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Impact of nutrient enrichment on productivity of coastal water along the SE Mediterranean shore of Israel - A bioassay approach

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ABSTRACT

The coastal waters of the southeastern Mediterranean-Sea (SEMS) are routinely enriched with naturally-occurring and anthropogenic land-based nutrient loads. These external inputs may affect autotrophic and heterotrophic microbial biomass and activity. Here, we conducted 13 microcosm bioassays with different additions of inorganic NO_3-(N), PO_4-(P) and $Si(OH)_4$-(Si) in different seasons along the Mediterranean coast of Israel. Our results indicate that cyanobacteria are mainly N-limited, whereas N or Si (or both) limit pico-eukaryotes. Furthermore, the degree to which N affects phytoplankton depends on the ambient seawater's inorganic N and N:P characteristics. Heterotrophic bacteria displayed no response in all treatments, except when all nutrients were added simultaneously, suggesting a possible co-limitation by nutrients. These results contrast the N + P co-limitation of phytoplankton and the P-limitation of bacteria in the open waters of the SEMS. These observations enable the application for a better science-based environmental monitoring and policy implementation along the SEMS coast of Israel.

1. Introduction

The euphotic layer of the southeastern Mediterranean Sea (SEMS) is considered a nutrient-poor marine environment (Thingstad et al., 2005; Krom et al., 2010; Herut et al., 2016a). The oligotrophic nature of the SEMS is mainly driven by a general anti-estuarine circulation and a relatively stable density stratification throughout most of the year, resulting in a very low supply of deep water nutrients to the euphotic zone (Pinardi and Masetti, 2000; Tanhua et al., 2013). These driving factors result in low phytoplankton biomass and low primary production rates in the SEMS offshore waters (Zohary and Robarts, 1998; Ignatiades et al., 2009; Rahav et al., 2013a; Tsiola et al., 2016). Previous studies showed that phytoplankton in the offshore SEMS are usually N and P co-limited, whereas heterotrophic bacteria are P limited (Zohary et al., 2005; Thingstad et al., 2005; Tanaka et al., 2007; Pitta et al., 2016).

The coastal waters of the SEMS are characterized by somewhat higher nutrient and algal biomass (chlorophyll-*a*) levels (Berman et al., 1984; Azov, 1986; Herut et al., 2000; Herut et al., 2016a, 2016b) and a higher primary production (Raveh et al., 2015; Rahav et al., 2016a) than the open water SEMS, yet remains oligotrophic (Berman et al., 1984; Azov, 1986; Yacobi et al., 1995; Herut et al., 2000). The shallow Israeli coastal waters of the SEMS are routinely enriched by naturally occurring terrestrial nutrient loads, mostly from coastal streams discharge (Herut et al., 2000), seawater desalination brine discharge (Belkin et al., 2015, 2017; Frank et al., 2017), high nitrates well water amelioration brines (Drami et al., 2011), activated sewage sludge disposal (Kress et al., 2016), cooling waters from power plants (Titelboim et al., 2016), sporadic accidental untreated sewage discharge and urban runoff (Rahav and Bar-Zeev, 2017). The external nutrient inputs from marine outfalls alone reached ~4200 ton N y^{-1} and ~1450 ton P y^{-1} at the Israeli coastal waters during 2004–2012 (Fig. S1), thereby introducing significant loads (Herut et al., 2015). Nonetheless, measurements of coastal water algal biomass and bacterioplankton productivity can be generally considered to be very low (Raveh et al., 2015). Previous studies conducted in the offshore waters of the SEMS showed that microbial dynamics and heterotrophic bacterial activity change in response to nutrient additions, specifically to N and P (Thingstad et al., 2005; Zohary et al., 2005; Lagaria et al., 2010; Pitta et al., 2016; Tsiola et al., 2017). This is expected to be the case for coastal waters as well. The primary motivation for this study was to help determine nutrient thresholds for Israeli coastal waters in order to achieve and maintain Good Environmental Status according to the Marine Strategy Framework Directive (MSFD) and the Ecosystem Approach (EcAp) adopted by the Barcelona Convention (Papathanassiou and Argyro, 2006; Ferreira et al., 2010). Where, several ecological objectives were set forth, among

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them the prevention of human-induced eutrophication and the adverse alterations of marine food webs, both pertinent to anthropogenic nutrient introduction (http://web.unep.org/unepmap/who-we-are/ecosystem-approach).

To this end, we experimentally tested the responses of microbial communities in Israeli Mediterranean coastal surface water to various nutrient-addition scenarios ($N\text{-}NO_3^-$, $P\text{-}PO_4^{-3}$, $Si\text{-}Si(OH)_4$) during different seasons and at different locations. We hypothesized that the coastal autotrophic and heterotrophic microbial communities will be bottom-up controlled (as in other oligotrophic environments) and therefore their abundance and activity should respond to external nutrient enrichment. We further hypothesized that this specific nutrient limitation would be different from those restricting phytoplankton and heterotrophic bacterial growth in the open waters of the SEMS, mostly due to differences in the ambient nutrient levels and ratios, and possibly due to the differences in microbial communities.

2. Material and methods

2.1. Water sample collection

Surficial seawater was collected along the Israeli coast (Fig. 1) from a depth of ca. 0.5 m on-board the R/V *Etzyona* and the R/V *Evon* between 2013 and 2016 (Table 1). Seawater was pumped into 1 or 4.5 L transparent Nalgene incubation bottles using a flow jet-pump (Model R4300-242). Subsamples of the seawater from each incubation bottle were collected for the determination of dissolved inorganic nutrients and chlorophyll-*a* concentrations, for picophytoplankton and heterotrophic bacterial abundance, and for measurements of primary and bacterial production rates.

