adaptation of the freshwater Cyanobacterium *Synechococcus* 6311 to salinity. *Plant Physiol* **87**: 767–775.

MacColl R (1998) Cyanobacterial phycobilisomes. *J Struct Biol* **124**: 311–334.

Magazzù G & Decembrini F (1995) Primary production, biomass and abundance of phototrophic picoplankton in the Mediterranean Sea: a review. *Aquat Microb Ecol* **9**: 97–104.

Martinez J & Azam F (1993) Aminopeptidase activity in marine chroococcoid cyanobacteria. *Appl Environ Microbiol* **59**: 3701–3707.

Martinez J, Riera M, Lalucat J & Vives-Rego J (1989) Thymidine incorporation into algal DNA from axenic cultures of *Synechococcus*, *Chlorella* and *Tetraselmis*. *Lett Appl Microbiol* **8**: 135–138.

Moore LR, Goericker R & Chisholm SW (1995) Comparative physiology of *Synechococcus* and *Prochlorococcus*: influence of light and temperature on growth, pigments, fluorescence and absorptive properties. *Mar Ecol Prog Ser* **116**: 259–275.

Nübel U, Garcia-Pinchel F & Muyzer G (1997) PCR primers to amplifying 16S rRNA genes from cyanobacteria. *Appl Environ Microbiol* **63**: 3327–3332.

Olson JR, Chisholm SW, Zettler ER & Ambrust EV (1990) Pigments, size, and distribution of *Synechococcus* in the North Atlantic and Pacific Oceans. *Limnol Oceanogr* **35**: 45–58.

Ong LJ & Glazer AN (1991) Phycoerythrins of marine unicellular cyanobacteria. I. Bilin types and locations and energy transfer pathways in *Synechococcus* spp. phycoerythrins. *J Biol Chem* **266**: 9515–9527.

Palenik B (2001) Chromatic adaptation in marine *Synechococcus* strains. *Appl Environ Microbiol* **67**: 991–994.

Paoli A & Del Negro P (2006) Bacterial abundances in the Gulf of Trieste waters from 1993 to 2004. *Biol Mar Medit* **13**: 141–148.

Paoli A, Celussi M & Del Negro P (2005) Attività fosfatasica di *Synechococcus*, isolato nel Golfo di Trieste. *Inform Bot Ital* **37**: 590–591.

Paoli A, Del Negro P & Fonda Umani S (2006) Temporal variability in bacterioplanktonic abundance in coastal waters of the Northern Adriatic Sea. *Chem Ecol* **22** (**S1**): S93–S103.

Paoli A, Celussi M, Valeri A, Larato C, Bussani A, Fonda Umani S, Vadrucci MR, Mazziotti C & Del Negro P (2007) Picocyanobacteria in Adriatic transitional environments. *Estuar Coast Shelf Sci* **75**: 13–20.

Porter KG & Feig YG (1980) The use of DAPI for identifying and counting aquatic microflora. *Limnol Oceanogr* **25**: 943–948.

Qin S, Zhao FQ & Tseng CK (2005) Evidence for positive selection in phycoerythrin genes of red algae and cyanobacteria *Prochlorococcus* and *Synechococcus*. *Photosynthetica* **43**: 141–146.

Scanlan DJ & West NJ (2002) Molecular ecology of the marine cyanobacteria genera *Prochlorococcus* and *Synechococcus*. *FEMS Microbiol Ecol* **40**: 1–12.

Sidler WAA (1994) Phycobilisome and phycobiliprotein structures. *The Molecular Biology of Cyanobacteria* (Bryant DA, ed), pp. 139–216. Kluwer Academic Publishers, Dordrecht.

Simon M & Azam F (1989) Protein content and protein synthesis rates of planktonic marine bacteria. *Mar Ecol Prog Ser* **51**: 201–213.

Six C, Thomas J, Thion L, Lemoine Y, Zal F & Partensky F (2005) Two novel phycoerythrin-associated linker proteins in the

marine cyanobacterium *Synechococcus* sp. strain WH8102. *J Bacteriol* **187**: 1685–1694.

Smith DC & Azam F (1992) A simple, economical method for measuring bacterial protein synthesis rates in sea water using ^{3}H-leucine. *Mar Microb Food Webs* **6**: 107–114.

Steeman-Nielsen E (1952) The use of radioactive ^{14}C for measuring organic production in the sea. *J Cons Int Explor Mer* **18**: 117–140.

Talarico L (1990) R-phycoerythrin from *Audouinella saviana* (Nemaniales, Rhodophyta). Ultrastructural and biochemical analysis of aggregates and subunits. *Phycologia* **29**: 292–302.

Talarico L (1996) Phycobiliproteins and pycobilisomes in red algae: adaptive responses to light. *Underwater Light and Algal Photobiology* (Figueroa FL *et al*., eds), pp. 205–222. CSIC, Barcelona, Spain.

Talarico L & Kosovel V (1982) Spectral analysis of the biliproteins from *Gracilaria verrucosa* (Huds.) Papenfuss (*Gigartinales*, *Florideophyceae*). *Photosynthetica* **16**: 184–190.

Talarico L & Maranzana G (2000) Light and adaptive responses in red macroalgae: an overview. *J Photochem Photobiol B: Biol* **56**: 1–11.

Talarico L, Rascio N, Dalla Vecchia F & Maranzana G (1998) Some observations on phycobilisomes of *Pterocladiella capillacea* (Gelidiales, Rhodophyta). *Plant Biosystems* **132**: 87–96.

Tandeau de Marsac N (1977) Occurrence and nature of chromatic adaptation in cyanobacteria. *J Bacteriol* **130**: 82–91.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F & Higgins DG (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **24**: 4876–4882.

Ting CS, Rocap G, King J & Chisholm SW (2002) Cyanobacterial photosynthesis in the oceans: the origins and significance of divergent light-harvesting strategies. *Trends Microbiol* **10**: 134–142.

Toledo G, Palenik B & Brahamsha B (1999) Swimming marine *Synechococcus* strains with widely different photosynthetic pigment ratios form a monophyletic group. *Appl Environ Microbiol* **65**: 5247–5251.

Wilbanks SM & Glazer AN (1993) Rod structure of a phycoerythrin II-containing phycobilisome. Complete sequence and bilin attachment site of a phycoerythrin γ subunit. *J Biol Chem* **268**: 1236–1241.

Wood AM, Phinney DA & Yentsch CM (1998) Water column transparency and the distribution of spectrally distinct forms of phycoerythrin-containing organisms. *Mar Ecol Prog Ser* **162**: 25–31.

Zubkov MV, Fuchs BM, Tarran GA, Burkill PH & Amann R (2003) Mesoscale distribution of dominant bacterioplankton groups in the northern North Sea in early summer. *Aquat Microb Ecol* **29**: 135–144.