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Missing nitrogen fixation in the Benguela region

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ABSTRACT

Opposing opinions on the importance of nitrogen fixation in the northern Benguela upwelling region provoked us to investigate the magnitude of nitrogen fixation in front of northern Namibia and southern Angola. Measurements of nitrogen fixation rates using the ^{15}N method at 66 stations during seven cruises from 2008 to 2014 showed that, in general, the ^{15}N content in the biomass did not increase after tracer incubation with $^{15}N_2$, indicating that no nitrogen fixation occurred. Correspondingly, the filamentous nitrogen-fixing cyanobacterium *Trichodesmium* was almost not present. The abundant picocyanobacteria did obviously not perform nitrogen fixation to a significant degree. The artificial improvement of conditions for nitrogen fixation in mesocosm experiments, including phosphate and iron additions and a warmer temperature, failed to induce nitrogen fixation. A plausible explanation of these findings is a lack of conditioned cells for nitrogen fixation in the Benguela region.

1. Introduction

Nitrogen is an essential nutrient such that low concentrations may limit the growth of marine phytoplankton. However, diazotrophic prokaryotes are relatively insensitive to shortages of inorganic and organic nitrogen because of their ability to use atmospheric dinitrogen in an energy-requiring enzymatic process referred to as “nitrogen fixation.” Nitrogen fixation in tropical and subtropical regions of the oceans has been well described (Capone et al., 1997; Zehr et al., 2001; Fernández et al., 2010) but it has also been demonstrated in nutrient-replete and coastal areas (Mulholland et al., 2012; Subramaniam et al., 2013).

The Benguela region is a coastal area of intensive nitrogen losses due to denitrification and anammox reactions (Tyrrell and Lucas, 2002; Kuypers et al., 2005). Thus, a balanced nitrogen budget would imply the need for substantial levels of nitrogen fixation (Deutsch et al., 2007). The determination of nitrogen fixation levels in the northern Benguela upwelling region was the focus of our study.

The Benguela upwelling system extends roughly from Cape Agulhas (~34°S) to the Angola-Benguela Frontal Zone, with variable positions between 14°S and 19°S. It is subdivided at 26°S by the Lüderitz upwelling cell into southern and northern parts, which have distinct characteristics (Hutchings et al., 2009). During earlier cruises off northern Namibia and southern Angola in April/May and August/September 2000 (Wasmund et al., 2005a), low molar ratios of inorganic N:P (N:P ratio) of 5.2 in the South Atlantic surface water and 1.3 in the subtropical surface water were determined. A general map of excess phosphate over nitrate in the Atlantic Ocean created by Moore et al. (2009) shows a region of relatively high phosphate surplus, termed P* by Deutsch et al. (2007), just in front of Namibia. Data on the N:P ratios and the P* values measured during several cruises in the northern Benguela upwellling were recently published (Flohr et al., 2014). The surplus of phosphate in those regions would be expected to provide diazotrophic cyanobacteria with a competitive advantage.

The most conspicuous nitrogen fixer in tropical and subtropical seas is *Trichodesmium*. This cyanobacterium is able to form large blooms, covering up to 90,000 km^2 and comprising roughly 10^7 trichomes ml^{-1}, according to the literature compiled by Carpenter and Capone (1992). However, there is no evidence of bloom formation in the eastern part of the Atlantic Ocean. This apparent absence of *Trichodesmium* from upwelling areas might be caused by the low temperature of the respective waters and a shortage of the trace metals required for nitrogen fixation, such as iron, molybdenum, and vanadium (Rueter et al., 1992; Marino et al., 2003; Berman-Frank et al., 2007; Bellenger et al., 2011; Hu et al., 2012).

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Conversely, in North Atlantic coastal waters, unicellular diazotrophic cyanobacteria are among the most abundant diazotrophs (Mulholland et al., 2012). Because they are widespread and penetrate into deep layers of the oceans, they strongly contribute to total nitrogen fixation (Montoya et al., 2004; Hamersley et al., 2011). Zehr et al. (2001) identified the expression of the nitrogenase-coding gene *nifH* in different unicellular cyanobacteria. Another potential contributor to global nitrogen fixation is a symbiosis between cyanobacteria (*Richelia, Calothrix*) and diatoms (*Rhizosolenia, Hemiaulus, Chaetoceros*), as reported by Villareal (1992), Carpenter et al. (1999), Foster et al. (2009) and Sohm et al. (2011b). Recently, a symbiosis between the widely distributed nitrogen-fixing cyanobacterium UCYN-A and a prymnesiophyte was described by Thompson et al. (2012).

A compilation of studies on nitrogen fixation by Capone et al. (1997) revealed the gap in knowledge regarding the southern and eastern Atlantic Ocean. Also recent studies have concentrated on the North Atlantic (Carpenter et al., 2004; Mills et al., 2004; Voss et al., 2004; Capone et al., 2005; Reynolds et al., 2007; Montoya et al., 2007; Benavides et al., 2011; Fernández et al., 2013). Consequently, in the maps of the world-wide database prepared by Luo et al. (2012) there are no data for the Benguela region. Only Staal et al. (2007) included the Benguela region in their north–south transect across the Atlantic Ocean, but they found no evidence of nitrogen fixation there, whereas Sohm et al. (2011a) measured nitrogen fixation rates in the Benguela region of up to ~8 nmol N l^{-1} d^{-1}. Moreover, the influence of upwelling on the magnitude of nitrogen fixation is still under debate. Benavides et al. (2013) found very low, but Subramaniam et al. (2013) found very high nitrogen fixation rates during upwelling. After these contradicting results, our measurements may help to answer the question whether significant nitrogen fixation occurs in the Benguela region or not and whether seasonal and regional patterns occur.

