# Monthly changes in the abundance and biomass of picoplankton (cyanobacteria *Synechococcus* & heterotrophic bacteria) in the Cilician Basin (Eastern Mediterranean)

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#### **ABSTRACT**

Monthly changes in the abundance and biomass of picoplankton

(heterotrophic bacteria & cyanobacteria Synechococcus) in the Cilician

Basin (Eastern Mediterranean)

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Within the content of this thesis, it was aimed to understand the changes in the biomass and abundance of heterotrophic bacteria and marine cyanobacteria *Synechococcus* in time with respect to multitude of ambient physical, chemical and biological factors. For this, monthly samples from discrete depths in the offshore (0-20-40-60-80-100-125-150-175-200 m) and in the near shore (surface and 10 m) stations over a period of one year in the Cilician Basin (eastern Mediterranean) were collected via rosette sampler. Epifluorescent microscope and the image analysis system were used to estimate abundance and biomass of both groups.

Coastal station was more abundant and had much higher bacterial (heterotrophic bacteria) and cyanobacterial (*Synechococcus*) biomass than the offshore station as it receives substantial amount of freshwater from the nearby Lamas River throughout the year. The surface annual averages for bacterial and cyanobacterial abundance and biomass were 9.6 x 10<sup>6</sup> cells ml<sup>-1</sup> - 56.5 µg C 1<sup>-1</sup>

and  $4.0 \times 10^5$  cells ml<sup>-1</sup> - 24.1 µg C l<sup>-1</sup>, respectively, at the coastal station. The surface annual averages for bacterial and cyanobacterial abundance and biomass were  $8.1 \times 10^6$  cells ml<sup>-1</sup> – 49.1 µg C l<sup>-1</sup> and  $2.1 \times 10^5$  cells ml<sup>-1</sup> – 10.6 µg C l<sup>-1</sup>, respectively, at the offshore station. Bacterial population always found to exceed *Synechococcus* abundance within the water column. In general, bacterial and cyanobacterial abundance and biomass tend to decrease with depth. On a

seasonal basis, bacterial population was found excessively dominant at the

surface or near-surface waters during the second half of the year. Synechococcus

were also found more abundant during late summer and autumn.

cyanobacterial abundance and biomass.

Temperature and nitrate concentration seemed to affect efficiently the abundance of both populations in the area. Based on Spearman Rank Correlation analysis, highly significant correlations between bacterial abundance as well as biomass and ambient temperature were observed at both stations. However, a significant correlation was found between *Synechococcus* and temperature only at the offshore station. Significant negative correlations are found between nitrate and bacterial abundance and biomass at both stations and between *Synechococcus* abundance and biomass only at the offshore station. At the offshore station, salinity was also found to be positively correlated with the bacterial and

**Key Words:** Heterotrophic bacteria, *Synechococcus*, abundance, biomass, Cilician Basin

# Kuzeydoğu Akdeniz, Kilikya Baseni'nde Pikoplanktonun (heterotrofik bakteri ve cyanobakteri *Synechococcus*) Bolluk ve Biyokütlelerindeki Aylık Değişimler

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Yüksek lisans tez çalışması kapsamında, denizel heterotrofik bakteri ve cyanobakteri *Synechococcus*'un bolluklarında ve biyokütlelerinde zamana bağlı değişimlerin, çevresel fiziksel, kimyasal ve biyolojik faktörlerle etkileşimlerinin incelenmesi amaçlanmaktadır. Bu amaçla, bir yıl boyunca, Kuzeydoğu Akdeniz'de, Kilikya Baseni kıta sahanlığında belirlenmiş iki istasyonda bir yıl boyunca aylık örnekler toplanmıştır. Örnekler, açık istasyonda on farklı derinlikten alınmıştır (0-20-40-60-80-100-125-150-175-200m). Daha sığ olan kıyı istasyonunda ise sadece yüzey ve 10m derinlikten örnekleme yapılmıştır. Her iki grubun bolluk ve biyokütlelerin saptanmasında Epifloresan mikroskop ve görüntü analiz sistemi kullanıldı.

Kıyı istasyonunda, yıl boyunca, önemli miktardaki tatlı su girdisi nedeniyle, açık istasyona oranla daha fazla bakteriyel ve cyanobakteriyel (*Synechococcus*) biyokütle tespit edilmiştir. Kıyı istasyonu, yüzey sularında yıllık ortalama bolluk ve biyokütle bakteriler için 9.6 x  $10^6$  hücre ml<sup>-1</sup> - 56.5 µg C l<sup>-1</sup> ve cyanobakteriler için  $4.0 \times 10^5$  hücre ml<sup>-1</sup> - 24.1 µg C l<sup>-1</sup> düzeyindedir. Açık istasyonda ise bu değerler, sırasıyla,  $8.1 \times 10^6$  hücre ml<sup>-1</sup> - 49.1 µg C l<sup>-1</sup> ve  $2.1 \times 10^5$  hücre ml<sup>-1</sup> -

10.6 μg C l<sup>-1</sup> olarak tespit edilmiştir. Su kolonunda bakteri populasyonu bolluğu,

Synechococcus bolluğuna oranla her zaman daha yüksek gözlenmiştir. Yıl

boyunca, genel olarak hem bakterilerin hem de cyanobakterilerin bollukları ve

biyokütleleri derinlikle birlikte azalma eğilimi göstermiştir. Mevsimsel olarak

bakıldığında, özellikle yılın ikinci yarısında bakteri populasyonu, yüzey

sularında ve yüzeye yakın derinliklerde baskın olarak bulunmaktadırlar.

Synechococcus populasyonu da yaz mevsiminin ikinci yarısında ve sonbahar

aylarında daha fazla sayıda gözlenmiştir.

Sıcaklık ve nitrat konsantrasyonundaki değişimler, her iki populasyonun da

çalışma alanındaki bolluklarını önemli düzeyde etkileyen faktörler olarak

gözlenmiştir. Spearman Rank Korelasyonu analizi sonuçlarına göre, bakteri

bolluğu ve biyokütlesi ile sıcaklık arasında her iki istasyonda da önemli bir ilişki

bulunmuştur. Öte yandan benzer bir ilişki Synechococcus ve sıcaklık arasında

sadece açık istasyonda bulunmuştur. Nitrat ile bakteriyel bolluk ve biyokütle

arasında her iki istasyonda negatif bir ilişki bulunmakla beraber, Synechococcus

ile nitrat arasında negatif ilişki sadece açık istasyonda bulunmuştur. Aynı şekilde

açık istasyonda bakteri ve cyanobakteri bolluğu ve biyokütlesi tuzluluk arasında

pozitif ilişki kaydedilmiştir.

Anahtar Kelimeler: Heterotrofik bakteri, Synechococcus, bolluk, biyokütle,

Kilikya Baseni

X

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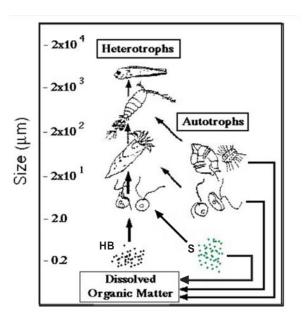
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#### 1. INTRODUCTION

Historically oceanographers viewed the structure of marine life as a simple grazing chain. The classic planktonic food web starts with phytoplankton that provided food for microzooplankton, which in turn reaches to top predators. This simple chain, later, replaced by a more complex network, with an addition of "microbial loop" (Pomeroy, 1974; Azam, *et al.*, 1983).

Both autotrophic and heterotrophic bacteria play a very important role in planktonic marine microbial food webs. They represent a significant part of plankton biomass and their activity has a large impact on ecosystem metabolism and function (Azam, 1998). They play a key role in the cycling of nutrients (Furhman, 1992). In microbial loop, heterotrophic bacteria, analogous to photosynthetic picoplankton, are at the base (Figure 1.1) (Ramaiah, 2005). Therefore, the microbial loop should not be considered separately from the rest of the microbial food web (Sherr and Sherr, 1988).



**Figure 1.1.** Simple representation of a partial aquatic food chain, where 'HB' stands for heterotrophic bacteria and 'S' for *Synechococcus* (modified from Thingstad, 2000).

Bacteria with cell diameters from 0.2 to 2 µm are termed picoplankton, and include phototrophic cyanobacteria and heterotrophic bacteria, drifting in the water column (van den Hoek, *et al.*, 1995). They are found in both seawater and freshwater and occupy a range of ecological niches in aquatic systems.

The bacterial production occurring in the euphotic layer prevents the organic matter from being exported and transports it through the food web (Carlson, et al., 1999). The role of marine microbes are stated to be as recyclers in eutrophic waters and a direct trophic link in oligotrophic waters as the dominant cell size in the phytoplankton community shifts to smaller forms with decreasing nutrient concentration (Munn, 2004). It has been clearly established that the functional effects of the microbial loop largely depend on the environmental conditions such as the trophic state, growth-limiting factors, and the size distribution (Legendre and Rassoulzadegan, 1996). Research in microbial ecology is therefore essential to understand the bio-geochemical cycles occurring in aquatic ecosystems. Three major characteristics distinguish microbes from other organisms, large population sizes, short generation times, and high dispersal capabilities (Dolan, 2005). Heterotrophic bacteria and the photosynthetic Synechococcus are found almost everywhere in the upper ocean. In temperate waters, generally, both groups are most abundant in summer and least so in winter (Li, 1998). Like other small plankton, the picoplankton are preyed upon by zooplankton (usually protozoan), and their numbers are controlled through infection by bacteriophages. The major consumers of the bacteria are small organisms like ciliates and flagellates (Valiela, 1995).

The effect of this microbial component as a whole was disregarded (Valiela, 1995). One reason is the agar-based culture, which does not fully support the growth of bacteria (Munn, 2004). Another reason may be the use of filters with larger pore size than the picoplankton (van den Hoek, *et al.*, 1995). After the development of direct observations and chemical analyses of bacteria with new methods and technologies, it is understood that they found in huge numbers and are competitors for substrates (Hobbie and Williams, 1981). The biomass of the marine bacteria can far outweigh that of the metazoan organisms (Sherr and

Sherr, 1988). Many are saprophytes, and obtain energy by consuming organic material produced by other organisms. This material may be dissolved in the medium and taken directly from there, or bacteria may live and grow in association with particulate material such as marine snow. Many other bacteria species are autotrophic, and these include cyanobacterial groups as *Synechococcus*. Bacteria also play roles in ecological pathways such as nitrogen fixation, nitrification, denitrification, remineralisation and methanogenesis (Valiela, 1995). It may be concluded therefore, that the control of processes observed on large scales is a result of events on microscale (Azam, *et al.*, 1993).

#### 1.1. General Characteristics and Biology of Heterotrophic Bacteria

Heterotrophic bacterial community is one of the basic components of the microbial loop (Valiela, 1995). Marine heterotrophic bacteria in general are between 0.1 to 4 µm in length and important recyclers in eutrophic waters and direct trophic links in oligotrophic waters (Munn, 2004). They have large population sizes with short generation times, thus they have high dispersal capabilities. They are abundant in water column with their high growth rate (Ducklow, *et al.*, 1992).

It is difficult to come up with a uniform classification for bacteria. Classification seeks to describe the diversity of bacterial species by naming and grouping organisms based on similarities. Bacteria can be classified on the basis of cell structure, cellular metabolism or on differences in cell components such as DNA and pigments (Cohan, 2002). While these schemes allowed the identification and classification of bacterial strains, it was unclear whether these differences represented variation between distinct species or between strains of the same species (Munn, 2004). Cell structure of heterotrophic bacteria are illustrated in figure 1.2 and a TEM photograph is shown in figure 1.3.

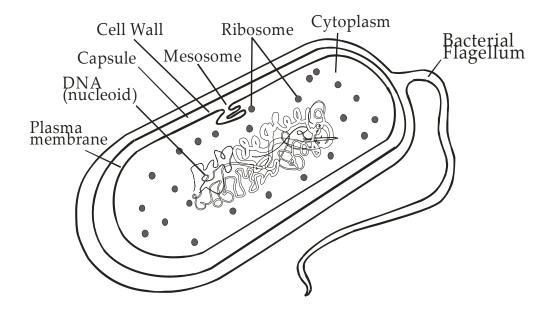
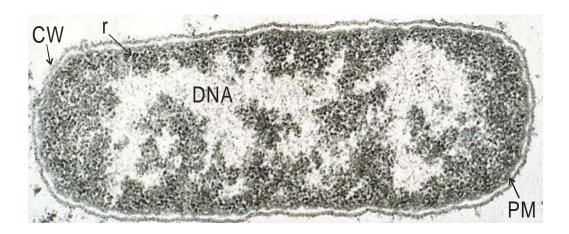


Figure 1.2. Structure of a heterotrophic bacteria (modified from Mariana Ruiz).



**Figure 1.3.** TEM photography of a heterotrophic bacteria, 'CW' is the cell wall, 'r' is ribosome, and 'PM' is the plasma membrane (Photograph from Cook S.).

Bacterial cells produce extra cellular enzymes to facilitate uptake of dissolved organic matter and solubilize particulate organic substances (van den Hoek, *et al.*, 1995). Bacteria are able to regulate their metabolic activity depending on the environmental conditions and they rapidly respond to changes (Hobbie and Williams, 1981). Temperature affects bacterial abundance and activity; in cold waters, for example bacteria are less effective at using organic substrates for growth (Shiah and Ducklow, 1994a).

Planktonic, free living bacteria are considered to be the largest bacterial community in the sea (Ramaiah, 2005). All microbes are potentially found everywhere (Dolan, 2005). The microenvironments of pelagic bacteria, long considered as homogeneous, are now believed to be heterogeneously structured in terms of the spatial distribution of organic matter (Azam, 1998). For instance, Bell and Mitchell (1972) defined a zone where the organic material released after the lyses of the algal cells, namely phycosphere, and stated the bacterial chemotaxis towards this zone. However, in general, the numbers of bacteria in the sea generally maximum at the sea surface where they are associated with a high concentration of organic material (Sieburth, 1971). Bacterial abundance in the water column of the world oceans is estimated as 10<sup>30</sup> cells ml<sup>-1</sup> (Pomeroy, et al., 1990). On the other hand, values reaching to 108 bacteria per milliliter may be found at the sea surface film; however,  $10^5$  or  $10^6$  cells per milliliter would be more common in the euphotic zone of the temperate waters (Li, 1998). Bacteria in the water column are generally aerobic, although the fecal pellets for instance may create suitable conditions for the anaerobic bacteria to be found in the oxygenated waters (Sieburth, 1971).

Bacteria are important in the metabolism of aquatic ecosystems. They obtain organic matter to support their metabolism from at least three sources; dissolved organic compounds, organic particulate matter, and by attacking to other larger living organisms (diatoms, dinoflagellates, etc.) (Valiela, 1995). Most bacteria are heterotrophic and they are extremely abundant and are responsible for the breakdown of large organic particles (Hobbie and Williams, 1981). Bacteria are vital in recycling nutrients and many important steps in nutrient cycles depend on bacteria (Furhman, 1992). One of the main effects of the bacterial activity on oligotrophic systems is the production of an excess biomass resulting from the particulate or dissolved organic carbon. This contribution is important since these particulate and dissolved carbon would otherwise be exported from the euphotic layer by a process of sedimentation or convection (Carlson, *et al.*, 1994). The other important effect of the heterotrophic bacteria within the microbial loop is that it contributes to the regeneration of mineral elements. Bacteria have lower carbon to nitrogen and carbon to phosphorus ratios than

other planktonic organisms (Lee and Fuhrman, 1987). Thus, they participate in the regeneration of the nutrient elements with the consumption by protozoa (Azam, *et al.*, 1983; Sherr and Sherr, 1988), the recycling of some of the nutrient elements that would otherwise be exported into the deeper layers.

