INVESTIGATING MARINE MICROBIAL REACTIONS USING NOVEL APPROACHES: GENETICS AND BIOGEOCHEMISTRY

DOCTOR OF PHILOSOPHY

IN

MARINE BIOLOGY AND FISHERIES
MIDDLE EAST TECHNICAL UNIVERSITY
INSTITUTE OF MARINE SCIENCES

BY

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MERSİN-TURKEY
MAY-2019

Approval of the thesis:

INVESTIGATING MARINE MICROBIAL REACTIONS USING NOVEL APPROACHES: GENETICS AND BIOGEOCHEMISTRY

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ABSTRACT

INVESTIGATING MARINE MICROBIAL REACTIONS USING NOVEL APPROACHES: GENETICS AND BIOGEOCHEMISTRY

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May 2019, 198 pages

Despite the many important roles, they play, bacteria are one of the least known components of the marine biogeochemical cycles. In order to determine the temporal dynamics of the species composition, and active metabolisms playing significant roles in the nitrogen cycling we used the power of novel genetic analyses and integrate biochemical measurements. Community dynamics were studied using 16S rRNA amplicon sequencing approach. Samples were collected with monthly intervals for one year from different depths of monitoring station (bottom depth 200 m) in the NE Mediterranean. mRNA expressions of genes that are responsible for nitrogen cycling were investigated seasonally using metatranscriptomics. In total, 2213 sequence variants were observed among which *Proteobacteria*, *Bacteroidetes* and *Cyanobacteria* were the mostly observed phylum. In the higher taxonomic level, SAR 11 clade belonging to *Pelagibacteriaceae* was highly dominant in our samples and its ecotypes show variation with depth. While Clade Ia more dominant in the surface waters, Clade Ib was abundant in the deeper waters. Seasonality found to be the main driver for patterns in bacterial communities. *Prochlorococcus* MIT 9313 was more abundant than *Synechococcus* CC9902 in the surface during fall. *Prochlorococcus* MIT 9313 contributes more to the formation of DCM in our region. Additionally, metatranscriptomics results showed that the nitrogen fixation is not the dominant process in the study region. Genus *Nitrospinae* are responsible for the high concentrations of nitrate in the deeper waters. We showed the importance of bacteria in the carbon and nitrogen cycling in the NE Mediterranean.

Keywords: 16S rRNA amplicon sequencing, Metatranscriptomics, Eastern Mediterranean, Nitrogen Cycle, Phosphate Cycle

ÖZ

DENİZEL MİKROBİYAL REAKSİYONLARIN YENİ YAKLAŞIMLAR KULLANILARAK ARAŞTIRILMASI: BİYOJEOKİMYA VE GENETİK

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Mayıs 2019, 198 sayfa

Oynadıkları birçok önemli rollere rağmen, bakteriler deniz biyojeokimyasal döngülerinin en az bilinen bileşenlerinden biridir. Tür kompozisyonunun zamansal dinamiklerini ve aktif metabolizmalarını tespit etmek için yeni genetik analizlerin gücü kullanılmış ve biyokimyasal ölçümler ile birleştirilmiştir. Topluluk dinamikleri, 16S rRNA amplikon dizileme yaklaşımı kullanılarak çalışılmıştır. Kuzey Doğu Akdeniz'deki izleme istasyonun (dip derinliği 200 m) farklı derinliklerinden bir yıl boyunca aylık aralıklarla örnekler toplanmıştır. Azot döngüsünden sorumlu olan genlerin mRNA ifadeleri metatranscriptomics yaklaşım kullanılarak mevsimsel olarak incelenmiştir. Toplamda, 2213 dizi varyantı gözlenmiştir ve bunların arasında *Proteobacteria*, *Bacteroidetes* ve *Cyanobacteria* en fazla gözlenen filumlardır. En yüksek taksonomi seviyesinde, *Pelagibacteriaceae* familyasına ait SAR 11 kladının örneklerimizde oldukça baskındı olduğu ve ekotiplerinin dağılımının derinliklerinde farklılık gösterdiği görülmüştür. Clade Ia yüzey sularında daha baskın iken, Clade Ib daha derin sularda bulunmaktadır. Mevsimsellik bakteri topluğunun dinamiklerini en iyi açıklayan güç olarak görülmektedir. *Prochlorococcus* MIT 9313, sonbaharda yüzeyde *Synechococcus* CC9902'den dahabaskındır. *Prochlorococcus* MIT 9313 bölgemizde Derin Klorofil Maksimum oluşumuna daha fazla katkıda bulunmaktadır. Ek olarak, metatranskriptomiks sonuçları, çalışma bölgesinde azot fiksasyonunun baskın süreç olmadığını göstermiştir. *Nitrospinae* cinsi, derin sulardaki yüksek nitrat konsantrasyonlarından sorumludur. Bu çalışmada, bakterilerin Kuzey Doğu Akdeniz'in karbon ve azot döngüsünde oynadıkları önemli rolleri göstermiş bulunmaktayız.

Anahtar Kelimeler: 16S rRNA amplikon dizileme, Metatranskriptomik, Doğu Akdeniz, Azot Döngüsü, Fosfat Döngüsü,

To My Family

Gülder Küçükavşar,
Mehmet Küçükavşar,
Sıla Küçükavşar,
Çağrı Deliceırmak,
Çağla Deliceırmak,
Sevgi Deliceırmak,
Cumhur Deliceırmak

for their love, support and trust in me

ACKNOWLEDGMENTS

This research was supported by Scientific and Technological Research Council of Turkey (TUBITAK) grants; CAYDAG- 115Y629 ("Investigating marine microbial reactions using novel approaches: genetics, biogeochemistry and modeling"). As well as, scientific cruises are supported by DEKOSIM Project ("Center for the Marine Ecosystem and Climate Research") founded by the Ministry of Development.

I would like to express my special appreciation and thanks to my advisor Associate Prof. Dr. Barış Salihoğlu, for the continuous support during my PhD study and immense knowledge.
My sincere thanks also go to my co-advisor Dr. Şadi Sinan Arkın. Your guidance helped me in all the time of research, especially in the very critical points.
Besides my advisors, Assoc. Prof. Dr. Arzu Karahan required to be mentioned in a very special way. Arzu Hocam, I owe you a deep sense of gratitude for your guidance and inspirations for completing this study even you do not have any legal responsibility on me. I am thankful that I had the chance to know you and you considered me as your own student. Each time we had brain storming on research related topics I learned a lot from you.
Before continuing, I need to stress that Dr. Şadi Sinan Arkın and Dr. Arzu Karahan are special for me and I need to mention a bit more about their contributions. They were there for me when I am stressful, down, or even unhappy and depressed. Thank you both for your all supports apart from study related obstacles. This study was only possible with your trust on me from the very beginning. Once again, please accept my sincere gratitude for the continuous support of my PhD study and related research, for your patience, motivation, and immense knowledge. Your guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisors and mentors for my PhD study.

