

# SHORT TERM TEMPORAL & SPATIAL FLUCTUATIONS IN MARINE CYANOBACTERIUM *SYNECHOCOCCUS* ABUNDANCE IN OLIGOTROPHIC DEEP SHELF WATERS (NORTHEASTERN MEDITERRANEAN)

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## ABSTRACT

Abundance of picoplanktonic marine cyanobacterium *Synechococcus* was monitored weekly over a year period at an oligotrophic deep shelf station in the northeastern Levantine basin (northeastern Mediterranean). In addition to abundance, ambient parameters such as; temperature, salinity, secchi disc depth, total suspended sediment, nitrate+nitrite, phosphate, chlorophyll and phytoplankton were also collected. Population was found most abundant during March & December ( $1.8 \times 10^4$  cells/ml) and June & August ( $1.4 \times 10^4$  cells/ml) whereas to the lowest counts were retained in October ( $9.6 \times 10^3$  cells/ml) and July ( $1.0 \times 10^4$  cells/ml). Low levels observed in July coincided with the initial phase of an extraordinary upwelling event that lasted for about 10 weeks. Average cell counts at surface ( $1.8 \times 10^4$  cells/ml) almost three fold that observed at 100m ( $6.4 \times 10^3$  cells/ml). In contrast to homogeneous temperature & salinity profiles, fluctuations in population abundance with depth was observed during winter convective mixing (January & February). Abundances remained below  $1.0 \times 10^4$  cells/ml below 50 m due to stratification observed during summer & autumn. Cell abundances ranged from a minimum of  $4.9 \times 10^3$  to a maximum of  $4.4 \times 10^4$  cells  $ml^{-1}$  with an annual mean level of  $1.8 \times 10^4$  cells  $ml^{-1}$  at surface. At the lower part of the euphotic layer abundances ranged from a minimum of  $3.0 \times 10^2$  to a maximum of  $2.8 \times 10^4$  cells  $ml^{-1}$  with an annual mean level of  $6.5 \times 10^3$  cells  $ml^{-1}$  at 100 m depth. Based on Spearman's rank correlation analysis, a highly significant correlation between *Synechococcus* abundance and ambient temperature was observed indicating the populations' affinity to elevated temperatures.

## KEYWORDS:

*Synechococcus*, abundance, spatial, temporal, Levantine Basin, northeastern Mediterranean

## INTRODUCTION

Following its first recognition in early 1980s [1] the picoplanktonic, phycoerythrin containing unicellular cyanobacterium *Synechococcus* at present is known to be a major contributor to the total phytoplankton biomass and chlorophyll, [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14], as well as to the pelagic food web of the oceans [15]. The group itself contributes up to an estimated 25% of photosynthetic carbon fixation [16] in oligotrophic large basins and accounts for 64% of the total photosynthesis in the north Pacific Ocean [14]. Its contribution to POC in the Arabian Sea was estimated to vary within 25 to 45% [17]. Moreover, highest concentrations of this group were reported for the same basin [18, 19, 20]. This group is important as a primary producer, especially in open ocean where  $>20 \mu m$  cells do not thrive [7]. Since Mediterranean is well known of its highly oligotrophic offshore waters it is critically important to know changes in time and space of its picoplanktonic constituents. Major aim of this study is to provide preliminary information about possible fluctuations in their abundance within the euphotic layer with respect to changing ambient physico-chemical properties in time in offshore waters of the north-eastern Mediterranean, well known of its' highly oligotrophic nature.

## MATERIALS AND METHODS

The sampling station is located offshore the Institute of Marine Sciences of Middle East Technical University (IMS-METU), situated on the northeastern coast of the Mediterranean (Figure 1). Coordinates of this shelf station are  $34^{\circ}22'E$  and  $36^{\circ}30'N$  and has a total depth of 150 m.

Weekly samplings were carried out on board R/V *Erdemli* of the Institute throughout the year 1998. Using closing bottles water samples were collected from five different depths (surface, 25, 50, 75 and 100 m depths within the euphotic zone) into 100 ml dark coloured polyethylene bottles and were

then preserved with 4% buffered formalin (final concentration) on board. 10 to 15 ml aliquots from each sample were filtered onto 25 mm diameter, black, polycarbonate, nuclepore membrane filters with a 0.2  $\mu\text{m}$  pore size. The filters were then placed onto glass slides using immersion oil and counted using a Nikon epifluorescence microscope at 1000X with a filter combination of B-2A and G-1A. Since the main light harvesting pigment of *Synechococcus* is phycoerythrin, it fluoresces orange to red when excited with green light. A minimum of 20 microscope fields were chosen at random and counted on each slide for their cell contents. In search of a possible relationship between the *Synechococcus* abundance and the ambient biological, physical and chemical variables, the Spearman's Rank Correlation analysis was applied.

