

RESEARCH ARTICLE

The Temporal Dynamics of Coastal Phytoplankton and Bacterioplankton in the Eastern Mediterranean Sea

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Abstract

This study considers variability in phytoplankton and heterotrophic bacterial abundances and production rates, in one of the most oligotrophic marine regions in the world—the Levantine Basin. The temporal dynamics of these planktonic groups were studied in the coastal waters of the southeastern Mediterranean Sea approximately every two weeks for a total of two years. Heterotrophic bacteria were abundant mostly during late summer and midwinter, and were positively correlated with bacterial production and with N₂ fixation. Based on size fractionating, picophytoplankton was abundant during the summer, whereas nano-micro-phytoplankton predominated during the winter and early spring, which were also evident in the size-fractionated primary production rates. Autotrophic abundance and production correlated negatively with temperature, but did not correlate with inorganic nutrients. Furthermore, a comparison of our results with results from the open Levantine Basin demonstrates that autotrophic and heterotrophic production, as well as N₂ fixation rates, are considerably higher in the coastal habitat than in the open sea, while nutrient levels or cell abundance are not different. These findings have important ecological implications for food web dynamics and for biological carbon sequestration in this understudied region.

Introduction

The Levantine Basin of the eastern Mediterranean Sea is one of the most oligotrophic marine environments in the world [1, 2, 3]. Dissolved inorganic nitrogen and phosphorus are usually observed at low [4] concentrations, suggesting severe nutrient limitation of autotrophic and possibly heterotrophic bacterial growth rates [5]. Concurrently, low chlorophyll *a* (Chl *a*) concentrations (<0.2 μg L⁻¹) [6, 7, 8], low primary production (~200 mg C m⁻² d⁻¹) [9, 10], and low N₂ fixation (<0.2 nmol N L⁻¹ d⁻¹) [8] rates are usually found at the surface. The phytoplankton biomass is often dominated by small pico-autotrophic cells (<3 μm) [5] due to their large surface area to volume ratios, which may allow them to utilize the low concentrations of ambient nutrients faster than larger microphytoplankton such as diatoms and dinoflagellates (>20 μm) [11, 12]. These pico-sized organisms are responsible for approximately 60% of the

Chl a and 65% of the primary annual production in the pelagic eastern Mediterranean Sea areas [13], making up as much as 80% of the biomass off the Israeli coastline during the early 1980s [14].

Heterotrophic bacteria also play an important role in the upper layers of the open oligotrophic Mediterranean Sea [15, 16], with bacterial abundance and production reaching $\sim 10^5$ cells mL^{-1} and $< 1 \mu\text{g C L}^{-1} \text{d}^{-1}$ respectively [5, 15]. Nevertheless, these organisms are responsible for the majority of the nutrient recycling in this low nutrient, low chlorophyll system [15, 17], as is typical for other oligotrophic environments [18, 19].

The coastal waters of the eastern Mediterranean Sea are considered to be more productive environments than the open sea, mainly due to occasional water runoff from land, wind, precipitation and anthropogenic pressure. These inputs can greatly influence microbial production and diversity. Yet, our understanding of the seasonal changes in the abundance and production of phytoplankton and bacteria in the easternmost oligotrophic coast of the Levantine Basin is incomplete, and most of the existing research was conducted 20 to 30 years ago, at stations located a few kilometers off the Israeli coast [1, 14, 20]. Since then, the surface temperature in the Levantine Basin (eastern Mediterranean Sea) rose by $\sim 3^\circ\text{C}$ [21], which may alter microbial populations (whether positively or negatively) and thus affect the bottom of the food web.

In this study, we have measured the surface temporal distribution and production of phytoplankton and heterotrophic bacteria and of N_2 fixation rates in the coastal ultraoligotrophic waters of the Levantine Basin for two consecutive years (April 2013 to April 2015). Our objectives were: (i) to describe the seasonal development of autotrophic and heterotrophic bacterial abundance at a Levantine Basin coastal station, (ii) to quantify the *in-situ* daily primary production and bacterial production and determine the relative importance of cell size to this process, (iii) to determine the role of diazotrophy (dinitrogen fixation) in this nutrient-poor environment, and (iv) to identify the factor(s) that affects the abundance and the production of these microorganisms in the Levantine Basin.

Materials and Methods

Study site and sampling

This study was conducted between April 2013 and April 2015 in the coastal waters of Tel Shikmona (Haifa), approximately 50 m from the Israel Oceanographic and Limnological Research (IOLR) Institute ($32^\circ 49' 34\text{N}$, $34^\circ 57' 20\text{E}$) (bottom depth ~ 5 m). Surface water (2 m) samples were collected by pumping, approximately every two weeks, except for in the case of inorganic nutrients, which were measured monthly. The pumped seawater was distributed into three acid-cleaned Nalgene bottles (4.6 L each) and was immediately brought to the lab for further subsampling for the different analyses described below. The temperature was measured using an *in situ* HOBO Pendant Temperature data logger (model UA-002-64, Onset Computer Corporation) mounted on the rocky bottom at the same depth. Salinity was measured using a Yellow Spring Instruments YSI 6000. Note that no specific permissions were required for the operation of this experiment and thus this study did not involve endangered or protected species

Inorganic nutrients

Water samples were collected monthly in 15-mL acid-washed plastic scintillation vials and placed immediately in a -20°C freezer until analysis. Nutrient content was determined from 1–2 plastic scintillation vials using the segmented flow Seal Analytical AA-3 system described by Krom et al. [22] and Kress & Herut [23] within 3–6 months of collection. The limits of

detection (twice the standard deviation of the blank) were 0.08 μM for nitrate+ nitrite, 0.008 μM for phosphate, and 0.05 μM for silicic acid.

Chlorophyll a (Chl a)

Seawater was collected into two 300–500 mL bottles and passed through 0.7- μm glass fiber filters in order to isolate all phytoplankton Chl a, and 3- μm glass fiber filters (Pall Inc) were used to separate the nano and microphytoplankton (hereafter referred to collectively as nano-microphytoplankton) contribution of Chl a from the smaller-size phytoplankton. Filters were stored at -20°C , in a light-tight box. Samples were extracted overnight in 5 mL of 90% acetone at 4°C in the dark within 1 week after collection. Chl a concentrations were determined using a Luminescence Trilogy Spectrofluorometer with a 436-nm excitation filter and a 680-nm emission filter [24]. The differences in fluorescence between the two filter types (0.7 and 3 μm) (i.e., total phytoplankton minus the contribution of nano-microphytoplankton) were defined as the pico-phytoplankton Chl a contribution. The phytoplankton size distribution upon which the described analyses were performed is based on Chisholm [25]. We are aware that the use of glass fiber filters may not be as quantitative as polycarbonate membranes may be for specific cell sizes. However, due to the extraction method, glass fiber filters were chosen.

Pico-phytoplankton and bacterial abundance

Duplicate pico-phytoplankton samples (1.8 mL), taken from the same bottles the Chl a samples were drawn from, were fixed with 50% glutaraldehyde (6 μL , Sigma-Aldrich G7651) and stored at -80°C until analysis, usually performed within 1 week or less. Pico-phytoplankton abundance was determined using an Attune® Acoustic Focusing Flow Cytometer (Applied Biosystems) equipped with a syringe-based fluidic system and 488 and 405-nm lasers. Taxonomic discrimination was based on the orange fluorescence of phycoerythrin (585 nm) and the red fluorescence of Chl a (630 nm) [26], on side-scatter (SSC, a proxy of cell volume) [27], and on forward-scatter (FSC, a proxy of cell size) [28]. A 1- μm bead (Polysciences) was used as a standard [29]. Samples were fast-thawed at 37°C in a water bath, and the abundance of pico-phytoplankton (i.e. *Synechococcus*, *Prochlorococcus* and small eukaryotes) was determined. *Prochlorococcus* abundance was usually very low throughout the study period, whereas *Synechococcus* comprised the majority of the picophytoplankton in most of our samples (S1 Table). Thus, *Synechococcus* and *Prochlorococcus* were merged and are henceforth referred to as “cyanobacteria”.

For heterotrophic bacterial abundance, 300 μL of the fixed water samples used for the pico-phytoplankton and nano-microphytoplankton determination were separately incubated at room temperature with the nucleic acid stain SYTO9 (1:10⁵ vol:vol) for 10 min in the dark [26] and then run at a low flow rate of 25 $\mu\text{L min}^{-1}$ using a discrimination threshold of green fluorescence (520 nm).