Heterotrophic bacteria obtain their energy requirements via the oxidation of organic matter. These bacteria account for a large proportion of the total planktonic biomass, and their numbers increase with the decreasing nutrient levels (Cho and Azam, 1990). The dissolved organic matter, which is partly excreted by the phytoplankton and partly produced by the feeding activities of the zooplankton (phytoplankton debris, excretions, fecal droppings), is efficiently scavenged by the heterotrophic bacteria (Azam, et al., 1983). The resulting bacterial biomass is consumed by heterotrophic nanoplankton and thus re-enters the food web. The other important effect of the microbial loop is that it contributes to the regeneration of mineral elements. As the bacteria have lower carbon to nitrogen and carbon to phosphorus ratios than other planktonic organisms (Lee and Fuhrman, 1987), and do not usually release these elements themselves. However, since they are actively consumed by protists, they participate in the regeneration of the nutrient elements. As Kirchman and Williams (2000) suggest, carbon is the crucial for examining the fate of primary production in the oceans, in assessing climate change, in studies concerning carbon cycle and for quantifying the standing stocks and production rates of various microbial communities in the marine ecosystems.

Data from many aquatic systems show that bacterial production increases as phytoplankton production increases (Hobbie and Cole, 1984). Bacteria are more productive relative to phytoplankton (up to 5:1 in terms of carbon) in nutrient poor waters, and become less productive (less than 1:1) in eutrophic waters where there are larger populations of phytoplankton (Cole, *et al.*, 1988).

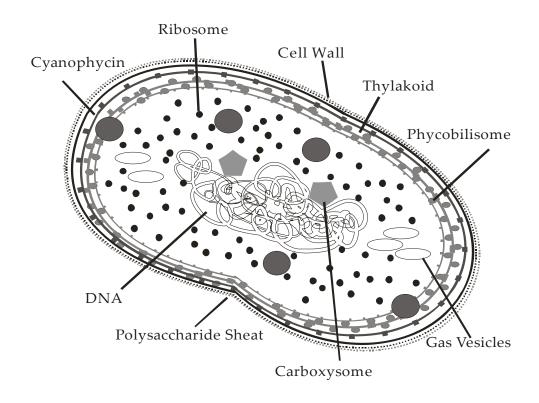
Most laboratory techniques for growing bacteria use high levels of nutrients to produce large amounts of cells in a short time. However, in natural environments nutrients are limited, thus bacteria cannot reproduce indefinitely. This nutrient limitation has led the evolution of different growth strategies (van den Hoek, *et al.*, 1995). Bacteria are *r*-strategists, which can grow extremely rapidly when nutrients become available. Nonetheless, not all taxa are everywhere in significant quantities. Where a microbe is found is where it is able to reproduce. This depends on a balance between the presence and quantities of resources it requires and the mortality it suffers from biological or physical interactions (Dolan, 2005). Bacterial activity in the water column is strongly affected by availability of nutrients and organic matter. Phytoplankton is major source of the organic matter (Valiela, 1995). Therefore, supplies of nutrients and organic matter, and abundance of bacteria and phytoplankton are linked (Ducklow and Carlson, 1992). It is also well established that phosphorous limitation play a key role in the control of bacterial production in Mediterranean Sea (Thigstad and Rassoulzadegan, 1995).

#### 1.2. General Characteristics and Biology of Synechococcus

Small organisms were rather more active than was realized. Production by small chlorophytes and coccoids often exceeds that by larger eukaryotic phytoplankton (Valiela, 1995). Three major groups of organisms constitute photosynthetic picoplankton; picoplanktonic eukaryotes, cyanobacteria belonging to the genus *Prochlorococcus*, and cyanobacteria belonging to the genus *Synechococcus* (Falkowski and Raven, 1997). Each picoplanktonic population occupies a specific ecological niche in the oceanic environment (Partensky, *et al.*, 1999).

Synechococcus is one of the most important components of the prokaryotic autotrophic picoplankton in the world's oceans. The organism was first described in 1979 (Johnson and Sieburth, 1979; Waterbury, et al., 1979). They had been overlooked previously for several reasons, principally because they are small; all < 2  $\mu$ m and most about 1  $\mu$ m in diameter. The main photosynthetic pigment in *Synechococcus* is chlorophyll a, while its major accessory pigments

are phycobilliproteins (Johnson and Sieburth, 1979; Waterbury, et al., 1979). Cyanobacterial thylakiods include alternate layers of phytobilisomes, particles of protein binding photosynthetic accessory pigments including phycoerythrin. This pigment has a characteristic orange fluorescence in blue light that is strong enough to mask the red fluorescence of the chlorophyll. This makes cyanobacterial readily countable in an epiflourescence microscope. Oceanic cyanobacterial are coccoid, which is spherical belonging to a subgroup termed chroococcoid cyanobacterial. Most of the cyanobacterial in marine phytoplankton are very similar and are all classed together in are genus *Synechococcus* (Miller, 2003). Cell structure of *Synechococcus* is illustrated in figure 1.4 and a TEM photograph is shown in figure 1.5.



**Figure 1.4.** The cell structure of *Synechococcus* (modified from Falkowski and Raven, 1997).

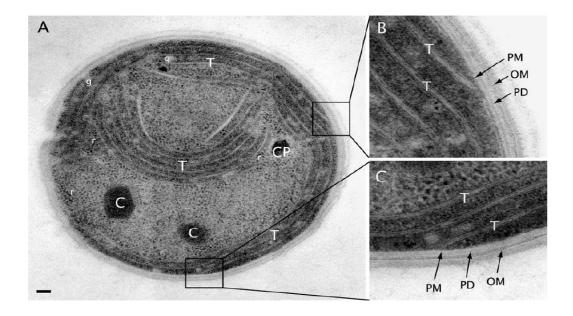


Figure 1.5. TEM photography of *Synechococcus*,(A); (B) and (C) are the enlargements of the boxed areas in 'A', showing close proximity of thylakoid membrane and plasma membrane. *T* Thylakoid membranes; *C* carboxysome; *CP* cyanophycin granule; *g* glycogen granule; *r* ribosomes; *PM* plasma membrane; *OM* outer membrane; *PD* peptidoglycan layer. Bar: 100 nm (Photograph from Michelle Liberton, R. Howard B., John H., Robin R., and Himadri B.)

In terms of nutrient acquisition, *Synechococcus* are able to utilize nitrate, nitrite, ammonium, urea, and some amino acids (Moore, *et al.*, 1995). Under nitrogen deprivation, *Synechococcus* will degrade the major light-harvesting pigment protein phycoerythrin as an internal nitrogen source (Wyman, *et al.*, 1985). Phosphorus utilization is via the uptake of phosphate and numerous organic phosphorus sources (Scanlan, *et al.*, 1997) as well as of novel organic sources of nitrogen and phosphorus, such as cyanates and phosphonates (Palenik, *et al.*, 2003). However, small changes in salinity seemed to cause spatial heterogeneity in cell concentrations (Cho and Azam, 1990).

Coccoid *Synechococcus* are abundant in world oceans with cells between 0.6 and 1.6 µm in size (van den Hoek, *et al.*, 1995). They are gram-negative cells with highly structured cell walls that may contain projections on their surface (Perkins, *et al.*, 1981). Significant as primary producers of the food web, especially in oligotrophic waters and open ocean (Azam, *et al.*, 1983; Li, *et al.*, 1992). They have low sinking rates with high growth rates.

It has been widely shown that the population exhibit characteristic synchronous division cycle, with cell division generally occurring near dusk (Sweeney and Borgese, 1989; Johnson, et al., 1996; Vaulot, et al., 1996; Jacquet, et al., 1998b; Jacquet, et al., 2001). Consequently, Synechococcus number can vary dramatically over the course of just a few hours or days (Vaulot, et al., 1996; Jacquet, et al., 1998a; Vaulot and Marie, 1999). Scientific classification of the Synechococcus is as follows:

Kingdom: Bacteria

Division: Cyanobacteria

Order: Synechococcales

Family: Synechococcaceae

Genus: Synechococcus (Nägeli, 1849)

All marine *Synechococcus* strains appear to be obligate photoautotrophs (Waterbury, *et al.*, 1986). Marine *Synechococcus* are traditionally not thought to fix nitrogen (Munn, 2004).

The picoplanktonic, chroococcoid cyanobacteria, *Synechococcus*, are now known to be major contributors to the total photosynthetic biomass in the oceans (Johnson and Sieburth, 1979; Waterbury, *et al.*, 1979; Li, *et al.*, 1983; Platt, *et al.*, 1983; Glover, *et al.*, 1986; Iturriaga and Mitchell, 1986; Li, *et al.*, 1992). Synechococcus is regarded as an important component of the phytoplankton in highly oligotrophic Mediterranean Sea (Li, *et al.*, 1993; Magazzu and Decembrini, 1995; Vaulot, *et al.*, 1996; Agawin and Agusti, 1997; Jacquet, *et al.*, 1998b; Agawin, *et al.*, 2000).

Synechococcus contribute from 15% to 25% and occasionally up to 45% of p (POC) in the oligotrophic waters of the Arabian Sea (Burkill, et al., 1993). In oligotrophic oceans, this group contributes up to 25% of photosynthetic carbon fixation (Waterbury, et al., 1986). There have been few studies of picoplankton distributions in the highly contrasting Turkish seas. Recently, new data have become available on pigments, size, distribution, growth and diurnal variability

of *Synechococcus* from Turkish coastal waters (Uysal, 2000, 2001, 2006; Uysal and Koksalan, 2006). Comparison of results of a time series study conducted on phytoplankton during 1997-1998 (Uysal, *et al.*, 2003) and on *Synechococcus* during 2002-2003 (Uysal, *et al.*, 2004) at the offshore station indicated that the contribution of *Synechococcus* to total phytoplankton biomass in terms of carbon varied significantly. During phytoplankton blooms this percentage ratio decreased to as low as 0.2%. During steady state (under normal conditions), it increased up to 60%. Minimum and maximum phytoplankton and *Synechococcus* biomasses ranged between about 3-1875 and 0.6-5.1 μgC 1<sup>-1</sup>, respectively in the Levantine shelf waters (Uysal, 2006).

Synechococcus has been observed to occur at concentrations ranging between a few cells per ml to 10<sup>6</sup> cells per ml in virtually all regions of the oceanic euphotic zone (Waterbury, et al., 1986). Cells are generally much more abundant in nutrient rich environments than in the oligotrophic ocean and prefer the upper part of the euphotic zone (Partensky, et al., 1999). Synechococcus has also been observed to occur at high abundances in environments with low salinities and/or low temperatures. In the oligotrophic waters Synechococcus is apparently present (Olson, et al., 1990; Campbell and Vaulot, 1993; Li, 1995). Vertically Synechococcus is usually relatively equally distributed throughout the mixed layer and exhibits an affinity for the higher light regime (Vaulot, et al., 1995). Below the mixed layer cell concentrations rapidly decline. Vertical profiles are however strongly influenced by hydrologic conditions and can be very variable both seasonally and spatially. Vertical distribution of the bacterial community is primarily determined and controlled by the light and nutrients (Margalef, 2001). The apparent consistency of the prokaryotic community composition from Arctic to Antarctic waters may correspond with relative consistency throughout all seasons and in their ecological role (Dolan, 2005).

The factors controlling the abundance of *Synechococcus* still remain poorly understood, especially considering that even in the most nutrient deplete regions of the central gyres, where cell abundances are often very low, population

growth rates are often high and not very drastically limited (Partensky, et al., 1999). Despite the uncertainties, it has been suggested that Synechococcus distribution throughout the water column is generally controlled by three main factors, namely temperature, nitrate availability and light conditions (van den Hoek, et al., 1995; Falkowski and Raven, 1997). However, the factors that regulate long term and large scale variations of Synechococcus abundance (e.g. nutrients, light, temperature) are different from those that result in short-term and small scale variations (e.g. grazing and advection) (Binder, et al., 1996). It is clearly shown that, there is at least a relationship between ambient nitrogen concentrations and Synechococcus abundance (Blanchot, et al., 1992; Partensky, et al., 1999). One environment where Synechococcus thrives particularly well are coastal plumes of major rivers (Paul, et al., 2000; Wawrik, et al., 2003; Wawrik, et al., 2004; Wawrik and Paul, 2004). Such plumes are coastally enriched with nutrients such as nitrate and phosphate, which drives large phytoplankton blooms. High productivity in coastal river plumes is often associated with large populations of Synechococcus. Although, most of the primary production suggested to be respired within the food web, it is also the ultimate food source for metazoan food web (Sherr and Sherr, 1988).

Both *Synechococcus* and heterotrophic bacteria are most abundant in summer and least in winter. More particularly, cell abundance at individual locations can evidently track water temperature throughout the year (Li, 1998).

It is easy to study them under epiflourescent microscope, flowcytometer and easy to culture (Falkowski and Raven, 1997). Epiflourescence microscopy played an important role in the discovery of *Synechococcus* (Johnson and Sieburth, 1979; Waterbury, *et al.*, 1979). The application of epiflourescence microscopy to the examination of natural water samples revealed that high abundances of bacteria exist in seawater (Hobbie, *et al.*, 1977). Image analyses provided more information regarding size and biomass (Sieracki, *et al.*, 1985).

#### 1.3. General Characteristics of the Mediterranean Sea

The Mediterranean is an enclosed sea, which reaches to a depth of 5093 m (Helenic Trench) as its deepest point with a mean depth of 1470 m (Mojetta, 1996). It covers an area of about 2.5 million square kilometers and almost four million cubic kilometers of volume. The Strait Gibraltar is the only interaction point of the Mediterranean Sea with Atlantic Ocean. Less saline Atlantic water enters to the Mediterranean through this strait, and saline water leaves from mid depths (Mojetta, 1996). The incoming Atlantic waters are nutrient poor, where as the outgoing waters are the nutrient rich Mediterranean deep waters (Krom, *et al.*, 1991). The water exchange between the Atlantic Ocean and the Mediterranean Sea is balanced the loss from great rate of evaporation than inflow from rain (Özsoy, *et al.*, 1989). Most of the nutrient input to the Mediterranean is via precipitation, river runoff, and atmospheric input.

The study area was located at the Cilician Basin, at the northeastern part of the Mediterranean Sea. Although the eastern part of the Mediterranean is considered to have limited riverian input in relative to the western part, the sampling area is close to the major rivers (Göksu and Seyhan) of the basin. In addition, the region also has a number of little or seasonal rivers. One of these local rivers is the nearby Lamas River and the study area receives fresh water over most of the year. Uysal et al. (2004) showed the effects of this river at the first station of the study. It is claimed that especially the first 2 or 3 meter of the water column is affected by the fresh water input coming from the Lamas River. However, the production is generally very low at the eastern part of the Mediterranean (Yılmaz and Tuğrul, 1998). The Cilician Basin is regarded as one of the most important regions of freshwater influence in the eastern Mediterranean (Uysal and Köksalan, 2006). In addition to the river input, appreciable amounts of nutrients are supplied via the underground freshwater sources as well as from the upwelling events. The sea surface temperatures are very high throughout the basin, which enhances the evaporation, resulting an increase in the salinity of the surface waters. There exists an imbalance between the evaporation and the precipitation and runoff (Yılmaz and Tuğrul, 1998).

The main current influencing the Cilician Basin is the Mid-Mediterranean Jet (MMJ) (Robinson and Coauthors, 1991; Özsoy, et al., 1993). This current is an extension of the Atlantic Water entering from the Gibraltar, leading to mesoscale eddies and dynamical structures in the basin (Robinson, et al., 1991; Özsoy, et al., 1993). There are four main water masses in the northeastern Mediterranean Sea. Going from surface to bottom, the vertical alignment of these masses are Levantine surface water, modified Atlantic water, Levantine intermediate water, and eastern Mediterranean deep water. Levantine surface water is the uppermost mixed layer, followed by Atlantic water, which originates from Atlantic Ocean. This water mass can be identified from the salinity minimum (38.5-39 psu). The third layer is the Levantine intermediate water with maximum salinity of them all (39.1-39.3 psu and 15.5 °C) (Robinson, et al., 1991; Özsoy, et al., 1993; Malonette-Rizzoli, et al., 1999). Levantine deep water has a typical salinity of 38.7 psu with a temperature value of 13.6 °C (Ünlüata, 1986; Özsoy, et al., 1989; Özsoy, et al., 1993). Among these water bodies, Levantine deep water is the only water mass that nutrient content is relatively high (Salihoğlu, et al., 1990). Levantine intermediate water is formed during the February and March with the cooling of the surface saline waters. From the late winter to early spring, sea surface temperatures dropped to a minimum value, thus, the density of the water mass increases. The water mass with higher density sinks to a level of equal density within the water column (Özsoy, et al., 1993). Levantine intermediate water is generally stays between 200-700 m depth (Malonette-Rizzoli, et al., 1996). These sinking waters carry nutrients to deeper parts, which is the one reason for the eastern part of the Mediterranean to be the poorest water body.