Temperature and salinity profiles for the station are obtained using a Sea-Bird model CTD probe which contains sensors, batteries and tape recording units. Nutrient subsamples from the bottle casts were stored in 50-100 ml HDPE bottles that were pre-cleaned with 10% HCl. Bottles for nitrate and phosphate analysis were kept frozen ( $-20^{\circ}\text{C}$ ), whereas those for silicate were kept cool ( $+4^{\circ}\text{C}$ ) in the dark until analysis. The nutrient measurements were carried out by using a Technicon model two-channel autoanalyzer; the methods followed were very similar to those described in [21, 22]. The detection limits achieved, using low concentration samples, were 0.02  $\mu\text{M}$  and 0.05  $\mu\text{M}$  for phosphate ( $\text{PO}_4\text{-P}$ ) and nitrate+nitrite ( $\text{NO}_3\text{+NO}_2\text{-N}$ ), respectively. Five liters of seawater were filtered through the GF/F filters (previously dried at  $105\pm 5^{\circ}\text{C}$  for three hours and pre-weighted) for their TSS (Total Suspended Sediment) contents. The filtrates were then kept at  $105\pm 5^{\circ}\text{C}$  overnight and weighted again and the difference was used in the calculations. One liter of seawater was filtered through GF/F filters and extracted into 90% acetone

solution for the assessment of Chl-*a* concentration. The fluorescence intensity of clear extracts was then measured by the standard fluorometric method [23] using a Hitachi F-3000 model fluorometer.

## RESULTS AND DISCUSSION

**Changes in temperature and salinity with depth.** Time profiles of temperature and salinity along depth over a year period are provided in Figures 2 and 3. Sea surface temperature varied in the range  $16.02 - 30.07^{\circ}\text{C}$  over the year, being coldest on 31 March and warmest on 18 August. Sea surface salinity ranged from 38.48 (recorded on 21 May) to 39.43 (recorded on 24 September). Both parameters displayed homogeneous profiles from surface to the bottom during winter (December, January & February) as a result of winter convective mixing. The water column mean temperature for December, January and February were 21.58, 17.98 and  $16.84^{\circ}\text{C}$ , respectively. The water column mean salinity remained stable during winter as well (39.19, 39.16 and 39.11 for December, January and February). With the onset of spring (March, April and May), a gradual warming of the surface waters as well as decrease in surface salinity (effecting top 25 m depth layer) were observed. Spring is the period when melting snow waters of the Taurus mountains merge with basin waters efficiently. During summer (June, July & August), with increasing irradiance, surface waters started to warm up and the temperature gradient became much wider with depth. Compared to lower depths, slight increase in near-surface salinity due to evaporation was observed. Surface mixed layer (SML) observed to be around 20 m thick in September deepened with further cooling and mixing in October & November. Just below SML lied the thermocline.

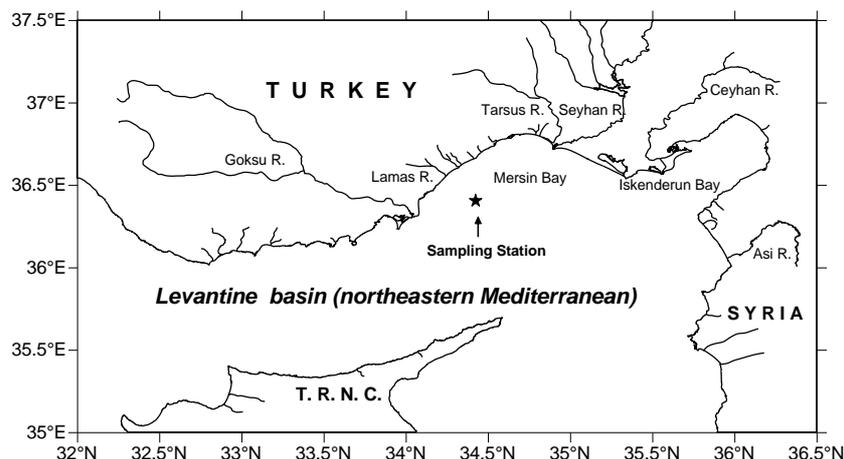
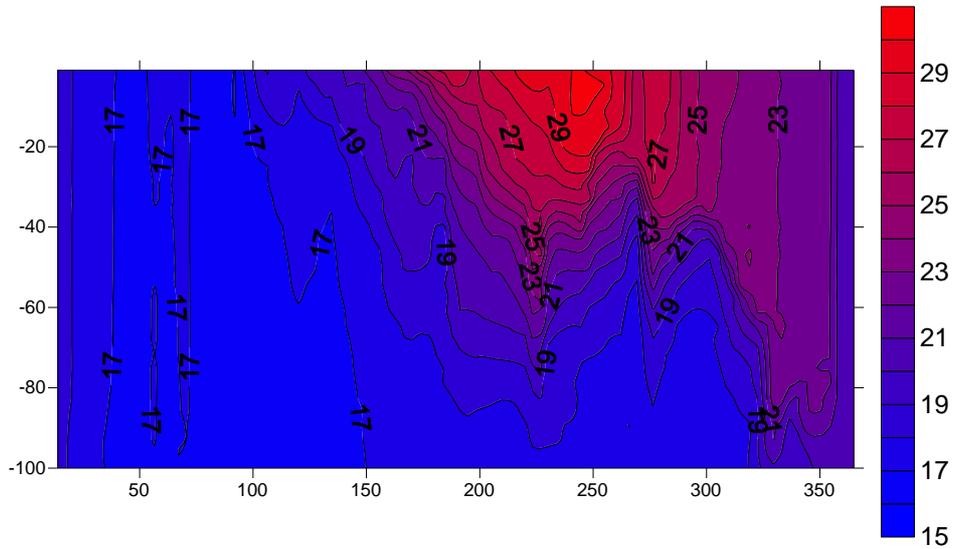
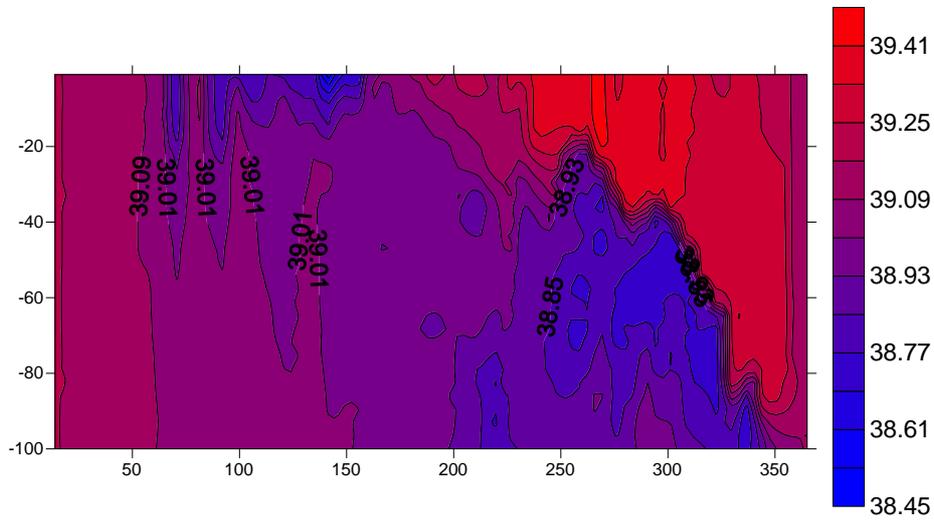