Primary production (PP)

The photosynthetic carbon fixation rates were estimated using the ¹⁴C incorporation method [30]. Water samples were analyzed in triplicates with dark and zero-time controls. Samples (50 mL) collected at 09:00 AM were added to polycarbonate bottles (Nalgene) containing 5 μCi of $\text{NaH}^{14}\text{CO}_3$ (Perkin Elmer) and incubated for 4–5 h under ambient natural illumination and temperature. To determine the quantity of the added radioactivity, 50 μL of each sample were immediately mixed with 50 μL of ethanolamine and stored for analysis. The incubations were terminated by filtering the spiked seawater onto GF/F filters in order to separate the total primary production or onto a 3- μm glass fiber filter in order to determine the contribution of

nano-microphytoplankton to primary production at <50 mmHg. The filters were incubated overnight in 5 mL scintillation vials containing 50 μ l of 32% HCl in order to remove excess ^{14}C -bicarbonate. After adding 5 mL of scintillation cocktail (Ultima-Gold) to each vial, the radioactivity was measured using a TRI-CARB 2100 TR (Packard) liquid scintillation counter.

Bacterial production (BP)

Bacterial production was estimated using the [4,5- ^3H]-leucine incorporation method (Amersham; specific activity: 160 Ci mmol^{-1}) [31]. Three aliquots (1.7 mL each) from each water sample (i.e. a total of nine tubes for each measurement) were incubated with 100 nmol leucine L^{-1} for 4–5 h at an ambient temperature in the dark. Preliminary experiments indicated that this was a saturating level of ^3H -leucine and that incorporation was linear during this time (data not shown). A triplicate addition of trichloroacetic acid (TCA) served as a control. The incubations were terminated with 100 μ L of cold (4°C) TCA (100%), followed by the microcentrifugation protocol [32]. After adding 1 mL of scintillation cocktail (Ultima-Gold) to each vial, the samples were counted using a TRI-CARB 2100 TR (Packard) liquid scintillation counter. We used a conversion factor of 3 kg C mol^{-1} per mole leucine incorporated, assuming an isotopic dilution of 2.0 [33].

Dinitrogen (N_2) fixation

Dinitrogen fixation rates were measured in triplicates using the newly developed $^{15}\text{N}_2$ -enriched seawater protocol [34]. $^{15}\text{N}_2$ enriched seawater was prepared by injecting 1:100 (vol:vol) $^{15}\text{N}_2$ gas (99%) into degassed (MiniModule G543) and filtered (Sterivex 0.2 μm) seawater collected at the study site. The enriched seawater stock was shaken vigorously in order to completely dissolve the $^{15}\text{N}_2$ gas and aliquots (225 mL) were then added to the triplicate experimental bottles (4.6 L). Following 24 h of incubations under ambient light and temperature, the samples were filtered through pre-combusted (450°C, 4.5 h) 25 mm GF/F filters and dried in an oven at 60°C overnight. The samples were then analyzed using a CE Instruments NC2500 elemental analyzer interfaced to a Thermo-Finishingan Delta Plus XP isotope ratio mass spectrometer (IRMS). For isotope ratio mass spectrometry, a standard curve to determine N mass was performed with each sample run.

The percentage of N_2 fixation contributed to the primary production was calculated based on the averaged particulate organic carbon (POC) and nitrogen (PON) concentration found in the eastern Mediterranean Sea, as described in the details in Yogev et al. [35]. Based on previous studies in this system, the average measured POC:PON ratio was used (i.e. 8) instead of the “Redfield” ~6.6 ratio, as it better characterizes this system [35].

Statistical analysis

Data is displayed as an average, with error bars signifying one standard deviation ($n = 2$ to 3). The differences between coastal and compiled open sea data were evaluated using a one-way analysis of variance (ANOVA), followed by a Fisher LSD multiple comparison post hoc test with a confidence of 95% ($\alpha = 0.05$), performed using the XLSTAT software.

Results

The temporal variability of autotrophic and heterotrophic bacteria

The seasonal characteristics of temperature and of inorganic nutrients in the nearshore waters of Tel Shikmona between April 2013 and April 2015 are shown in Fig 1 and Table 1. Temperatures were as low as ~16°C in wintertime (January and February of 2014 and 2015) and

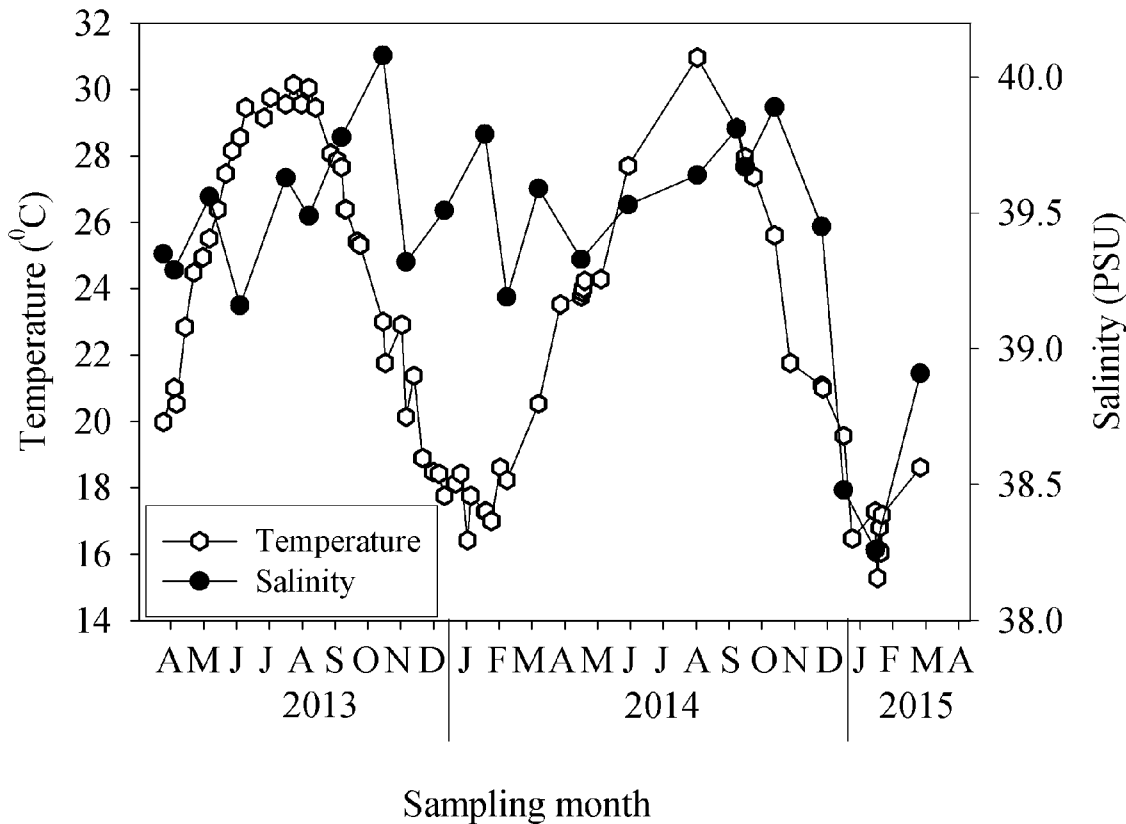


Fig 1. The seasonal surface (2 m) variability of temperature (open circle) and salinity (black circle). Data were collected between April 2013 and April 2015 at the study site off the eastern Mediterranean coast.

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increased to $>30^{\circ}\text{C}$ during the summer months (August and September of 2013 and 2014). The level of salinity did not exhibit any distinct seasonal variability and was usually > 39.0 PSU, except during the winter of 2015 when lower salinity levels were recorded (Fig 1). The concentrations of inorganic nutrients (e.g., nitrate+ nitrite, phosphorus, and silicic acid) throughout the study period were in the nano-molar range and did not reveal any distinct seasonal trends (Table 1). Dissolved NO_2+NO_3 were low ($0.20\pm 0.11 \mu\text{M}$) overall, especially during the early summer and autumn of 2014 ($<0.10 \mu\text{M}$), though they were somewhat higher during January and February of 2015 ($> 1 \mu\text{M}$). Phosphorus concentrations were low throughout the sampling period ($0.02\pm 0.01 \mu\text{M}$), with the highest concentrations measured during both winters ($\sim 0.08 \mu\text{M}$) and the lowest measured at the end of both summers (below the detection limit). Exceptionally high phosphate concentrations were recorded following a sewage effluent discharge during February 2015 (Table 1). The concentration of silicic acid ($\text{Si}(\text{OH})_4$) was the lowest during midwinter (February 2014, $0.30 \mu\text{M}$) and the highest during June 2014 ($2.39 \mu\text{M}$).