The nutrient salts are generally relatively high in concentration during winter months within the euphotic zone. Phosphate concentrations for example are found to be  $0.03~\mu M$  and nitrate concentrations are measured in a range of 0.6-  $0.8~\mu M$ . However, these values reduce to nearly to the detection limits as the

stratification occurs (Yılmaz and Tuğrul, 1998). Eastern Mediterranean is generally considered to be a phosphate limiting area because of the high N:P ratio (29-27:1) (Krom, *et al.*, 1991). Production at this area thus, relies on the regenerated material. Only 30 percent of the total production is new production and the more than half of this new production is depend on the vertical advection of the nutrients (Bingel, *et al.*, 1993; Yılmaz and Tuğrul, 1998). The productivity of the area is also highly influenced by the current regime of the basin together with the riverian input, resulting a local and seasonal production (Azov, 1991).

The microbial processes dominate the ecological and biogeochemical dynamics in Eastern Mediterranean where the picoplankton communities dominate primary production in the basin, suggesting that the most material and energy transfer is through the microbial loop (Malonette-Rizzoli, *et al.*, 1996). In Aquatic environment, the presence of the microbial community should be considered in both spatial and temporal dimensions (Pinhassi and Hangström, 2000). Information on abundance and activity of *Synechococcus* and heterotrophic bacteria is very useful to recognize their varied roles.

It has by now been clearly established that the functional effects of the microbial loop largely depend on the environmental conditions like the trophic state, growth-limiting factors, size distribution, etc. (Legendre and Rassoulzadegan, 1996). Research in microbial ecology is therefore essential to understanding the bio-geochemical cycles occurring in aquatic ecosystems.

Phytoplankton investigations conducted earlier in the Cilician Basin shelf waters mainly focused on the qualitative and quantitative aspects of diatoms, dinoflagellates and coccolithophorids among others, and their relationships to environmental factors (Eker and Kideys, 2000; Eker-Develi, *et al.*, 2003; Uysal, *et al.*, 2003; Yılmaz, *et al.*, 2003). All these studies dealing with phytoplankton overlooked heterotrophic bacteria and *Synechococcus*, the bacterioplankton and the picoplankton. Oligotrophic nature of the region makes these two groups even

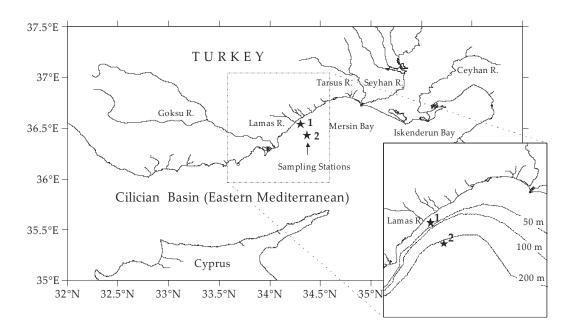
more important. It was the aim of this study to provide baseline information on bacterial and *Synechococcus* dynamics in a rapidly changing environment with respect to several physical, chemical as well as biological parameters.

The present study on marine heterotrophic bacteria and *Synechococcus* aimed to provide baseline information on the dynamics of picoplankton community with respect to ambient biological, physical and chemical parameters in the Cilician Basin.

#### 2. MATERIAL AND METHODS

#### 2.1. Study Area:

The sampling site is located offshore of the Institute of Marine Sciences of the Middle East Technical University (IMS-METU), which is on the northeastern coast of the Mediterranean Sea (Figure 2.1). Seawater samples were collected, on a monthly basis, from two stations between February 2005 and January 2006, onboard R/V Lamas and R/V Bilim-2. The sampling stations are located at 34°15.680' E, 36°33.580' N (Station # 1) and at 34°21' E, 36°26' N (Station # 2) in the northern Cilician Basin.



**Figure 2.1.** Location of the sampling stations.

First station is located near the coastline. This shallow station, which has a total depth of 20 m, is under great influence of coastal dynamics as well as the freshwater input from the relatively small, local Lamas River (Uysal, *et al.*, 2004) throughout the year. This river drains a large basin (1055 km<sup>2</sup>) and the annual average discharge is about 6.7 m<sup>3</sup>sec<sup>-1</sup>, which also greatly differs from nearby streams flowing only for short period throughout a year (Okyar, 1991).

Second station is located at the shelf-break. Surface waters of this station may receive freshwater from local major rivers (Seyhan, and Göksu) time to time. Having greater depths compared to the first station, late summer – autumn stratification and winter mixing were observed at this station.

#### 2.2. Sampling

Water samples, for the measurement of both biological and chemical parameters, were captured from standard depths (Table 2.1) using five liter Niskin closing bottles. Physical parameters were obtained and recorded *in situ* by using a Sea-Bird Electronics model CTD profiler. Secchi Disk depth was also measured at both stations.

**Table 2.1**. Sampling depths and selected parameters for both stations.

	Station # 1 (20m)	Station # 2 (210m)
Sampling Depths (m)	0, 10	0, 20, 40, 60, 80, 100, 125, 150, 175, 200
Biological parameters	heterotrophic bacteria and Synechococcus	
Chemical parameters	phosphate (PO <sup>-3</sup> <sub>4</sub> -P), nitrate + nitrite (NO <sup>-</sup> <sub>3</sub> -N+NO <sup>-</sup> <sub>2</sub> -N), silicate (Si), and dissolved oxygen (DOW)	
Physical parameters	Temperature, salinity and density	

#### 2.2.1. Physical Parameters

Changes in temperature and salinity with depth were measured using a Sea-Bird Electronics model CTD probe. This system contained sensors, batteries and internal data recording units (sea ram memory). The data recorded during the casts were later processed by the computers in the Institute laboratories. Temperature and salinity profiles were drawn from the processed downcast data. A 20 cm diameter Secchi disk was used for Secchi disk depth measurements.

#### 2.2.2. Chemical Parameters

Samples for chemical parameters are also drawn in parallel to biological parameters from the closing bottles.

**Nutrient salt** samples (phosphate (PO<sup>-3</sup><sub>4</sub>-P), nitrate + nitrite (NO<sup>-3</sup>-N+NO<sup>-2</sup>-N) and silicate (Si)) were collected into 100 ml high-density polyethylene bottles that were pre-cleaned with 10% HCl. Samples for phosphate and nitrogen salts were kept frozen (-20°C), whereas those for silicate were kept cool (+4°C) in the dark until analysis. The nutrient measurements were carried out by using a Technicon model two-channel auto-analyzer; the methods followed were very similar to those described by (Strickland and Parsons, 1972). The detection limits achieved, using low concentration samples, were 0.02 μM and 0.05 μM for phosphate and nitrate + nitrite, respectively.

**Dissolved oxygen** samples for measurements, were transferred to specially produced 150 ml glass bottles with covers (pre-washed with argon gas and kept with covers until the time of sampling) using a plastic pipette to avoid formation of air bubbles while draining. MnCl<sub>2</sub> and KOH/KI were added immediately into samples to capture the dissolved oxygen. Dissolved oxygen measurements were done according to Winkler titration method by using international trade mark Methrohm (Hydro-Bios) 645 Multi-Dosimat Oxygen Auto-Titrator Analyzer and the sensitivity of this method is about ±0.05 ppm (Uysal, *et al.*, 2004).

#### 2.2.3. Biological Parameters

The sampling and the analysis of the biological parameters were explained in the following sections.

#### 2.2.3.1. Sample Collection and Filtration

For biological measurements, water samples, taken from the standard depths using closing bottles, were transferred to the 50 ml polycarbonate bottles (Murphy and Haugen, 1985). They immediately preserved by an addition of 1.25 ml of 25% glutaraldehyde to a final concentration of about 0.625%, and refrigerated until filtration. Aliquots from each preserved sample was filtered onto 0.2 µm black, 25 mm diameter, nucleopore membrane filters (Hobbie, et al., 1977). To make sure that an even distribution would be achieved for counting and measurement purposes, pre-wetted Millipore backing filters (type RA 1.2 µm) were placed under nucleopore filters. Depending on the amount required, 15 to 20 ml aliquots from each sample were filtered onto nuclepore membrane filters. Acridine Orange (3,6-bis(dimethylamino)acridine, AO), which is a commonly used fluorochrome for epifluorescence microscopy, was used for the staining of the RNA and DNA of the specimen (Hobbie, et al., 1977). 200µl of AO was added to the last 5 ml of the filtration volume (Uysal, et al., 2004). Filtration performed at 125 mm-Hg vacuum pressure (Murphy and Haugen, 1985). The filters were then mounted on glass slides using low fluorescence immersion oil. Cell counts were performed immediately after this step. In cases where this was not achieved, slides were kept frozen at -18°C for later counts.

Sampling was conducted consistently during the same periods of day between 11:00 and 12:00 hours at the second station and between 15:00 and 16:00 hours at the first station.

#### 2.2.3.2.Direct Counts and Cell number calculations

Cell counts were done in the laboratory using a Nikon EFD3 epifluorescence microscope at 1500X with a filter combination of B-2A (blue excitation-DM 505, EX 450–490, BA 520) and G-1A (green excitation-DM 575, EX 546/10, BA 580) (Uysal, 2001). Under blue excitation, *Synechococcus* appears to be yellowish orange and the heterotrophic bacteria appears to be bright green, whereas, under green excitation *Synechococcus* can be viewed bright reddish orange with no clear image of heterotrophic bacteria (Caron, 1983). Both the *Synechococcus* and the heterotrophic bacteria were counted and recorded on the same slides. Cells were counted in at least 30 randomly chosen microscope fields to enumerate a minimum of 400 cells (Sherry and Wood, 2001). During cell counts of both *Synechococcus* and heterotrophic bacteria, 1500X magnification were used.

Direct counts of the *Synechococcus* through filters were transformed into cell numbers in unit volume using the following equations:

$$N = MF \times Avg. / V$$
 , where (1)

MF: Multiplication factor

Avg.: Average cell number counted on all microscope fields

V : Total volume filtered (ml)

MF (Multiplication Factor) was calculated from the following equation,

$$MF = FA/A$$
 , where (2)

FA: Total filtered area (2.1 cm<sup>2</sup>)

A : Area of a single field

As a result MF for 100X = 29012

Area of a single field was calculated as follows,

$$A = \pi r^2 \qquad , where \qquad (3)$$

 $\pi: 3.1416$ 

r: Objective Radius (0.096 mm for 100X)

# 2.2.3.3.Image Analyses and estimation of cell volumes

Image analyses system is composed of an epiflourescence microscope, a digital camera, and a computer with appropriate software. The analyses of the images were done using a commercial software (Image – Pro Plus 5.0.) appropriate for counting and measuring purposes, with digital color images taken under the 100X magnification by using another software program (ACT-1 ver. 2.51, Nikon) on each slide. Four photographs were taken randomly from each slide under blue excitation for cell size measurements. After necessary settings and adjustments on each picture, the software, automatically counts the cells and provide the required size measurements (major axis, area, perimeter, and length).

Mean cell volume was estimated using image analysis of Acridine Orange (slide) preparations (Ducklow, *et al.*, 1995). Cell volumes were estimated from measurements of length, width, area and perimeter for each cell using biovolume algorithms (Sieracki, *et al.*, 1989; Ducklow, *et al.*, 1995). Calculation of the biovolume is as follows:

$$V = b^2 x a x \pi/6$$
, where (4)

V: volume of the cell

b: axis (minor) of the cell

a: axis (major) of the cell

 $\pi : 3,1416$ 

$$b = (A/(\pi x a)/2) x 2$$
 ,where (5)

b: axis (minor) of the cell

A: area of the cell

a: axis (major) of the cell

 $\pi : 3,1416$ 

Then, mean volume and mean area for heterotrophic bacteria and *Synechococcus* were calculated separately.

Bacterial biomass was calculated as the product of bacterial abundance and equivalent amount of carbon per cubic micron of bacterial volume. 77 fg C per cubic micron of bacterial volume is used for carbon biomass estimation (Carlson, *et al.*, 1999). In case of *Synechococcus*, 123 fg C per cubic micron of its volume is used (Waterbury, *et al.*, 1986). (fg denotes femtogram.)

# 2.3. Statistical analysis

The relationships between the biological, physical and chemical parameters were analyzed in order to understand the level of the interactions and the possible outcomes of those relations. At the beginning, it was checked whether the data were normally distributed or not. This was done by the test of randomness (index of dispersion) testing the  $s^2/\bar{x}$  ratio, which should be '1' for an ideal normally, distributed data set.

$$I = s^2 (n-1) / \overline{x}$$
, where (6)

I: Index of dispersion

s<sup>2</sup>: sample variance

n: Sample size

 $\bar{x}$ : The sample mean

As a second step, Spearman rank-order correlation analysis was done between the ambient parameters and the cell counts, in order to understand whether there is relationship between variables or not. The formula for the Spearman rankorder correlation coefficient is as follows:

$$r_s = \left[\sum (y - \overline{y})(z - \overline{z})\right] / \sqrt{\sum (y - \overline{y})} \sum (z - \overline{z}), \text{where}$$
 (7)

 $\overline{y}$ : mean rank of the sample from variable 1,

 $\overline{z}$ : mean rank of the sample from variable 2,

Degrees of freedom = n-2, where n = sample size.

If  $r_s \ge r_s$  critical: significant result and if  $r_s \le r_s$  critical: non-significant result.

#### 3. RESULTS

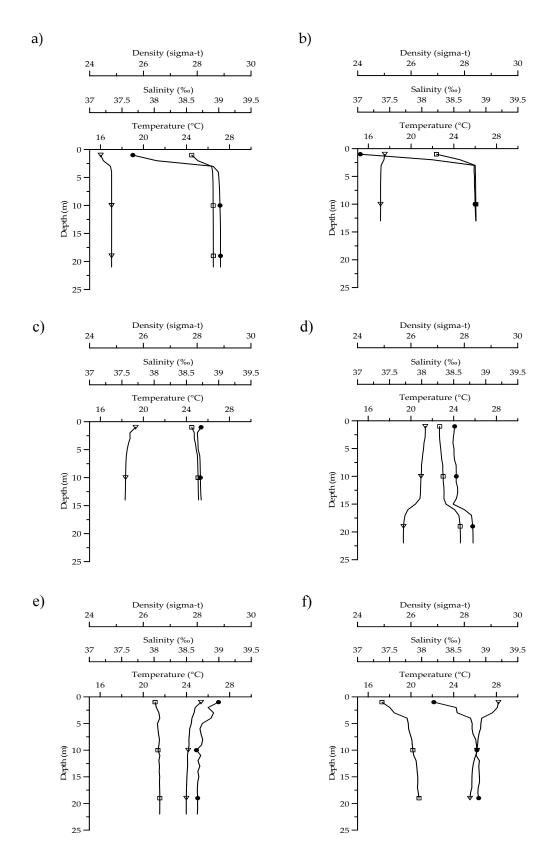
In the following sections, annual monitoring of environmental factors (physical and chemical) that influence biomass and abundance of bacteria and cyanobacteria, together with their changes in time and space were interpreted.

## 3.1. Physical Parameters

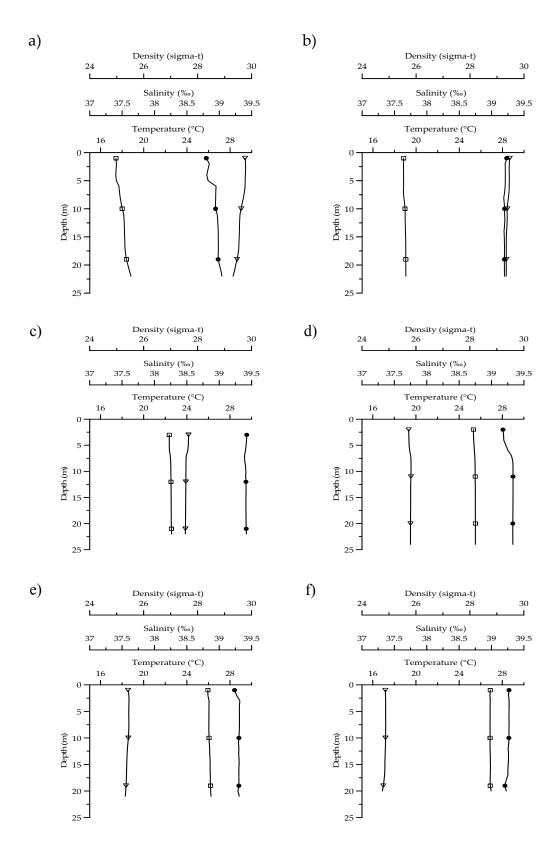
Monthly changes in temperature, salinity, and density at the stations were presented in this part, together with the Secchi disk depths.