FIGURE 1

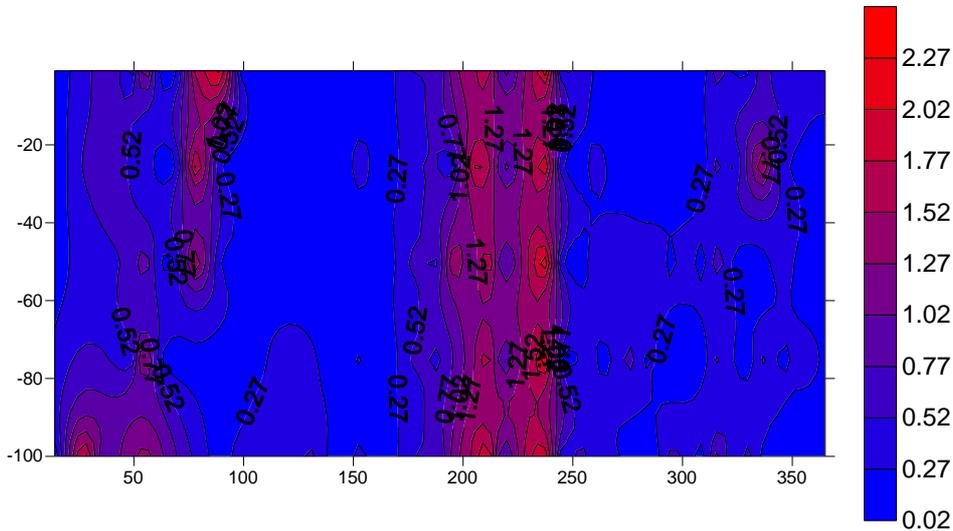
Location of the offshore station in the northern Levantine basin (northeastern Mediterranean).



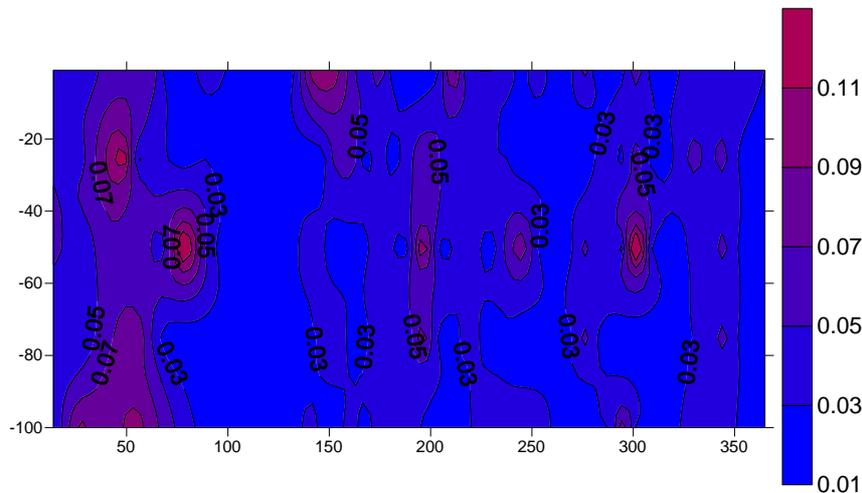
**FIGURE 2**  
Changes in temperature (°C) with depth over the year at the offshore station.



