NUTRIENTS AND SAHARAN DUST EVENTS CONTROL UNICELLULAR DIAZOTROPHIC CYANOBACTERIA DEVLOPMENT IN OLIGOTROPHIC MEDITERRANEAN SEA

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Introduction

The Mediterranean Sea has been described to harbor scarce concentration of diazotrophic cyanobacteria (3.5 cell mL^{-1}; Le Moal and *al.*, 2011) as well as low rates of nitrogen fixing activity (0.052 ± 0.031 nmol-N L^{-1}d^{-1}; Ibello et *al.*, 2010; Bonnet et *al.*, 2011). These organisms are largely dominated at 99.9 % by pico-planktonic cells (< 3 µm in size) identified as unicellular diazotrophic cyanobacteria from group A (UCYN-A) in both Mediterranean basins (Man-Aharonovich et *al,*. 2007; Le Moal and *al.*, 2011). Despite their low abundance, Mediterranean UCYN community can reach exceptional concentrations (5.3 x 10^3 cells mL^{-1}, Le Moal and Biegala, 2009) and significant N_2 fixation rates (129 nmol-N L^{-1}d^{-1}, Rees et *al.*, 2006). Atmospheric nutrient inputs have been suggested to be at the origin of such impressive phenomena.

Saharan dust depositions occur regularly over the oligotrophic Mediterranean Sea with an increasing gradient from north to south (Volpe et *al.*, 2009). This atmospheric deposition is recognized as a potential source of nutrients such as Fe, P and N (Loye-Pilot et *al.*, 1990; Guieu et *al.*, 2010b; Pulido-Villena et *al.*, 2010), which have been recently recognized as limiting factors for the development of UCYN through Pacific and Atlantic oceanic waters (Moisander et *al.*, 2011 and Turk-Kubo et *al.*, 2012; Langlois et *al.*, 2012).

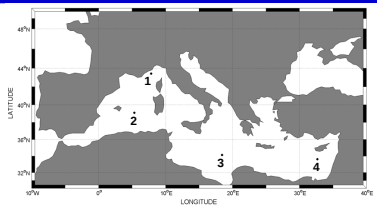
In the Mediterranean Sea the limiting factors to UCYN development are still unknown, although a recent study demonstrated that Saharan dust significantly stimulated nitrogen fixations (Ridame et *al.*, 2011) and among suspected nutrients phosphorous was identified as the key limiting element.

The aims of this work are:

- ❑ To assess impact of Saharan dust additions on UCYN concentrations.
- ❑ To identify witch of the P, Fe and N nutrients containing Saharan Dust are limiting UCYN development.
- ❑ To identify the stimulated UCYN population if any.

To reply to these aims, three series of microcosm experiments were performed in the North, Western, Central and Eastern Mediterranean Sea, which involved either dust or P, Fe and N nutrient additions within different kind of microcosms (Table 1).

Methods



Sampling and microcosm experiments: Water samples from 0,2-3 µm fraction was collected across the Mediterranean Sea, Three nutrient-enrichment experiments (Expt A, B and C) was performed. Experimental conditions are summarized in the following table.

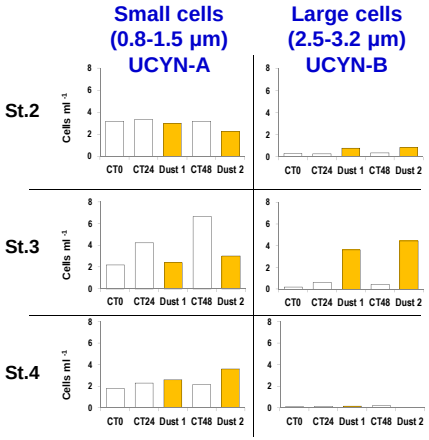
Expt	Date	Stations	Oceanographic cruise	Water sampled depth (m)	Seawater collected volume (L)	Nutrient added (nM)
A	July 2009	St.1	DYFAMED	8	4	+P: 1000 +Fe: 3 +N : 3000
B	July 2008	St. 2, 3 and 4	BOUM	8	4.5	+P: 30 +Fe: 2
C	July 2008	St. 2, 3 and 4	BOUM	8	20	+P: 100 +N: 1600 at St.2 and St.3 and 3200 at St.4

TSA-FISH and Cell count: Cells were hybridized using TSA-FISH technique (Tyramide Signal Amplification-Fluorescent In Situ Hybridization) according to Biegala and Raimbault (2008)'s protocol with the 16S rDNA Nitro821 probe (Mazard et *al.*, 2004). For hybridized cells count, epifluorescence microscopy was used.