### 3.1.1. Temperature

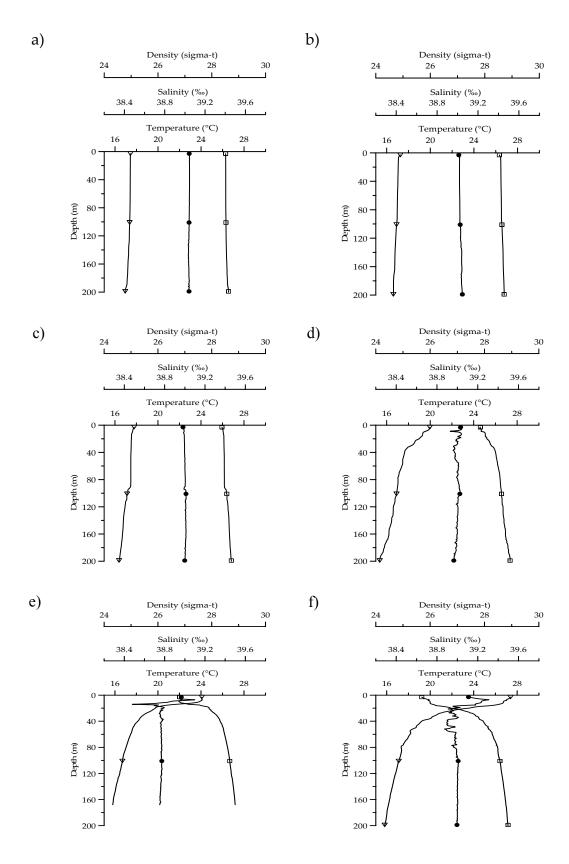
Surface temperature of the coastal shallow station ranged between 16 - 29.4 °C, being coldest in February and hottest in August, over the course of the study period. In February, intrusion of colder fresh water from the nearby local Lamas river to the top few meters at surface was evident, with low surface temperature and low salinity values at the first station (Figure 3.1a). A reverse situation was observed during March and April where the top few meters had slightly higher temperatures compared to lower depths (Figure 3.2b & c). In May, a temporary thermocline formation was observed at around 15 m depth. The top 15 m also had the lowest salinities compared to the rest of the year. This was mainly due to the increasing Lamas river input enriched with melting snow waters of the Taurus Mountains during spring. In June, the water column temperature showed almost a uniform distribution with depth. In July, in contrast to the increase in temperature of the surface waters, an apparent decrease in salinity due to Lamas river input was observed. However, in general, an increase in average water column temperature from February towards August and a decrease from September to January was clearly illustrated in Figure 3.3 and 3.4. Changes in profiles were minimum during autumn and winter. Temperature of the water column reached its initial low level of 17.1 °C in January.



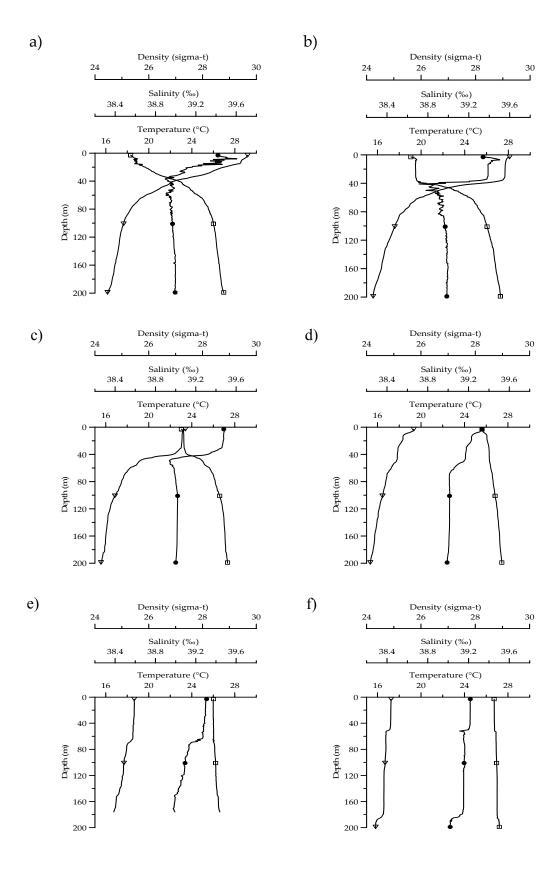
**Figure 3.1.** Monthly temperature (—¬¬—), salinity (—¬—), and density (—¬—) profiles of first station; February (a), March (b), April (c), May (d), June (e), July (f).



**Figure 3.2.** Monthly temperature (—¬¬), salinity (—¬), and density (—¬) profiles of first station; August (a), September (b), October (c), November (d), December (e), January (f).



**Figure 3.3.** Monthly temperature (—¬¬—), salinity (—¬—), and density (—¬—) profiles of second station; February (a), March (b), April (c), May (d), June (e), July (f).



**Figure 3.4.** Monthly temperature (—¬¬), salinity (—•—), and density (—¬) profiles of first station; August (a), September (b), October (c), November (d), December (e), January (f).

Surface and bottom values of the second station was summarized together with the average values of temperature in the Table 3.1. Over the year, sea surface temperature varied in the range 17.25 – 29.18 °C at the second station (Figure 3.3 and 1.4) being coldest in January (Figure 3.4f) and hottest in August (Figure 3.4a). The temperature profiles for the water column during January, February and March were nearly the same from surface to bottom due to winter mixing (Figure 3.3a and b). Except winter, the shelf waters were stratified throughout the year. Surface waters start to warm up during spring and summer. Thermocline forms at about 15 m depth during June (Figure 3.3e). This further deepens to a depth of 20 m in July (Figure 3.1f). In August, surface temperature reaches a maximum of 29.18 °C in August (Figure 3.4a). A well defined surface mixed layer extending to a depth of 40 m was observed in September. This was also true for the October with cooler surface mixed layer. With further cooling of the surface waters in late autumn and early winter, the stratification weakened and the thermocline perished. Shelf waters became uniformly mixed from surface to the bottom with the onset of strong winds and heavy storms during winter.

3

**Table 3.1.** The surface, bottom, and water column averages for each month for the temperature, salinity and density at second station.

	Temperature (°C)				Salinity (psi	1)	Density		
Date	Surface	Bottom	Average	Surface	Bottom	Average	Surface	Bottom	Average
10 February 2005	17.43	16.94	17.31	39.04	39.04	39.04	28.51	28.63	28.54
25 March 2005	17.26	16.61	16.88	39.01	39.05	39.03	28.53	28.71	28.64
21 April 2005	17.78	16.35	17.11	38.98	39.00	39.00	28.38	28.74	28.56
13 May 2005	19.97	15.34	17.02	39.03	38.97	39.00	27.85	28.95	28.57
12 June 2005	24.07	15.78	17.58	38.96	38.75	38.77	26.81	28.87	28.44
2 July 2005	27.39	15.80	18.18	39.11	38.99	39.00	25.69	28.87	28.26
2 August 2005	29.18	16.13	19.50	39.42	38.99	39.02	25.32	28.79	27.91
7 September 2005	28.12	15.59	19.55	39.34	38.98	39.04	25.62	28.91	27.89
27 October 2005	23.40	15.55	18.05	39.48	39.00	39.09	27.21	28.93	28.37
27 November 2005	19.33	15.32	16.69	39.33	38.99	39.06	28.25	28.98	28.71
29 December 2005	18.65	16.74	17.90	39.31	38.99	39.14	28.41	28.65	28.47
30 January 2005	17.25	15.83	16.73	39.22	39.02	39.16	28.69	28.88	28.77

### **3.1.2. Salinity**

Compared to the offshore station, almost no monthly trend in salinity profiles of the shallower first station was observed. Throughout the study, the surface salinity ranged between 37 and 39.5 psu at the first station. The minimum and maximum values were recorded in March and October, respectively (Figure 3.1b and Figure 3.4c). The invasion of the freshwater was observed at the top few meters of the water column at the first station in February and March (Figure 3.1a and b). Considering the water column averages, it could be concluded that the station received input from local Lamas River most intensively between March and July. The average water column salinity remained higher in the second half of the year (from August to December as well as in January and February. The freshwater input from the nearby Lamas River appeared to play a major role in the heat as well as the salinity budget of the water column at the sampling site. Since the total depth was about 20 m, it was difficult to talk about stratification at the site. An apparent temporary thermocline as well as a halocline formation could not be observed throughout the year.

Salinity of the surface waters ranged from a minimum of 38.96 in June to a maximum of 39.48 in October at the second station (Figure 3.3e and Figure 3.4c). On the other hand, the salinity of the bottom waters at 200 m depth stayed constant at around 39 psu. Salinity profiles resembled much of a straight line during February, March and April due to mixing of the water column. (Figure 3.3a, b, and c). In May, a slight increase in salinity of the surface waters was observed in parallel to increase in temperature (Figure 3.3d). Compared to previous months, besides abrupt fluctuations in salinity of the surface waters, an apparent decrease in the average water column salinity was observed during June. This reduction however was replenished during July. In August, a further increase in salinity of the stratified surface waters in parallel to increase in temperature was observed. Below 40 m the salinity of the water column remained stable. A sharp halocline was observed at around 40 m depth in September and October (Figure 3.4b and c).

In October, surface salinity reached a maximum level of 39.48 psu. Stratification continued to weaken during November and December. From October to December, a deepening of the halocline from about 40 m to almost 70 m was observed. Salinity profile in January appeared more homogenous due to winter mixing. The salinity values measured through out the study period at the surface, bottom, and the water column averages were shown in the Table 3.1.

## **3.1.3. Density**

The monthly density profiles for the first station were shown in Figure 3.1 and 3.2. Density of the surface waters ranged from a minimum of 24.97 in August to a maximum of 28.75 in January at the first station. In general, the surface densities remained lower compared to those at lower depths during the first half of the year. During the second half of the year, a rather more homogenous distribution with depth was observed. Density of the water column was lowest during the hottest period of the year (August and September).

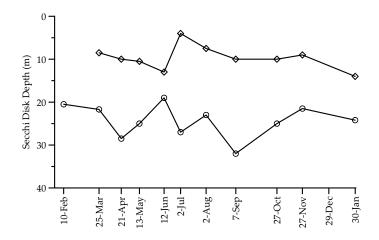
The monthly density profiles for the second station were shown in Figure 3.3 and 3.4. Density of the surface waters ranged from a minimum of 28.32 in August to a maximum of 28.69 in January at the second station (Figure 3.4). Against the fluctuations in water column densities the near-bottom densities stayed almost the same over the year. Changes in water column density profiles were minor from February to April. Starting from May, warming of the surface waters towards summer led for a decrease in density of the surface waters till August. Formation of a well-defined surface mixed layer was observed in September and October. With the onset of winter conditions (cooling of surface waters, strong winds) the density of the surface waters continued to increase from November until January in parallel to weakening stratification. A homogenous water column density was established from January till April. Density values of the second station were also shown in the Table 3.1, for surface and bottom waters, together with the water column averages.

## 3.1.4. Secchi Depths

The Secchi disk depths were generally measured at noon (11:00 - 12:00 am) at the second station and in the afternoon (15:00-16:00) at the first station. In February, at the first station, the secchi disk depth could not be measured, since the arrival to the first station was after the sunset. It was also could not be measured during the December cruise due to the high waves.

The annual average Secchi disk depth (SDD) was 9.65 m, being the lowest (4 m) in July and the highest (14 m) in January at the first station. Data was missing for February, since arrival to the station was after sunset. Secchi disk depths increased towards June, followed by a minimum value in July. In September and October, secchi disk depths were measured the same as 10 m. Except a slight decrease in November, it increased towards the end of the study period.

The Secchi disk depth varied between 19 m and 32 m at the second station (Figure 3.5). The minimum and maximum values were recorded in June and in September, respectively. The average Secchi disk depth was 24.3 m throughout the study period. This average level was exceeded during April, May, July, September, and October. For the rest of the period, Secchi disk depths remained below the average value.



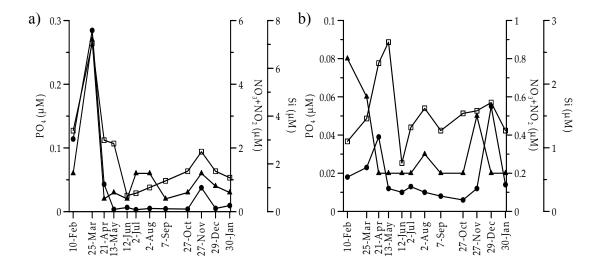
**Figure 3.5.** Changes in the Secchi disk depths of both first  $(-\circ-)$  and second  $(-\circ-)$  stations throughout the study.

#### 3.2. Chemical Parameters

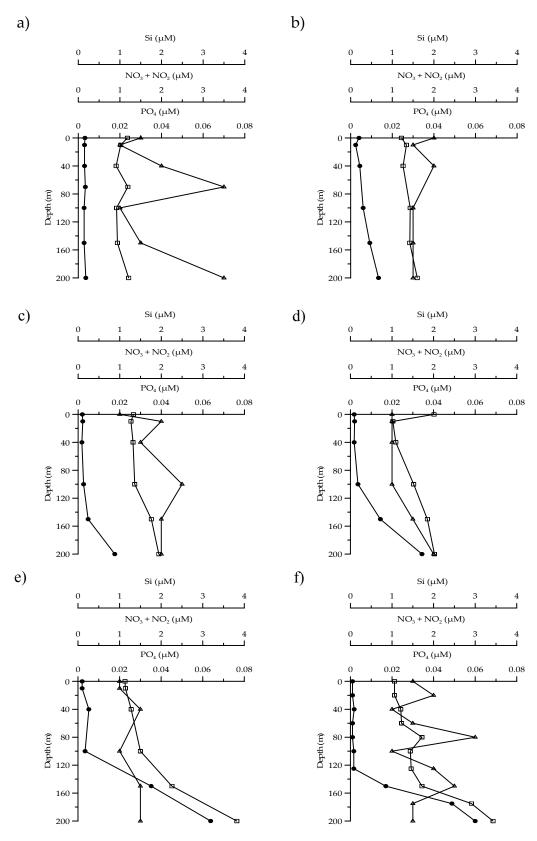
Monthly changes in nutrient salts (phosphate, nitrate, and silicate) and dissolved oxygen measurements were presented in this part for each station.

#### 3.2.1. Nutrient Salts

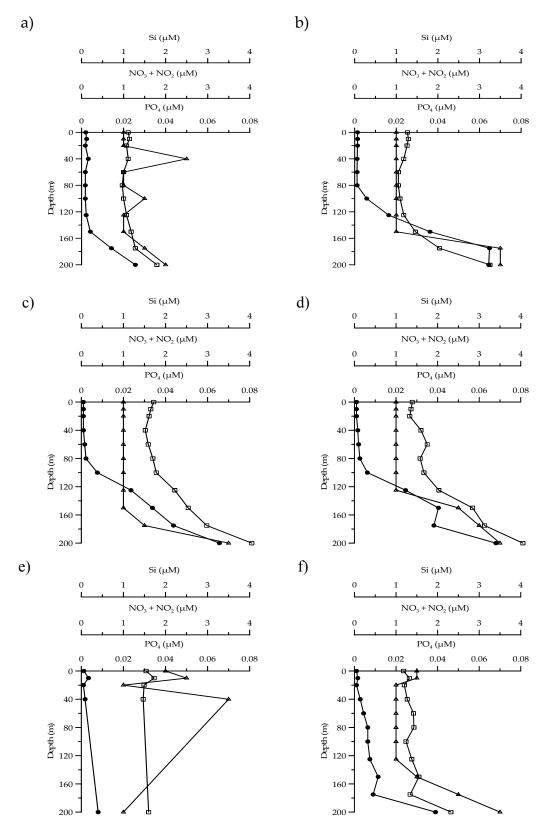
**Phosphate** concentration at the surface of the first station, increased significantly to a level of 0.27  $\mu$ M in March which was then followed by a sharp decline (0.02  $\mu$ M) in April (Figure 3.6a). This minimum concentration was also obtained in June. Despite the slight increases in July and August, it again decreased to 0.02  $\mu$ M in September. The concentrations again continued to increase with a peak level observed in November, then again decreased to its initial low surface concentration in January. At 10 m depth, phosphate concentration made a peak in February (0.08  $\mu$ M). It gradually declined to 0.02  $\mu$ M in the following two months and stayed at that level throughout the study period, except the higher levels obtained in August and November.