**FIGURE 3**  
Changes in salinity (psu) with depth over the year at the offshore station.

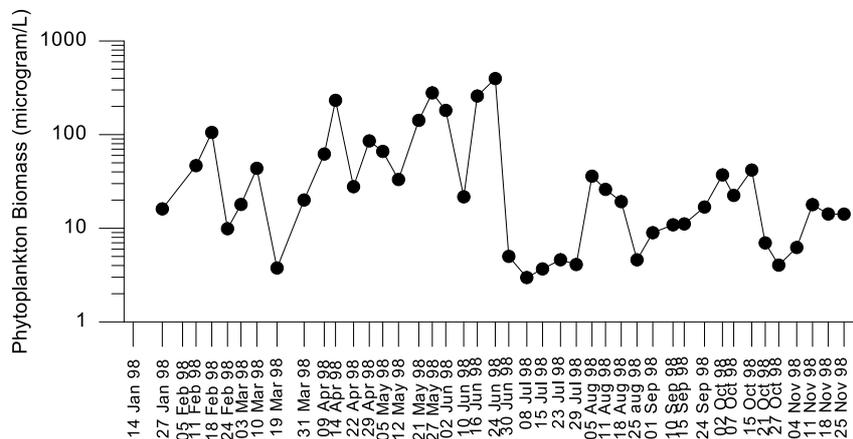


**FIGURE 4**  
Changes in nitrite+nitrate concentrations (µM) with depth over the year at the offshore station.



**FIGURE 5**

Changes in phosphate concentrations ( $\mu\text{M}$ ) with depth over the year at the offshore station.



**FIGURE 6**

Changes in phytoplankton biomass ( $\mu\text{g/L}$ ) at surface over the year at the offshore station.

**Changes in nutrient concentrations in time with depth.** Since the station was partly influenced from the local river runoffs and partly from the nutrient depleted offshore waters a wide range in both nitrite+nitrate and phosphate concentrations was observed throughout the year (Figures 4 and 5). Surface nitrite+nitrate and phosphate concentrations varied in the range 0.02 – 2.23 and 0.02 – 0.11  $\mu\text{M}$  with annual surface averages of 0.52 and 0.04  $\mu\text{M}$ , respectively. Unusual remarkable high levels observed near-surface at both during summer associated with an upwelling event that lasted for about 10 weeks. Over the year water column (Surface to 100 m) average for nitrate and phosphate were 0.51 and 0.04  $\mu\text{M}$ , respectively.

**Changes in phytoplankton biomass at surface in time.** Total phytoplankton biomass ranged from 3 to 396  $\mu\text{g l}^{-1}$  with an annual average value of 56.4  $\mu\text{g l}^{-1}$  at the surface (Figure 6). Biomass yields were maximal during April - June and minimal during the initial phase of the upwelling event ob-

served in July. Increased freshwater discharge during spring from local perennial rivers has stimulated significantly the primary production at near-surface waters in the basin. Diatoms were the major component of the surface flora [24]. Diatoms' contribution to the bulk phytoplankton have been also reported to be significant from the Cilician shelf waters [25] as well as from the neighboring Iskenderun bay area [26, 27] and the Sea of Marmara [28]. In addition to diatoms, dinoflagellates and coccolithophorids were the other important constituents of the phytoplankton assemblage in the offshore. Overall, diatoms, dinoflagellates and coccolithophorids made up 79, 14 and 5 percent of the annual total surface phytoplankton biomass, respectively.

**Changes in total chlorophyll concentration in time.** Over the year surface chlorophyll concentration varied in the range 0.05 – 0.65  $\mu\text{g l}^{-1}$  with an annual average value of 0.2  $\mu\text{g l}^{-1}$  (Figure 7). Based on annual averages, chlorophyll content of the 25 m

depth layer was measured least compared to surface and 50 m. Total chlorophyll concentrations fluctuated between  $0.036 - 0.43 \mu\text{g l}^{-1}$  and  $0.068 - 0.45 \mu\text{g l}^{-1}$  with annual mean levels of  $0.1$  and  $0.2 \mu\text{g l}^{-1}$ , at 25 and 50 m depths, respectively. Parallel to phytoplankton biomass, altered chlorophyll concentrations were observed during the period March to June. This was also the period when significant fluctuations between chlorophyll content of different depths were observed.

