5-2013

Springtime Contribution of Dinitrogen Fixation to Primary Production Across the Mediterranean Sea

E. Rahav
B. Herut
A. Levi
Margaret Mulholland
Old Dominion University, mmulholl@odu.edu
I. Berman-Frank

Follow this and additional works at: http://digitalcommons.odu.edu/oeas_fac_pubs
Part of the Biogeochemistry Commons, Marine Biology Commons, and the Oceanography Commons

Repository Citation
Rahav, E.; Herut, B.; Levi, A.; Mulholland, Margaret; and Berman-Frank, I., "Springtime Contribution of Dinitrogen Fixation to Primary Production Across the Mediterranean Sea" (2013). OEAS Faculty Publications. Paper 29.
http://digitalcommons.odu.edu/oeas_fac_pubs/29

Original Publication Citation

This Article is brought to you for free and open access by the Ocean, Earth & Atmospheric Sciences at ODU Digital Commons. It has been accepted for inclusion in OEAS Faculty Publications by an authorized administrator of ODU Digital Commons. For more information, please contact digitalcommons@odu.edu.
Springtime contribution of dinitrogen fixation to primary production across the Mediterranean Sea

E. Rahav¹, B. Herut², A. Levi¹, M. R. Mulholland³, and I. Berman-Frank¹

¹Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat Gan 52900, Israel
²Israel Oceanographic and Limnological Research, National Institute of Oceanography, Haifa 31080, Israel
³Department of Ocean, Earth and Atmospheric Sciences, Old Dominion University, 4600 Elkhorn Avenue, Norfolk, Virginia 23 529-0276, USA

Correspondence to: I. Berman-Frank (ilana.berman-frank@biu.ac.il)

Received: 6 December 2012 – Published in Ocean Sci. Discuss.: 4 January 2013
Revised: 5 April 2013 – Accepted: 5 April 2013 – Published: 14 May 2013

Abstract. Dinitrogen (N₂) fixation rates were measured during early spring across the different provinces of Mediterranean Sea surface waters. N₂ fixation rates, measured using ¹⁵N enriched seawater, were lowest in the eastern basin and increased westward with a maximum at the Strait of Gibraltar (0.10 to 2.35 nmol N L⁻¹ d⁻¹, respectively). These rates were 3–7 fold higher than N₂ fixation rates measured previously in the Mediterranean Sea during summertime and we estimated that methodological differences alone did not account for the seasonal changes we observed. Higher contribution of N₂ fixation to primary production (4–8 %) was measured in the western basin compared to the eastern basin (∼2 %). Our data indicates that these differences between basins may be attributed to changes in N₂-fixing planktonic communities and that heterotrophic diazotrophy may play a significant role in the eastern Mediterranean while autotrophic diazotrophy has a more dominant role in the western basin.

1 Introduction

The Mediterranean Sea (MS) is frequently described as a “blue desert” with low phytoplankton biomass and primary production (Berman et al., 1984; Bosc et al., 2004; Ignatiades et al., 2009; Siokou-Frangou et al., 2010). The low primary production is due to the low concentration and supply of dissolved nutrients in surface waters during most of the year and this is exacerbated during spring through late fall when the water column is thermally stratified. Compounding the problem, is the export of underlying, nutrient-rich intermediate-depth water to the North Atlantic Ocean through the Strait of Gibraltar (Moutin and Raimbault, 2002; Krom et al., 2010).

Dissolved inorganic nitrogen (NO₃⁻, NO₂⁻, NH₄⁺) is considered the proximate limiting nutrient for primary productivity in many oceanic regions (Falkowski, 1998), especially in low nutrient, low chlorophyll (LNLC) environments. While traditionally the MS has been considered phosphorus (P) limited (Krom et al., 1991; Thingstad et al., 1998), more recent publications demonstrate nitrogen (N) limitation or N and P co-limitation across the two sub-basins within the MS (Thingstad et al., 2005, Tanaka et al., 2011). Diazotrophs (i.e. N₂ fixers) are likely to have an advantage in N-limited environments because they are able to utilize the abundant dissolved N₂, unavailable to most organisms, as an N source for growth (Capone and Montoya, 2001; Zehr and Ward, 2002).

