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Nitrate reducing activity pervades surface waters during upwelling

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ABSTRACT

Nitrate reducing activity (NRA) is known to be mediated by microaerophilic to anaerobic bacteria and generally occurs in the sub-surface waters. However, we hypothesize that NRA could become prominent in the surface waters during upwelling. Hence, we examined nitrification and nitrate reduction along with hydrographic and environmental parameters off Trivandrum and Kochi, south-west-India in June 2010. Shoaling isolines of temperature, density, and nutrients revealed the onset of upwelling off Trivandrum. Shoaling of these signatures was absent in the northern transect off Kochi. The degree of nutrient consumption (DNC) was low emphasizing the presence of newly upwelled water off Trivandrum. A significant increase in NRA ($df = 1$, $p < 0.05$) was observed off Trivandrum than at Kochi. Moreover, as hypothesized, NRA at Trivandrum was pronounced at the surface with a maximum rate of 0.85 (± 0.02) μmol L^1 h^{-1} nearshore which was ~29× higher than that at Kochi. Further, an inverse relationship between NRA and NO_3^- concentration ($n = 34$, $r = -0.415$, $p < 0.01$) suggested transformation of the upwelled nutrient. Nitrification/NRA was ~10× lower at 0.28 off Trivandrum indicating a discernible shift towards reduction. Such contribution from bacterial activity could be a response towards restoration of homeostasis.

1. Introduction

Upwelling is a process which occurs in three sequential stages — (1) the offshore transport of surface water and its replacement by cold, nutrient rich sub-surface water (Krishna, 2008) (2) the productive phase wherein an increase in phytoplankton standing stock (Habeebrehman et al., 2008; Sawant and Madhupratap, 1996) occurs due to nitrate and ammonium uptake while enhanced bacterial production (Carvalho and Gonzalez-Rodriguez, 2004) is a result of heterotrophic carbon assimilation and (3) the oligotrophic relaxing or waning phase which results in nutrient depletion accompanied by decrease in phytoplankton biomass (Rodriguez et al., 1992). In the Indian Ocean region, the main upwelling zone is located along the Somali coast in the western Arabian Sea (Schott and McCreary, 2001). The south-west coast of India also experiences seasonal upwelling during the summer monsoon (June–September). Analyses of hydrographic characteristics have revealed the presence of strong upwelling signatures off the southern tip of the Indian peninsula by May which progress towards the north with time (Antony and Unnikrishnan, 1992). The upwelling phenomenon along the south west coast of India is highly localized with different forcing mechanisms operating at various latitudes. These are longshore wind stress, coastally trapped Kelvin waves and the offshore propagating Rossby waves (Smitha, 2010).

Until now, some of the upwelling related studies in the coastal Arabian Sea have focused on observing hydrographic signatures to delineate the process (Jyothibabu et al., 2008; Muraleedharan and Prasannakumar, 1996), the commencement and cessation of upwelling (Antony and Unnikrishnan, 1992) and, biological response to physical and chemical changes in the environment (Habeebrehman et al., 2008; Jyothibabu et al., 2008; Madhupratap et al., 1990; Subina et al., 2012). So far, very little attention had been given to appreciate the contribution made by bacteria and the different geomicrobial processes they mediate. In the microbial grid of interactions, the nitrogen (N) cycle is an important hub as far as upwelling is concerned as transformation of the element into various oxidation states can affect key ecosystem processes such as decomposition and primary production. Nitrogen is one of the main limiting nutrients in the oceans (Dufour et al., 1999). Increased concentration of NO_3^- from upwelled waters in the euphotic zone supports higher primary production which in turn could cascade to the tertiary trophic level. Moreover, low — oxygen (minimum values close to the hypoxic threshold of 1.4 mL L^{-1}) (Roegner et al., 2011) and high NO_3^- content (typically between 5 and 15 μmol L^{-1}) in freshly upwelled waters (Smith and Codispoti, 1980) has a potential to induce occurrence of alternate respiratory pathways like denitrification. As upwelling progresses, nutrient draw-down results in an N deficit in the water column. During the waning stage of

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upwelling, a rapid depletion of oxygen in the coastal waters due to degradation of high phytoplankton biomass could further intensify dissimilatory pathways in the N cycle i.e. nitrate reduction and denitrification. Though a few reports on N transformations are available from upwelling regions elsewhere (Alvarez-Salgado et al., 1996; Chen et al., 2004a,b; Fernandez and Farías, 2012; Kuypers et al., 2005; Sohm et al., 2011), there is a lack of information from the Indian Ocean region.

In this study, we focused on the first of the three sequential phases of upwelling. We aimed to understand the following (i) delineating the characteristics and intensity of upwelling in the waters off Trivandrum (southern-most location) during June, 2010 and compare it with Kochi located towards the north (ii) to understand whether nutrient draw-up during upwelling causes a discernible shift towards reduction in the N cycle i.e. nitrate reduction activity prevails over nitrification (iii) examine the environmental factors influencing the dominant process (nitrification v/s nitrate reduction) in freshly upwelled waters and most importantly (iv) to verify the hypothesis that nitrate reducing activity (NRA) could become prominent even in the surface waters during upwelling. Hydrographic observations in the present study indicated the commencement of upwelling off Trivandrum and its absence off Kochi. The pre-dominance of NRA in the waters off Trivandrum strengthens our hypothesis of a discernible shift towards reduction in the N cycle and its spread in the surface waters. Though previous research has shown the dominance of NO_3^- reduction in upwelled waters, we have been able to delineate the microbial contribution to such a shift in response to changes in the water column chemistry for the first time.