**Figure 3.6.** Annual nutrient salts changes of first stations at 0 m (a) and 10 m (b) throughout the study,  $PO_4$  (--),  $NO_3+NO_2$  (--), and Si (--), note scales.



**Figure 3.7.** Monthly PO<sub>4</sub> (—•—), NO<sub>3</sub>+NO<sub>2</sub> (—•—), and Si (—•—) profiles of second station; February (a), March (b), April (c), May (d), June (e), July (f).



**Figure 3.8.** Monthly PO<sub>4</sub> (—•—), NO<sub>3</sub>+NO<sub>2</sub> (—•—), and Si (—•—) profiles of second station; August (a), September (b), October (c), November (d), December (e), January (f).

The annual mean concentration for phosphate in the water column was 0.03 µM in the second station. Phosphate concentrations ranged between a surface minimum of 0.02 µM to a maximum of 0.07 µM measured at deeper depths during winter. In February, phosphate concentration showed two peaks at 75 and 200 m and lows at 10 and 100 m depths respectively (Figure 3.7a). The near-bottom concentration was as high as 0.07 µM. Compared to February, phosphate concentration was more homogenously distributed in the water column during March. The surface concentration was low in April and increased slightly with depth while fluctuating. In May, phosphate concentration was 0.02 µM at first 100 meters, then increased to 0.04 µM at 200m (Figure 3.7d). Phosphate concentration in June showed minor highs (40 and 150 m) and lows (surface, 10 and 100 m) with depth. Although the surface and bottom concentrations were the same, concentrations fluctuated significantly with depth in July. In August, it showed a major peak at 40 m and two more increases at 100 and 200 m, 150 the PO<sub>4</sub> concentrations made peaks at 40 m, 100 m, and after 150m depths. In both September and October, the phosphate concentrations were as low as 0.02 µM from surface to 150 m depth, and increased sharply to 0.07 µM below. Similar profile was also observed in November. The top 125 meters of the water column had a concentration of 0.02 μM phosphate and then increased to a value of 0.07 μM at 200 m depth. In December, due to harsh weather conditions and related technical problems the depths between 40-200 m could not be sampled (Figure 3.8e). December was the only month having relatively high surface values with lower bottom concentrations when the second half of the year was considered. At the end of the study period, the surface concentration reached its initial value of 0.03 µM (Figure 3.8f). Phosphate values remained at 0.02 µM between 20 and 125 m and continued to increase towards bottom in January.

The **nitrate** concentration at the surface of the first station, ranged between a minimum of  $5.69 \,\mu\text{M}$  in March and a maximum of 0.06 in July. Initially, very high nitrate concentrations were obtained in February and March (Figure 3.6a). In March, an enormous increase in all three nutrients indicating freshwater input from local Lamas River to the station was observed (see also Figure 3.1b for surface

freshwater intrusion to the site). From May to October, surface concentrations remained very low. Another small pulse in nutrient levels was also observed in November. Compared to surface, concentrations remained remarkably lower at 10 m depth (Figure 3.6b). Only two major increases, one in April (0.39  $\mu$ M) and the other one in December (0.55  $\mu$ M) were observed at 10 m throughout the year.

In the offshore station, nitrogen concentration showed a minimum during winter  $(0.05 \mu M)$  and a maximum at 200 m in November  $(3.40 \mu M)$  at the surface waters (Figure 3.7 and 3.8). Despite the low levels of nitrogen observed at surface, especially in summer and autumn, deep maximum values measured in the water column. Except February, an increase in nitrate concentration with depth was observed over the year. In February, the water column average was as low as 0.15 μM. Beginning from March, nitrate concentrations started to increase with depth. In March, nitrate concentration continued to increase below 10 m towards the bottom (Figure 3.7b). In April, nitrate concentration was stable at the first 40 m and then showed a gradual increase at lower depths (Figure 3.7c). Same pattern was observed in May, except from that the increasing trend had a larger magnitude than it observed in April (Figure 3.7d). In June, there existed a slight increase in the concentration at 40 m and a decrease at 100 m (Figure 3.7e) which was further followed by a distinguished increase towards the bottom. In July, the top 125 m was almost denuded of nitrate with wealthy of nitrate stock present underneath (Figure 3.7f). The 40 m peak was very weak but still existed. In August, a gradual decrease in the bottom concentration compared to previous and following months was observed (Figure 3.8a). Concentrations remained almost the same from surface the 80 m and then increased gradually with depth in September (Figure 3.8b). Nitrate profiles were almost the same for the autumn period except for a slight decrease observed at 175 m during November. Since December had some missing data, only concentrations for five sampling depths were plotted (Figure 3.8e). From the available data, a slight increase from surface to bottom could be observed. In January, a gradual increase in middepths with a slight decrease in the concentration at 175 m depth (Figure 3.8f) was observed.

Silicate concentrations measured as the maximum and minimum at the surface of the first station were 7.04  $\mu$ M and 0.66  $\mu$ M, respectively (Figure 3.6). The average Si concentration was 2.28  $\mu$ M at the surface of the first station (Figure 3.6a). Much higher silicate concentrations were measured from February to May might be due to increasing freshwater input from the Lamas river. A rapid decline in June followed by a steady increase in silicate concentration at surface till November. It than slightly decreased in December and January. The annual average for silicate at 10 m depth was 1.55  $\mu$ M, which was much lower than the surface average (Figure 3.6b). Concentrations at 10 m showed an appreciable increase from February to May (to a highest concentration of 2.66  $\mu$ M was reached in May at 10 m). This high value followed by a very sharp decline in June (0.76  $\mu$ M). For the rest of the period silicate concentrations remained at annual average levels with minor fluctuations.

In the offshore station, the silicate concentration ranged between 0.91  $\mu$ M and 4  $\mu$ M in the water column during the study period (Figure 3.7 & Figure 3.8). The annual average for the water column was 1.57  $\mu$ M. From February to July, a gradual increase in the silicate content of the water column was observed. Silicate concentrations were much higher at lower depths in June and July. Silicate seemed to be depleted homogenously in the water column with slightly higher concentrations near the bottom in August. Profiles remained almost unchanged during the autumn. The annual maximum of Si also reached in November at 200 m (Figure 3.8d). Significant fluctuations in silicate content with depth were observed in December. Silicate concentration was almost homogenously distributed at the top 175 m in January with a relatively higher concentration at 200 m (Figure 3.8f).

# 3.2.2. Dissolved Oxygen

Dissolved oxygen (DOW) concentrations at the first station ranged between 200.5  $\mu$ M and 256.3  $\mu$ M at the surface and from 200.1  $\mu$ M to 250.3  $\mu$ M at 10 m (Figure 3.9). DOW content of the water column made peak in March. Overall, a decreasing trend in concentrations at both depths was observed from March to September. The surface DOW content was at its lowest level of 200.5  $\mu$ M in September. Concentrations then continued to increase gradually till January.

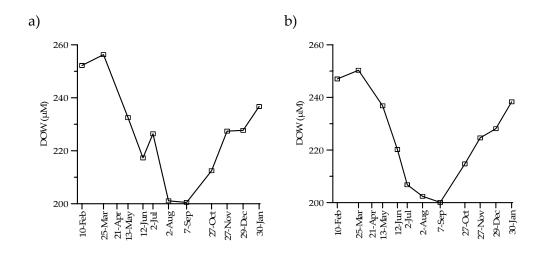
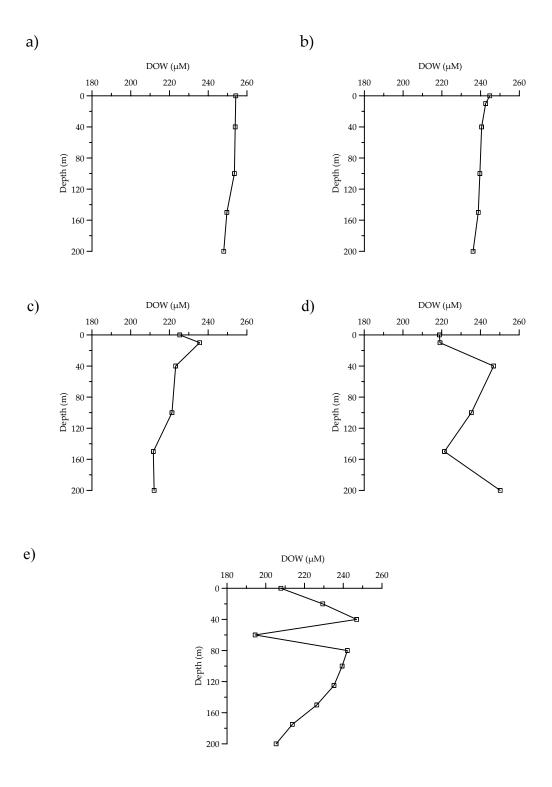
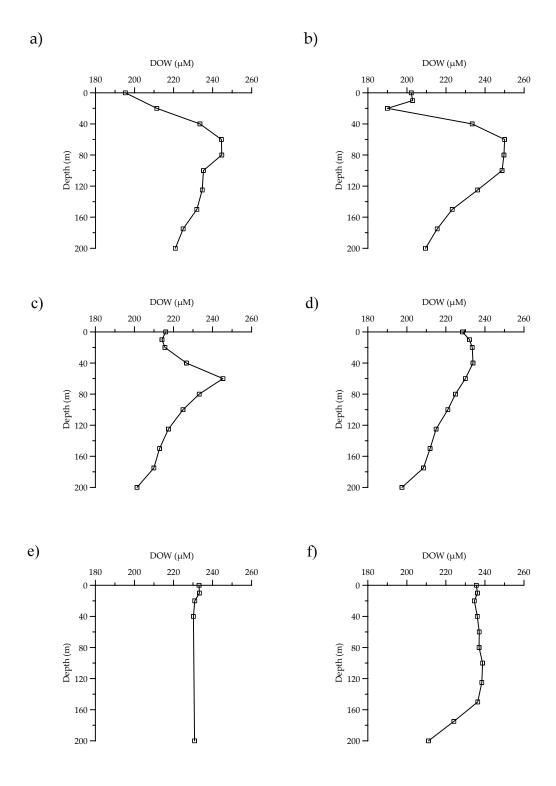


Figure 3.9. Annual DOW (——) profiles of first station at 0 m (a) and 10 m (b)

The maximum DOW concentration was  $254.2~\mu M$  in February at the surface, and the minimum value was  $190~\mu M$  in September at 20~m depth (Figure 3.10~and 3.11). The annual average for the water column was  $228~\mu M$ . From February to May, DOW content of the water column was high and decreased slightly with depth. Significant fluctuations with depth were observed in June and July. DOW submaximum was observed till October. A slight increase was observed at the upper layers, from surface to 40~m in November (Figure 3.11d). The values then gradually declined towards the bottom to its lowest level for the whole study period. In December, regardless of the data gap, surface and bottom values were very close to each other (Figure 3.11e). In January, the DOW concentration was nearly the same from surface to 150~m depth (Figure 3.11f) and declined below.



**Figure 3.10.** Monthly DOW (—•—) profiles of second station; February (a), March (b), May (c), June (d), July (e), note no data in April.



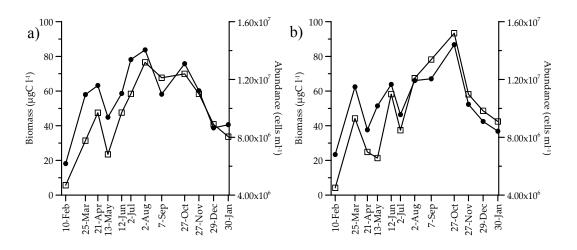
**Figure 3.11.** Monthly DOW (—•—) profiles of second station; August (a), September (b), October (c), November (d), December (e), January (f).

#### 3.3. Biological Parameters

The abundance and biomass distribution of both heterotrophic bacteria and *Synechococcus* were presented in this part at the first and second station.

### 3.3.1. Heterotrophic Bacteria

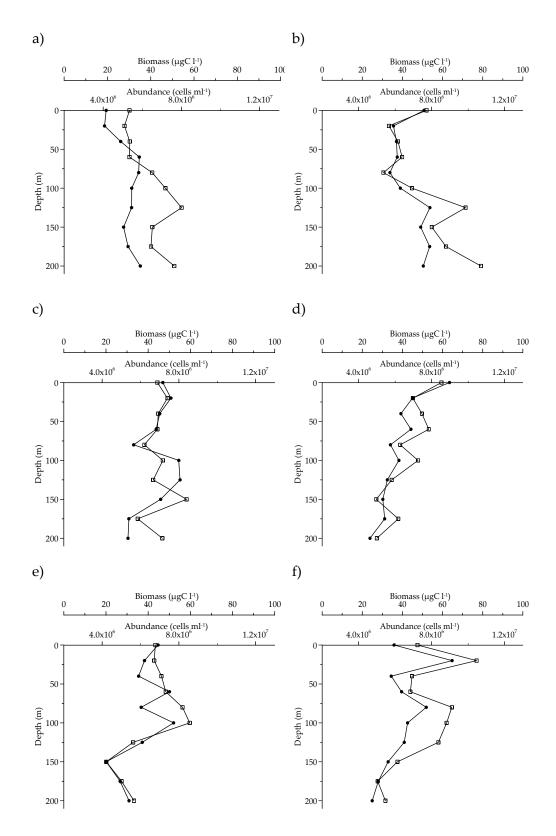
At the first station, the minimum and maximum biomass values ranged between 18.2 μg C l<sup>-1</sup> at surface in February and 86.7 μg C l<sup>-1</sup> at 10 m in October, respectively (Figure 3.12). To a lowest and highest abundance values were obtained in February (4.5x10<sup>6</sup> cells ml<sup>-1</sup>) and October (1.5x10<sup>7</sup> cells ml<sup>-1</sup>) at 10 m depth. The annual average for surface biomass was 56.5 μg C l<sup>-1</sup> and the maximum abundance value obtained was 9.6x10<sup>6</sup> cells ml<sup>-1</sup> (Figure 3.12a). Both the biomass and abundance values remained above the annual average value for most of the year. At 10 m, heterotrophic bacteria were more abundant from late summer to early winter (Figure 3.12b). Biomass values were also had high values during this period. Both parameters showed a sharp increase in March, just after the very low levels of biomass and minimum abundance values observed in February.



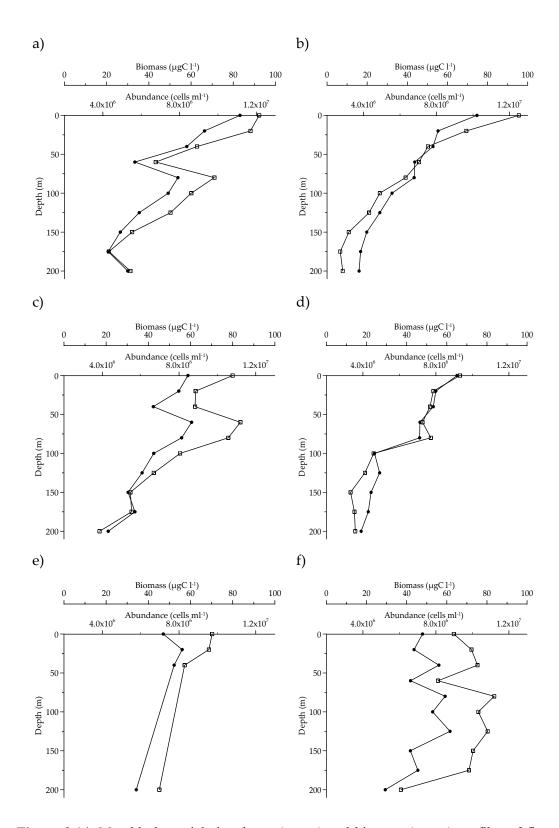
**Figure 3.12.** Changes in bacterial biomass (—•—) and abundance (—•—) in time at surface (a) and at 10 m depth (b) at first station.