Prokaryotic dinitrogen (N₂) fixation is now recognized as a globally important input of new oceanic N (reviewed in Gruber, 2008) that can be subsequently transferred to other planktonic groups (Mulholland et al., 2004; Mulholland and Capone, 2009). However, reported rates of N₂ fixation from the MS are limited to a few studies from the last six years and most are restricted to surface waters and the summer season. Typical rates of N₂ fixation during summer from both the eastern and western basins of the MS are generally low, ranging from undetectable to ∼0.15 nmol N L⁻¹ d⁻¹ (Ibello et al., 2010; Ridame et al., 2011; Yoge et al., 2011; Rahav et al., 2013); however, N₂ fixation rates at the central zone of the Ligurian Sea station in the NW Mediterranean (DYnamique des
Flux de matière en Méditerranée-DYFAMED) are higher ranging from 2–17 nmol N L$^{-1}$ d$^{-1}$ (Garcia et al., 2006; Sandroni et al., 2007).

Diazotrophs contributing to N$_2$ fixation in the MS have been partially characterized (Man-Aharonovich et al., 2007; Bar Zeev et al., 2008; Le Moal and Biegala, 2009; Le Moal et al., 2011; Yogev et al., 2011). In the MS organisms expressing $nifH$, a gene encoding part of the nitrogenase complex, include unicellular cyanobacteria, diatom-diazotroph assemblages, proteobacteria, methanogenic archaea, anaerobic bacteria, and purple sulfur bacteria. (Man-Aharonovich et al., 2007; Yogev et al., 2011). The filamentous cyanobacterium *Trichodesmium* has been sporadically observed in extremely low abundances (Yogev et al., 2011); one bloom of this genus was recorded from the Aegean Sea near Lesvos Island (Spataris et al., 2012).

The contribution of N$_2$ fixation to new primary productivity in the MS was mostly examined during the stratified period in summer and appears to vary between the eastern and western basins. In the western basin, N$_2$ fixation was shown to contribute up to 35 % to new primary production during the stratified period (Bonnet et al., 2011), while in the Levantine basin and the eastern Mediterranean Sea (EMS), N$_2$ fixation contributed only $\sim$0.5–2 % to the new production (Yogev et al., 2011, Rahav et al., 2013). Yearly variability in the contribution of N$_2$ fixation to new primary productivity was also observed in the DYFAMED station ranging from 1 % to 28 % (Sandroni et al., 2007).

Here we present N$_2$ fixation and carbon uptake rate measurements from surface waters collected from a transect across the Mediterranean Sea during spring (before summer stratification). We calculate the contribution of diazotrophy to primary production in spring and compare these with similar measurements made during the stratified summer period to provide a more comprehensive seasonal assessment of N$_2$ fixation in the Mediterranean Sea. Additionally, we assessed the relative contribution of heterotrophic versus autotrophic diazotrophy during springtime across the MS.

2 Material and methods

2.1 Sampling locations

This research was carried out aboard the R/V *Meteor* (cruise M84/3) between 4 and 28 April 2011. Eight stations were sampled along an east to west transect across the Mediterranean Sea, each representing a different water mass with associated mesoscale characteristics. Stations included: the NW Levantine basin (St. 290), the anti-cyclonic Shikmona eddy (St. 294), the Ionian Sea (St. 304), the Adriatic Sea (St. 312), the Tyrrenian Sea (St. 316), the Alboran Sea (St. 333), Strait of Gibraltar (St. 338), and the Gulf of Cadiz (St. 339) (Fig. 1 and Table 1). Seawater samples collected east of the Sicily strait were defined as eastern Mediterranean (EMS) stations, whereas samples collected to the west of Sicily strait were defined as western Mediterranean (WMS) stations. More details on the physical, chemical and biogeochemical characteristics of the water column during the cruise can be found in Tanhua et al. (2013a, b).