Besides my advisors, I would like to thank the rest of my thesis committee: Prof. Dr. Zahit Uysal, and Assoc. Prof. Dr. Mustafa Yücel for their insightful comments and encouragement, but also for the hard questions which direct me to widen my research from various perspectives. I am also grateful to Prof. Dr. Gülşen Altuğ and Assist. Prof. Dr. Donato Giovanelli to contribute to this thesis with scientific discussions, comments and advices.
I would like to express my gratitude to Dr. Hasan Örek, Dr. Yeşim Ak Örek and Asist. Prof. Dr. Devrim Tezcan for organizing scientific cruises.

Phytoplankton identifications and heterotrophic cell counts were conducted by Dr. Leona Julia Boran. I would like to thank her for this invaluable contribution.

Ismail Akçay also deserves a special thank. Thank you for helping me to control the quality of the chemical data and your friendship.

I would like to thank to Kerem Gökdağ and Mehmet Özalp for sampling and counting micro-plastic particles.

Data that was presented in the first chapter were collected by the collaborative work of researchers and technicians of IMS-METU. I am grateful to all of them. I would like to thank to Ismail Akçay, Şehmuz Başduvar, Merve Açıkyol, Pınar Kalegeri for their help with chemical sampling and measurements. I also would like to thank to Volodymyr Myroshnychenko for providing help regarding the CTD data.

Dr. Mohammed Abdul Latif, I have learned a lot from our discussion. I am thankful for your contributions to the physical data analysis and interpretations.

It was a long journey and it was not only my advisors or thesis committee members that I learned from. Before I decided to study in the field of marine sciences, I have been a lot on the diving boats and I thought this was an enough experience to handle harsh cruises. I am grateful to Dr. Hasan Örek that he thought me how to work during the research cruises. It was always full with fun besides the disciplined field work.

Yeşim Ak Örek, you need a special mention too. Yeşim Hocam, whenever I came to a deadlock, I knocked your door. It was not because your office was the one closes to our office but because you're the one that I trust to take advices. I have a lot to learn from you.

Throughout the sampling period, each month, it was long long days and nights to process samples and I share these tiresome moments with Dr. Valeria Ibello. Valeria, I am so glad that these days has passed since it was a bit exhausting. I am not sure if I can convince myself to do blabla-tion experiments again. But I am thankful that I learned a lot during these days from you and thank you for your support during the analysing step of chemical data and your contribution for the writing of the first chapter of this thesis.

Along this journey, the most important support I took was from my beloved one, Çağrı. I am happy that I have you in my life. Thank you for being there whenever I needed a support and

a hug. Thank you for your patience, I said I will only have 2 years left to finish when we first met but it took ages. But I am sure you will take your revenge.

It has been more than five years and it was not always work! I am lucky that I have Fatima Nur Oğul and Leona Boran to have fun and enjoy the life. I have already missed the moments we share.

I would like to thank to my office-mates; Begüm, Batıkan, Saba. Thank you for your patience when I was walking along the office like a crazy and, also for dancing like crazy as we were not in the office.

Elanur Yılmaz and Didem Molla, you know what I am saying…

Last but not the least, this paragraph will be devoted to say how I am thankful to my family and will be written in Turkish so that they can understand. “Annecim, babacım ve başımın tatlı belası kardeşim, iyi ki varsınız. Bana olan inancınız için çok teşekkür ederim. Her başım sıkıştığında yanımda oldunuz, maddi ve manevi. Bu yolu bitirebilmemdeki en büyük dayanağım hep siz oldunuz. Sizi çok seviyorum. “

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LIST OF ABBREVIATIONS

AMC:	Asian Minor Current
AOU:	Apparent Oxygen Utilization
AW:	Atlantic Water
BL:	Biogenic Layer
CC:	Cilician Current
CCA:	Canonical Correspondence Analysis
Chl:	Chlorophyll
CLBC:	Central Levantine Basin Current
CTD:	Conductivity Temperature Density
DCM:	Deep Chlorophyll Maximum
DL:	Deep Layer
DON:	Dissolved Organic Nitrogen
DOP:	Dissolved Organic Phosphorus
EM:	Eastern Mediterranean
EMDW:	Eastern Mediterranean Deep Water
EMS:	Eastern Mediterranean Sea
EMT:	Eastern Mediterranean Transient
ETS-200 m:	Erdemli Time Series 200 m Station
ETS:	Erdemli Time Series
EZ:	Euphotic Zone
EZD:	Euphotic Zone Depth
FL:	Free-living
IMS-METU:	Institute of Marine Sciences
IP:	Inorganic Phosphorus
LIW:	Levantine Intermediate Water
LSW:	Levantine Surface Water
MAW:	Modified Atlantic Water
ML:	Mineralization Layer
MLD:	Mixed Layer Depth
MP:	Micro-plastic
mRNA:	Messenger RNA
NGS:	Next Generation Sequencing
OM:	Organic Matter
OTU:	Operational Taxonomic Unit
PAR:	Photosynthetic Active Radiation
PCoA	Principle Coordinates Analysis
Pi:	Orthophosphate
POC:	Particulate Organic Carbon
POM	Particulate Organic Matter

PON:	Particulate Organic Nitrogen
POP:	Particulate Organic Phosphorus
PP:	Particulate Phosphorus
RIN:	RNA Integration Number
SOC:	Saturated Oxygen Content
SST:	Sea Surface Temperature
TP:	Total Phosphorus
WM:	Western Mediterranean
WMS:	Western Mediterranean Sea

GENERAL INTRODUCTION

Prokaryotes were the only life forms on planet Earth for 3 billion years. Not only throughout these 3 billion years but also after the emergence of other life forms, prokaryotes shaped the environment. Evolution of todays' cyanobacteria-like prokaryotes oxygenated the atmosphere and afterwards all planet earth has become habitable to other life forms. Throughout the earth's history, prokaryotes showed high resilience coping with changing environmental conditions, they evolve different strategies from heterotrophy to autotrophy and, survived under variety of conditions both anoxic to oxic. It is crucial to understand how they interact with their environment in the regional and global scale especially under the circumstances of climate change.