Monthly changes in bacterial abundance and biomass with depth at second station were displayed in Figure 3.13 and 3.14. The minimum, maximum and water column averages for each month were given in Table 3.2.

Bacterial abundance and biomass varied distinctly in time at the stations throughout the study period. Bacterial abundance increased gradually from 5.3 ×  $10^6$  cells ml<sup>-1</sup> at surface to  $8 \times 10^6$  cells ml<sup>-1</sup> at 125 m depth in February. This was followed by a decrease in numbers down to 175 m  $(6.4 \times 10^6 \text{ cells ml}^{-1})$  and again with an increase both in numbers and biomass towards the bottom. Despite the increase in numbers, the biomass did not increase simultaneously at lower depths. Compared to February a much greater population was observed at surface waters in March. The abundance profile below 80 m mimicked that of February except with a much greater magnitude in March. In April, the abundance profile showed minor fluctuations from surface to a depth of 125 m. A sharp decrease from a peak level of  $8.4 \times 10^6$  cells ml<sup>-1</sup> at 150 m to  $5.9 \times 10^6$  cells ml<sup>-1</sup> at 175 was observed at near bottom. In May a gradual decrease in bacterial abundance as well as biomass from surface  $(8.5 \times 10^6 \text{ cells ml}^{-1})$  to bottom  $(5 \times 10^6 \text{ cells ml}^{-1})$  with minor fluctuations was observed. Bacterial abundance was found higher at mid-depths in June. A sharp reduction in numbers as well as in biomass from 100 m to 150 m was an important feature of this month. Below this depth, the population continued to increase towards the bottom. Two submaxima, one at 20 m and the other at 80 m depths were observed in July. In general, higher biomass and abundance values were obtained at lower depths during the first half of the study period.



**Figure 3.13.** Monthly bacterial abundance (—•—) and biomass (—•—) profiles of first station; February (a), March (b), April (c), May (d), June (e), July (f).



**Figure 3.14.** Monthly bacterial abundance (—•—) and biomass (—•—) profiles of first station; August (a), September (b), October (c), November (d), December (e), January (f).

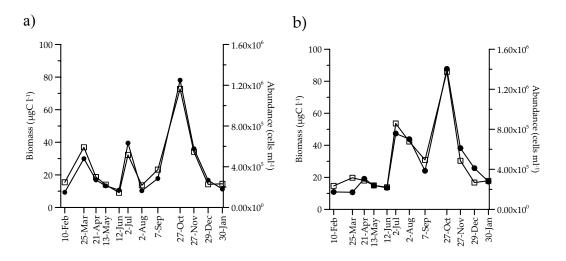
Bacterial population was found excessively dominant at the surface or near-surface waters during the second half of the year. Bacterial abundance and biomass profiles showed similar trends from surface to bottom in August. In addition, at surface, bacterial biomass reached the highest level of the year (83.1 µg C 1<sup>-1</sup>). In the following month, bacterial abundance reached a peak level of  $1.25 \times 10^7$  cells ml<sup>-1</sup> at the surface. An appreciable decline from a very high surface value to a very low bottom value was observed in September. In October, the surface maximum followed by a secondary maximum (1.1 x 10<sup>7</sup> cells ml<sup>-1</sup>) at 60 m depth, and underneath, a gradual decrease in abundance and biomass towards bottom was observed. Changes were less pronounced in the top 80 m in November. A sharp reduction in both bacterial abundance and biomass from 80 m (7.7 x 10<sup>6</sup> cells ml<sup>-1</sup>) to 100 m depth (4.6 x 10<sup>6</sup> cells ml<sup>-1</sup>) was also observed in this month. Minor changes were observed below 100 m depth. Since the sampling depths were few for December, one can only suggest that the population was slightly more abundant at near-surface waters than the bottom. Although it coincided with the mixing of the water column major fluctuations in abundance and biomass were observed with depth in January. The population was found more abundant at middepths and less at the bottom.

**Table 3.2.** The minimum, maximum and water column averages for each month for the heterotrophic bacteria and *Synechococcus* at second station.

	Heterotrophic Bacteria						Synechococcus					
	Biomass		Abundance		Biomass		Abundance					
Date	max	min	avg	max	min	average	max	min	avg	max	min	average
10 Feb												
2005	34.70	18.67	28.79	$8.01 \times 10^6$	$5.09x10^6$	$6.34 \times 10^6$	10.72	4.46	7.34	$2.47x10^5$	$1.19 \times 10^5$	$1.76 \times 10^5$
25 Mar												
2005	53.70	33.77	44.03	$1.07 \text{x} 10^7$	$5.36 \times 10^6$	$7.56 \times 10^6$	30.79	3.81	12.32	$3.38 \times 10^5$	$9.66 \times 10^4$	$2.30 \times 10^5$
21 Apr												
2005	55.11	30.43	43.69	$8.40 \times 10^6$	$5.86 \times 10^6$	$6.95 \times 10^6$	15.53	2.11	8.60	$3.01 \times 10^5$	$7.46 \times 10^4$	$1.81 \times 10^5$
13 May												
2005	63.46	23.83	38.19	$8.54 \times 10^6$	$4.97x10^6$	$6.63 \times 10^6$	12.15	4.61	7.51	$1.69 \times 10^5$	$7.10 \text{x} 10^4$	$1.12x10^5$
12-Jun-												
05	52.04	20.10	37.21	$8.57 \times 10^6$	$4.22 \times 10^6$	$6.52 \times 10^6$	15.01	3.28	10.01	$2.54 \times 10^5$	$7.83 \times 10^4$	$1.37x10^5$
2 Jul												
2005	64.76	24.94	39.57	$1.05 \times 10^7$	$5.04 \times 10^6$	$7.44 \times 10^6$	13.06	2.32	8.11	$2.75 \times 10^5$	$7.22 \times 10^4$	$1.48 \times 10^5$
2 Aug											_	
2005	83.18	26.49	45.65	$1.21 \text{x} 10^7$	$4.32 \times 10^6$	$8.08 \times 10^6$	7.54	0.03	4.90	$1.59 \times 10^5$	$7.15 \times 10^2$	$9.66 \times 10^4$
7 Sep											_	_
2005	74.71	15.98	38.11	$1.25 \times 10^7$	$2.73x10^6$	$6.10 \times 10^6$	9.07	1.99	5.06	$1.84 \times 10^5$	$4.65 \text{x} 10^4$	$1.07 \times 10^5$
27 Oct												
2005	58.77	20.97	43.65	$1.12 \times 10^7$	$3.85 \times 10^6$	$7.99 \times 10^6$	17.93	2.13	6.64	$3.08 \times 10^5$	$5.38 \times 10^4$	$1.33 \times 10^5$
27 Nov												
2005	65.07	17.31	37.69	$9.32 \times 10^6$	$3.33x10^6$	$5.90 \times 10^6$	25.16	0.01	4.95	$3.40 \times 10^5$	$3.01 \times 10^2$	$8.30 \times 10^4$
29 Dec												
2005	56.04	34.28	47.41	$9.73x10^6$	$6.97x10^6$	$8.64 \times 10^6$	15.83	0.12	11.49	$2.86 \times 10^5$	$2.75 \times 10^3$	$2.00 \times 10^5$
30 Jan												
2006	59.18	29.32	48.01	$1.12x10^7$	$6.08x10^6$	$9.56 \times 10^6$	16.95	4.66	14.22	$2.86 \times 10^5$	$7.71 \times 10^4$	$2.16 \times 10^5$

### 3.3.2. Synechococcus

Since the first station was too shallow, and only two depths were sampled, changes in abundance and biomass of *Synechococcus* at surface and at 10 m depth in time were illustrated in Figure 3.15a and b. At surface, *Synechococcus* concentrations were in the range  $1.4 \times 10^5 - 1.1 \times 10^6$  cells ml<sup>-1</sup>. The annual average for this depth was  $4 \times 10^5$  cells ml<sup>-1</sup> with a maximum of  $1.1 \times 10^6$  cells ml<sup>-1</sup> recorded in October. Major peaks were observed in March, July and October. Despite the three major peaks observed at surface, only two peaks were observed at 10 m depth. Population was found most abundant during July, August, October and November at 10 m depth.

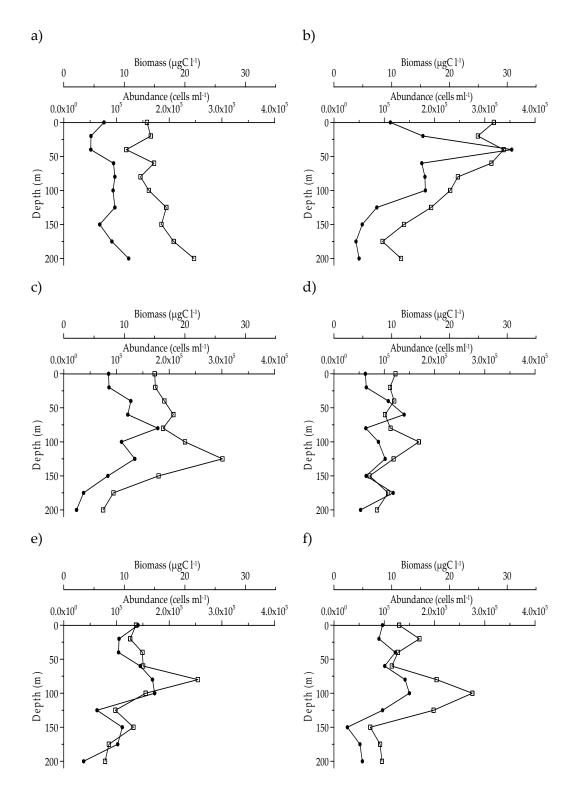


**Figure 3.15.** Changes in *Synechococcus* biomass (—•—) and abundance (—•—) in time at surface (a) and at 10 m depth (b).

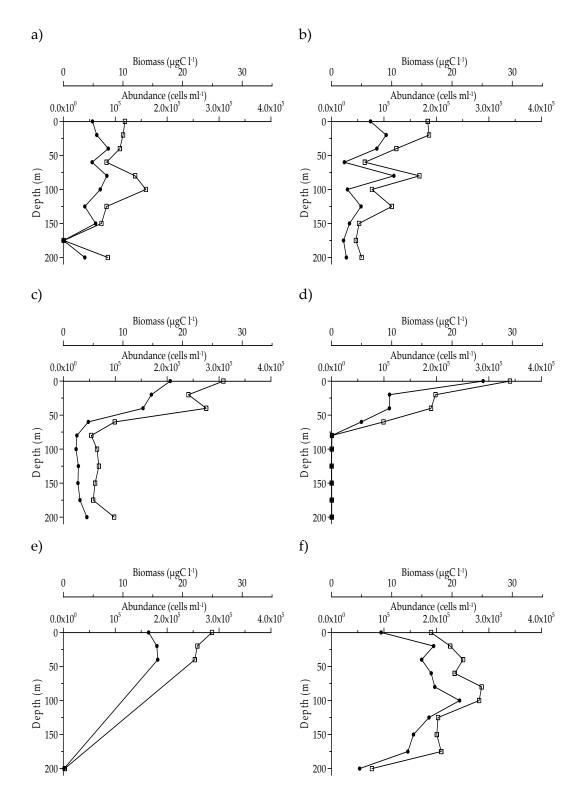
Monthly changes in *Synechococcus* abundance and biomass with depth at second station were displayed in Figure 3.16 and 3.17. The minimum, maximum and water column averages for each month were given in Table 3.2.

*Synechococcus* abundance increased gradually from  $1.6 \times 10^5$  cells ml<sup>-1</sup> at surface to  $2.5 \times 10^5$  cells ml<sup>-1</sup> at 200 m depth in February with minor fluctuations at mid-depths. The situation reversed in the following month and much higher

counts at surface waters were obtained in March. Cell counts made a peak at 40 m depth  $(3.4 \times 10^5 \text{ cells ml}^{-1})$ . Below 40 m, both the abundance and biomass values decreased significantly. In April, the population showed a submaximum at 125 m where the maximum for biomass was at 80 m depth. Similarly in May, to a maximum abundance and biomass were reached at 100 and 60 m depths, respectively. Compared to May, the magnitude of variation in cell numbers and biomass with depth was greater in June and July. The maxima for abundance and biomass were observed at 80 and 100 m depths respectively. The maxima for both parameters occurred at 100 m depth in July. Population was almost twice as much at 100 m depth than that observed at the surface. A gradual increase in population abundance and biomass from 150 m to 200 m was also observed in this month. The magnitude of variation with depth was less in August compared to July. Most of the population was found at 100 m depth and it almost disappeared at 175 m depth. With the onset of autumn, the surface population became more abundant and cell counts below 80 m depth were negligible in October and in November. Due to lack of intermediate depths, it is hard to suggest the same for December. In January, an increase in population from surface to middepths (80-100 m) and a decrease towards bottom was observed.



**Figure 3.16.** Monthly *Synechococcus* abundance (—•—) and biomass (—•—) profiles of first station; February (a), March (b), April (c), May (d), June (e), July (f).



**Figure 3.17.** Monthly *Synechococcus* abundance (—•—) and biomass (—•—) profiles of first station; August (a), September (b), October (c), November (d), December (e), January (f).

#### 3.4. Statistical Analyses

Initially each parameter was checked whether the data was distributed normally or not. This was done by applying the test of randomness for each parameter. Since none of the data set was normally distributed, for the correlation analyses, non-parametric Spearman rank-order correlation test was applied to look for relationships among biological parameters and ambient physical and chemical variables.

Highly significant correlations (p < 0.01) between bacterial abundance as well as biomass with temperature and density were found at the first station (Table 3.3). Bacterial abundance correlated positively with temperature and negatively correlated with density. Bacterial biomass was also found to be negatively correlated (p < 0.05) with Secchi disk depth. However, no correlation with salinity was found at the first station. On the other hand, both bacterial biomass and abundance were found highly positively correlated (p < 0.01) with temperature and salinity and negatively correlated with density at the second station, but no significant relation with Secchi disk depth was observed. *Synechococcus* abundance and biomass showed no correlation with any of the physical parameters at the first station. However, highly significant positive correlations (p < 0.01) between *Synechococcus* abundance and biomass with temperature and salinity were found at the second station. Density was negatively correlated with the *Synechococcus* abundance and biomass at p < 0.01 level. Secchi disc depth showed no correlation with *Synechococcus*.

**Table 3.3.** Correlations between the biological parameters and the physical parameters at station # 1 & #2 during study period.