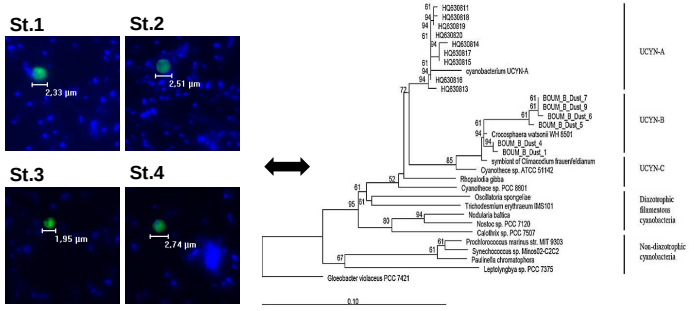
Phylogenetic analyses were performed at St.3 after Saharan dust enrichment in B microcosm experiments to identify large Nitro821-targeted cells. The 16S rDNA of UCYN was amplified with Nitro821 according to Mazard et *al.*, (2004).

Results

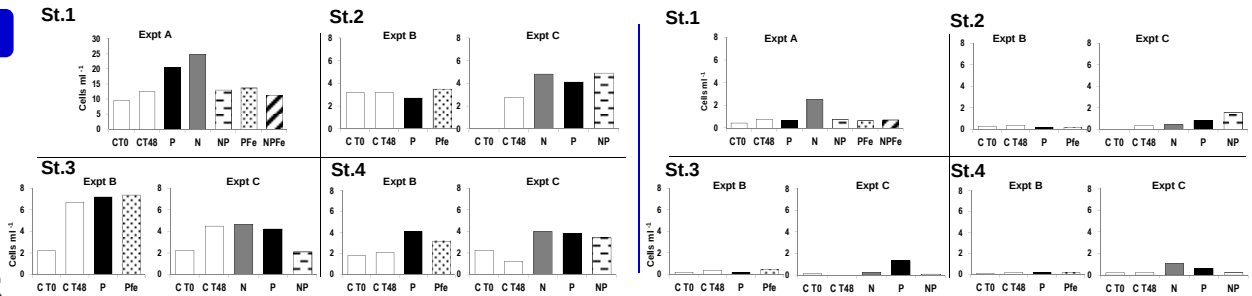
Response of Pico-planktonic UCYN to Saharan Dust addition:



Crocosphaera watsonnii targeted by Nitro821 probe and primer



Response of Pico-planktonic UCYN to nutrients addition:



Discussion

Similarly as Le Moal et *al.* (2011), Nitro821 probe detected two cell types, small cells (0.8-1.5 µm) affiliated to UCYN-A, and unidentified larger cells (2.5-3.2 µm). In this study we revealed for the first time that larger cells were *Crocosphaera watsonii* (99 % identity on 16S rDNA), a well-known UCYN-B so far undetected in the Mediterranean Sea. Although this population was very scares (< 1 cell ml^{-1}) its phylogenetic identification was made possible due tosignificant stimulations (10.2 times) after dust additions. While changes in biomass and metabolic rates may not be synchronized for a given population, our results are surprisingly coherent with Ridame et al. (2011) who demonstrated that nitrogen fixation was significantly enhanced by Saharan dust additions through the Mediterranean Sea.

Despite their differences, microcosm experiment revealed that both UCYN-A and B were clearly stimulated by N and P in the Western and Eastern Mediterranean basins, while the Central basin did not show any significant stimulation. In contrast, the combination of N and P did not show any co-limitation. Our results are consistent with recent literature which demonstrated that both nutrients are able to stimulate UCYN in the North Atlantic (Turk-Kubo et *al.*, 2012; Langlois et *al.*, 2012). Little information on the nutrient assimilation mechanism by UCYN-A are available as they have not been cultured yet. In contrast, *Crocosphaera watsonii* cultures experiment reported that these cyanobacteria have a robust capacity for scavenging phosphorus in oligotrophic systems and are efficient in assimilating NH_4^+ and NO_3^- while simultaneously fixing N_2 (Dekaezemacker and Bonnet, 2011).The addition of Fe combined with P, N or both did not show any stimulation across the Mediterranean Sea. This result contrasts with South Pacific Ocean data which indicated that UCYN were clearly Fe limited (Moisander et al., 2011). However this lack of effect in the Mediterranean Sea can be explained by the accumulation of dissolved iron in the surface water during the stratification period due to recurrent atmospheric iron deposition (Guieu et *al.*, 2010b).

Overall both UCYN populations from the Mediterranean Sea were not limited by the same nutrients. When UCYN-A were mainly limited by N and P, UCYN-B were strongly stimulated by Saharan dust addition. However, within dust particles, the element responsible of such stimulation remains unknown.

Conclusions

- ❑ Saharan dust addition stimulated particularly the growth of UCYN-B cells. Phylogenetic tree of 16S rDNA confirm that these cells were affiliated at 99% to the cultivated strain *Crocosphaera watsonni*
- ❑ UCYN-A largely dominated the Med Sea and their development was mainly stimulated by Phosphorus and Nitrogen.

References

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