The total chlorophyll *a* (Chl *a*) levels (filtered onto $0.7 \mu\text{m}$ GF/F) showed clear seasonal dynamics, with the lowest concentrations measured during summer ($\sim 0.10 \mu\text{g L}^{-1}$) and the peak concentrations measured during wintertime ($\sim 0.55 \mu\text{g L}^{-1}$) (Fig 2A). The nano-microphytoplankton Chl *a* (filtered onto a $3 \mu\text{m}$ filter) concentrations displayed similar seasonal patterns, ranging from $0.01 \mu\text{g L}^{-1}$ in July and August of 2013 to $0.45 \mu\text{g L}^{-1}$ in January 2014 (Fig 2A). The relative contribution of nano-microphytoplankton Chl *a* was high during both winters ($\sim 60\%$) and significantly lower during both summers, usually below 30% (Table 2). The

Table 1. The chemical characteristics of surface seawater (~2 m deep) sampled between April 2013 and April 2015. The limits of detection (twice the standard deviation of the blank) were 0.08 μM for nitrate + nitrite, 0.008 μM for phosphate, and 0.05 μM for silicic acid. BDL: below detection limit, NA: not available.

Sampling period	NO_2+NO_3 (μM)	PO_4 (μM)	$\text{Si}(\text{OH})_4$ (μM)
2013			
Apr.	0.29	0.04	1.72
May	0.28	0.04	1.01
Jun.	0.20	0.02	1.26
Jul.	0.21	0.03	1.54
Aug.	0.26	BDL	1.16
Sept.	0.37	0.01	1.32
Oct.	BDL	BDL	0.64
Nov.	BDL	0.02	0.84
Dec.	0.30	0.01	2.12
2014			
Jan.	0.26	0.03	0.64
Feb.	0.24	0.02	0.30
Mar.	0.19	0.04	0.59
Apr.	NA	0.04	0.76
May	0.12	0.02	0.67
Jun.	0.10	0.01	2.39
Jul.	0.26	0.01	1.71
Aug.	0.36	0.02	1.30
Sept.	0.23	0.03	1.81
Oct.	0.16	BDL	0.94
Nov.	0.20	0.02	1.56
Dec.	0.24	BDL	1.00
2015			
Jan.	1.21	0.05	1.46
Feb.*	1.37–2.49	0.08–0.29	1.12–2.18
Mar.	0.64	0.01	0.86
Apr.	0.14	0.01	0.90

*A range provided using both the routine sampling and the measurements taken during the sewage effluent discharge.

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abundance of cyanobacteria (i.e., *Synechococcus* and *Prochlorococcus*) and picoeukaryotes derived from flow-cytometric analyses was overall throughout both 2014 and 2015 (Fig 2B, S1 Table), and followed the same pattern as Chl a (Fig 2A). Concurrent with the abundances, the contribution of picophytoplankton Chl a was highest during the summer (57–91%) and the lowest during winter (<50%, Table 2).

Similar to Chl a, photosynthetic carbon fixation rates (i.e. primary production) also exhibited seasonal patterns throughout the sampling period, with the highest rates measured in winter and the lowest in midsummer (Fig 2C). The relative contribution of nano-microphytoplankton to the total primary production was largely dependent on the period of sampling (Table 2). In general, nano-microphytoplankton made a greater contribution to the total primary production levels during winter (up to 87%), while picophytoplankton primary production became the primary contributor under the ultraoligotrophic summer conditions (up to 97%).

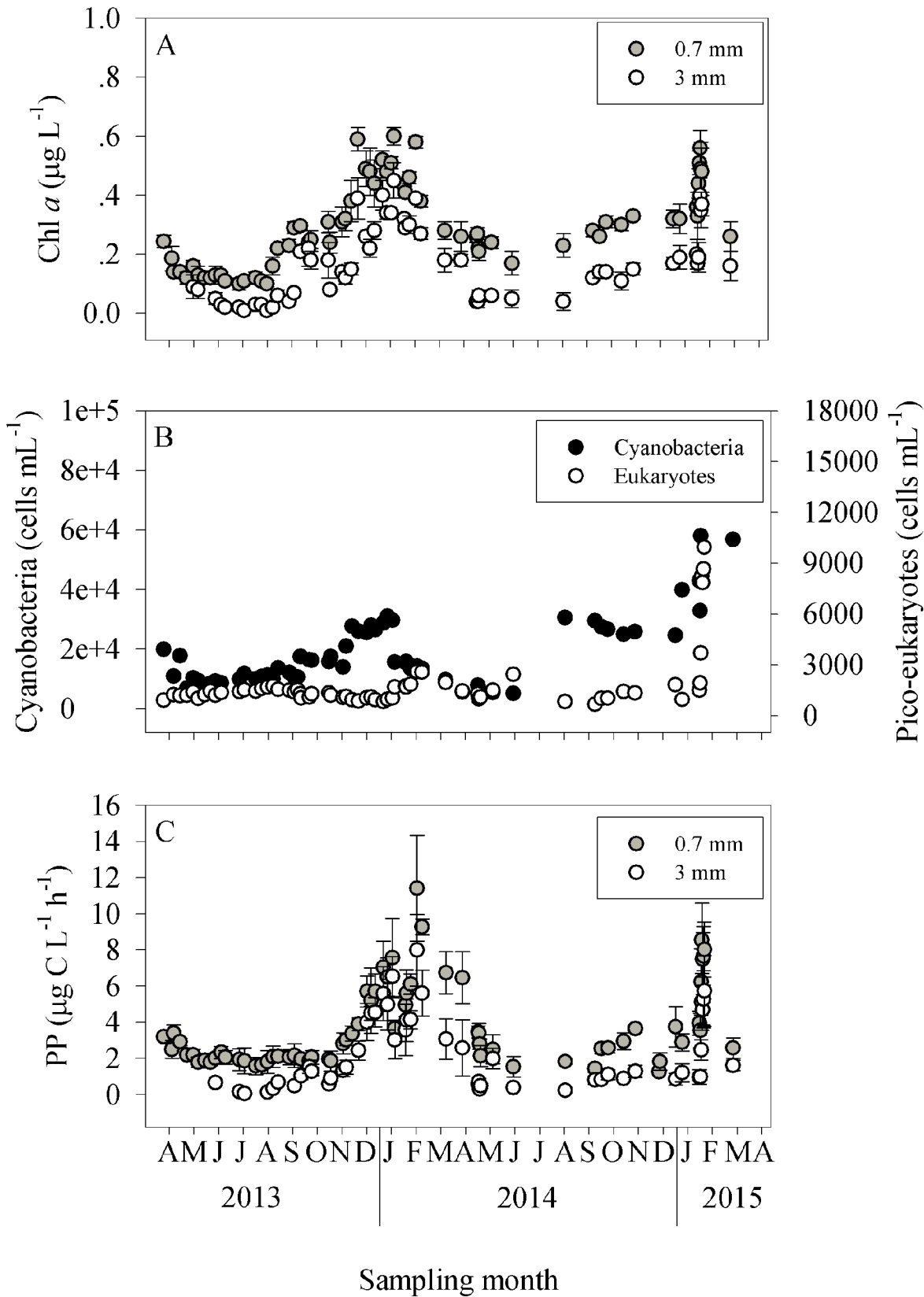


Fig 2. The temporal dynamics of autotrophic bacterioplankton in the coastal eastern Mediterranean Sea. Data presented are for Chl *a* (A) pico-phytoplankton (B) and primary production, PP (C) between April 2013 and April 2015.

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Table 2. The average and range (in parenthesis) of chlorophyll a (Chl a) and the primary production (PP) distribution for each size fraction as a percentage of the total values obtained from Tel Shikmona from between April 2013 and April 2015. NA: not available. The raw data can be found in the supplementary (S1 Table).