2.2 Experimental design

Subsurface seawater (6–8 m depth) was collected using a low pressure pump and placed in triplicate 4.6 L polycarbonate Nalgene bottles. NaH$^{13}$CO$_3$ (Sigma) was added to obtain an enrichment of approximately 1 % of the ambient dissolved inorganic carbon (460 µL of 200 mmol L$^{-1}$ NaH$^{13}$CO$_3$) (Mulholland and Bernhardt, 2005). $^{15}$N$_2$ uptake measurements were measured using a newly developed $^{15}$N-enriched seawater protocol (Mohr et al., 2010). Enriched seawater was prepared by first degassing filtered (0.2 µm) natural seawater collected at the same site and depth using a
significant difference between the rates (24 h incubations (conducted in parallel) and obtained no significant difference with the rates ($R^2 = 0.91$ $n = 24$ $P < 0.05$, see Supplement Fig. S1). The incubations under ambient irradiance (representative of a full diel cycle) record the activities of both autotrophic and heterotrophic diazotrophs. Whereas, we assume that the 48 h dark incubations reflected the activity of mainly heterotrophic diazotrophs who do not require light energy for dinitrogen fixation. We estimated heterotrophic contribution to N$_2$ fixation to primary productivity. The percent contribution of N$_2$ fixation to primary productivity was calculated based on the measured particulate carbon (POC) and nitrogen (PON) in each sample. Although the measured POC and PON are representative of the whole planktonic community and are not specific to diazotrophs, our previous experience in the EMS suggests higher POC : PON ratio than the conventional 106 : 16 Redfield ratio (Yoge et al., 2011, Rahav et al., 2013) and thus were used to calculate the % contribution.

### 2.3 Physical measurements

Measurements of temperature and salinity were taken at each station along the cruise track using an in situ conductivity, temperature and depth (CTD) sensor (Seabird 19 Plus).

### 2.4 Inorganic nutrients

Nutrient concentrations were determined for the same seawater used for the N$_2$ fixation measurements. Duplicate water samples were collected in 15 mL acid-washed plastic scintillation vials from surface (6–8 m) using a low pressure pump (see Sect. 2.1) and immediately frozen at $-20°C$. Nutrients were determined in the laboratory ~4 months after the cruise using a segmented flow Skalar SANplus System Instrument as detailed in Kress and Herut (2001). The precision of the nitrate + nitrite, orthophosphate and silicic acid measurements were 0.02, 0.003 and 0.06 µM, respectively.

### Table 1. Physical and chemical characteristics of the surface seawater (6–8 m) of the MS stations sampled during April 2011. BD- below detection limit; MLD- mixed layer depth.

<table>
<thead>
<tr>
<th>Station number</th>
<th>Location</th>
<th>Position</th>
<th>Temperature (°C)</th>
<th>Salinity</th>
<th>MLD (m)</th>
<th>NO$_2$+NO$_3$ (µM)</th>
<th>PO$_4$ (µM)</th>
<th>Si(OH)$_4$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>290</td>
<td>Levantine basin</td>
<td>34°20'N, 27°30'E</td>
<td>17.0</td>
<td>39.0</td>
<td>46</td>
<td>0.86 ± 0.05</td>
<td>0.05 ± 0.01</td>
<td>1.10 ± 0.18</td>
</tr>
<tr>
<td>294</td>
<td>Shikmona Eddy</td>
<td>34°00'N, 34°25'E</td>
<td>18.1</td>
<td>39.0</td>
<td>49</td>
<td>0.07 ± 0.01</td>
<td>0.05</td>
<td>0.97 ± 0.06</td>
</tr>
<tr>
<td>304</td>
<td>Ionian Sea</td>
<td>35°36'N, 17°15'E</td>
<td>17.1</td>
<td>38.3</td>
<td>30</td>
<td>0.39 ± 0.09</td>
<td>0.01</td>
<td>0.79 ± 0.17</td>
</tr>
<tr>
<td>312</td>
<td>Adriatic Sea</td>
<td>41°15'N, 18°00'E</td>
<td>14.7</td>
<td>38.5</td>
<td>28</td>
<td>0.54 ± 0.16</td>
<td>0.02 ± 0.02</td>
<td>0.95 ± 0.17</td>
</tr>
<tr>
<td>316</td>
<td>Tyrrenian Sea</td>
<td>38°36'N, 11°30'E</td>
<td>16.2</td>
<td>37.2</td>
<td>21</td>
<td>0.63 ± 0.01</td>
<td>0.02 ± 0.02</td>
<td>0.81 ± 0.32</td>
</tr>
<tr>
<td>333</td>
<td>Alboran Sea</td>
<td>36°06'N, 2°48'E</td>
<td>36.2</td>
<td>36.3</td>
<td>45</td>
<td>0.56 ± 0.23</td>
<td>0.24 ± 0.18</td>
<td>0.61 ± 0.08</td>
</tr>
<tr>
<td>338</td>
<td>Strait of Gibraltar</td>
<td>35°57'N, 4°45'E</td>
<td>17.8</td>
<td>36.3</td>
<td>44</td>
<td>1.39 ± 0.84</td>
<td>0.07 ± 0.02</td>
<td>0.48 ± 0.13</td>
</tr>
<tr>
<td>339</td>
<td>Gulf of Cadiz</td>
<td>35°54'N, 7°00'E W</td>
<td>17.7</td>
<td>36.4</td>
<td>38</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