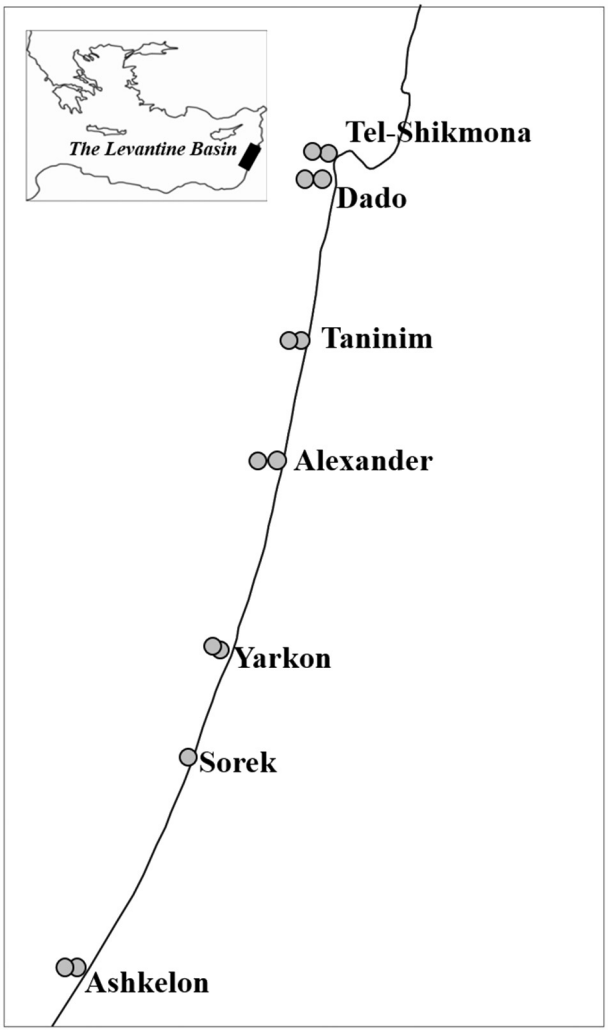

Fig. 1. Sampling locations along the SEMS shore from 2013 to 2016. Descriptions of the sampling sites, dates and the type of treatments undertaken are detailed in Table 1.

2.2. Nutrient addition experiments

A total of 13 microcosm experiments were conducted, each with 3–5 different nutrient addition treatments in triplicates. These experiments involved 39 different chemical manipulations during different seasons and years, and at different sites along the Israeli coast (Table 1). Seawater was filtered through 125-μm mesh net to remove large-size grazers (Kress et al., 2005) into acid-washed polycarbonate Nalgene bottles (1 or 4.5 L). Microcosm bioassays included the following treatments: [1] no addition, [2] 50–100 nM K_2HPO_4 (P), [3] 200–2000 nM $NaNO_3$ (N), [4] 600–2500 nM $Si(OH)_4$ (Si), [5] N + P and [6] N + P + Si (A) (Table 1). A few microliters of ultra-clean nutrient standards (Mercury, each standard was 1000 mg L^{-1}) were used for the different additions, therefore did not dilute any of the samples. The microcosm bottles were incubated for 24 h on-deck or in a coastal pool with running seawater in order to maintain the ambient temperature. The incubation pool/tanks were covered with an illumination net in order to maintain natural light (representing full dial cycle). In most cases no significant seasonal or spatial differences were observed in the responses of the microbial communities to the nutrient additions and therefore all of the experimental results were pooled. The results shown in Figs. 2 and 3 are the percent changes in each treatment from their corresponding controls.

The response of biomass and activity of phytoplankton and heterotrophic bacteria to increasing N additions was investigated in dedicated microcosm experiments during August 2014 (Table 1). Where, gradually increasing N additions were made from 0 to 2000 nM and incubations lasted for 24 h. These experiments were carried out at contrasting sites based on their ambient water characteristics. Two stations were located at 7 m bottom depth across from the Yarkon stream mouth and near the high nitrate well water amelioration plant outfall near Ashqelon (Fig. 1). At these stations the ambient NO_3 was > 1000 nM and NO_3:PO_4 was > 31. Similar experiments were also carried out at four additional stations, where $NO_3 < 500$ nM and NO_3:PO_4 was 4–12 and bottom depths varied between 6 and 30 m.

2.3. Inorganic nutrients determination

Samples were collected in 20-mL acid-washed plastic scintillation vials, immediately frozen (− 20 °C) and kept frozen until analysis. Nutrients were measured with a Seal Analytical AA-3 system (Kress et al., 2014; Ozer et al., 2016). The precision for $NO_2 + NO_3$, PO_4 and $Si(OH)_4$ was ± 0.02 μM, 0.003 μM and 0.06 μM, respectively. The limits of detection (two times the standard deviation of the blank) for $NO_2 + NO_3$, PO_4 and $Si(OH)_4$ were 0.08 μM, 0.008 μM and 0.03 μM, respectively. The quality of the nutrient measurements was confirmed by the results of the inter-comparison exercises (JAPAN, QUASIMEME).

2.4. Chlorophyll-a (Chl-a) determination

Samples (300 mL) were filtered through a Whatman GF/F filter (0.7-μm pore size), extracted overnight at 4 °C in 90% acetone in the dark and then determined by the non-acidification method (Welschmeyer, 1994) using a Trilogy fluorometer (Turner Designs) with 436 nm excitation and 680 nm emission filters.

2.5. Picophytoplankton and heterotrophic bacterial abundance

Samples (1.8 ml) were fixed with 6-μL glutaraldehyde (Sigma-Aldrich G7651), frozen in liquid nitrogen and stored at − 80 °C until analysis. *Synechococcus* and *Prochlorococcus* (hereafter referred to as