**Changes in Total Suspended Sediment (TSS) contents in time.** The annual surface mean for the total suspended sediment (TSS) was  $4.2 \text{ mg l}^{-1}$  with minimum and maximum levels of 2 and  $9.85 \text{ mg l}^{-1}$  recorded on 19 March and 16 June, respectively (Figure 8). TSS content of the top 50 m increased parallel to highs observed in phytoplankton biomass and eventually in total chlorophyll content during late spring and early summer. The an-

nual mean for 25 m depth was  $3.9 \text{ mg l}^{-1}$  with minimum and maximum levels of  $1.16$  and  $7.6 \text{ mg l}^{-1}$  recorded on 27 January and 18 November, respectively. Compared to surface and 25 m depth layers, fluctuations in weekly TSS amounts were more stable at 50 m depth layer. The annual average TSS value was  $3.9 \text{ mg l}^{-1}$ , being lowest ( $1.56 \text{ mg l}^{-1}$ ) on 18 February and highest ( $7.68 \text{ mg l}^{-1}$ ) on 18 November.

**Changes in Secchi Disk Depth (SDD) in time.** The annual average SDD was 25.5 m, being lowest (11.1 m) on 31 March and highest (41.3 m) on 30 June (Figure 9). SDD measurements retained remarkably high levels during July - August as a result of invasion of the shelf with nutrient rich & particle devoid Atlantic deep waters. Low SDD values observed during spring and early summer have coincided with high phytoplankton biomass levels obtained in the meantime.

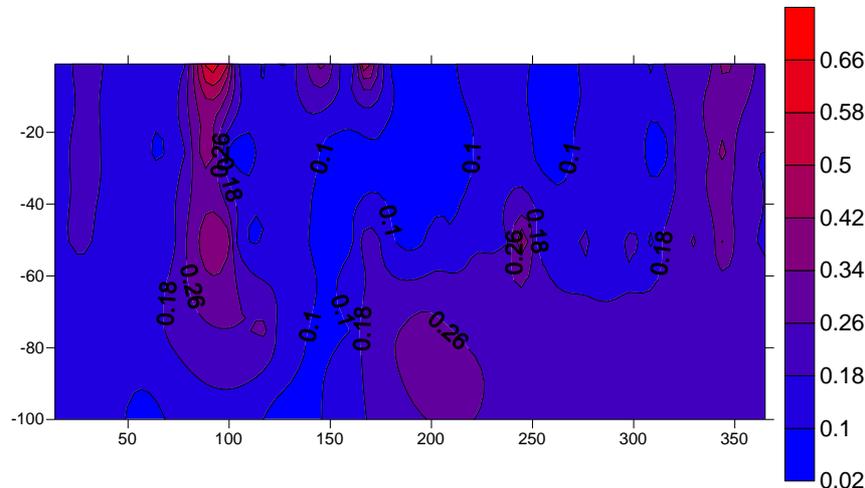


FIGURE 7

Changes in total chlorophyll concentrations ( $\mu\text{g/L}$ ) with depth over the year at the offshore station.

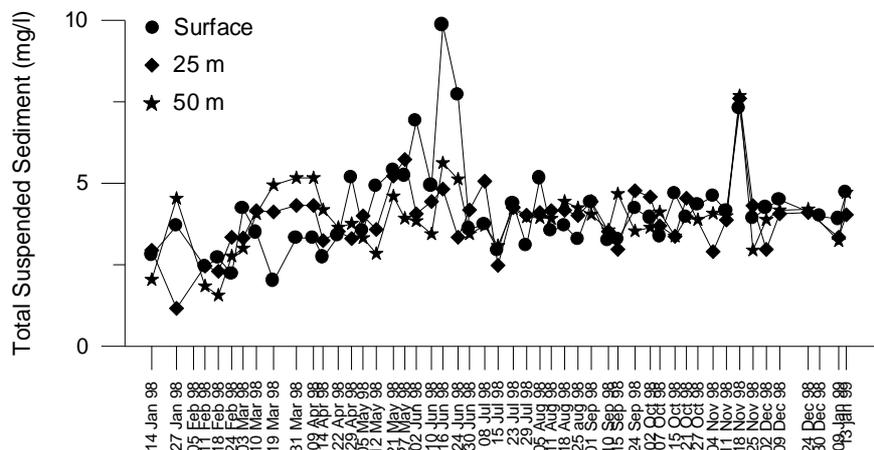


FIGURE 8

Changes in total suspended sediment concentrations ( $\text{mg/L}$ ) at discrete depths (Surface, 25 and 50 m) over the year at the offshore station.