E. Rahav et al.: Springtime contribution of dinitrogen fixation to primary production

www.ocean-sci.net/9/489/2013/

for 10 min, subsequently frozen in liquid nitrogen, and kept. Glutaraldehyde (Sigma G-5882) retained at room temperature for immediate fixation with 23 µL of 25 % stock (585 nm and 630 nm, respectively). Samples of 1.8 mL were processed for flow cytometry. Taxonomic discrimination was based on the peaks across the MS (n = 104). The limits of quantification were 0.075 µM, 0.008 µM and 0.07 µM for nitrate+ nitrite, orthophosphate and silicic acid, respectively (note that for silicic acid the limit of quantification is similar to the precision). For full nutrients profiling of samples collected from Niskin bottles see Tanhua et al. (2013b).

### 2.5 Chlorophyll a extraction

Duplicate seawater samples (500 mL) taken twice a day across the MS (n = 94) were filtered onto glass fiber filters. The filters were stored at −20 °C in a dark box until analysis within 2–3 days. Samples were extracted in 5 mL 90 % acetone overnight, at 4 °C in dark. Chlorophyll a (Chl a) concentrations were determined with a Turner Designs (TD-700) fluorometer, using a 436 nm excitation filter and a 680 nm emission filter (Holm-Hansen, 1965). A blank filter was also stored in 90 % acetone under the same conditions as the samples.

### 2.6 Picophytoplankton abundance

The abundance of picophytoplankton was determined by flow cytometry. Taxonomic discrimination was based on the following parameters: cell side scatter – a proxy of cell volume; forward scatter – a proxy of cell size; and orange and red fluorescence of phycocerythrin and of chlorophyll a (585 nm and 630 nm, respectively). Samples of 1.8 mL were fixed immediately at room temperature with 23 µL of 25 % gluteraldehyde (Sigma G-5882) retained at room temperature for 10 min, subsequently frozen in liquid nitrogen, and kept at −80 °C until analyzed. Samples were fast thawed at 37 °C, and counted using a FACScan Becton Dickinson flow cytomter, fitted with an Argon laser (488 nm) for 10 to 15 min or until 30 000 cells were counted (Vaulot et al., 1989). Pico/nano phytoplankton carbon (C) biomass was calculated from cell counts assuming 175 fg C cell−1 for Synechococcus cells 53 fg C cell−1 for Prochlorococcus cells, and 2100 fg C cell−1 for pico-eukaryotes (Campbell and Yentsch, 1989).