Photoautotrophic activities transform the inorganic carbon, nitrogen and phosphorus into organic compounds. Organic matter produced in the surface of the oceans undergone various transformation during its journey from surface to deep ocean. Marine bacteria play important role in this journey by interacting with organic matter and changing its composition. Decomposition of organic matter by bacteria while sinking recycles inorganic nutrients back to the marine environment. These inorganic nutrients are used by other organisms and, photoautotrophic organisms which, produce organic matter again. Despite the many important roles that they play, bacteria are one of the least known components of the marine biogeochemical cycles. The main reasons for this are their complex community structures and the fact that most marine bacteria cannot be cultured successfully in the laboratory yet. Recently developed methods of 16S rRNA amplicon sequencing and *metatranscriptomics* allow a detailed study of bacterial communities without the need to culture them. Using these methods, one can study the community structures and the abundances of bacteria as well as their active metabolisms.

The goal of this study is to determine the temporal dynamics of the community composition, the structure and the abundances of the bacteria that play important roles in the biogeochemical cycles of the Mediterranean as well as determining the active metabolism of bacteria perform using 16S rRNA amplicon sequencing and metatranscriptomics approaches.

In order to achieve these goals, 16S rRNA amplicon sequencing analysis was performed on samples taken from a regular monitoring station near Erdemli to determine the species composition, the community structure and the abundances of the bacteria that play significant roles in the cycling of nitrogen and phosphorus in the Mediterranean. In addition, metatranscriptomics analysis was performed on samples taken seasonally from the monitoring station to elucidate the periods in which the bacterial metabolisms are active.

The work that was performed is the first attempt in the seas surrounding Turkey and in the northern part of Eastern Mediterranean to study marine bacteria that cannot be cultured in the laboratory. In addition, despite the fact that the genetic analyses that will be performed in this project are planned for only a year, knowledge gained in this project is intended to provide the methodological framework for longer genetic studies, which investigate the interaction between marine microbes and climate change in the region.

This thesis includes four main chapters. In Chapter I, physical, chemical and biological data which were collected by the collaborative work of METU IMS research stuff under the framework of monitoring studies were used to identify key process in the region. In Chapter II, 16S rRNA samples collected for one year with monthly intervals were analysed using 16S rRNA amplicon sequencing approach. These novel data paved the way to a new understanding of the temporal dynamics of the species composition, the community structure and the abundances of the bacteria. In Chapter III, differences between bacterial community considered as free-living and particle attached was determined. In the last chapter, Chapter IV, metatranscriptomics approach was used to present active metabolisms of bacteria. Marker genes for nitrogen cycle and alkaline phosphatase activity was investigated in order to understand nitrogen and phosphate cycle dynamics in the region.

CHAPTER I

CHARACTERISTICS OF THE STUDY REGION

As part of the present study, a year-long sampling programme was implemented at monthly intervals at the 200 m Erdemli Time Series (ETS) station in the Eastern Mediterranean. In addition to the genetic sampling and analysis described in the following chapters, a large number of physical, chemical and biological parameters were measured at the station. In this chapter, these properties are described and interpreted to provide the foundations for the genetic analyses that is the main focus of this thesis.

Highlights

- Lateral transport from the Levantine Intermediate Water (LIW) or Modified Atlantic Water (MAW) is not found to be predominant at the station based on the analysis of temperature and salinity data. In some periods, the station is affected by the fresh water input from Lamas River depending on the dominant wind direction and speed. There were no Lamas River flux observed from salinity profiles during year-long study period.
- Nutrient concentrations in the photic zone are well depleted except for the periods of vertical mixing. Lower N to P ratio than Redfield ratio can be concluded that surface waters are N and P co-limited.
- Nitrate concentrations were clearly showing different behaviours in three distinct periods.
- Formation of deep chlorophyll maximum was present during fall, spring and summer.
- Positive Apparent Oxygen Utilization values, especially below the mixed layer depth, indicates the degradation processes if the lateral fluxes ignored.
- Heterotrophic bacteria and prokaryotic autotrophs were clearly dominating the study area by abundance.
- *Emiliania huxleyi*, throughout the year and depths, were the most dominant eukaryotic phytoplankton. Their dominance is the results of their ability to survive under N and P limited conditions.

1.1. General Features of the Mediterranean

1.1.1. Physical properties

Mediterranean is a concentration basin where evaporation exceeds precipitation and is connected to the Atlantic Ocean by the narrow and shallow Strait of Gibraltar, to the Black Sea by the Turkish Strait System and to the Red Sea through the Suez Canal. It is composed of two basins, Western and Eastern basins, which are connected by the Strait of Sicily (sill depth 500 m- Bergamasco and Malanotte-Rizzoli, 2010). Eastern part of the Mediterranean is larger in volume and area. The present study has been conducted in the north-eastern part of the Mediterranean Sea which is defined as Levantine Basin. Eastern Mediterranean Sea includes other sub-basins which are the Adriatic Sea, the Aegean Sea and the Ionian Basin. Ionian Sea has the deepest point with the depth of 5267m, which is known as Calypso Deep.
As Mediterranean is connected to the Atlantic Ocean through Gibraltar Strait, main circulation is described in terms of surface inflow from Atlantic Ocean which ends up in the eastern basin and flows back as an intermediate water forming in the Levantine Basin. Atlantic water follows cyclonic circulation in the Western Mediterranean and generates mesoscale and larger eddies in the open sea that transports AW (Atlantic Water) to the interior basin (Tanhua et al., 2013). Levantine basin is well known by its comparably higher heat flux than the western basin which results in a density gradient between two basins, thus basin-scale circulation is driven by the thermohaline forcing. Heat loss in the Levantine Basin is compensated by the inflow of the fresh surface Atlantic water (Malanotte-Rizzoli et al., 2014). While Atlantic water flows towards the eastern Mediterranean, transformation of surface waters to intermediate waters and deep water occurs in areas where downward movement of water is supported by oceanic conditions and air-sea interactions. Such processes resulted in the exchange of physical and biochemical properties between surface and deep waters. Deep water is formed in the Gulf of Lions in the western basin during cooling and evaporation in winter. Southern part of Adriatic Sea contributes to the deep-water formation in the Eastern Mediterranean. Levantine Intermediate Water (LIW) is described as a salinity maximum layer at depths around 200-500m and potential density of 29.0-29.1 kg/m^3 (Malanotte-Rizzoli and Hecht, 1988). East of Rhodes and possibly the Cretan Sea is reported to be the formation area of LIW.