Sampling period	Chl a (% of the total)		PP (% of the total)	
	Picophytoplankton (0.7–3 μm)	Nano-microphytoplankton (> 3 μm)	Picophytoplankton (0.7–3 μm)	Nano-microphytoplankton (> 3 μm)
2013				
Apr.	NA	NA	NA	NA
May	44	56	NA	NA
Jun.	50± 16 (38–62)	50± 16 (38–62)	68	32
Jul.	82± 6 (77–91)	18± 6 (9–23)	95± 4 (92–97)	5± 4 (3–8)
Aug.	79± 9 (73–90)	21± 9 (10–27)	92	8
Sept.	80± 7 (73–88)	20± 7 (12–27)	76± 8 (67–84)	24± 8 (16–33)
Oct.	22± 12 (28–29)	78 ± 12 (71–72)	34± 15 (17–47)	66± 15 (53–83)
Nov.	54± 12 (42–67)	46± 12 (33–58)	58± 11 (51–70)	42± 11 (30–49)
Dec.	52± 12 (34–63)	48± 12 (37–66)	33± 15 (13–37)	67± 15 (63–87)
2014				
Jan.	29± 6 (23–36)	71± 6 (64–77)	19± 4 (14–21)	81± 4 (79–86)
Feb.	31± 4 (26–35)	69± 4 (65–74)	29± 3 (27–32)	71± 3 (68–73)
Mar.	29	71	39	61
Apr.	33± 3 (31–36)	67± 3 (64–69)	57± 4 (58–64)	43± 4 (36–42)
May	80± 6 (71–85)	20± 6 (15–29)	70± 28 (78–89)	30± 28 (11–22)
Jun.	75	25	75	25
Jul.	71	29	NA	NA
Aug.	83	17	87	13
Sept.	57	43	44	56
Oct.	50± 6 (46–55)	50± 6 (45–54)	62± 7 (57–67)	38± 7 (33–43)
Nov.	59± 6 (55–63)	59± 6 (37–45)	67± 4 (65–70)	33± 4 (30–35)
Dec.	NA	NA	NA	NA
2015				
Jan.	45± 3 (41–47)	55± 3 (53–59)	68± 12 (33–42)	32± 12 (58–77)
Feb.	38± 13 (23–57)	62± 13 (43–77)	50± 19 (29–75)	50± 19 (25–71)
Mar.	38	62	36	64
Apr.	39± 2 (37–41)	61± 2 (59–63)	39	61

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The abundance of heterotrophic bacteria ranged between 4.3×10^5 and 1.4×10^6 cells mL⁻¹, with the highest abundances measured during February 2015, when an anthropogenic sewage input was recorded, and the lowest during the autumn (November 2013) (Fig 3A). Bacterial production followed the same trend as the bacterial abundance, with the highest rates measured during midsummer and midwinter ($\sim 1 \mu\text{g C L}^{-1} \text{d}^{-1}$), before they decreased by a factor of 2–4 during the spring and autumn of both years ($\sim 0.5 \mu\text{g C L}^{-1} \text{d}^{-1}$, Fig 3B).

Dinitrogen (N₂) fixation rates exhibited a seasonal variability similar to that measured for heterotrophic bacterial abundance and bacterial production (Fig 4). The lowest N₂ fixation rates were measured during spring and autumn, ranging between 0.1 and 0.2 nmol N L⁻¹ d⁻¹ (Fig 4). In contrast, 2–4 fold higher N₂ fixation rates were recorded during the winter and early summer ($\sim 0.4 \text{ nmol N L}^{-1} \text{d}^{-1}$, Fig 4), concurrent with the temporal dynamics of bacterial abundance and bacterial production temporal dynamics (Fig 3). New production due to N₂ fixation equaled 10± 7% of the total primary production, with a larger contribution during wintertime (>8%) and the smallest contribution during autumn (<3%).

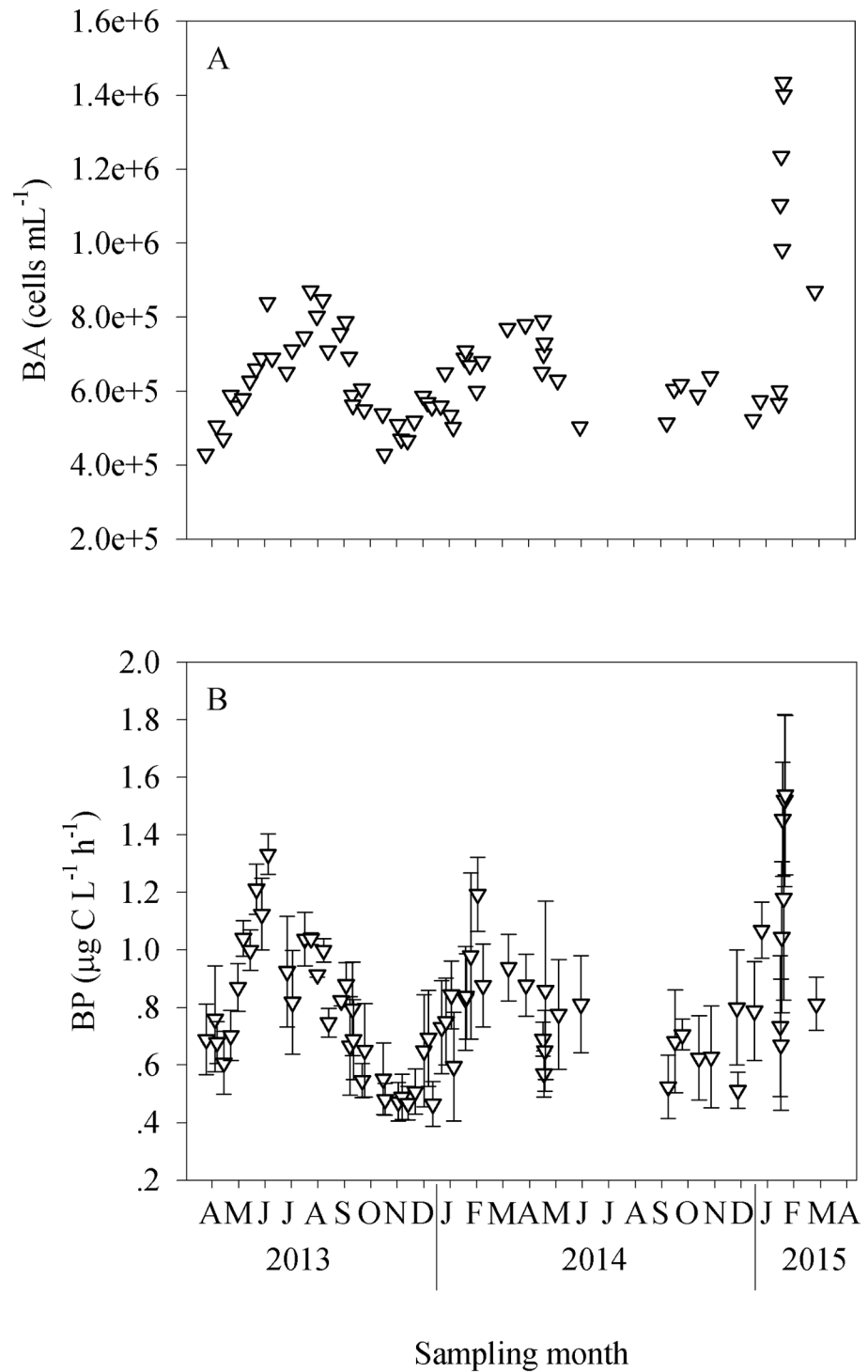


Fig 3. The temporal dynamics of heterotrophic bacterioplankton in the coastal eastern Mediterranean Sea. Data presented are for bacterial abundance, BA (A) and bacterial production, BP (B) between April 2013 and April 2015.

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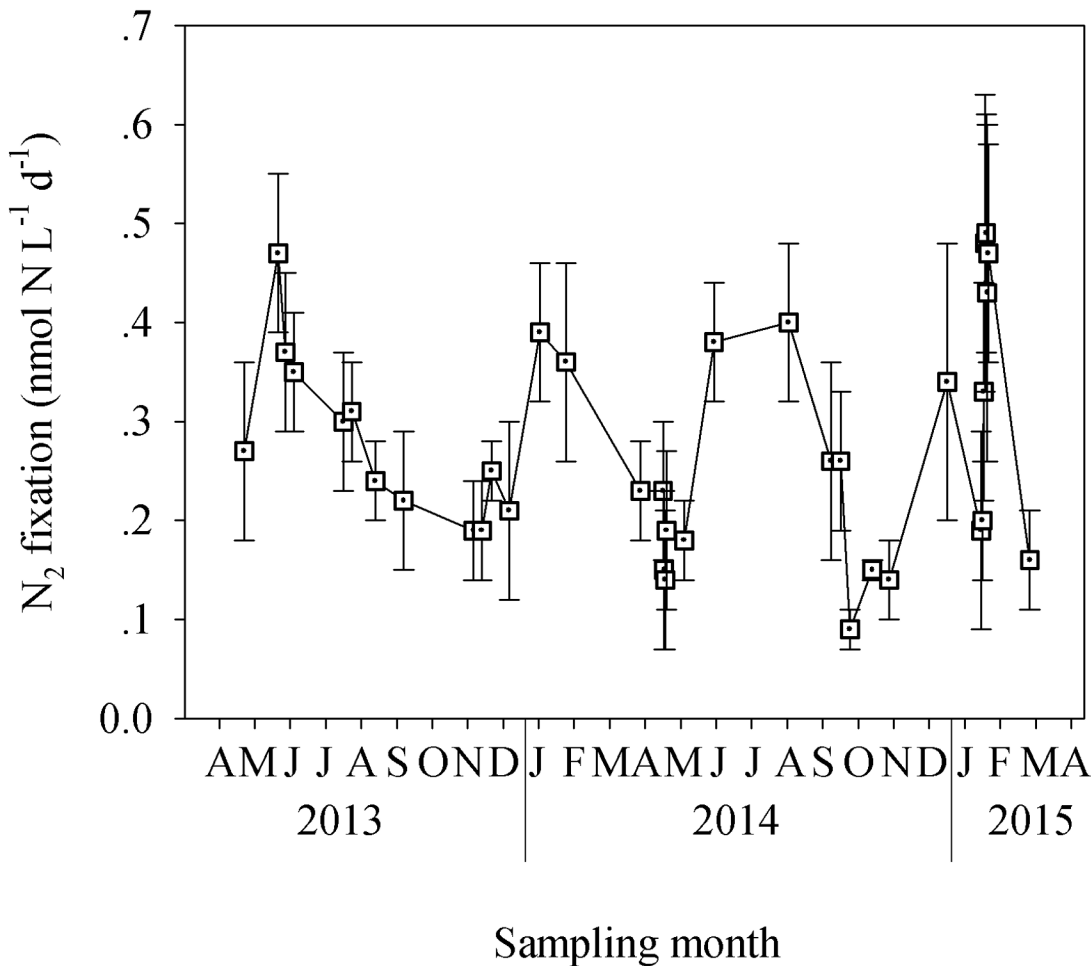