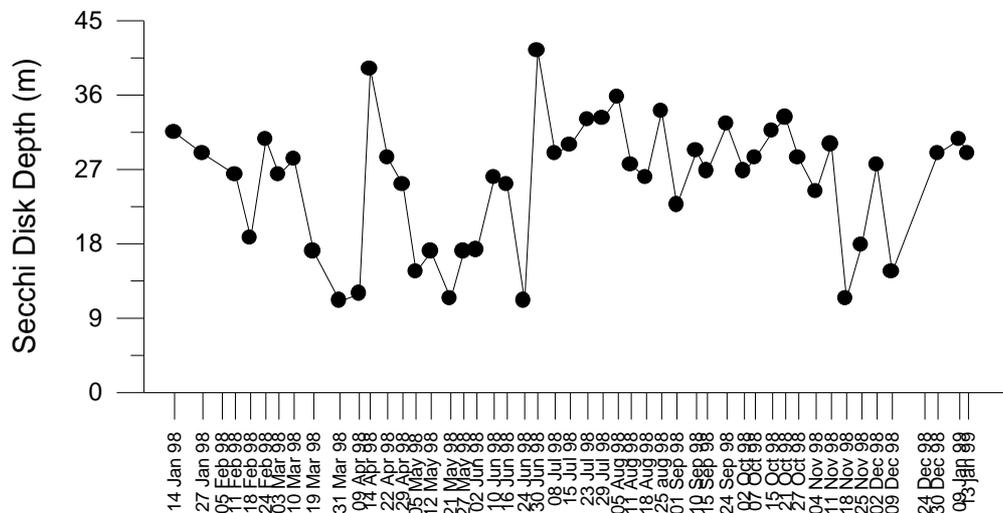


FIGURE 9

Weekly changes in Secchi Disk Depth (m) over the year at the offshore station.

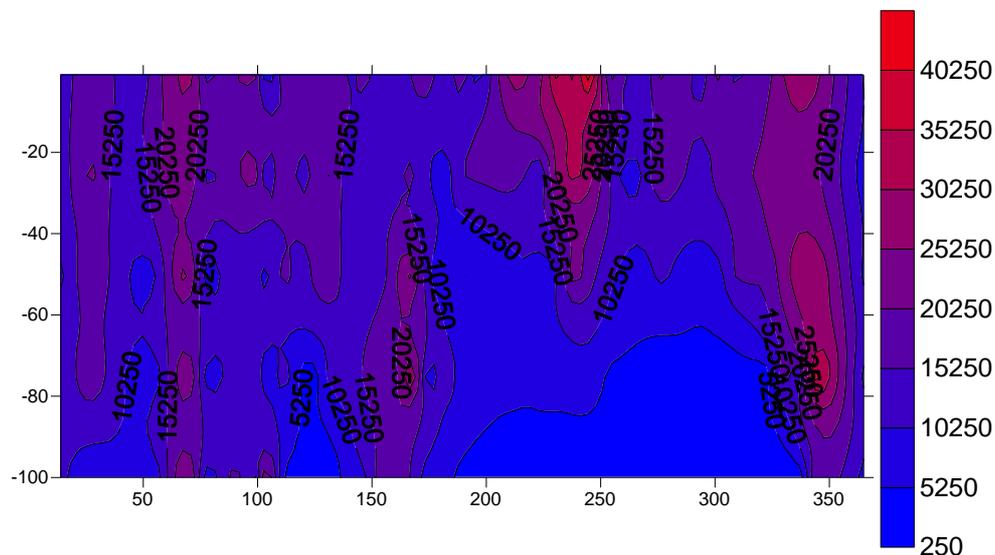


FIGURE 10

Changes in *Synechococcus* abundance (cells/ml) with depth over the year at the offshore station.

**Changes in *Synechococcus* abundance in time with depth.** Time profiles of *Synechococcus* abundance along depth over a year period are provided in Figure 10. Cell counts varied from a minimum of  $3.0 \times 10^2$  cells  $\text{ml}^{-1}$  retained at 100m depth in late October to a maximum of  $4.4 \times 10^4$  cells  $\text{ml}^{-1}$  retained at surface in early December with an annual water column (from surface to 100m depth) mean level of  $1.3 \times 10^4$  cells  $\text{ml}^{-1}$ . Annual water column average observed for the offshore station remained much lower than those retained earlier for the middle ( $2 \times 10^4$  cells  $\text{ml}^{-1}$ ) and the shallow shelf ( $3.7 \times 10^4$  cells  $\text{ml}^{-1}$ ) station located on an inshore-offshore transect in the same basin [10, 11]. Based on water column mean abundances the population was found most abundant during December (onset of winter convectonal mixing) and March (onset of spring phytoplankton bloom). On the other hand,

bulk occurrences of cells at discrete depth layers varied in time.

Surface *Synechococcus* abundances ranged from a minimum of  $4.9 \times 10^3$  to a maximum of  $4.4 \times 10^4$  cells  $\text{ml}^{-1}$  with an annual mean level of  $1.8 \times 10^4$  cells  $\text{ml}^{-1}$  at the offshore station. Based on monthly averages, the population was found most abundant during August followed by December, March and September, all with almost equal densities where to a minimum was met in June. Surface abundances retained peak levels on 1 September ( $4.4 \times 10^4$  cells  $\text{ml}^{-1}$ ), 18 August ( $3.8 \times 10^4$  cells  $\text{ml}^{-1}$ ), and on 10 March ( $3.0 \times 10^4$  cells  $\text{ml}^{-1}$ ). These peak levels were followed by rapid decreases in cell numbers in the following week.