		Station # 1			
		Temperature (°C)	Salinity (psu)	Density (sigma-t)	Secchi Disc (m)
Heterotrophic Bacteria Abundance	φ σ Ν	0.726(**) 0.000 22	0.396 0.068 22	-0.549(**) 0.008 22	-0.431 0.214 10
Heterotrophic Bacteria Biomass	φ σ Ν	0.732(**) 0.000 22	-0.034 0.879 22	-0.708(**) 0.000 22	-0.681(*) 0.030 10
Synechococcus Abundance	φ σ Ν	0.173 0.440 22	0.108 0.633 22	-0.259 0.244 22	-0.411 0.238 10
Synechococcus Biomass	φ σ Ν	0.368 0.092 22	0.259 0.244 22	-0.278 0.210 22	-0.399 0.254 10

Station # 2						
Temperature (°C)	Salinity	Density	Secchi			
	(psu)	(sigma-t)	Disc (m)			
0.503(**)	0.390(**)	-0.436(**)	0.396			
0.000	0.000	0.000	0.228			
111	111	111	11			
0.590(**)	0.326(**)	-0.526(**)	0.232			
0.000	0.000	0.000	0.492			
111	111	111	11			
0.378(**)	0.432(**)	-0.306(**)	-0.087			
0.000	0.000	0.001	0.800			
111	111	111	11			
0.353(**)	0.282(**)	-0.272(**)	-0.337			
0.000	0.003	0.004	0.311			
111	111	111	11			

 $\phi$  : Correlation coefficient  $(r_s)$ 

 $\sigma$ : Significance level (p-level)

N : Sample size

\* Correlations are significant at p<0.05

\*\* Correlations are significant at p<0.01

Bacterial abundance and biomass showed no correlation with phosphate and silicate at the first station (Table 3.4). On the other hand, significant negative correlations were found between bacterial abundance and nitrate as well as with dissolved oxygen. Same negative correlation was also observed between bacterial biomass and aforementioned parameters. *Synechococcus* abundance and biomass showed no correlation with chemical parameters at the first station. At the second station, bacterial abundance and biomass were negatively correlated (p < 0.01) with phosphate, nitrate and silicate. No correlation was observed with dissolved oxygen. Phosphate showed no correlation with *Synechococcus* abundance and biomass at the second station. *Synechococcus* abundance and biomass were found negatively correlated (p < 0.01) with nitrate.

Abundance also found positively correlated with silicate and negatively correlated with dissolved oxygen. *Synechococcus* biomass showed negative correlation (p < 0.01) with silicate and positive correlation with dissolved oxygen. At first station, bacterial abundance was found positively correlated (p < 0.01) with *Synechococcus* abundance (p < 0.05) (Table 3.5). At the second station, bacterial biomass and abundance were highly correlated with *Synechococcus* abundance and biomass.

**Table 3.4.** Correlations between the biological parameters and the chemical parameters at station # 1 & #2 during study period.

		Station # 1				
		PO4	NO3+NO2	Si	DOW	
Heterotrophic	φ	-0.191	-0.547(**)	-0.391	-0.801(**)	
Bacteria Abundance	σ N	0.371 24	0.006 24	0.059 24	0.000 22	
Heterotrophic	φ	-0.042	-0.529(**)	-0.289	-0.636(**)	
Bacteria Biomass	σ N	0.844 24	0.008 24	0.170 24	0.001 22	
Synechococcus	φ	0.026	-0.108	0.223	-0.224	
Abundance	σ	0.905	0.615	0.296	0.316	
	N	24	24	24	22	
Synechococcus	φ	-0.190	-0.296	0.122	-0.409	
Biomass	σ	0.373	0.160	0.571	0.059	
	N	24	24	24	22	

Station # 2							
PO4	NO3+NO2	Si	DOW				
-0.320(**)	-0.616(**)	-0.311(**)	0.209				
0.002 89	0.000 89	0.003 89	0.057 84				
-0.368(**)	-0.667(**)	-0.310(**)	0.149				
0.000 89	0.000 89	0.003 89	0.177 84				
-0.058	-0.551(**)	0.395(**)	0.351(**)				
0.590	0.000	0.000	0.001				
89	89	84	89				
-0.137	-0.535(**)	-0.342(**)	0.309(**)				
0.199	0.000	0.001	0.004				
89	89	89	84				

N : Sample size

 $<sup>\</sup>begin{array}{l} \phi \ : Correlation \ coefficient \ (r_s) \\ \sigma : Significance \ level \ (p\text{-level}) \end{array}$ 

<sup>\*</sup> Correlations are significant at p<0.05

<sup>\*\*</sup> Correlations are significant at p<0.01

**Table 3.5.** Correlations between the biological parameters with each other at station # 1 & #2 during study period.

		Station # 1				Station # 2			
		Bacterial Abundance	Bacterial Biomass	Synec Abundance	Synec Biomass	Bacterial Abundance	Bacterial Biomass	Synec Abundance	Synec Biomass
Bacterial Abundance	φ	1.000				1.000	0.877(**)	0.522(**)	0.448(**)
	σ N	24				114	0.000 114	0.000 114	0.000 114
Bacterial Biomass	φ		1.000				1.000	0.512(**)	0.454(**)
	σ N		24				114	0.000 114	0.000 114
Synechococcus Abundance	φ	0.468(*)	0.386	1.000				1.000	
	σ N	0.021 24	0.062 24	24				114	
Synechococcus Biomass	φ	0.400	0.370		1.000				1.000
	σ	0.053	0.075						•
	N	24	24		24				114

 $\phi$ : Correlation coefficient ( $r_s$ )

 $\sigma$ : Significance level (p-level)

N : Sample size

\* Correlations are significant at p<0.05

<sup>\*\*</sup> Correlations are significant at p<0.01

### 4. DISCUSSION

The Eastern Mediterranean has some of the world's most optically clear waters (Berman, et al., 1985; Ediger and Yılmaz, 1996a). The depth of subsurface chl-a maximum layer is found as deep as 120 meters, and in some cases, it is deeper than the compensation depth (Ediger and Yılmaz, 1996b). Since the waters flowing through Gibraltar are nutrient depleted Atlantic surface waters, both nitrate and phosphate are limited in the Mediterranean. Although the eastern Mediterranean is characterized by its oligotrophy (Azov, 1991) due to phosphate limitation (Krom, et al., 1991), the Cilician Basin could be regarded as one of the most important region of freshwater influence in the Mediterranean.

First station is located near the coastline. This shallow station, which has a total depth of 20 m, is under great influence of coastal dynamics as well as nearby Lamas River (Uysal, *et al.*, 2004). This river drains a large basin (1055 km²) relative to nearby small streams and the average annual discharge is about 6.7 m³ sec⁻¹ which also greatly differs from nearby streams flowing for only short periods throughout a year (Okyar, 1991). The study area receives freshwater from the Lamas River over most of the year. In addition to the river input, appreciable amounts of nutrients are supplied via the underground freshwater sources as well as from the upwelling events (Uysal and Köksalan, 2006). Significant fluctuations in nitrate and phosphate concentrations were observed over most of the year. Fluctuations observed during winter are mainly due to storms and turbulences that result in mixing of the water column. High nitrate and phosphate levels observed at the surface were due to intense Lamas River input during spring (Uysal and Köksalan, 2006).

Second station placed at the edge of the continental shelf (about 210 m). At this station, the influence of Lamas River was not observed, additionally, due to the depth of the station, the stratification within the water column can easily be observed (Uysal, *et al.*, 2004). However, this station was suggested to be under

the influence of the Seyhan River (Uysal and Köksalan, 2006) regards to its wide drainage area (17498 km<sup>2</sup>) and great discharge (193,1 m<sup>3</sup> sec<sup>-1</sup>) (Olson, *et al.*, 1990) coupled with the general westerly circulation pattern of the Cilician Basin (Özsoy, *et al.*, 1989).

In surface waters of diverse marine habitats, the annual average abundances of both heterotrophic bacteria and Synechococcus were found to be directly related to annual average temperature (Li, 1998). More particularly, cell abundance at individual locations can evidently track water temperature throughout the year. Li (1998) find a critical temperature value of 14°C on an annual worldwide basis, that, the abundance of bacteria is predicted with reasonable accuracy by temperature in regions where the average is less than that critical value. This extends the view developed at shorter time scales, that in cold waters, temperature is the dominant factor influencing the processes that set bacterial abundance (Hoch and Kirchman, 1993; Shiah and Ducklow, 1995). At higher temperatures, other factors apparently become dominant and affect abundance by varying degrees in different habitats. It appears that at the annual scale, the role of substrate supply in setting the net bacterial abundance is evident only where average temperatures exceed 14°C (Li, 1998). The surface temperature in this study ranged between 16 – 29.4°C throughout the year, being coldest in February and warmest in August. Thus, the physical and chemical parameters, together with the biological factors, should be considered for changes in bacterial abundance in time.

Several factors may affect bacterial abundance in a water body. These may be the ambient physical, chemical, and biological factors together with the differences in grazing pressures among sites or timing schedule of the sampling during the day (Uysal, *et al.*, 1998). Synechococcus have been shown to exhibit varying degrees of diurnal periodicity in cell division rates both in culture (Campbell and Carpenter, 1986) and in incubation experiments (Carpenter and Campbell, 1988). Heterotrophic bacteria and *Synechococcus* also appear to be directly related to temperatures below, but not above, 14°C (Li and Wood,

1988). The same temperature is significant with respect to the global distribution of nitrate that its concentrations are never high where the temperature is above approximately 14°C (Levitus, *et al.*, 1993).

Heterotrophic bacteria and *Synechococcus* are the dominant communities in the upper layer of the Mediterranean Sea, which may be related to their high affinities for phosphate (Mountin and Raimbault, 2002), in a phosphate limited area.

## 4.1. Heterotrophic Bacteria

Bacterial abundance in the water column of the world oceans is estimated as  $10^{30}$  cells ml<sup>-1</sup> (Pomeroy, *et al.*, 1990). On the other hand, typical mean numbers for bacterial abundance estimates in eutrophic lagoons and estuaries ( $10^7$  cells ml<sup>-1</sup>), coastal zones ( $10^6$  cells ml<sup>-1</sup>), and the open ocean ( $10^5$  cells ml<sup>-1</sup>) are set by the magnitude of the flux of dissolved organic matter (Ducklow and Carlson, 1992). Some examples of the abundance and biomass data were given in Table 4.1.

In the present study, the abundance of heterotrophic bacteria ranged between  $4.5 \times 10^6$  and  $1.5 \times 10^7$  cells ml<sup>-1</sup>, obtained at first station, at 10 m, in February and October, respectively. The average bacterial abundances for the coastal and offshore stations were  $9.7 \times 10^6$  and  $7.3 \times 10^6$  cells ml<sup>-1</sup>, respectively. To a maximum bacterial abundance ( $1.25 \times 10^7$  cells ml<sup>-1</sup>) was reached in September, at surface in the second station.

 $\textbf{Table 4.1.} \ \ \text{Heterotrophic Bacterial abundance and biomass measurements from the world oceans.} \ \ (^{\bigstar}: \mu\text{g/l})$ 

	Heterotrophic	Bacteria	References			
Location	Abundance Biomass		Abundance		D:	
	(cells ml <sup>-1</sup> x $10^5$ )	$(mg C m^{-2})$	Abundance		Biomass	
NE Mediterranean Sea 2005-2006	73	41(*)	Present study			
NE Mediterranean Sea 2002-2003	8.0	5.19	Uysal et. al., 2004			
Red Sea (Gulf of Aqaba) 1999	6.5		Grossart and Simon, 2002			
Red Sea 1999	8.0		Grossart and Simon, 2003			
Subarctic Pacific 1988	8000		Kirchman et. al., 1993			
Baltic Sea 1995	15.8		Pinhassi and Hangstrom, 2000			
Hawaii 1991-1992		1.35				
Hawaii 1993	6.0	1.63	Campbell et. al., 1997		Campbell et. al.,	
Hawaii 1990-1994		1.42			1997	
SE Mediterranean Sea 1999	2.9 - 5.1		Christaki et. al., 2001			
Baltic Sea	14.5		Andersson et. al., 1994			
Baltic Sea	12.6		Wikner and Hangstrom, 1991			
Baltic Sea	8.8		Hangstrom et. al., 1979			
Baltic Sea	25.9		Kuparinen and Kuosa, 1993			
Baltic Sea	19.8		Zimmerman, 1977			
NW Mediterranean Sea (Spain)	21.2		Zdanowski and Figuerias, 1997			
NW Mediterranean Sea (Villefrenche)	7.3		Mostajir et. al., 1995	Li,		
N Atlantic	10.0	1000	Duclow, 1984, 1986	1998	Duclow, 2000	
NW Mediterranean Sea (Spain)	4.8		Satta et. al., 1996			
Hawaii	4.8		hahana.soest.hawaii.edu/hot			
Equatorial Pacific	7.3	1467	usjgofs.whoi.edu/jg/dir/jgofs/eqpac		Duclow, 2000	
Arabian Sea	11.0	1148	usjgofs.whoi.edu/jg/dir/jgofs/arabian		Ramaiah et. al.1996	
Indian Ocean	16.0		Wiebinga et. al., 1997			

Bacterial abundance was found higher from summer to early winter. In a similar study conducted earlier at the first station revealed that the lowest bacterial abundance was achieved during the winter mixing period, where the water temperature was low (Uysal, *et al.*, 2004). Bacterial abundances ranged from a minimum of 5.85 x 10<sup>5</sup> cells ml<sup>-1</sup> at 10 m in December to a maximum of 4.06 x 10<sup>6</sup> cells ml<sup>-1</sup> at surface in September during that period. Bacterial biomass was also found lowest (3.05 μg C l<sup>-1</sup>) in December and highest (32.3 μg C l<sup>-1</sup>) in September, respectively. Bacterial biomass for this study ranged between a minimum of 18.2 μg C l<sup>-1</sup> at the surface, in February and a maximum of 86.7 μg C l<sup>-1</sup> at 10 m depth, in October. Increase in bacterial abundance and biomass within three years is notable.

At the offshore station, the variation of both abundance and biomass in the water column within the study period was remarkable. In September, to the highest density of bacterial community was reached (1.25 x 10<sup>7</sup> cells ml<sup>-1</sup>) at surface waters. On the other hand, to a minimum value of 2.73 x 10<sup>6</sup> cells ml<sup>-1</sup> at 175 m was also reached in the same month. The average abundance value throughout the study period was 7.31 x 10<sup>6</sup> cells ml<sup>-1</sup>. The second station was monitored previously during the years 2002 - 2003 by Uysal et. al. (2004). During this previous study, to a minimum (1.71 x 10<sup>5</sup> cells ml<sup>-1</sup> at 175 m) and maximum (1.66 x 10<sup>6</sup> cells ml<sup>-1</sup> at surface) bacterial abundance were reached in October and September, respectively. The range for the bacterial biomass was between 10.7  $\mu g$  C I<sup>-1</sup> and 0.99  $\mu g$  C I<sup>-1</sup>, at surface in September and at 175 m in November, respectively during 2002 and the biomass fluctuated with depth. The bacterial biomass in the present study is found relatively higher during the warm season. Biomass ranged between 15.98 µg C l<sup>-1</sup> at 200 m in September and 83.18 μg C l<sup>-1</sup> at surface in August. Biomass was greater during late summer and early autumn compared to rest of the year. The most important ecological role of the heterotrophic bacteria is that they directly take up inorganic phosphate (Pomeroy, et al., 1990). Since the region is phosphate limited (Krom, et al., 1991), the recycling of that element is crucial. As implied by Pomeroy et. al. (1990), the ability of direct uptake of inorganic phosphate by bacteria is very

important for flagellates, ciliates and other higher trophic organisms. Bacteria are also dominant in most of the main processes of nitrogen cycle transformations. Uptake and transformation of inorganic forms of nitrogen into organic forms, regeneration and release of inorganic forms (primarily as ammonia) from organic forms, oxidation of ammonium and nitrite in nitrification, reduction of nitrite or nitrate to gaseous forms, in denitrification, and reduction of nitrogen to ammonia (Ramaiah, 2005). Temperature, like essential chemical elements and organic substrates, is always a potentially limiting factor. It affects all chemical and biochemical processes. A strong positive correlation between bacterial production and temperature is regularly observed under natural conditions (Shiah and Ducklow, 1994a; Shiah and Ducklow, 1994b). At the seasonal scale, temperature emerges as the dominant influence of heterotrophic bacterial processes in winter and spring (Hoch and Kirchman, 1993; Shiah and Ducklow, 1995). In Cilician Basin, bacterial abundance and biomass had a significant positive correlation with temperature throughout the study period. At both stations, increase in bacterial abundance and biomass coupled with increase in temperature of the seawater. The role of substrate would be important in the summer, when metabolic rates rise with temperature (Shiah and Ducklow, 1994a; Shiah and Ducklow, 1994b, 1995). There were small decreases in numbers in late spring at first station and in early summer at the second station, which may be due to an increase in numbers of protozoa. Bacteria are stated to be under the effect of grazing during that warm period (Christaki, et al., 2001). Species specific grazing in bacteria would maintain a relatively high diversity and allow replacement of one species by another in the ecosystem (Legendre and Rassoulzadegan, 1996). It is shown that the relationship between bacterial production and temperature during summer coincided with changes in the species composition of the bacterial community (Pomeroy and Wiebe, 2001). This suggests that not only direct effects of temperature and nutrient concentrations on the growth of the species present, but also indirect effects through changes in the community structure of the bacterial assemblage (Pinhassi and Hangström, 2000). This phenomenon would explain the excess biomass observed in early autumn at first station and at particular depths especially in autumn at the second station.