Interannual variability of atmospheric forcing over the area is proposed to be the driving mechanisms of the place where LIW forms in the basin (Lascaratos et al.,1999). From the formation areas, east of Rhodos and Cretan Sea, LIW bifurcates to the east, to Levantine Basin, and west, to the Ionian Sea. LIW thus flows through the Adriatic Sea where it contributes to the formation of deep water (Tanhua et al., 2013). Together with the deep water forming in the Adriatic Sea, Aegean Sea is reported to be another region where deep water forms and this

process is known as the Eastern Mediterranean Transient (EMT) (Bergamasco and Malanotte-Rizzoli, 2010, Robinson et al, 2001). Aegean originated deep water is warmer and saltier than Adriatic originated water. Cold winters together with the intrusion of high-salinity waters into the Aegean Sea is the causing process of Aegean originated deep water formation. Adriatic–Ionian Bimodal Oscillation System (BiOS- described in details: (Gačić et al., 2011)) effects the thermohaline characteristics of the LIW, and can explain the switches between Adriatic and Aegean as source regions for the Eastern Mediterranean Deep Water (EMDW).

Levantine basin possesses different water masses (Figure 1). In the surface, Levantine Surface Water (LSW) is present within the mixed layer during highly stratified seasons and is characterized by highest temperatures and salinities. Below the surface layer, relatively cooler and fresher Modified Atlantic Water flows through the entire Mediterranean Sea and reaches the Levantine Basin with the salinity of 38.6 psu (Özsoy et al., 1993). Sub-surface salinity minimum shows the existence of AW under the LSW which is more saline and warmer (Bingel et al., 1993). Levantine Intermediate Water (LIW) which is said to form locally in the northern Levantine Basin (Özsoy et al., 1993) is present at intermediate depths.

There are four major gyres in the Eastern Mediterranean which are Rhodes (cyclonic), the west Cyprus (cyclonic), Mersa Matruh (anticyclonic) and the Shikmona (anticyclonic). An important surface circulation for the study area is Central Levantine Basin Current (CLBC), entering into the Levantine, flows eastward between the Rhodes and Mersa Matruh. CLBC first bifurcates at the west of Cyprus, and flows northward to join Asian Minor Current (AMC). The second branch of the CLBC continues to flow along the east of Cyprus. This branch bifurcates once again at the east of Cyprus and joins to the westerly flowing Cilician Current (CC) along the southeastern Turkish coast. The CC enters to the study area from the east and continues to the west to feed the Asian Minor Current (Bingel et al., 1993; Roussenov et al., 1995) (Figure 2).

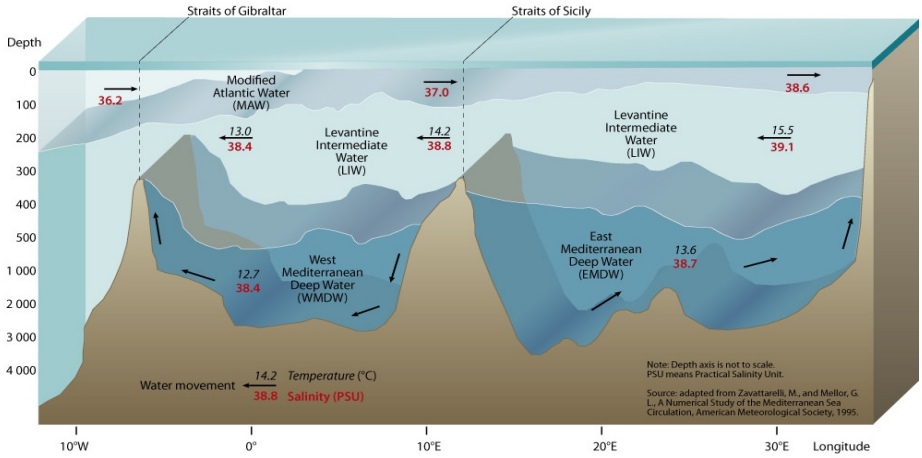

Figure 1. Mediterranean Sea water masses: vertical distribution. Figure was taken from: http://www.grida.no/resources/5885

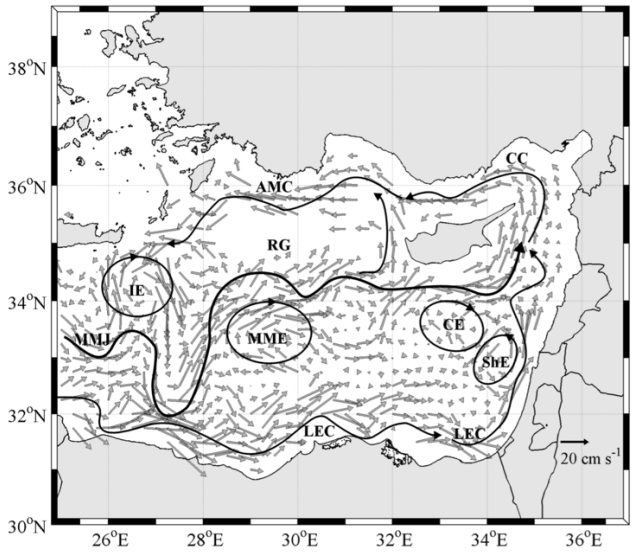

Figure 2. Main currents and eddies in the Levantine Basin. Black lines denote the main currents and sub-basin eddies for which names are RG: Rhodes Gyre, CE: Cyprus Eddy, IE: Ierapetra Eddy, LEC: Libyo-Egyptian Current, AMC: Asian Minor current, MME: Mersa Matruh Eddy, ShE: Shikmona Eddy, CC: Cilician Current, MMJ: Mid Mediterranean Jet. Mean absolute geostrophic velocities between the period 1992 and 2010 are represented as grey arrows. (Figure is taken from (Malanotte-Rizzoli et al., 2014))