Below the surface, cell concentration ranged from a minimum of  $6.1 \times 10^3$  to a maximum of  $3.2 \times 10^4$  cells  $\text{ml}^{-1}$  with an annual mean level of

$1.7 \times 10^4$  cells  $\text{ml}^{-1}$  at 25 m depth. Based on monthly averages, the population was found most abundant during August, December, March and November where the minimal levels were observed in February and June at this depth. Changes in abundance in time at this depth have mimicked those observed at surface.

Abundance ranged from a minimum of  $5.2 \times 10^3$  to a maximum of  $3.0 \times 10^4$  cells  $\text{ml}^{-1}$  with an annual mean level of  $1.3 \times 10^4$  cells  $\text{ml}^{-1}$  at 50 m. Maximal counts were observed on 9 December ( $3 \times 10^4$  cells  $\text{ml}^{-1}$ ), 2 December ( $2.8 \times 10^4$  cells  $\text{ml}^{-1}$ ) and on 10 March ( $2.9 \times 10^4$  cells  $\text{ml}^{-1}$ ). Compared to surface and 25 m depth layer, significant fluctuations were eminent from January to April at 50 m which can be considered unusual particularly for the period of intense winter convectional mixing. Based on monthly averages the population was also found very abundant in November & December at 50 m. Nutrient fluxes (mainly the nitrogen and phosphorus) from both the surface and deeper parts to the mid depths as a result of winter convectional mixing could have promoted population growth at mid depths (see Figures 4-5).

At 75 m depth layer, cell concentration ranged from a minimum of  $1.9 \times 10^3$  to a maximum of  $3.8 \times 10^4$  cells  $\text{ml}^{-1}$  with an annual mean level of  $1.0 \times 10^4$  cells  $\text{ml}^{-1}$ . Maximal counts were observed on 9 December ( $3.8 \times 10^4$  cells  $\text{ml}^{-1}$ ), 16 June ( $3.3 \times 10^4$  cells  $\text{ml}^{-1}$ ) and 10 March ( $2.7 \times 10^4$  cells  $\text{ml}^{-1}$ ). Winter-spring populations have been found to be more abundant than summer-fall populations at this depth.

At 100 m depth layer, cell concentrations ranged from a minimum of 295 cells  $\text{ml}^{-1}$  to a maximum of  $2.8 \times 10^4$  cells  $\text{ml}^{-1}$  with an annual mean level of  $6.5 \times 10^3$  cells  $\text{ml}^{-1}$ . Maximal counts were observed on 10 March ( $2.8 \times 10^4$  cells  $\text{ml}^{-1}$ ), 14 April ( $2.7 \times 10^4$  cells  $\text{ml}^{-1}$ ) and on 9 December ( $2.0 \times 10^4$  cells  $\text{ml}^{-1}$ ). Keeping slightly higher figures at 75 m compared to 100 m, both depths have displayed similar abundance patterns over the year.

Based on monthly water column mean abundances the population was found most abundant during March and December. During late winter, spring and early summer (from February to June) shelf waters are enriched by nutrients via precipitation, winter convectional mixing as well as by local rivers' runoff. Winter convectional mixing aids for homogeneous distribution of both dissolved and particulate substances within the water column in the shelf. Although *Synechococcus* compete with other phytoplankton groups (mainly diatoms, dinoflagellates and coccolithophorids etc.) for available nutrients presence of sufficient amount of nutrients during winter & spring enable all flora bloom to a certain level in the shelf. In the case of *Synechococcus*, due to their large surface-to-volume ratio, it takes a relatively short time to adapt shortage of nutrients compared to larger cells. In terms of nutri-

ent acquisition, *Synechococcus* are able to utilize nitrate, nitrite, ammonium, urea, and some amino acids [29]. Under nitrogen deprivation, *Synechococcus* will degrade the major light-harvesting pigment protein phycoerythrin as an internal nitrogen source [30]. Phosphorus utilization is via the uptake of phosphate and numerous organic P sources [31] as well as of novel organic sources of N and P, such as cyanates and phosphonates [32]. Phosphorus stress on this group during the summer months was demonstrated from the Red Sea [33]. Distribution of *Synechococcus* among water bodies generally controlled by three main factors, namely the temperature, nitrate availability and light conditions [34]. But the factors that regulate long term (and broad scale) variations in the population of *Synechococcus* (e.g. nutrients, light, temperature) are different to those that result in short-term (and small scale) variations (e.g. grazing and advection) [35].

Extremely high nitrate levels (up to  $2 \mu\text{M}$ ) observed in the whole water column between 15 July and 25 August in shelf waters was considered a very unique event for the region. In general, the basin waters are regarded as nutrient poor and the average nitrate concentration for the surface waters is around  $0.2 \mu\text{M}$ . During this period, 38.9 salinity waters occupied the entire shelf indicating an intense advection of nutrient-rich Atlantic deep waters to the entire shelf area [10,11]. Indeed, this low salinity water in the Levantine Basin may hold elevated nitrate levels up to  $4\text{--}6 \mu\text{M}$  [36]. This nutrient-rich water stayed in the shelf for about 10 weeks leading to a "high nutrient, low chlorophyll" case. This was also evident from the very low phytoplankton biomass, total chlorophyll-*a* concentrations and low TSS contents (Figures 6, 7, 8) as well as inversely from the very high secchi depth readings (Figure 9) observed during this period. As these offshore water masses were advected from deep below the euphotic zone waters, they initially do not contain any live photosynthetic cells at all. Therefore, it takes a relatively longer time for both the picoplankton and net-phytoplankton to redevelop shortly after and to further flourish in such water masses. In the case of *Synechococcus*, due to their large surface-to-volume ratio, it takes a relatively short time to adapt such shortcomings compared to larger cells. Abundances at 75 and 100 m depths seemed to be least effected from the upwelling. Almost stable and low levels retained at these depths indicate upward flux of the deep waters to the shelf throughout the event.