We performed nitrogen fixation measurements with rather high spatial and temporal resolution during several cruises between 2008 and 2014, with the aim of identifying the spatio-temporal patterns of nitrogen fixation off northern Namibia and southern Angola. In case we found significant nitrogen fixation, the conditions for nitrogen fixation were checked, supported by mesocosm experiments with manipulated growth-stimulating factors such as temperature and availability of phosphorus and/or iron.

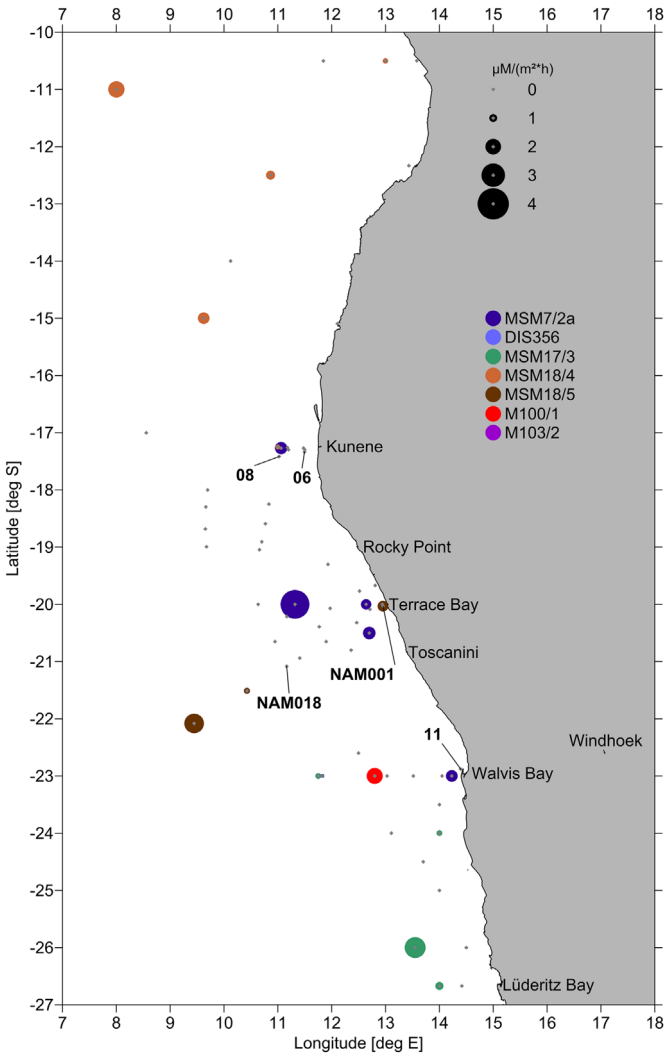

Fig. 1. Investigation area showing the stations where nitrogen fixation rates were measured. The size of the points reflects the rates, measured in μM m^{-2} h^{-1} (0–40 m depth; only positive values are shown). Small gray points indicate "zero" or negative values of nitrogen fixation. The colors of the points indicate the cruise. Stations where mesocosm experiments were started are indicated by the station numbers.

2. Methods

2.1. Sampling

The measurements were carried out during seven research cruises to the Benguela region, located off the coast of northern Namibia and southern Angola (Table 1 and Fig.1). Sixty-six stations were sampled.

Since diazotrophic cyanobacteria may inhabit the entire photic zone, samples were taken from different depths at each station by means of a rosette sampler combined with a CTD SBE911+ and a log quantum scalar irradiance sensor (QSP-2350). The sampling depths were chosen according to the ambient light intensities, which should roughly resemble the fixed light intensities given in the incubator. On most cruises, only standard depths (5, 10, 20, 30, 50 m) were sampled. Water was used from those depths that best fitted the required depth. Thus, in many cases, bottles for 100%, 50%, and 25% light intensities were filled with water from a depth of 5 m, because the surface water was well mixed and narrow depth ranges could not be sampled separately. Phytoplankton

Table 1
List of cruises and information on the method used to measure nitrogen fixation.

No.	Cruise name	Research vessel	Period	Nitrogen fixation method
1	MSM07/2	Maria S. Merian	19.02.–19.03.2008	Montoya et al. (1996)
2	D356	Discovery	10.09.–13.10.2010	Montoya et al. (1996), Mohr et al. (2010)
3	MSM17/3a	Maria S. Merian	30.01.–10.02.2011	Mohr et al. (2010)
4	MSM18/4	Maria S. Merian	24.07.–20.08.2011	Mohr et al. (2010)
5	MSM18/5	Maria S. Merian	22.08.–20.09.2011	Mohr et al. (2010)
6	M100/1	Meteor	01.09.–01.10.2013	Montoya et al. (1996), Mohr et al. (2010)
7	M103/2	Meteor	21.01.–11.02.2014	Montoya et al. (1996)