The following supplement accompanies the article

Effect of sunlight on prokaryotic organic carbon uptake and dynamics of pigments relevant to photoheterotrophy in the Adriatic Sea

Mauro Celussi*, Alessandra A. Gallina, Josephine Ras, Michele Giani, Paola Del Negro

*Corresponding author: mcelussi@ogs.trieste.it

Aquatic Microbial Ecology 74: 235–249 (2015)

SUPPLEMENTARY MATERIAL

The supplementary material includes:
- Cyanobacterial community structure Materials and methods and Results (Fig. S1, Table S1)
- Background information: temperature and salinity data description
- Table S2 summarizing all sampling points for each parameter

Cyanobacterial community structure

MATERIALS AND METHODS

Samples for cyanobacterial community structure analysis were collected in surface waters at stations A04, A11, A16, A20, A23 in February and at stations A19, A20, A23 in October. 1.5 l seawater samples were filtered onto 47 mm Ø, 0.2 µm polyethersulfone membrane filters (Supor 200, PALL Corporation) and filters were frozen at –20 °C until DNA extraction which was subsequently performed according to Boström et al. (2004). Cyanobacterial 16S rRNA genes were PCR-amplified using the primer set CYA 349F (plus a GC clamp) and CYA 781R (equimolar mixture of CYA 781Ra and CYA 781Rb) described by Nübel et al. (1997). Briefly, PCR protocol consisted of an initial denaturation step at 94 °C for 5 min followed by 35 amplification cycles (1 min at 94 °C, 1 min at 60 °C, 1 min at 72 °C) and a final extension step consisting of 10 min at 72 °C. PCR products were visualised on 1% (wt/vol) agarose gel stained with ethidium bromide using UV transilluminator to confirm the presence of the proper length products. Denaturing Gradient Gel Electrophoresis (DGGE) was run as previously described (Celussi et al. 2008). Each sample was first run in triplicate (PCR products from 3 different reactions) and then used for community structure analysis if a Sørensen Index ≥0.98 among replicates was detected. DGGE banding patterns were used to determine cyanobacterial community structure. Bands in different lanes corresponding to the same Operational Taxonomic Unit (OTU) were identified by using the EquiBands applet (Huber & Peduzzi 2004). The polyacrylamide gel slices containing the bands of interest were excised using a sterile scalpel and eluted in 100 µl of MilliQ water overnight at -20 °C followed by a freeze-thaw cycle. After 1 h at 37 °C, 1 µl of elution was used to reamplify the extracted nucleic acid. The reamplified PCR fragments were separated by DGGE under identical conditions as described above. Reamplified (without the GC clamp) amplicons were purified using the QIAquick PCR purification kit (Qiagen) according to the supplier's instructions, sequenced using ABI Prism Big Dye dye-terminator chemistry (Applied Biosystems) at the 'BMR Genomics' facility at the Padova University (www.bmr-genomics.it) and sequences were aligned to known sequences in the GenBank database using BLAST (Altschul et al. 1997). All sequences were submitted to the RDP

program CHECKCHIMERA to identify possible chimeras within the 16S sequences. Sequences have been deposited in GenBank under the accession number from GQ272344 to GQ272353.

RESULTS

DGGE fingerprinting highlighted from 1 to 9 Operational Taxonomic Units (OTUs) in each sample with only one band being present in every sample (Fig. S1). A total of 10 among the sharpest bands were excised and sequenced and results indicated that the cyanobacterial community was composed both by *Synechococcus*-like and *Prochlorococcus*-like organisms (Table S1). *Synechococcus* ribotypes were widely distributed along the whole transect during both cruises whereas *Prochlorococcus* 16S rRNA genes were found only in February in the central and southern basins of the Adriatic Sea. Winter cyanobacterial assemblages were composed of a higher number of ribotypes and spatial differences in community structure were more pronounced than in October when only CYADR1, CYADR2 and an unidentified ribotype were found.

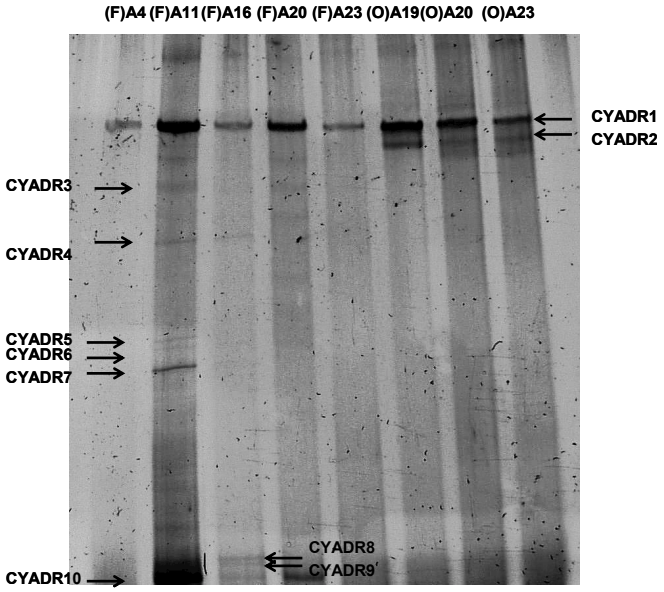

Fig. S1. DGGE profiles of cyanobacterial PCR-amplified 16S rRNA gene fragments (c. 400 bp) from samples collected during February (F) and October (O), with a 20-50% denaturing gradient. Arrows indicate the bands that were excised and used for sequencing

Table S1. Identification of the representative phylogenetic groups obtained by cyanobacterial DGGE band sequencing.