1.1.2. Biogeochemical properties

Mediterranean Sea is surrounded by highly populated land and coastal regions are productive due to land base nutrient sources compared with the open sea where primary production is controlled by other natural factors. Despite the productivity of coastal areas, Mediterranean Sea is classified as oligotrophic. Especially primary production in the Eastern Mediterranean Sea is approximately half of other oligotrophic seas (I Berman-Frank and Rahav, 2012; Krom, Emeis, and Van Cappellen, 2010). Main reason of such low primary production is the nature of the anti-estuarine circulation in the Mediterranean. Atlantic water that flows in Mediterranean is depleted in inorganic nutrients but is enriched in organic nutrients (Béthoux et al., 1998), whereas deeper outflow to Atlantic Ocean carries high concentrations of nutrients (Béthoux et al., 2002). Anti-estuarine circulation results in constant export of nutrients out of the basin. Especially, in the EMS formation of Levantine Intermediate water results in the sinking of available nutrients from surface to deep water and thus, leaves the Mediterranean by outflow to Atlantic water. Apart from nutrient input from the coastal sources, the Eastern Mediterranean has one of the highest deposition fluxes of mineral dust from atmosphere owing to its close location to arid regions (Koçak et al., 2004). Dissolved inorganic nitrogen and PO_4^{-3} contribution from the atmosphere to the north-eastern Levantine Basin is ~90% and ~60% respectively compared to the riverine fluxes (Koçak et al. 2010).

There were studies to attempt to explain the possible reasons of low primary production. At first, especially after the study of Krom et al., (1991), it was a widely accepted fact that the whole basin is P limited. Possible reasons of such limitation are discussed by Krom et al., (2010) who conclude that nutrient inputs to the EMS has high N to P ratio compared to the Redfield ratio and denitrification rates are low within the oligotrophic basin (Krom et al., 2010). Thus, primary production is limited by the P available for organisms, as a result of its unusual anti-estuarine circulation in which there is a net export of nutrients from the basin at the straits of Sicily within the LIW. However, P- addition experiments fail to increase phytoplankton abundances until NH_4^+ was added to the system (Zohary et al., 2005). Despite the widely accepted P-limitation hypothesis over the primary production, Zohary and colleagues (2005) shows that in fact phytoplankton in the surface waters of Eastern Mediterranean are N and P co-limited during summer, while bacteria show the evidence of P-limitation. Euphotic zone of eastern Mediterranean water column possesses low concentrations of nitrate and phosphate (0.1-0.3μM and 0.02-0.03μM, correspondingly) (Yilmaz and Tugrul, 1998), except for the upwelling events in the cyclonic regions. In the deep water, N to P ratio is 28:1, exceeding the Redfield ratio (Kress and Herut, 2001b). It is critical to stress here that such P limitation might be due to following possible reasons: either system lacks P; the preferential removal of P relative to N; or system receives an excess of N, all resulting in unbalance of nutrients in the system. It is suggested by Krom et al. (2005) that

the P limitation of the Eastern Mediterranean is due to the lack of P within the system. Lower denitrification rates in the region are also suggested as another reason why nitrate is not used. The process, which is responsible for the loss of N from the system are slow, and as a result, the ratio of N:P is found higher than the Redfield Ratio (Krom et al., 2005). Since the renewal time for the Mediterranean waters are short and thus, water column has high oxygen content, possibly resulting in low denitrification rates.

In general, nutrient concentrations in the Eastern Mediterranean are lower than the western basin thus, two basins show distinct biogeochemical features (Figure 3). Deep water concentrations of nitrate and phosphate in the western basin is twice as high as eastern basin (Pujo-Pay et al., 2011) (Figure 3).

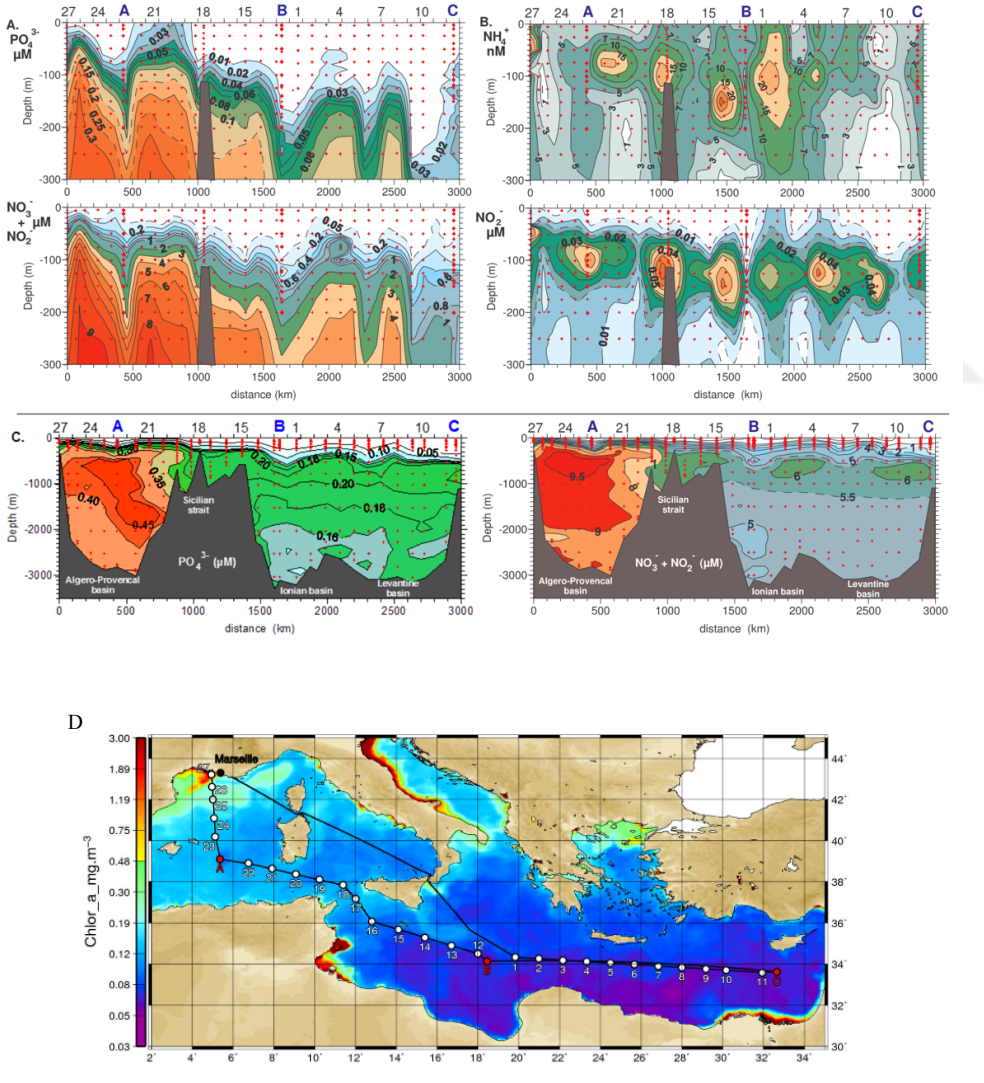

Figure 3. Nutrient profiles of BOUM transect. Surface vertical section of (A) phosphate, nitrate + nitrite and (B) ammonium and nitrite along the transect. (C) for whole water column, (D) the location of the points A, B, and C along the transect (Figure was taken from (Pujo-Pay et al., 2011)).