Fig 4. The temporal dynamics of N₂ fixation in the coastal eastern Mediterranean Sea. Data was collected from April 2013 to April 2015, following 24 h incubations under ambient light and temperature.

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The relationship between temperature and autotrophic and heterotrophic bacteria

The temporal distributions of Chl a and primary production correlated negatively (Pearson correlation, $p < 0.001$ and $p < 0.0001$ respectively) with temperature, and thus the largest algal biomass and the highest algal production were recorded during winter when the water temperature were the coldest (Fig 5A and 5B). In contrast, bacterial abundance, bacterial production and N₂ fixation exhibited a different temporal pattern, with minimal abundances/rates during spring and autumn, when the surface water temperature was ~22°C, and maximal values during winter and summer (Fig 5C, 5D and 5E).

The coastal versus the open Levantine surface waters

In order to better understand the spatial dynamics of inorganic nutrients, production and N₂ fixation rates in the Levantine Basin, we compared the data available from different studies conducted in the open (non-coastal) eastern Mediterranean Sea [3, 35, 36, 37, 38, 39, 40, 41] to the results obtained in this study (coastal). While inorganic nutrient concentrations were

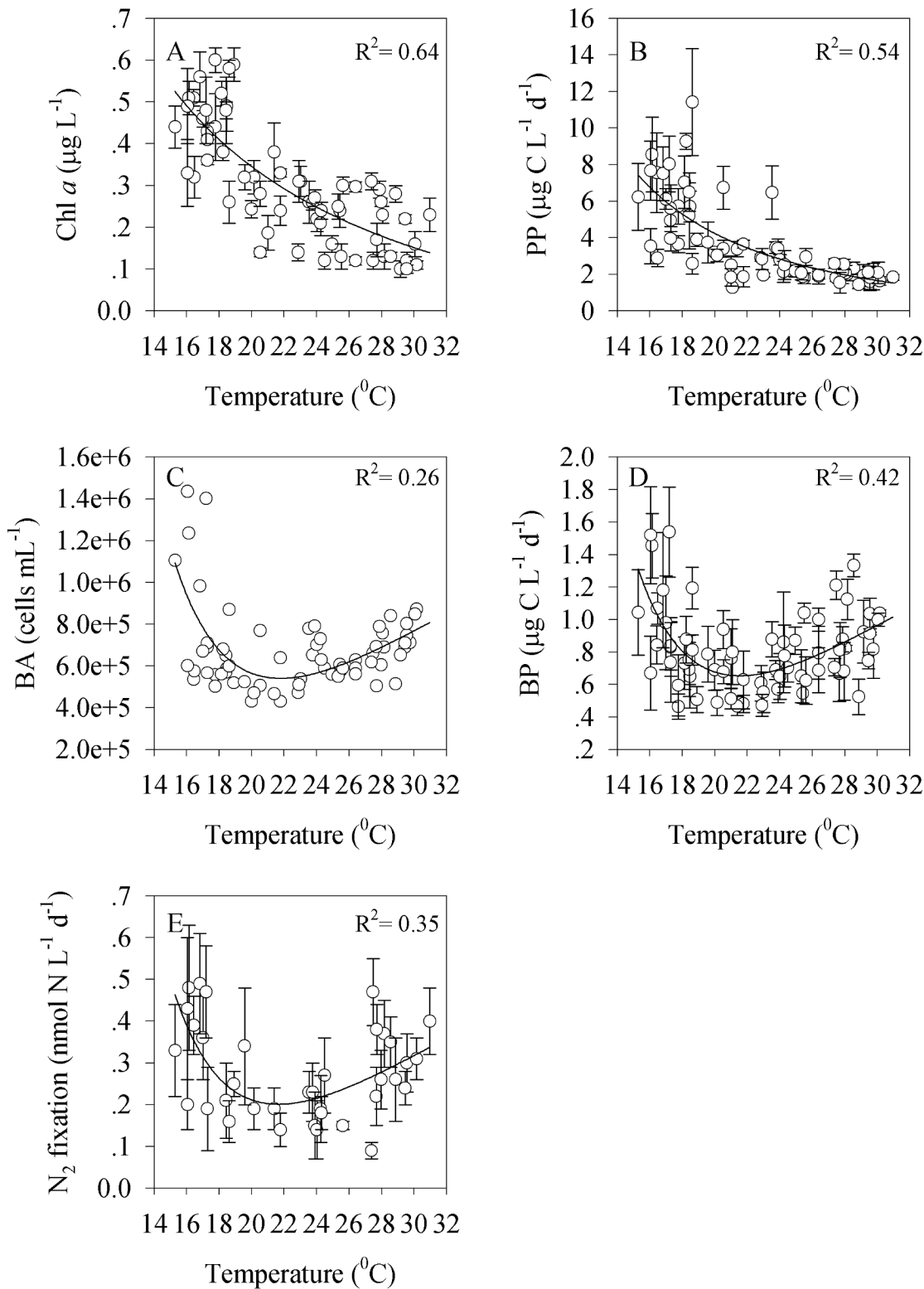


Fig 5. The relationship between temperature and bacterioplankton in the coastal eastern Mediterranean Sea. Data presented are for total Chl *a* (A), primary production (B), bacterial abundance (C), bacterial production (D) and N₂ fixations (E) during the April 2013 to April 2015 sampling period.

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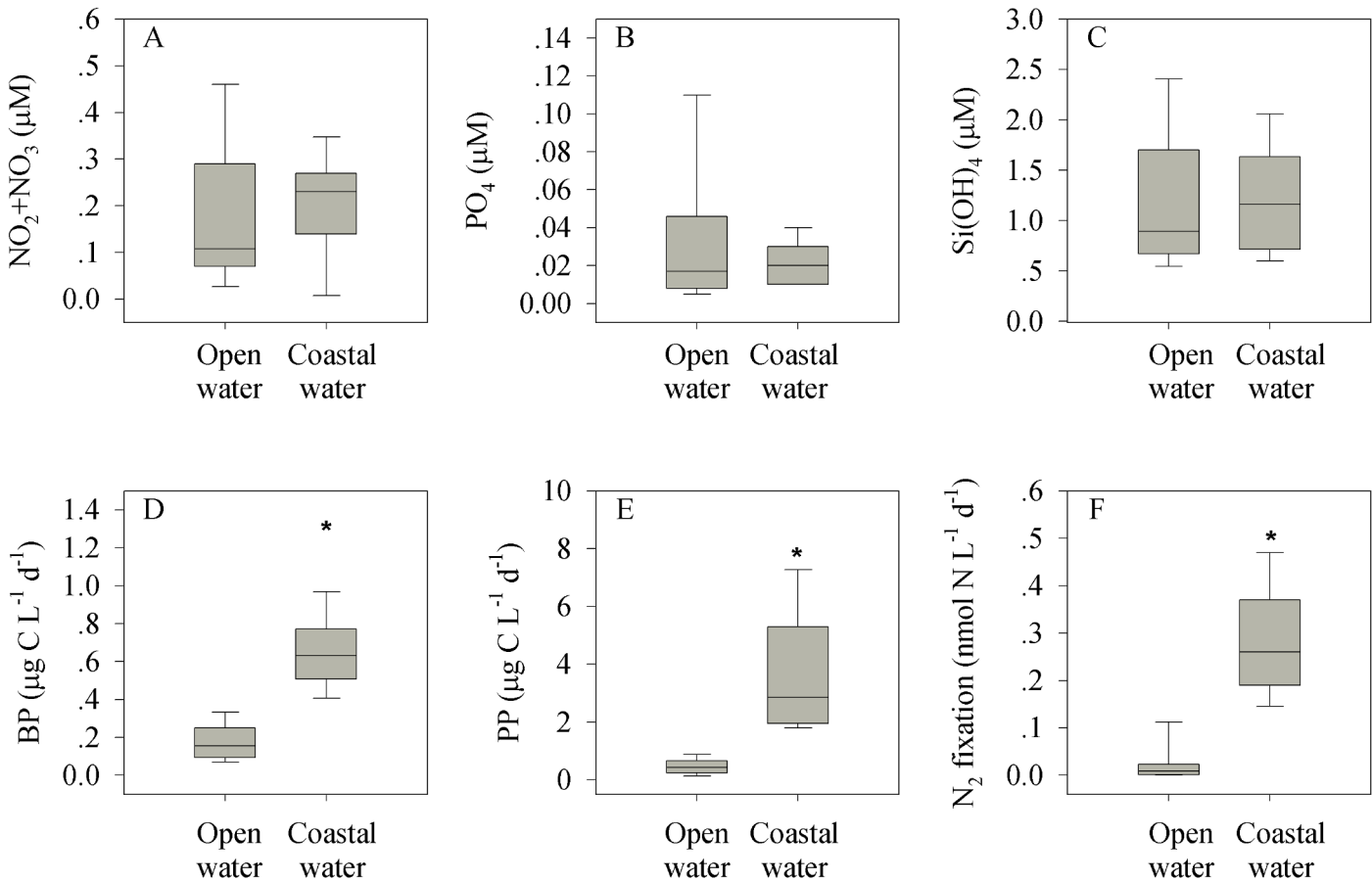