Band	Closest relative	GenBank Acc #	% Identity
CYADR1	Unc. *Synechococcus*	EU249939	97
CYADR2	*Synechococcus* sp.	FJ497728	95
CYADR3	Unc.*Prochlorococcus*	EU361166	95
CYADR4	Unc. *Prochlorococcus*	EU361166	95
CYADR5	Unc. *Prochlorococcus*	DQ187919	95
CYADR6	Unc. *Synechococcus*	FM995186	96
CYADR7	Unc. *Prochlorococcus*	DQ187919	97
CYADR8	Unc. *Synechococcus*	EU249939	96
CYADR9	Unc. *Synechococcus*	AY664204	99
CYADR10	Unc. *Synechococcus*	EU249939	97

Background information: temperature and salinity data description

In February a north-to-south increasing temperature gradient was observed without any pronounced vertical stratification due to winter mixing (Fig. 2). From station A01 to station A03 temperature was on average 9.6 ± 0.6 °C (all stations and depths), increasing to 11.9 ± 0.9 °C from station A04 to station A12 (all stations and depths). Southern stations mean temperature (from A13 to A23, all depths) was 13.7 ± 0.4 °C. Summer heating was recorded in surface waters during the October cruise, with temperature as high as 19.5 ± 0.7 °C at surface (average of all surface data), decreasing to ~ 15°C at the 100 m isobath. Below 200 m temperature ranged between 11.3 (at the bottom of the Middle Adriatic Pit, station A10) and 14.2 °C.

Salinity values (data not shown) were very stable south of station A12, being between 38.6 and 38.8 during both cruises. The effect of freshwater dilution was observed at the northernmost stations. In February the lowest salinity values were recorded at station A02 (water column average = 37.8 ± 0.2) whereas at station A01 and between station A03 and A11 freshets influence was minor (38.4 ± 0.1, water column average). In October the influence of freshwater from the Po river was more pronounced and the lowest salinity value was recorded at surface at station A02 (35.8). In this period freshwater inputs affected all northern stations A01 - A07, especially in the upper layer where salinity was on average 37.5 ± 0.7. Below 10 m and in the central sector of the Adriatic, intermediate salinity values were found (38.4 ± 0.2).

Table S2: Sampling stations and depth for pigment and dissolved organic carbon (DOC) analyses. Bold text indicates October samples. s = surface

Station	Pigments	DOC	Total Prokaryotes and *Synechococcus*
A01		s,5,10,20,24 / **s,10, 20**	s,5,10,20,24 / **s,10,20,24**
A02	s,5,10,15,32 / **s,10,20,33**	s,10,15,20,32 / **s,10,20,33**	s,5,10,15,32 / **s,10,20,33**
A03	s,10,20,30,39 / **s,11,20,30,45**	**s,10,20,30,45**	s,10,20,39 / **s,11,20,30,45**
A04		s,10,20,50,57 / **s,10,22,48,61**	s,10,20,57 / **s,10,22,48,61**
A05		**s,11,21,30**	s,10,30
A06		s,10,20,50 / **s,11,21,50,71**	s,10,62
A07	s,11,20,40,50,87 / **s,10,20,49,60,80**	s,11,20,40,50 / **s,10,20,49,60,80**	s,20,87 / **s,10,20,49,60,80**
A10	s,10,20,49,100 / **s,10,25,51,100**	s,10,20,49,100,200,221,250 / **s,10,25,51,100,200,237**	s,10,20,49,100,250 / **s,10,25,51,100,237**
A11		**s,11,31,51,100,165**	s,168 / **s,165**
A12		**s,11,25,52,100,152**	s,179 / **s,152**
A14		s,10,20,50,100,150 / **s,10,20,50,70,101,142**	s,20,100,150 / **s,10,20,50,70,101,142**
A15		s,10,20,50,100,200,300,500,650,842,980 / **s,10,20,30,60,100,200,300,500,749,950**	s,983 / **s,948**
A16	s,10,20,51,100,200 / **s,11,21,31,53,102,200**	s,10,20,51,100,200,300,500,750,1000,1185 / **s,11,21,31,53,102,200,300,500,750,1000,1139**	s,20,51,100,500,1185 / **s,11,21,31,53, 102,200,300,500,750,1000,1139**
A17		s,10,20,50,100,200,300,500,732,1000,1215 / **s,10,20,30,65,101,200,300,500,750,1000,1204**	s,1215 / **s,1204**
A18		s,10,20,50,100,200,300,460,600,1040 / **s,10,20,40,50,80,100,200,300,500,750,933**	s,20,50,100,200,1040 / **s,10,20,40, 50,80,100,200,300,500,750,933**
A19		**s,10,21,36,51,61,100,200,300,500,1000,1034**	s,20,50,100,200,1040 / **s,10,20,35, 50,60,100,200,300,500,750,1000,1035**
A20	s,10,20,50,75,100,128 / **s,10,20,36,51,60,100**	**s,10,20,36,51,60,100,200,300,500,839**	s,20,100,200,845 / **s,20,36,51,60,100,200,300,500,750,840**
A22		s,10,20,50,100,200,230,270,300,360 / **s,10,21,46,60,102,202,293**	s,360 / **s,290**
A23		s,10,20,50,100,130,200,300,375,500,700,887 / **s,10,20,32,51,63,100,200,300,500,750,853**	s,887 / **s,20,32,51,63,100,200,300,500,750,853**
A24		s,10,20,50,100,200,300,500,750,1000,1044 / **s,10,20,50,100,200,300,500,750,1000,1011**	**s,100,1011**

LITERATURE CITED

Altschul SF, Madden TL, Scheaffer AA, Zhang J, Zang 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.

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.

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.

Huber F, Peduzzi P (2004) Online tool for analysis for denaturing gradient gel electrophoresis profiles. Appl Environ Microbiol 70:4390-4392.

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