### 3 Results

#### 3.1 East–west distribution of physical, chemical and phytoplankton parameters

The physical, chemical and biological parameters of the surface waters at each station are provided in Tables 1 and 2. Overall, surface temperatures and salinities increased from west to east from 14.7 to 18.1 °C and 36.3 to 39, respectively. NO₂⁻ +NO₃⁻ (DIN) increased from east to west from below detection in the Ionian Sea to 1.39 µM at the Gulf of Cadiz station (Table 1). In contrast, Station 290 (NW Levantine Basin) had high surface concentrations of DIN (0.86 µM), probably due to upwelling of deeper waters within the cyclonic Rhodes Gyre. Dissolved inorganic phosphorus (DIP) ranged from 0.01 to 0.24 µM in surface waters across the entire Mediterranean Sea (MS) (Table 1). Silicic acid (Si(OH)₄) concentration was lowest in the westernmost stations – at the entrance to the MS (0.44 µM), and increased toward the east with highest concentration observed at the easternmost station (1.10 µM) (Table 1).
Chlorophyll (Chl a) concentrations increased from east to west across the MS. Surface Chl a concentrations were ~0.03 µg L\(^{-1}\) at the eastern basin stations and up to 0.31 µg L\(^{-1}\) at the Strait of Gibraltar – the westernmost station (Fig. 1). *Synechococcus* dominated the picophytoplankton ranging from as low as 2.26 \(\times\) 10\(^6\) cells L\(^{-1}\) to 3.27 \(\times\) 10\(^7\) cells L\(^{-1}\) in the eastern and western basin, respectively (Fig. 2, Table 2). Using a cell : carbon conversion ratio of 175 fg C cell\(^{-1}\) (see methods), this represents a range of 396 ng C L\(^{-1}\) to 5723 ng C L\(^{-1}\). In the eastern basin, the picoeukaryote abundances (~2.1 \(\times\) 10\(^4\) to 7.5 \(\times\) 10\(^4\) cell L\(^{-1}\)) and biomass (44 to 158 ng C L\(^{-1}\)) were low except in the Levantine basin (Station 290) where higher abundances (4.36 \(\times\) 10\(^5\) cell L\(^{-1}\)) and biomass (916 ng C L\(^{-1}\)) were measured (Fig. 2, Table 2). *Prochlorococcus* abundances and biomass from the surface waters were generally low throughout the whole MS, especially at the Shikmona Eddy (Station 294) and the Ionian Sea (station 304) (Fig. 2, Table 2).

### 3.2 Primary productivity and N\(_2\) fixation rates

Photosynthetic carbon fixation rates ranged from 0.21 to 0.74 µg C L\(^{-1}\) d\(^{-1}\) in the eastern basin, and 0.76 to 1.39 µg C L\(^{-1}\) d\(^{-1}\) at the western Mediterranean stations. Much higher rates were measured at the Strait of Gibraltar (15.04 ± 1.6 µg C L\(^{-1}\) d\(^{-1}\)) and in the Gulf of Cadiz (8.22 µg C L\(^{-1}\) d\(^{-1}\)) (Table 2).

N\(_2\) fixation rates obtained across the MS exhibited a strong zonal gradient from the eastern to western basins (Fig. 3a and Table 2). The lowest N\(_2\) fixation rates were measured in the eastern basin, ranging from 0.10 ± 0.02 nmol N L\(^{-1}\) d\(^{-1}\) in the Ionian Sea, to 0.15 ± 0.01 nmol N L\(^{-1}\) d\(^{-1}\) at Station 290 (affected by the Rhodes Gyre) (Fig. 3a and Table 2). N\(_2\) fixation rates increased gradually toward the west ranging from 0.22 ± 0.03 in the Tyrrhenian Sea to 2.35 ± 1.12 nmol N L\(^{-1}\) d\(^{-1}\) at the westernmost station at the Strait of Gibraltar (Fig. 3a and Table 2). The springtime rates of N\(_2\) fixation at all stations were 3–7 fold higher than measurements published previously during summertime (Fig. 3b).

In addition to total N\(_2\) fixation (measured in light bottles under ambient diel irradiance), we examined N\(_2\) fixation rates in bottles incubated for 48 h in the dark. While some unicellular cyanobacteria fix N\(_2\) during the dark hours, they require light energy to fuel the process. We assumed that after 48 h in the dark, the contribution by these diazotrophs will be negligible and most N\(_2\) fixation would be due to heterotrophic diazotrophs that do not require light for the N\(_2\) fixing process (Postage, 1979). The N\(_2\) fixation rates from 48 h dark incubations showed a similar east-west trend as observed in light bottle incubations (Fig. 4a); within the eastern basin, N\(_2\) fixation in dark incubations were lowest at the easternmost Station 290 (0.11 ± 0.02 nmol N L\(^{-1}\) d\(^{-1}\)) and highest at Station 294 in the Shikmona Eddy (0.16 ± 0.01 nmol N L\(^{-1}\) d\(^{-1}\)) (Figure 4A). In the western basin N\(_2\) fixation rates in dark incubation bottles rates ranged from 0.20 ± 0.05 to 0.40 ± 0.11 nmol N L\(^{-1}\) d\(^{-1}\) (Fig. 4a).

We compared rates of light and dark N\(_2\) fixation (Fig. 4b) to estimate the relative contribution of autotrophic versus heterotrophic N\(_2\) fixation. In the western basin, light : dark estimates of N\(_2\) fixation were always > 1, suggesting the predominance of autotrophic N\(_2\) fixation. In the eastern basin light : dark N\(_2\) fixation rates were < 1 suggesting a preponderance of heterotrophic diazotrophs (Fig. 4).