After the winter mixing (Nov- Mar) that brings nutrients to the photic zone, there is an increase in the phytoplankton abundance (Siokou-Frangou et al., 2010). Development of summer thermocline also coincides with the low nutrient low chlorophyll conditions where DCM can also be observed. In such summer periods in the Eastern Mediterranean Sea, concentrations of ammonia are reported to be between 30–80 nM, whereas nitrate and nitrite <1–10 nM and phosphate <2–4 nM (Krom, Woodward, Herut, Kress, Carbo, et al., 2005). It was concluded that the presence of measurable ammonia in the photic zone especially when nitrate is close to or below detection limits suggests that grazing is an important process in this system since ammonia (and DON) are the first products of grazing activity (Krom et al., 2005; Thingstad et al., 2005). A strong nutricline which separates low concentrations of nutrients in the surface waters and high concentrations below the euphotic zone (EZ) is a characteristic in the eastern basin, although the nutricline deepens from the west to the east (Pujo-Pay et al., 2011).

Together with the inorganic N to P ratio, ratios of PON/POP and DON/DOP are also higher than the Redfield ratio. These PON/POP and DON/DOP ratios are reported to be 27–32:1 and ∼100:1, respectively (Krom et al., 2005) as shown in the Figure 4. In the relatively more productive western basin of Mediterranean Sea, POM concentrations range between 1.4 to 9.2 μm (Socal et al., 1999). In the anticyclonic area of the North-Eastern Mediterranean Sea, POC/PON molar ratio is reported to be ~7 during winter and 11.4 ±4.8 during summer stratification (Ediger et al., 2005) Thus, POC is ~100 times higher than the chlorophyll-a in winter whereas it is ~200 times higher in the summer period. Relatively high C/chl-a ratios in the POM from the nutrient depleted surface layer indicates that POM pool is mostly composed of the bacteria, detritus and zooplankton (Ediger et al., 2005). Chl-a in the basin exhibits a feature in which it peaks in subsurface waters (Deep Chlorophyll Maximum) except during the winter mixing (Arin et al., 2002; Estrada et al., 1993) and this unique feature was also confirmed in the Northern Levantine Basin (Ediger and Yilmaz, 1996; Ediger et al., 2005).

One important mass-balance modelling study of P and N suggests that the main contribution to the new production in the Mediterranean Sea is the lateral input of N and P (Figure 5: fraction of sources in the two basin). The reason of the oligotrophy in the Mediterranean Sea was also pointed out that for both the WMS and EMS the anti-estuarine circulation exports more readily bioavailable P than it brings in (Powley et al., 2017).

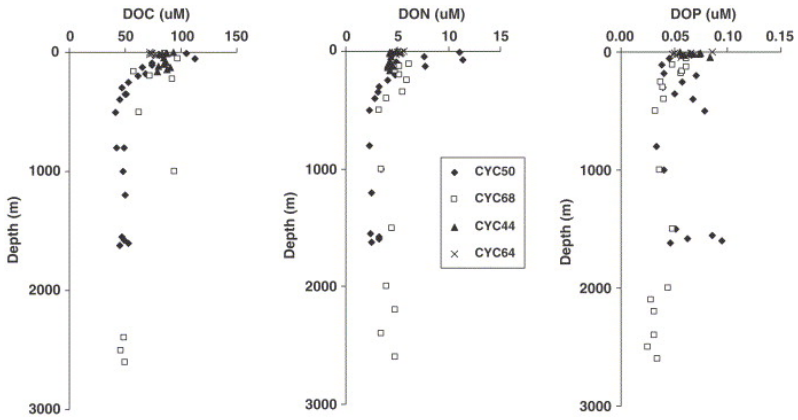

Figure 4. Depth profiles of DOC, DON and DOP from outside the Cyprus warm-core eddy. Figure was taken from (Krom et al., 2005, Nutrient cycling in the south east Levantine basin of the Eastern Mediterranean: Results from a phosphorus starved system).

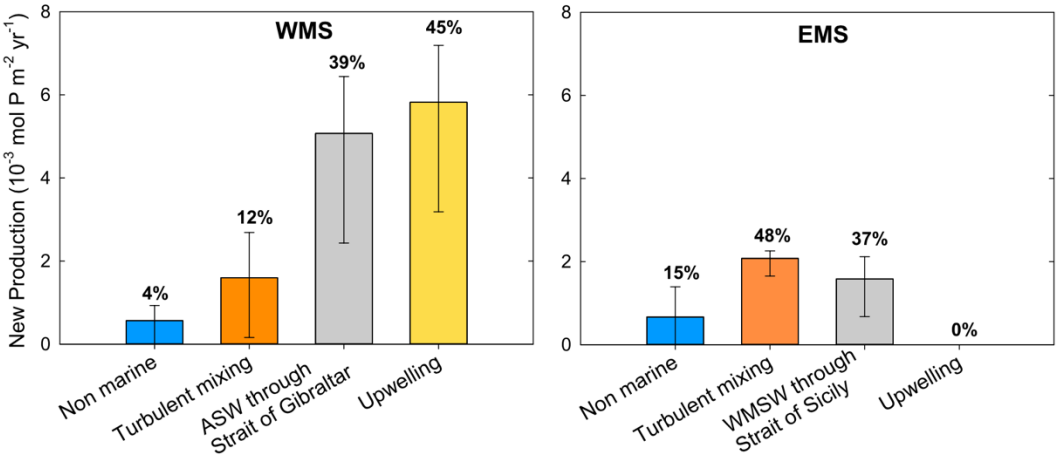

Figure 5. Source attribution of new production in the WMS and EMS. Figure was taken from Powley et al. (2017). The percentages represent the relative contribution of each source; for a total new production of 10.6×10^9 mol P/yr in the WMS and 5.8×10^9 mol P/yr in the EMS.