Fig 6. Comparison between the open and coastal Levantine Basin (eastern Mediterranean Sea) water. Box-plot distribution of NO₂+NO₃ (A), PO₄ (B), Si(OH)₄ (C), bacterial production, BP (D), primary production, PP (E) and N₂ fixation (F) in the open Levantine Basin (euphotic zone, bottom depth of stations >1000 m) and in the coastal site (this study). Data for the open sea were compiled from Yogev et al., [35]; Kress et al., [3, 36], Rahav et al., [37, 38, 39]; Bonnet et al., [40], Ibello et al., [41] and Rahav et al., unpublished.

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similar in both the coastal and the open water environments ($P > 0.05$, Fig 6A, 6B and 6C), autotrophic and heterotrophic bacterial production, as well as N₂ fixation were significantly ($P < 0.001$) higher by threefold to sevenfold in the coastal waters (Fig 6D, 6E and 6F). Furthermore, heterotrophic bacteria were present in the same order of magnitude in the coastal water as they were in the open Levantine (average 3.9×10^5 cells mL⁻¹), yet their averaged production rates were ~fourfold higher in the coastal ($0.66 \mu\text{g C L}^{-1} \text{d}^{-1}$) relative to the open sea ($0.17 \mu\text{g C L}^{-1} \text{d}^{-1}$, Fig 7A, Table 3) [42–53]. This means that the average bacterial cell-specific activity (i.e. the bacterial production per bacterial cell) was ~50 pg C cell⁻¹ d⁻¹ in the open sea, whereas it was approximately twofold, in the coastal study site ($106 \text{ pg C cell}^{-1} \text{d}^{-1}$). Finally, the relationship between bacterial and primary production was examined in both water types [42–53]. This relationship is a proxy for the flux of phytoplankton-derived carbon that passes through the microbial heterotrophic food web. While primary and bacterial production exhibited positive linear correlation in the open water of the Mediterranean Sea ($p < 0.001$), no such coupling was found in the coastal water ($p = 0.114$, Table 3, Fig 7B).

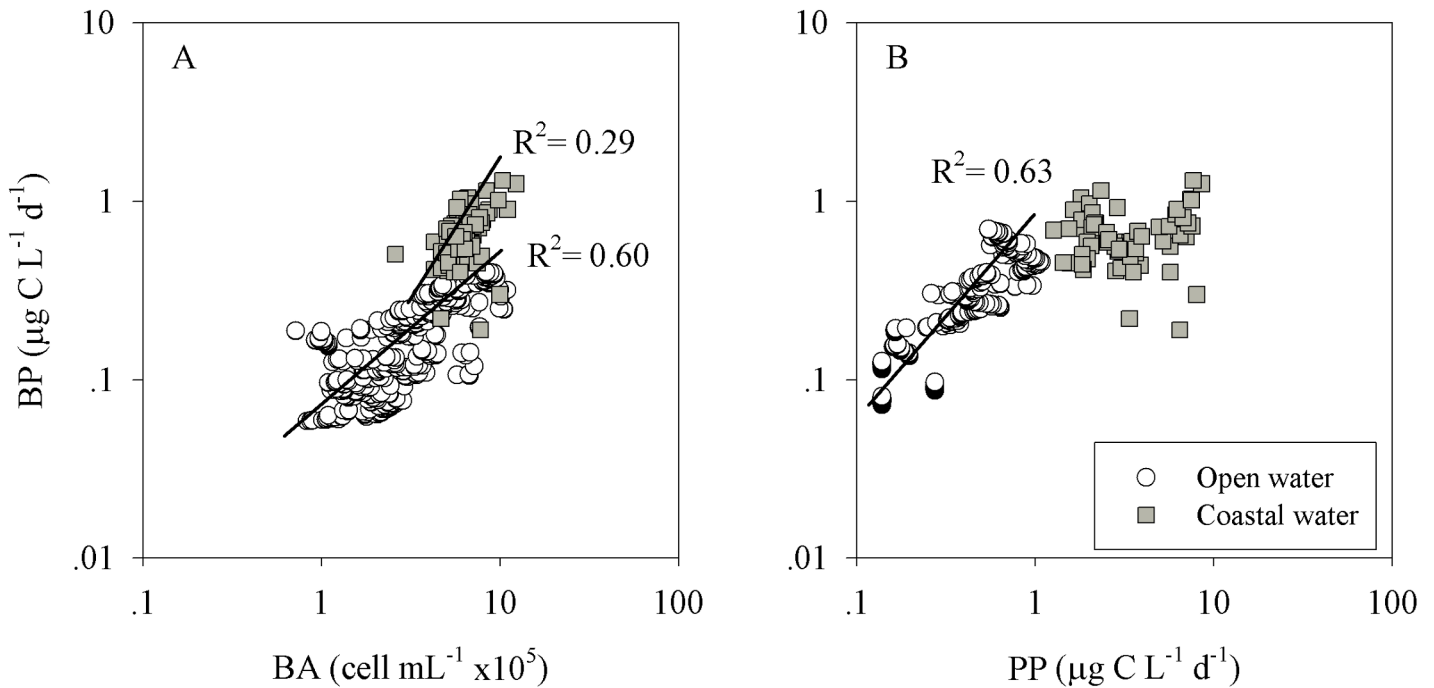


Fig 7. A log-log (base 10) relationship between bacterial production and bacterial abundance or primary production in the open and coastal Levantine Basin water. Data for the open sea stations (euphotic zone, bottom depth of stations >1000 m) were compiled from Zohary & Robarts [42], Zohary et al. [43, 44], Christaki et al. [45, 46], Turley et al. [47], Van Wambeke et al. [17, 48, 49], Ignatiades et al. [50], Kress et al. [51], Tanaka et al. [52, 53], Pulido-Villena et al. [15], and Rahav et al. [38]. A 95% confidence interval (CI) of the regressions is presented in Table 3.

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Discussion

Seasonal variability in the abundance and production of phytoplankton and bacteria

The seasonal dynamics of phytoplankton and bacterial properties are important to the ecology of all aquatic environments, as they are at the bottom of the food web and therefore hold a key role in nutrient recycling within the photic layers [54, 55, 56]. Their quantitative and qualitative variability can be associated with temperature-dependent physical processes such as stratification or mixing [57, 58], and with external nutrient inputs from natural and anthropogenic sources such as land runoff [59] and atmospheric dust depositions [60, 61].