### 3.3 The contribution of N\(_2\) fixation to primary productivity

We calculated the percent contribution of N\(_2\) fixation to total primary productivity during springtime based on rates of N\(_2\) fixation measured in the light bottle incubations and the associated C fixation estimated using an the average particulate C : N ratio obtained at each station (Table 2, and see Yogev et al., 2011; Rahav et al., 2013). New production due to N\(_2\) fixation was ~2 % of the total primary productivity at the EMS stations and increased by a factor of 2 to 4 in the western Mediterranean Sea (WMS), ranging from 3.5 % in the Adriatic Sea to 8.5 % in the Alboran Sea. The percent contribution of N\(_2\) fixation to primary production in the Gulf of
4 Discussion

This study provides the first springtime measurements of N\textsubscript{2} fixation in surface waters along an east–west transect across the Mediterranean Sea (MS). We focused sampling at representative stations from different water provinces in the MS (Fig. 1, Table 1). Our results yielded N\textsubscript{2} fixation rates in surface waters that are 3–7 fold higher (Fig. 3a, Table 2) than published rates from two summertime basin-wide N\textsubscript{2} fixation studies (Ibello et al., 2010; Bonnet et al., 2011), routine stations off the Israeli coast (Yoge et al., 2011), and a Levantine Basin transect (Rahav et al., 2013). Moreover, the gradient of increasing N\textsubscript{2} fixation rates from east to west coincide with the east-west gradient in surface Chl \textit{a} (Fig. 1) and primary productivity (Table 2).

Seasonal measurements of N\textsubscript{2} fixation rates in the MS have been made at two monitoring stations, one located west of the Israeli coastline (Levantine Basin) (Yoge et al., 2011) and the other off the coast of France, the DYFAMED station (Ligurian Sea) (Garcia et al., 2006; Sandroni et al., 2007). Rates of N\textsubscript{2} fixation in surface waters from the Levantine Basin were uniformly low (∼ 0.01 nmol N L\textsuperscript{-1} d\textsuperscript{-1}) and did not show any seasonality (Yoge et al., 2011). In contrast, at the WMS time series station (DYFAMED), higher rates of N\textsubscript{2} fixation were measured during April and August (4–7.5 nmol N L\textsuperscript{-1} d\textsuperscript{-1}, 10 m) relative to other months (< 2 nmol N L\textsuperscript{-1} d\textsuperscript{-1}, 10 m), which were associated with higher primary productivity rates (Sandroni et al., 2007).

The Shikmona Eddy (Station 294) and the Ionian Sea (Station 304), representing ultraoligotrophic conditions, had lower nutrient and Chl \textit{a} concentrations than the more productive cycloic Rhodes Gyre station (Station 290). Yet similar N\textsubscript{2} fixation rates were measured at all three stations (Fig. 3a, Table 2) and there was no correlation between N\textsubscript{2} fixation and primary production ($R^2 = 0.18$, $n = 9$, $t$ test, $P > 0.05$). This suggests that N\textsubscript{2} fixation is attributed mainly to heterotrophic bacteria or that diazotrophs and nondiazotrophic phytoplankton are limited or co-limited by different nutrients. Heterotrophic bacteria are known to compete for N with autotrophs in the nutrient-depleted surface waters of the EMS (Thingstad et al., 2005; Tanaka et al., 2007) and molecular fingerprinting suggests a highly diverse heterotrophic community of \textit{nifH} phylotypes (Man-Aharonovich et al., 2007; Yoge et al., 2011). Heterotrophic diazotrophs may outcompete other bacteria in an N-impoverished system because they can acquire N from the abundant N\textsubscript{2} pool. Evidence for heterotrophic diazotrophy was found in both surface and aphotic depths in the EMS (Rahav et al., 2013).