Biological N_2 fixation is a process that introduces new N to systems that are N-limited and fewer number of studies were conducted in the Mediterranean Sea showing N_2 fixation as another source of N to the system. This process is expected to be present where N is depleted in the system. On the other hand, studies reported the presence of N_2 – fixation where N is not

limited (Yogev et al., 2011). Low to moderate rates of N_2 – fixation were reported in the Western Mediterranean basin (Garcia et al., 2006; Sandroni et al, 2007). Across a west-east transect low rates of N_2 – fixation was also shown (Ibello et al., 2010). Rahav and colleagues, (2013) recently showed higher N_2 – fixation rates during summer in the western (higher in the Strait of Gibraltar) and eastern basin (2.35 and 0.10 nmol NL−1 d−1, respectively) compared to the previous studies. Furthermore, the role of the heterotrophic diazotrophs might be important in the Eastern Mediterranean and autotrophic diazotrophs have a more intense role in the western part of the basin (Rahav et al., 2013). Indicator gene of N_2 – fixation, *nifH*, was studied over 2 years in the Israel coast (representing characteristics of Levantine basin) and this study suggests that members of the Cyanobacteria and Proteobacteria were identified as N_2 – fixers. In another study, N_2 – fixation was reported to be the minor contributor to the new N input in the region studied (Yogev et al., 2011).

Another important question is how organisms take up and release phosphate, the concentrations of which are often close to the detection limit in the open Eastern Mediterranean waters. Orthophosphate (P_i) is the most preferred form of P for organisms. In the Mediterranean Sea, nanomolar Pi concentrations and seasonally changing turnover times (minutes to hours) were reported (Krom et al., 2005; Sebastián et al., 2012; Thingstad et al., 1998). Assimilation of phosphate and adenosine-59-triphosphate (ATP) by heterotrophic bacteria and cyanobacteria (both *Prochlorococcus* and *Synechococcus*) was evaluated in the Sargasso Sea (Michelou et al., 2011). Study concluded that *Synechococcus* uptake rate for P and ATP was 50-to-80 fold higher than other groups, thus no significant difference between ATP and phosphate uptake per cell by *Prochlorococcus* and heterotrophic bacteria was detected. However, due to the dominance of heterotrophic bacteria in terms of abundance, total uptake of P and ATP (per liter of seawater) was dominated by heterotrophic bacteria. One important study investigated the phosphate uptake rates of the phototrophic and heterotrophic groups along a transect from the Levantine Basin to Western Mediterranean in the euphotic zone (Talarmin et al., 2015). In total, heterotrophic bacteria contributed 82% (due to their high abundance) of the total Pi uptake by variable cellular rates (6.6 ± 9.3 amol P cell/1*h) between surface to 50 m depths. On the contrary, in the surrounding of DCM, cyanobacteria were the major contributors of P_i uptake (up to 72%). *Prochlorococcus* and *Synechococcus* cells during their higher abundance was observed takes up 40 and 402 amol P per cell/h, respectively (Talarmin et al., 2015).

1.1.3. Plankton Community

Dominance of picoplankton (cell size ranges between 0.2-2 μm, mainly composed of cyanobacteria) in the whole basin (Magazzu and Decembrini, 1995) is reported and this feature is related with the low biomass values in the MS (Ignatiades et al., 2002; Tanaka et al., 2007). Especially, picoplankton outcompetes other phytoplankton groups (that are bigger cell sized) for nutrients in the surface waters of the southern Levantine basin (Yacobi et al., 1995). *Synechococcus* are found to be the dominant group when summer stratification results in low nutrient conditions in the surface (J.-C. Marty and Chiavérini, 2002) while low light adapted *Prochlorococcus* are found to thrive in deeper waters (Li, Zohary, Yacobi, and Wood, 1993; Partensky, Hess, and Vaulot, 1999; Yacobi et al., 1995) but also high light adapted ones can be found in the surface waters in the summer (Moore et al., 1998). *Coccolithophores* are found to be the more abundant in the EM than the WM (Ignatiades et al., 2009) where generally the most represented species are *Emiliania huxleyi* in autumn and winter (Gačić et al., 2002). *Cryptophytes* generally show increase in their presence during winter and spring (Marty et al., 2002; Vidussi et al., 2001). Diatom increase is reported in EMS during winter (Gačić et al., 2002; Wassmann et al., 2000). Diatoms often dominate in eutrophic environments such as in upwelling regions, river mouths, spring blooms and blooms triggered by wind-mixing events (Ragueneau et al., 2000). Ignatiades and colleagues (2009) showed that actually in the Levantine basin diatoms are less important in the formation of DCM (Ignatiades et al., 2009) compared to the WMS (Marty and Chiavérini, 2002).

Unicellular microbes are the unseen majority of marine life (Whitman et al., 1998) and known to be dominant in terms of abundance, metabolic activity and diversity (Azam and Malfatti, 2007b). Bacteria consume almost half of the primary production in the oligotrophic EMS, especially along the depths that are between the sea surface and the depth of the deep chlorophyll maximum (Turley et al., 2000). Prokaryotic primary producers, especially picoplanktonic *Synechococcus* spp., are dominant with respect to the total photosynthesis that occurs in the oceans (Waterbury et al., 1979). *Synechococcus* spp. is able to use different N sources such as ammonium and nitrate (Moore et al., 2002; Zohary et al., 2005). This ability is probably the main reason why these species are dominant. They play a crucial role of organic carbon production in the Mediterranean ecosystem (Agawin et al., 1998). Furthermore, if phosphorus (P) in form of PO_4 is available, *Synechococcus* is able to outcompete eukaryotic phytoplankton species (Moutin et al., 2002; Tanaka et al., 2007). However, recent findings on the global distribution of picocyanobacteria showed that nutrients were not the significant factor impacting the distribution at local scale but rather it is modulated by PAR (Photosynthetic Active Radiation) in a positive and non-linear fashion (Flombaum et al., 2013).

In this chapter, dynamic structure of the ETS-200 m station in terms of physical, biological and chemical features is discussed using available one-year long time series data. **It was aimed to understand underlying physical and biological characteristic of the study region and connect these properties with the range of features described aforementioned studies in the region. This chapter was also aimed to establish the environmental setting for the -omics studies described in the Chapter II, III and, IV. Results of this chapter were used to interpret the -omics studies outcomes in the correct context.**

In order to achieve these goals, we participated in the collection of water samples required for inorganic nutrients, particulate organic carbon, particulate organic nitrogen, total phosphorus and plankton samplings. Furthermore, produced data was controlled in respect to quality, visualized and, interpreted.