It has been suggested on theoretical grounds that substrate concentration normally should not be limiting to heterotrophic bacteria in the upper mixed layer (Thingstad and Lignell, 1997). At least some heterotrophic bacteria have the ability to adjust their growth rate and body size according to the substrate concentration present and in the absence of temperature limitation, may grow at maximum rates in both eutrophic and oligotrophic environments (Pomeroy and Wiebe, 2001). Meanwhile, experimental and observational data suggest that limiting temperatures can affect substrate utilization by heterotrophic bacteria (Pomeroy and Wiebe, 2001). On the other hand, high nutrient salt concentration within the water column would enhance the growth of primary producers and thus the organisms of the higher trophic levels. This would create a predation pressure on the heterotrophic bacteria.

No correlation was found between bacteria and phosphate, as well as silicate at the first station. Nitrate and dissolved oxygen showed negative correlation with bacterial abundance and biomass. As the nitrogen and oxygen concentration declined in the water column, bacterial abundance and biomass increased, which was occurred most commonly during the warm period. At the offshore station, the bacterial abundance and biomass were found to be negatively correlated with phosphate, nitrate, and silicate. No correlation was found between bacteria and dissolved oxygen at that station. Similar to the coastal station, the bacterial abundance and biomass had high values during the summer and early autumn. Although the values tend to increase towards the end of the study period, in general, the values were lower during winter period compared to rest of the year. Reduced bacterial production may be resulted from inhibition by low temperature, high substrate concentration, and a lag phase prior to the onset of bacterial division, which may last from a day to a month (Pomeroy and Wiebe, 2001).

# 4.2. Synechococcus

Synechococcus is one of the main components of the microbial loop, with a cell size of roughly 1 μm that regulates the biogeochemical cycles (Burkill, et al., 1993). It is important as a primary producer especially in the open ocean where >20 μm cells cannot thrive (Glover, et al., 1986). Synechococcus possesses high specific growth rates (Bienfang and Takahashi, 1983; Landry, et al., 1984) and its ecological success has been attributed to the increased efficiency of light-harvesting and nutrient uptake due to their small size and their negligible sinking rate (Glover, et al., 1988). Synechococcus is also regarded as an important component of the phytoplankton in highly oligotrophic Mediterranean Sea (Li, et al., 1993; Magazzu and Decembrini, 1995; Vaulot, et al., 1996; Agawin and Agusti, 1997; Jacquet, et al., 1998a; Agawin, et al., 2000).

Synechococcus form one of the major groups of the autotrophic picoplankton community in the upper layer of the Mediterranean Sea, which might be related to their high affinities for phosphate. The relative abundance of Synechococcus in marine environments, as well as transient blooms already observed in the open ocean (Glover, et al., 1988), might be related to a high potential phosphate uptake capacity relative to other photosynthetic and heterotrophic microorganisms (Mountin and Raimbault, 2002).

Usually, open ocean waters (oligotrophic) exhibit lower *Synechococcus* abundances than eutrophic coastal waters. Their concentrations may range from a few hundred cells per milliliter in oligotrophic systems to millions in nutrient rich coastal waters (Gasol, *et al.*, 1997). Counts from the eutrophic, mesotrophic and oligotrophic regions of the northeastern Atlantic Ocean yielded 2.2, 1.7 and  $1.3 \times 10^5$  cells ml<sup>-1</sup>, respectively (Lantoine and Neveux, 1997). The highest abundances of *Synechococcus* were always found in the mixed layer within the water column, whereas the maximum abundances were frequently observed at the surface (Murphy and Haugen, 1985; Landry, *et al.*, 1996) and near the deep

chlorophyll maximum, particularly in oligotrophic waters (Iturriaga and Marra, 1988). Synechococcus abundances were reported in the range  $1.7 - 13 \times 10^3$  cells ml<sup>-1</sup> in the stratified northwest Mediterranean (Agawin and Agusti, 1997). Again in the northwestern Mediterranean (Bay of Blanes), Synechococcus abundances increased from winter levels of  $5 \times 10^2$  to  $7 \times 10^4$  cells ml<sup>-1</sup> towards summer (Agawin, et al., 1998). Some other examples of Synechococcus abundance and biomass observations were given in Table 4.2. The stations sampled in the present study were observed previously in 1998 by Uysal and Köksalan (2006) and 2002-2003 period by Uysal et.al. (2004). In 1998, the observed Synechococcus density was 3.9 x 10<sup>4</sup> cells ml<sup>-1</sup>, reaching to a maximum (1.5 x 10<sup>5</sup> cells ml<sup>-1</sup>) in September at the first station. The maximum value observed at the offshore station was 4.3 x 10<sup>4</sup> cells ml<sup>-1</sup>, where the average abundance was 1.8 x 10<sup>4</sup> cells ml<sup>-1</sup>. Average cell counts determined in the following study between 2002 and 2003 had similar observed abundances. The maximum Synechococcus abundances observed were 1.62 x 10<sup>5</sup> (in July, at surface) and 7.51 x 10<sup>4</sup> cells ml<sup>-1</sup> (in July, at 20 m), at the first and the second station respectively. The maximum abundances reached during this period were 11.59 (in July, at surface) and 6.31 µg C l<sup>-1</sup> (in July, at 20 m), respectively (Uysal, et al., 2004).

**Table 4.2.** *Synechococcus* abundance and biomass measurements from the world oceans

	Synecho	coccus				
Location	Abundance (cells ml <sup>-1</sup> x 10 <sup>3</sup> )	Biomass	References			
NE Mediterranean Sea 2005-2006	152	8.43 µg/l	Present Study			
Black Sea 1994	45.0		Uysal, 2000; Uysal, 2001			
Black Sea 1996	109.0 44.0 100.0		Uysal, 2006			
Black Sea 2001			Uysal, 2006			
Marmara Sea 2000			Uysal, 2006			
Aegean Sea 2000	17.2		Uysal, 2006			
NE Mediterranean Sea 1998	39.0 15.4		Uysal and Koksalan, 2006			
NE Mediterranean Sea 2000			Uysal, 2006			
NE Mediterranean Sea 2002-2003	20	1.9 μg/l	Uysal et. al., 2004			
NE Mediterranean Sea 2003	22.5	$0.6 - 5.1 \mu g/l$	Uysal, 2006			
Adriatic Sea (Cesenatico)	42.193		Vanucci et. al., 1994			
NW Mediterranean Sea (Naples)	14.38		Modigh et. al., 1996			
NW Mediterranean Sea (Sardinia)	5.543		Andreoli et. al., 1989	Li, 1998		
Equatorial Pacific	12.063		usjgofs.whoi.edu/jg/dir/jgofs/eqpac	L1, 1996		
Arabian Sea	94.157		usjgofs.whoi.edu/jg/dir/jgofs/arabian			
Indian Ocean	127.704		Veldhuis et. al. 1997			
NW Mediterranean Sea (Villefranche) 1996	43.0		Jacquet et. al., 1998			
Subarctic Pacific 1988	6.0		Kirchman et. al., 1993			
Hawaii 1991		0.04mgC/m <sup>2</sup>				
Hawaii 1992-1993	6.0	$0.05 \text{ mgC/m}^2$	Campbell et. al., 1997			
Hawaii 1994						
Hawaii 1990-1994		$0.05 \text{ mgC/m}^2$				
SW Mediterranean Sea 1998		2-10 μg/l	Arin et. al., 2002			
SE Mediterranean Sea 1999	3.7 - 9.3		Christaki et. al., 2001			

The first station was a shallow one, having a total depth of 20 m. It is under the influence of nearby Lamas River. At surface waters of the first station, Synechococcus concentrations were in the range  $1.4 \times 10^5 - 1.1 \times 10^6$  cells ml<sup>-1</sup>. The annual average for this depth was  $4 \times 10^5$  cells ml<sup>-1</sup> with a maximum of  $1.1 \times$ 10<sup>6</sup> cells ml<sup>-1</sup> recorded in October. In March, July and October, three major peaks of both biomass and abundance were observed. However, only two peaks were observed at 10 m depth. Community was found most abundant during July, August, October and November at 10 m depth. The observed fluctuations in abundances are due to many factors that are characteristic of the shallow coastal waters. At this shallow station, the environmental factors, like temperature, salinity, nutrient availability, light regime and riverian input are changing rapidly. Since the sampling station is located nearby the Lamas River, freshwater input to the sampling site governs most of the biological, chemical and physical processes. The low Secchi disk depths readings throughout the sampling period are mainly resulting from phytoplankton blooms and suspended sediment carried by the Lamas River.

Synechococcus frequently forms transient blooms lasting up to a week under favorable conditions (Glover, et al., 1988; Iturriaga and Marra, 1988). Due to monthly sampling intervals in this study, some blooms most probably were missed. As reported by Li (1998), Synechococcus was found more abundant during summer and autumn indicating the strong response to increasing water temperature in the long term. Although Synechococcus abundance and biomass were not found to be correlated with any of the ambient physical and chemical parameters at the coastal station, the synergistic effects of the environmental changes would affect the community. As a response to nutrient pulses, transient increases in abundance were also revealed in the region. In March, for example, very distinct nutrient input at surface waters was resulted in high abundance and biomass values. At that month, the temperature was quite low and the salinity of the surface waters was the minimum record of the study period. This may imply that, the strong fresh water input enriched with nutrient salts, enhances Synechococcus bloom. July also has relatively low salinities at the surface

however; high temperature values around 28°C. Although the nutrient values in this month was quite low, a very low Secchi disk depth (4 m) recorded and a relatively high Chl-a measurement (Zenginer, personal communication) might show a post bloom condition. The most distinct increase in cell numbers and biomass of *Synechococcus* was observed in October, coupled with low nutrient concentrations and highest salinity at that station.

Second station was located at the edge of the continental shelf. However, it is partly influenced by the two major rivers namely, Göksu and Seyhan. In June, the water column was dominated by the Atlantic water, which is defined by its low salinity value of 38.8 psu. At the second station, the minimum and maximum Synechococcus abundance and biomass values ranged between  $3.01 \times 10^2 - 3.40 \times 10^2 = 3.40 \times 10^2$  $10^5$  cells ml<sup>-1</sup> and 0.01 - 30.79 ug C l<sup>-1</sup>, respectively. The averages for abundance and biomass were  $1.52 \times 10^5$  cells ml<sup>-1</sup> and  $8.43 \ \mu g \ C \ l^{-1}$ , respectively. The values are also quite large when they are compared with the results of another study done in 2003. Previously the average Synechococcus abundance was found as 2.25 x 10<sup>4</sup> cells ml<sup>-1</sup> and the minimum and maximum biomass ranged between 0.6 - 5.1 μg C l<sup>-1</sup> at the station (Uysal and Koksalan, 2006). Synechococcus was found more abundant during late summer and autumn, and least during winter. A similar pattern was found, which was only relatively earlier; that Synechococcus was more abundant during summer and early autumn (Köksalan, 2000). The maximum biomass was observed in March, at 40 m depth. Abundance was also very high; 3.38 x 10<sup>5</sup> cells l<sup>-1</sup>, which is very close to the maximum value (3.40 x 10<sup>5</sup> cells 1<sup>-1</sup>). This month, the water column was thoroughly mixed and the nutrient salts were at average levels. The distinct increase in cell density and biomass may also be due to a strain shift in the Synechococcus community. Distinct seasonal shifts in community composition have been reported from the Bermuda time-series study site (Morris, et al., 2005). Seasonal shifts may be related to physical dynamics of the water column and to the mixing patterns (Dolan, 2005). Another possibility of the high measurements might be the post bloom conditions. At this month, the surface chlorophyll was quite high (Zenginer, personal communication), showing the productivity of the water.

The nutrient salt measurements, taken throughout the study, supported the phosphate limited conditions of the basin, which was clearly stated by Krom et al. (1991). Higher phosphate and lower nitrate concentrations observed only in July at 80 m, indicating the preferential utilization of nitrate by existing flora at the site. However, no correlation between Synechococcus and phosphate was found during the study. Nitrate was found to be negatively correlated with Synechococcus. Nitrate was low at the upper layers of the water column throughout the study period. The nitrogen concentration at surface waters declined towards the end of the year, however, the average values of the water column increased. As the values increase with depth from late summer to early winter, the Synechococcus abundance and biomass were decreased distinctly through bottom during this period. In terms of nutrient acquisition, Synechococcus are able to utilize nitrate, nitrite, ammonium, urea, and some amino acids (Moore, et al., 1995). Under nitrogen deprivation, Synechococcus will degrade the major light-harvesting pigment protein phycoerythrin as an internal nitrogen source (Wyman, et al., 1985).

In fact, it is difficult to suggest a direct correspondence between *Synechococcus* abundances and other environmental variables. It is known that *Synechococcus* make transient blooms when the nitrate concentration increases suddenly (Thingstad and Rassoulzadegan, 1995). Due to the greater surface-to-volume ratio of *Synechococcus* in comparison with other organisms, a quick responding mechanism to the available nutrient salts favors the community (Landry, *et al.*, 1996). However, the major peaks of both abundance and biomass did not coincide with nutrient maxima. Significant increases in abundance and biomass mostly occurred during low nutrient concentrations. It may be possible that at diminished nutrient levels *Synechococcus* may be more favorable compared to other phytoplankton (Uysal, 2000). Relationship with other planktonic groups based on seasonality and different water bodies is also important in understanding the dynamics of *Synechococcus*. For example, there is a seasonal shift between eukaryotic phytoplankton in winter and autotrophic picoplankton in summer (Agawin, *et al.*, 1998).

There may be other factors affecting cell concentrations other than physicochemical ones. This may be due to differences in grazing pressure among sites or timing of the sampling schedule during the day. Data gathered from field and microcosm experiments conducted on board R/V Bilim-II in the Black Sea have shown that synchronous division occurs between noon and midnight with an apparent grazing pressure from midnight to noon along the southern Black Sea coast (Uysal, et al., 1998). Similar results have also been obtained from the Arabian Sea (Sherry and Uysal, 1995). Significant differences were also observed in the acclimated growth rates of *Synechococcus* clones from the Black Sea (Uysal, 2001). Clonal isolates from deeper parts of the euphotic zone exhibited higher growth rates compared to clones from the surface mixed layer. This may also be regarded as a factor affecting heterogenous abundance distribution of this group. Studies of isolates and field samples suggest that physiologically and genetically different *Synechococcus* groups may exist at the same site (Palenik, 1994).

#### 5. CONCLUSION

Direct microscope observations revealed some features related to size and fluorescence yields of the cells. The cells were smaller and fluoresced pale yellow in the upper layers of the water column and they were bigger and fluoresced orange at lower depths.

Coastal station was more abundant and had much higher bacterial and cyanobacterial (*Synechococcus*) biomass than the offshore station as it receives substantial amount of freshwater from the nearby Lamas River throughout the year. On a seasonal basis, bacterial population was found excessively dominant at the surface or near-surface waters during the second half of the year. *Synechococcus* were also found more abundant during late summer and autumn. Bacterial population always found to exceed *Synechococcus* abundance within the water column. In general, bacterial and cyanobacterial abundance and biomass tend to decrease with depth. Especially, during summer and autumn, stratified water column leads a sharp decrease in abundance of both populations with depth. In contrast, minor fluctuations in abundance and biomass distribution of the bacterial and cyanobacterial populations within the water column were observed during the winter mixing period.

Temperature and nitrate concentration seemed to affect efficiently the abundance of both populations in the area. Based on Spearman Rank Correlation analysis, highly significant correlations between bacterial abundance as well as biomass and ambient temperature were observed at both stations. However, a significant correlation was found between *Synechococcus* and temperature only at the offshore station. Significant negative correlations are found between nitrate and bacterial abundance and biomass at both stations and only between *Synechococcus* abundance and biomass at the offshore station. At the offshore station, salinity was also found to be positively correlated with the bacterial and cyanobacterial abundance and biomass.

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