Higher DIN (Table 1) and Chl \textit{a} concentrations were measured in the more productive WMS compared to the EMS (Fig. 1, Table 2). Concurrently, N\textsubscript{2} fixation rates in the WMS were also higher (ANOVA, $P < 0.05$) ranging from 0.22 to 0.86 nmol N L\textsuperscript{-1} d\textsuperscript{-1} (Fig. 3a, Table 2) and correlated with PP ($R^2 = 0.82$, $n = 12$, $t$ test, $P < 0.05$), suggesting photoautotrophic associated N\textsubscript{2} fixation. Indeed, relatively high diatom abundances were detected in surface waters of the WMS (> 100 cells L\textsuperscript{-1}) associated with a small spring bloom (A. Oviedo, personal communication, 2013). \textit{Richelia intracellularis}, a symbiotic N\textsubscript{2} fixing cyanobacterium, has been found associated with diatoms in the EMS previously (Bar-Zeev et al., 2008) and may have contributed to N\textsubscript{2} fixation in the WMS.

The highest N\textsubscript{2} fixation rates during this spring transect were observed at the westernmost station in the Strait of Gibraltar (Fig. 3a, Table 2). Moreover, these springtime N\textsubscript{2} fixation rates were 7-fold higher than those measured previously during summer by Ibello et al. (2010) (2.35 nmol N L\textsuperscript{-1} d\textsuperscript{-1} vs. 0.3 nmol N L\textsuperscript{-1} d\textsuperscript{-1}, respectively). These differences suggest seasonality of N\textsubscript{2} fixation and/or the abundance or activity of diazotrophic populations, or seasonal exchange of water and resident planktonic populations between the eastern Atlantic Ocean and the MS through the Strait of Gibraltar.

During this study N\textsubscript{2} fixation rates were only measured in surface waters (upper 6–8 m) and therefore depth-integrated N\textsubscript{2} fixation rates could not be calculated. It is therefore conceivable that many autotrophic and heterotrophic diazotrophic groups populating other depths, such as the deep Chl \textit{a} maximum (DCM), were not accounted for in our rate measurements. In addition, seasonal changes in the vertical

---

**Fig. 3.** Seasonal variations of N\textsubscript{2} fixation in the surface waters of the Mediterranean Sea. (A) Springtime rates measured in this study. (B) Summer data compiled from Rahav et al., 2013; Yoge et al., 2011; Ibello et al., 2010; Bonnet et al., 2011.
distribution of diazotrophic microbes were not considered here. For example, a recent study from the eastern basin found no statistical difference in N$_2$ fixation rates measured in water collected from below the pycnocline at the DCM compared to surface waters during the stratified period, while during the winter mixing period, when the water column was mixed up to 150 m, the N$_2$ fixation rates were 2–3 fold higher at the DCM than in surface waters (Yogev et al., 2011).

Another methodological contribution to the higher N$_2$ fixation rates during spring throughout the MS was our use of the newly enriched ($^{15}$N$_2$) seawater addition method (Mohr et al., 2010) rather than the gas bubble $^{15}$N$_2$ addition method (Montoya et al., 1996). The gas bubble enrichment method may underestimate N$_2$ fixation rates by a factor of 2 or more in some circumstances (Großkopf et al., 2012; Wilson et al., 2012). Our preliminary comparison of both methods in MS waters demonstrated a 2–3 fold increase in rates using the enriched seawater method ($n = 18$). However, in long incubations (> 24 h), the underestimate of N$_2$ fixation using the bubble method was reduced because the gas bubble should have equilibrated within the first several hours of the incubation (Mohr et al., 2010; Mulholland et al., 2012). While it is impossible to convert from one method to another using a constant conversion factor, if we assume a two-fold underestimate of previously reported summer N$_2$ fixation rates, we still observe significant seasonal differences in N$_2$ fixation rates between the early spring and fully stratified summer periods. This suggests that methodological differences alone cannot account for the seasonal changes we observed.

We examined the relative contribution of autotrophic and heterotrophic diazotrophs to the measured N$_2$ fixation rates using parallel natural light and dark bottle incubations. It has generally been assumed that diazotrophy in surface waters is dominated by photoautotrophic cyanobacteria that use light energy to satisfy the energetic demands of N$_2$ fixation and to acquire carbon (Karl et al., 2002). Yet, research demonstrates that the abundant and widely distributed unicellular group A cyanobacteria are photoheterotrophs (Zehr et al., 2008; Tripp et al., 2010). Further, many heterotrophic diazotrophs are present in surface waters (Riemann et al., 2010; Zehr and Kudela, 2011; Mulholland et al., 2012). Our results show that in the eastern basin stations, the ratio of light : dark bottle N$_2$ fixation was usually < 1 (Fig. 4b) suggesting that heterotrophic diazotrophs may be the dominant N$_2$ fixers, although we cannot exclude that some of the...
dark N₂ fixation was performed by unicellular cyanobacteria. In the western basin, this ratio was generally > 1 suggesting that autotrophic diazотrophs predominated (Fig. 4b). We acknowledge that some phototrophic diazотrophs fix N₂ during the dark, to avoid the inhibitory effects of oxygen, but we assume that our long incubation time in the dark (48 h) would have diminished their impact as they require light energy to fix N₂.