During summer, with increasing irradiance, the surface waters started to warm up and the temperature gradient became much wider with depth. This further prohibited upward downward flux of nutrients in the water column. Deficiency especially in phosphate (near detection limits) at near-surface waters observed during July &

November greatly restrained growth of larger cells. Highly reduced phytoplankton biomass and total chlorophyll levels were retained during this period. However enormous increases in *Synechococcus* abundances at top 25 m were observed during late July & August accompanied by an increase in phytoplankton during August. This sudden outburst in cell numbers resulted in a decrease especially in phosphate levels in the water column. Response of large sized cells including diatoms and dinoflagellates were minor at the initial phase of the upwelling event (during July) but it was major for both populations (*Synechococcus* and other large phytoplankters) throughout August. This eventually led for fluctuations in nutrient levels. Phosphate seemed to be utilized more efficiently than the nitrate at near-surface waters during this period. It is well known that *Synechococcus* make transient blooms when the nitrate concentration increases suddenly. This is due to the quick responding mechanism of *Synechococcus* over other organisms [37] having greater surface-to-volume ratio. Besides *Synechococcus*, other phytoplankters also responded to these high nutrients shortly after the *Synechococcus*, which brought the phenomenon of smaller species succeeding over the bigger ones in time.

With the onset of autumn a well defined surface mixed layer (SML) was formed at top 20 m in September. Underneath lied the thermocline to a depth of approximately 25 m. In October, the SML deepened to as much as 30 m due to continuing mixing and cooling processes. Below 30 m, a rather sharp decline in salinity and temperature was observed. In November, the SML covered almost the top 50 m, and a gradual decrease in both temperature and salinity below it was observed. Changes in the physical structure of the water column influenced both the biology and chemistry of the ambient waters as well. Abundance profiles almost mimicked temperature and salinity profiles during autumn all displaying decreasing trends with depth. With the onset of winter a homogeneous abundance profile is observed in December due to winter convective mixing. The water column mean abundances for September, October and November were  $1.1 \times 10^4$  cells  $\text{ml}^{-1}$ ,  $9.6 \times 10^3$  cells  $\text{ml}^{-1}$  and  $1.3 \times 10^4$  cells  $\text{ml}^{-1}$  respectively. Population displayed very high abundances during early winter at mid-depths reaching as high as  $3.8 \times 10^4$  cells  $\text{ml}^{-1}$  at 75 m on 9 December.

Spearman's rank correlation analysis results showed that *Synechococcus* abundance was positively correlated to water temperature ( $n = 168$ ,  $r_s = 0.271$ ,  $P < 0.01$ ), salinity ( $n = 168$ ,  $r_s = 0.416$ ,  $P < 0.01$ ), total chlorophyll ( $n = 146$ ,  $r_s = 0.208$ ,  $P < 0.05$ ) and negatively correlated to nitrate ( $n = 216$ ,  $r_s = -0.214$ ,  $P < 0.01$ ).

## CONCLUSION

It is well known that *Synechococcus* frequently forms transient blooms lasting up to a week under favourable conditions [38, 39]. Weekly samplings performed in this study appears relevant to document any significant temporal changes in abundance of this picoplanktonic group. Population was found most abundant during March & December and June & August in offshore waters of the northeastern Mediterranean. Similarly, the population has been found more abundant during summer and early autumn indicating the strong response to increasing water temperature in the long term [40]. Highly significant correlation observed between *Synechococcus* abundance and ambient temperature in this study indicates the populations' affinity to elevated temperatures. As a response to nutrient pulses, transient increases in abundance were also revealed time to time in the region. Annual water column average observed for the offshore station ( $1.3 \times 10^4$  cells  $\text{ml}^{-1}$ ) remained much lower than those retained earlier for the middle ( $2 \times 10^4$  cells  $\text{ml}^{-1}$ ) and the shallow shelf ( $3.7 \times 10^4$  cells  $\text{ml}^{-1}$ ) station located on an inshore-offshore transect in the same basin. In contrast to homogeneous abundance profiles retained during winter convective mixing at shallower shelf stations a rather heterogeneous profile was obtained at the offshore station during this period. The population responded quickly (a week later) to extreme nutrient loads to near-surface waters during the summer upwelling event followed by enormous increases in abundance throughout August. Near-surface highs in abundance were also reported elsewhere (41, 42).

## ACKNOWLEDGEMENTS

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