In the present study, we used high-frequency sampling for a duration of two years (April 2013 to April 2015) in order to determine the phytoplankton and bacterial properties of the

Table 3. The relationship between bacterial production (BP) and bacterial abundance (BA) or primary production (PP) in the open and coastal water of the Levantine Basin. Data for the open sea stations (euphotic zone, bottom depth of stations >1000 m) were compiled from Zohary & Robarts [42], Zohary et al. [43, 44], Christaki et al. [45, 46], Turley et al. [47], Van Wambeke et al. [17, 48, 49], Ignatiades et al. [50], Kress et al. [51], Tanaka et al. [52, 53], Pulido-Villena et al. [15], and Rahav et al. [38]. A 95% confidence interval (CI) is presented for the slope and the intercept.

		n	R ²	Slope	Intercept	P value
Open water	BP vs. BA	383	0.60	0.03± 0.00	0.07± 0.01	<0.001
	BP vs. PP	223	0.63	1.19± 0.06	0.10± 0.02	<0.001
Coastal water	BP vs. BA	65	0.29	0.07± 0.01	0.19± 0.10	<0.001
	BP vs. PP	66	0.03	1.80± 1.13	2.41± 0.79	0.114
All data	BP vs. BA	448	0.60	0.03± 0.00	0.06± 0.01	<0.001
	BP vs. PP	289	0.38	4.29± 0.32	0.49± 0.15	<0.001

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surface coastal waters of Tel Shikmona (eastern Mediterranean Sea) and to estimate some of the forcing factors that control their seasonality. While nutrient levels were usually very low (Table 1), the temperature varied greatly between the sampling periods (Fig 1), a fact which seems to explain the seasonal dynamics observed (Figs 2–5). Accordingly, temperature may affect primary and bacterial production rates [62, 63], increase or reduce algal and bacterial biomass [64], change species composition [65, 66], alter the timing and magnitude of annual blooms [65, 67] and influence sedimentation rates [68]. However, it should be noted that other physical and ecological processes that co-vary with the temperature (and nutrients) dynamics may also explain the variability and patterns of the phytoplankton and bacteria observed in this study. For example, an uncoupling between loss processes such as grazing or viral lysis and phytoplankton growth may result in an increase in phytoplankton during wintertime [69]. Thus, the increase in the abundance of cyanobacteria during the winter of 2014 and 2015 (Fig 2B), for example, may be explained by a weak coupling between grazers and phytoplankton and not by temperature or nutrient concentrations alone. Thus grazing rates and viral lysis in this system should be studied in relation to bacterioplankton spatial dynamics.

Picophytoplankton generally dominated the Chl a biomass according to their relative biomass and activity during summer (i.e. their portion out of all autotrophic biomass and activity, Table 2). Thus, autotrophic cyanobacteria such as *Synechococcus* and *Prochlorococcus* were most important to algal biomass during the summer months (Fig 2B, S1 Table), and were also the main contributors to primary production (Table 2, S1 Table). Similarly, picophytoplankton are the dominating phytoplankton in the upper layers of the pelagic eastern Mediterranean Sea waters [7, 39, 50, 70], as well as in other oligotrophic and subtropical environments [71, 72]. As water temperature declined during wintertime, larger autotrophic organisms (i.e., nano-microphytoplankton) became the major contributors to the Chl a biomass and total primary production (Table 2, S1 Table), even though the nutrient levels recorded were relatively low and did not substantially differ from the concentrations observed during the summer (Table 1). This means that either the balance between phytoplankton growth and loss was changed [69, 73], or that large autotrophs outcompeted smaller phytoplankton for the low levels of nutrients available or that other limiting elements were introduced by water mixing or land runoff. Indeed, following the sewage effluent discharge that occurred during February 2015, as evident from the increased nitrate and phosphate levels (Table 1) and the lower salinity (Fig 1), an immediate increase in large-sized Chl a and primary production was recorded (Fig 2), highlighting large size organisms benefit from the anthropogenic input more than small-size phytoplankton. Similarly, diatoms represent 37–58% of the >5 μm phytoplankton fraction during winter in the Cretan Sea [74], and 88% of the phytoplankton fraction at the shelf stations in the Levantine Basin [75]. This type of size-fractionated dynamic as was observed in this case between summer and winter, is common in many oceans [76], and likely occurs because of the imbalance processes detailed above [69], or because different species have an optimal nutrient uptake under different temperatures [77, 78, 79]. For example, PO_4 uptake kinetics performed by *Synechococcus* are faster compared to those performed by most bacteria and algae [80, 81], allowing them to outcompete other species under low phosphorus concentrations. Regardless of the autotrophic size-fraction, the absolute level of Chl a was low, even during winter and spring (Fig 2A), hinting at the existence of other constraints, such as organic and inorganic nutrient sources or top-down control interactions. Furthermore, while Chl a and cyanobacteria exhibited a winter bloom that started at the end of October 2013 and lasted until March 2014 during the first year, during the following winter these variables moderately increased only in December 2014, and they reached a maxima only following the sewage effluents in February 2015 (Fig 2A and 2B). During October 2013, the water temperature was $\sim 22^\circ\text{C}$, whereas at the same time in October 2014 it was higher than 26°C (Fig 1). This again suggests that

temperature is an important controlling factor not only for the overall biomass and algal size distribution (Fig 2A and 2B, Table 2), but also for the timing of the bloom [65, 67]. However, we cannot rule out the possibility that other factor/s may have been involved in the phytoplankton bloom lag, among them top-down pressure. More baseline data is required in order to better understand this possible interannual variability, as well as the seasonal patterns. Such temporal lag in phytoplankton bloom between the two winter periods can shift the abundance, growth and activity of other components of the food web (such as grazers) and also change the oxygen consumption in the water column [82].

Heterotrophic bacterial abundance and bacterial production displayed seasonal patterns different from those of autotrophs, with peaks in abundance and activity during summer and winter and minimal activity during spring and autumn (Figs 3 and 5). We speculate that this seasonal cycle can largely be explained as a physiological response to the ambient temperature that prevails during summer, and, to a lesser extent, to the somewhat higher concentration of inorganic nutrients during winter. Bacterial metabolic rates vary linearly and positively with temperature in many coastal waters, such as Chesapeake Bay [83], the equatorial Pacific Ocean [84] and the Northern Baltic Sea [85], and therefore it is not surprising that the highest heterotrophic production rates and abundance were recorded when the water temperature was maximal. During wintertime, other constraints must have positively affected the heterotrophic bacteria, possibly interactions with large-size phytoplankton alongside the somewhat higher nutrient levels recorded, especially following the sewage input (Fig 2C). However, it should be noted that even an input of nutrient/s might result in different responses of the microbial community. Thus, Shiah and Ducklow [83] showed that nutrient limitation had acted differently on bacterial abundance, production and growth rates in Chesapeake Bay, and that the limitation is dependent on the season. Similarly, Morán et al., [86] showed in a study performed in the southern Bay of Biscay (NE Atlantic) that the sources and substrates used by heterotrophic bacteria differed seasonally. Thus, it is possible that the temporal dynamics of heterotrophic bacteria may also be explained by factors other than temperature and resource supply. All of these issues, including studying whether different bacterial groups interact with other microbial autotrophs or heterotrophs in the oligotrophic eastern Mediterranean habitat and if so how, should be investigated. It is possible, for example, that bacteria (autotrophic and/or heterotrophic) in this region have unique metabolic strategies that help them utilize the few nutrients available using different interactions. It was recently shown that heterotrophic bacteria in the eastern Mediterranean waters thrive on microenvironments such as transparent exopolymer particles, and it was even hypothesized that diazotrophs are likely to benefit from these microenvironment due to their low oxygen levels and high carbon content [39].

Dinitrogen fixation showed the same seasonal pattern that was observed for bacterial production or bacterial abundance (Fig 4). This type of relationship between these variables implies that heterotrophic bacteria may be an important component-fixing dinitrogen [37, 39]. Unlike the open eastern Mediterranean Sea, where the highest N_2 fixation rates were measured during spring ($\sim 0.10 \text{ nmol N L}^{-1} \text{ d}^{-1}$) [37], coastal diazotrophy was the lowest in spring (Fig 4). This suggests that coastal diazotrophs and pelagic N_2 fixers do not necessarily belong to the same groups, and/or that they have different metabolic controls and limitations, hence the temporal differences in their activities. Furthermore, similar to our measured coastal N_2 fixation, which was the lowest during the autumn and the highest during summer, Foster et al., [87] reported undetectable rates during the fall and maximal rates during midsummer in the oligotrophic northern Red Sea. However, to date, our study is the first that shows full seasonal N_2 fixation patterns in the eastern Mediterranean Sea (in both the open and the coastal habitats), and additional research is needed in this region to better understand the role of diazotrophs in

providing new nitrogen in the coastal realm as well as studying interannual variability in N₂ fixation.