Phylogenetic characterizations of diazотrophs in surface waters across this Mediterranean transect are currently unavailable. However, a diverse group of auto- and heterotrophic diazотrophs have been reported from the eastern basin with ~40% of the nifH transcripts attributed to heterotrophic bacteria (Man-Aharonovich et al., 2007; Bar-Zeev et al., 2008; Yoge et al., 2011). In the WMS, unicellular cyanobacteria (including UCYN-A) are present in low abundances year round and short blooms of 2000–5000 cells ml⁻¹ have been reported from a coastal station off Marseille during June and July (Le Moal and Biegala, 2009). Another recent study suggested that cells < 0.7 µm in size, usually ignored during routine sampling, could contribute 50% of the N₂ fixation (Konno et al., 2010). In this study we used GF/F filters to measure planktonic N₂ fixation (nominal pore size of ~0.7 µm, see methods), as is a common practice. Thus, it is possible we could have missed N₂ fixation by very small bacteria diazotrophs and thereby underestimated total planktonic N₂ fixation.

Based on results from studies conducted during summer in the EMS, N₂ fixation accounted for only 0.7–2% of primary productivity at stations in the Levantine basin (Yoge et al., 2011, Rahav et al., 2013), but increased to ~6% in the more productive Rhodes Gyre and Cyprus Eddy (Rahav et al., 2013). Consistent with these results, during a summer transect across the Mediterranean (BOUM campaign), N₂ fixation accounted for 6 to 35% of new production at stations in the more productive western basin but only 0 to 0.3% at the more oligotrophic eastern basin (Bonnet et al., 2011). Our springtime results show higher N₂ fixation rates (2–4 fold) at both basins and a similar spatial trend. A higher contribution of N₂ fixation to primary production (4–8%) was measured in the western basin compared to the eastern basin (~2%, Fig. 5). These differences between the two basins are probably attributed to changes in N₂-fixing planktonic communities and other environmental aspects. Summertime data from the EMS demonstrated a significant positive correlation between N₂ fixation rates and bacterial production suggesting a higher involvement of heterotrophic diazотrophs in the ultraoligotrophic EMS (Rahav et al., 2013).

5 Conclusions

This study provides the first direct measurements of N₂ fixation rates in surface waters across the MS during springtime. N₂ fixation rates were measured using the newly modified ¹⁵N₂-uptake method (Mohr et al., 2010) during a spring transect and were 3–7 fold higher than measurements made in surface waters during the stratified summer period. Methodological differences cannot fully account for the higher rates of N₂ fixation observed during this cruise and we suggest that the higher rates are due to seasonal variability in primary productivity and environmental factors. N₂ fixation was higher and contributed more to total primary production in the western basin than in the eastern basin. While our data suggests that N₂ fixation rates across the MS are higher during spring than in the summer stratified period, our measurements were constrained to surface waters and thus we cannot provide depth integrated estimates of N₂ fixation during spring. We suggest that future investigations should include N₂ fixation rate measurements and phylogenetic identity of diazотrophs at both photic and aphotic depths to better constrain the contribution of N₂ fixation to N budgets as well as the total and new production within the Mediterranean Sea.

Supplementary material related to this article is available online at: http://www.ocean-sci.net/9/489/2013/os-9-489-2013-supplement.pdf.

Acknowledgements. Many thanks for the help provided by the captain and crew of the R/V Meteor. We would also like to thank Toste Tanhua, the Chief Scientist of this cruise, for allowing us to take part in this campaign. This research was supported by the Israel Science Foundation grant (996/08) to I. B-F and B. H. This study is in partial fulfillment of a Ph.D. thesis for Eyal Rahav from Bar Ilan University.

Edited by: T. Tanhua

References


www.ocean-sci.net/9/489/2013/