2. Methods

2.1. Study area and sampling

Stratified water column sampling was carried out along two cross shelf transects located off Trivandrum and Kochi, south-west coast of India (Fig. 1; Table S1). Water samples were collected using 10-L Go Flow bottles (General Oceanics, Miami, FL, USA) mounted on to a Conductivity Temperature Depth (CTD; Sea-Bird Electronics, SBE 911 Plus, USA) rosette onboard FORV *Sagar Sampada* (cruise no. 276; June, 2010).

2.2. Physico-chemical parameters

The vertical profile of temperature, salinity, density, and depth at each station was collected from the respective sensors of the CTD profiler. Hydrogen ion concentration (pH) at each section was measured using an *Orion* 4-Star Plus Benchtop pH/ISE Meter (Thermo Scientific, Beverly, MA, USA) after calibration with standard buffers (pH 4, 6.9 and 9; Merck). Dissolved oxygen (DO) content in the ambient water was determined using the traditional Winkler's titration (Winkler, 1888) method. A dosimeter (Metrohm 785 DMP Titrino, Switzerland) was used for the analysis. Nutrients (ammonium, nitrite, nitrate, phosphate and silicate) analyses were done onboard using a segmented flow *SKALAR* auto-analyzer (Skalar, Breda, The Netherlands; Model 51001-1) as described by Wurl (2009).

2.3. Microbiological studies

Bacterial productivity (BP) was determined by the incorporation of the nucleoside ^{3}H-thymidine into bacterial DNA (Smith and Azam, 1992). Seawater was collected in sterile polycarbonate bottles. Filtered samples (1.5 mL) were spiked with ^{3}H-thymidine (^{3}H-TdR) at a final concentration of 10 nM (specific activity = 52 Ci mmol^{-1}, BARC, Mumbai) and incubated under *in situ* conditions in the dark on deck for 2 h. Concentrations in kinetic experimental set up showed that isotopic dilution was negligible when using 10 nM thymidine. TdR incorporation was terminated by addition of 5% TCA (Trichloroacetic acid). The samples were kept at 4 °C for 30 min prior to centrifugation. The tubes were centrifuged for 10 min at 16,000 ×*g* after which the supernatant was pipetted and discarded. The pellet was washed with 5% TCA and vortexed briefly. Centrifugation of washed samples was repeated followed by removal of the supernatant. A 500 μL volume of scintillation cocktail was added to the samples which were left to stand overnight. Radioactivity was measured with a liquid scintillation counter (Model no. LS6500; Beckman Coulter International, Nyon, Switzerland) with external standards. BP was calculated as moles of TdR incorporated into DNA. The values obtained have been expressed as pmol L^{-1} h^{-1}.

The method of Hobbie et al. (1977) was used for the enumeration of total bacterial cells (TC) by epifluorescence microscopy. An aliquot of 5 mL water sample was fixed using 250 μL of buffered formalin (2%

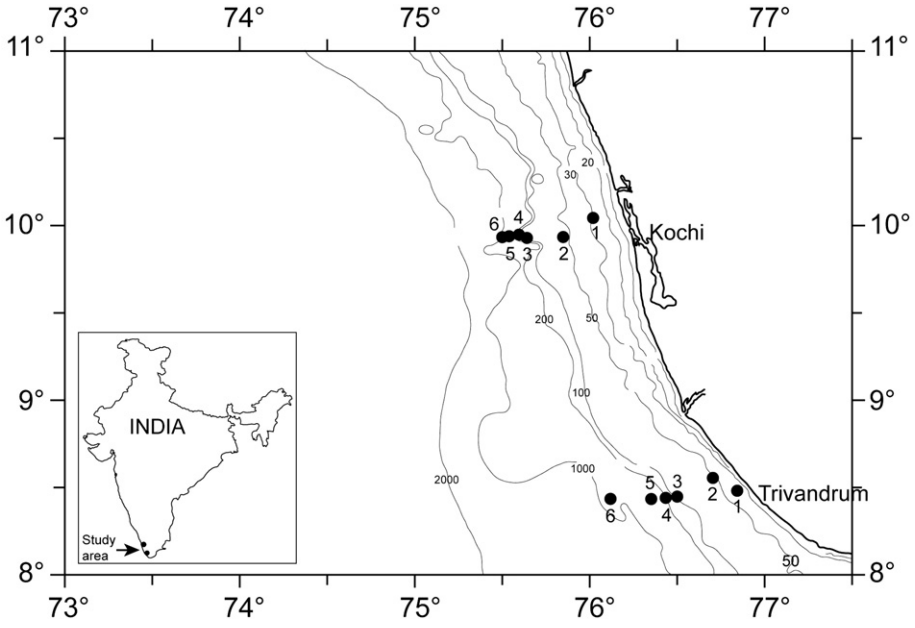

Fig. 1. Location of sampling sites off Trivandrum and Kochi.