Autotrophic and heterotrophic bacterial communities; pelagic vs. coastal eastern Mediterranean Sea waters

The phosphorus and silicic acid levels measured in our coastal study site were similar to those routinely found in the surface of the pelagic eastern Mediterranean Sea, whereas nitrate was found to have a 2-fold higher mean in the coastal areas compared to our study site (Fig 6A, 6B and 6C). These overall low coastal nutrient concentrations reflect the oligotrophic nature of the eastern Mediterranean Sea, and yet highlight the fact that microorganisms (autotrophic or heterotrophic) inhabit the coastal water are exposed to higher nitrate concentrations, possibly via N₂ fixation (Fig 4) or other outsource inputs such as ground water discharge or anthropogenic effluents. Contrary to the nutrient levels, primary production, bacterial production and N₂ fixation were all significantly ($P < 0.01$) higher in the coastal water compared to their concentrations in the open sea (Fig 6D, 6E and 6F and references therein). Furthermore, the contribution of N₂ fixation to new primary production, calculated based on the average particulate carbon to nitrogen ratio [35, 38], was ~twofold higher in the coastal stations than in the open sea. New production due to N₂ fixation was $10 \pm 7\%$ of the total primary production at the coastal station, whereas it usually ranges between 0.5 and 2% in the open eastern Mediterranean Sea [35]. The higher contribution of N₂ fixation to primary production in our study site is in agreement with measurements taken in more productive environments, such as the surface of the western Mediterranean Sea during spring (4–8%) [37], yet it was lower than the contribution reported in the core of an anticyclonic eddy (up to 35%) [40]. Our observations of the contribution of N₂ fixation to primary production in the coastal eastern Mediterranean Sea suggest that diazotrophy may be an important source of bioavailable nitrogen that may support, at least to some extent, the metabolic needs of the microbial community. This is in contrast to the open eastern Mediterranean Sea, where N₂ fixation is considered a negligible process [8, 35]. However, additional research is required to better understand the seasonal and interannual dynamics of diazotrophy and its relation with the microbial community in the eastern Mediterranean Sea.

The higher primary production, bacterial production and N₂ fixation rates found in the coastal waters (Fig 6D, 6E and 6F), unlike the insignificant differences in the inorganic nutrients (Fig 6A, 6B and 6C) or phytoplankton abundance [5], can also be explained by factors other than elevated nitrate levels, including a different supply of other limiting nutrients that were not measured here (organic nutrients such as dissolved or particulate organic carbon, nitrogen, phosphorus/ phosphonates), top-down interactions, and the temperature. Whatever the controlling factors are, it seems that the microbial communities that inhabit the coastal areas have different metabolic rates and limitations. This is well exemplified in the bacterial abundance and bacterial production correlations plotted in both of these systems (Fig 7A). While the bacterial abundances measured in the present study comply with those measured in other studies of the eastern Mediterranean Sea (Fig 7A, Table 3 and references therein), the bacterial production rates were usually higher compared to those measured in open seawater (Fig 6 and references therein). Thus the heterotrophic cell-specific carbon uptake (bacterial production per cell) was higher in the coastal realm compared to the uptake measured in the open eastern Mediterranean Sea water (Fig 7). This indicates that coastal bacterial populations channel dissolved organic carbon into biomass twice as fast as bacteria do in the open sea, or that they have faster growth rates, indicating that coastal heterotrophs are more active and may have different metabolic requirements. Indeed, relatively high dissolved organic substrate

concentrations are occasionally found in our coastal site [88] which can greatly contribute to bacterial proliferation in the eastern Mediterranean Sea [88, 89].

In order to further examine how nutrients affect the heterotrophic bacterial production rates, we inspected the relationship between bacterial production and bacterial abundance (Table 3, Fig 7). Assuming that bacterial production is a proxy for resource availability and that bacterial abundance is a proxy for bacterial biomass, the relationship between these two factors can indicate whether heterotrophic bacteria are a nutrient resource (bottom-up control) or whether they are controlled by predation (top-down) [18]. Based on the strong positive relationship (Table 3), we suggest that bacteria represent the prominent nutrient resource (bottom-up control) that controls the dynamics of abundance and production in this system. This trend was observed in both the coastal and the open eastern Mediterranean Sea waters (Table 3, Fig 7A and references therein), although the slope was shallower in open seawater sites (Table 3), indicating that the bacteria in these areas have slower carbon fixation rates per bacterial cell. However, to better characterize bacterial efficiency, bacterial growth rate measurements should be undertaken. Further, the results of a mesocosm (1-m³ bags) experiment conducted in May 2014 showed an insignificant difference ($P > 0.05$) in autotrophic (Chl a, primary production) and heterotrophic (bacterial abundance and production) variables between ambient and pre-filtered (63 μm) waters during 48 h incubations (S1 Fig). These findings suggest that micro-zooplankton had only a minor role in controlling phytoplankton and bacterial abundance/biomass and production during early summer, and indicate that the proliferation of these microorganisms is controlled by either nutrients (bottom-up control), physical properties (i.e. temperature), viral lysis or that they are grazed by small-size heterotrophic nano-flagellates. Moreover, when considering the constant presence of picophytoplankton in our study site throughout the year, and particularly during the summer months (Table 2), we are not surprised that micro-zooplankton are likely only minor contributors to grazing pressure and to limiting phytoplankton proliferation. The nutritional value of picophytoplankton is low compared to the larger phytoplankton (e.g., diatoms and dinoflagellates) that dominate the more productive habitats [46], and therefore fewer micro-zooplankton are expected to flourish, and the phytoplankton community is thus controlled more by nutrients (bottom-up) than by grazers (top-down).

The relationship between bacterial production and primary production

The relationship between bacterial production and primary production is often used as an indicator of the flux of phytoplankton-derived carbon through the microbial heterotrophic food web [89, 90]. In principle, a positive relationship between bacterial production and primary production suggests that the latter is an important source for dissolved organic carbon that nourishes bacteria [15]. For example, the published data from the open Levantine Basin, compiled here, indicated a positive correlation between these variables (Table 3, Fig 7B). However, this relationship was not detected in the coastal waters examined in this study (Table 3), suggesting that coastal bacterial populations depend less on phytoplankton primary production for dissolved organic carbon. This suggests that heterotrophic microbial recycling processes likely predominate the coastal waters and/or that coastal heterotrophic bacteria have other sources of bacterial nutrition, possibly atmospheric inputs [91], or other anthropogenic pollutants (such as sewage) they can use to maintain their metabolism. Alternatively, this uncoupling between primary production and bacterial production may suggest a more complicated pathway by which organic carbon made by phytoplankton is processed via the food web and released into the dissolved pool where it is available to bacteria. Whatever the mechanism is, it is important to note that our study encompass all seasons, whereas the data for open sea was

acquired mainly during the summer period. Therefore, in order to conduct a more seasonally unbiased comparison, further studies of the open sea-coastal water interactions should be conducted, focusing on seasonal variations in the eastern Mediterranean Sea with respect to vertical as well as integrated production. Moreover, more interannual studies should be carried out in order to distinguish between seasonal changes and long-term yearly variations.

Conclusions

We show that the annual autotrophic and heterotrophic microbial abundances and production in the coastal eastern Mediterranean Sea were largely impacted by temperature and were less affected by the basal inorganic nutrient concentrations. These temperature-dependent trends might be especially important when considering the previously documented warming in the region [21] and the frequent extreme weather events observed in the eastern Mediterranean Sea in the last three decades [92]. Assuming water temperatures will continue to rise, and that summers will become longer, picophytoplankton will likely predominate throughout the year. Under these circumstances, seasonal variations will diminish. Moreover, picophytoplankton transfer very little energy to high trophic levels [46], and therefore the zooplankton that feed on them are likely to prevail, whereas large-size zooplankton's abundance will most likely become scarce. These community shifts could have tremendous impacts on carbon sequestration in the eastern Mediterranean coast and are thus of great ecological interest. However, it is important to note that more studies are required in order to fully understand seasonal, and especially interannual, variability in bacterioplankton in the coastal easternmost Mediterranean Sea waters.

Finally, intrusion of coastal waters into the open sea, which is occasionally detected in the Levantine Basin through satellite observations [93], hyperspectral remote sensing surveys [60], or direct measurements [94], may export coastal bacteria and phytoplankton to the open sea, including potentially toxic cyanobacteria, diatoms, and dinoflagellates. Therefore, investigating the composition, abundance, and production of phytoplankton and bacteria in the coastal-open ocean interphase is essential if one is to understand the trophic balance in the eastern Mediterranean Sea.

Supporting Information

S1 Fig. Bottom-up and top-down interactions of autotrophic and heterotrophic microbial abundance and activity in the coastal eastern Mediterranean Sea- a mesocosm experiment.
(DOCX)

S1 Table. A summary table of all the data used to generate the results presented in this study.
(DOCX)

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Author Contributions

Conceived and designed the experiments: ER. Performed the experiments: OR ND GR ER. Analyzed the data: OR ER. Contributed reagents/materials/analysis tools: GR ER. Wrote the paper: OR GR ER.

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