1.2. Material and Method

Current study has been conducted in the Erdemli Time Series (ETS) regular monitoring station of 200 m water column depth (ETS-200) which is located at the coordinates 36º26.1694'N and 34º20.7556'E (Figure 6). Data that has been used in this chapter is collected under the framework of Middle East Technical University, Institute of Marine Sciences monitoring studies. *All the physical and biogeochemical data is produced by a collective effort of scientists, technicians and, crew members of R/V Bilim-2 and R/V Lamas.*

Physical Properties

Physical properties of the seawater collected in the ETS-200 station are salinity, temperature, density, oxygen saturation, which were measured *in situ* using a CTD - Seabird model-SBE 19- fixed to a rosette sampler. The precision of the CTD probe is ±0.005 °C for temperature and ±0.0005 S/m for conductivity. Salinity values were calculated automatically from *in situ* conductivity and temperature measurements.

Mixed layer is defined as a layer in which active turbulence has homogenized among some range of depths in the ocean. Within the mixed layer mass, momentum and energy is transferred. Mixed layer depth (MLD) has important implication on chemical and biological properties of water column. In order to calculate MLD, the density at the depth of 10 meters was taken as a reference. The depth where the change in the density was more than 0.03 kg m^{-3} defined as MLD (de Boyer Montégut, 2004).

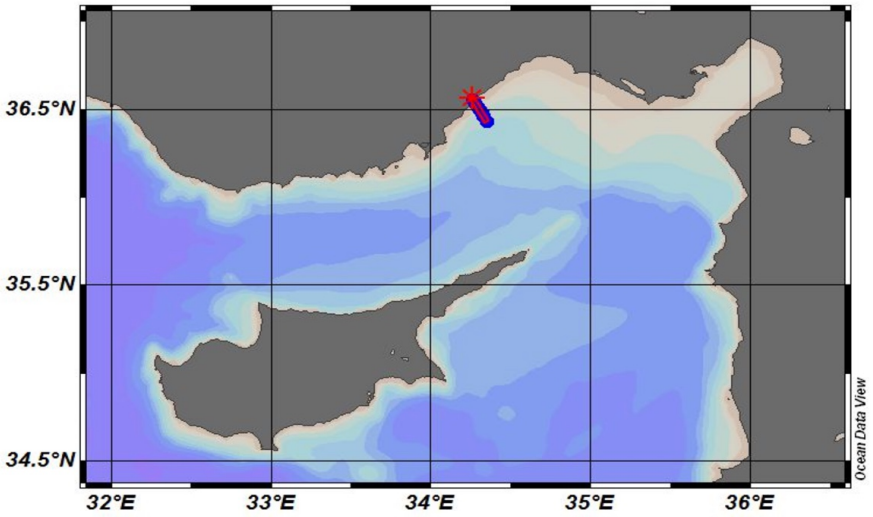

Figure 6. Location of Erdemli Time Series Stations. Stations of ETS were named after their total water column depth: ETS 20 m, ETS 50 m, ETS 75m, ETS 100 m, ETS150 m, ETS 175m, ETS 200 m and, ETS 500m. Study conducted in the relatively off shore station of ETS 200 m (8.7 nm away from the shore).

Calculation of Euphotic Zone depth

Photosynthetic Active Radiation data was used to approximate the euphotic zone (EZ) depth. EZ depth is defined as the depth where 1% of the surface PAR reaches. PAR sensor is located in the upper part of CTD rosette system while pressure sensor was located in the bottom part of the system. The distance between these two sensors was 1.15 meter. Due to this distance EZ depth was calculated from the depth of 1m. One important issue regarding the $PAR_{(1m)}$ data was also reported in the IMS-METU internal report regarding the issues in PAR data acquisition. Due to physical misalignment of sensors, the $PAR_{(1m)}$ can be contaminated with data obtained in air and some measurements do not contain data in the 1m depth. To overcome these issues extrapolation by polynomial equation of 6^{th} order obtained with curve-fitting based on 50 data points. PAR at 1m depth as suggested in the IMS-METU internal report. PAR_{1db} can be successfully used for estimating the depth of euphotic zone and the average error was reported to be below 10%. Accordingly, euphotic zone depth was calculated from the PAR data extrapolated to the 1m depth ($EZ_{(0)} = EZ_{(1m)}$).

Inorganic nutrients and Chlorophyll- a measurements

For nutrients, water samples were drawn from the Niskin bottles into high-density polyethylene bottles (HDPE) and deep frozen at -20 °C until they were analyzed after standard

calorimetric methods (Strickland and Parsons, 1972) using a four-channel auto-analyzer (Bran Luebbe). Detection limits for nitrate+nitrite, nitrite, phosphate and ammonium were 0.05 μM, 0.01 μM, 0.02 μM and 0.05 μM respectively. For chlorophyll-a measurements, seawater from corresponding depths was taken into brown polyethylene bottles and immediately filtered (1050-ml) on GF/F filters. Before the analysis filters were digested with 90% acetone (Strickland and Parsons, 1972) and a conventional spectrofluorometric method was used (HITACHI fluorescence spectrophotometer F-2500) to determine chlorophyll-a concentrations. Calibration was performed using commercially available standard curves for chlorophyll-a. Standard curves of inorganic nutrient measurements are given in Appendix A. It should be stated here that METU-IMS chemistry laboratories have successfully participated in the international QUASIMEME intercalibration tests.

Organic matter, Total Phosphate, Total Nitrogen sampling and measurements

POM sampling has been carried out at the same sampling depths as the 16S rRNA amplicon sequencing study (Chapter II): surface, 25 m, 50 m 100 m 150 m and 200 m. In total, two liters of sea water was sampled with brown polyethylene bottles and directly filtered on GF/F filters for each POC and PON measurement. Organic carbon and nitrogen were measured by dry combustion method using Carlo Erba model 1108 CHN analyzer. Total phosphate and total nitrogen were measured calorimetrically after using published methods for digestion and extraction of N from labile and refractory pools (Ediger et al., 2005).

Dissolved organic phosphate was calculated from the direct measurements of total phosphate (TP), particulate phosphate (PP) and inorganic phosphate (IP) according to the equation given below.

$$DOP = TP - (PP + IP)$$

Calculation of saturation oxygen content, percent oxygen saturation, and apparent oxygen utilization

Oxygen concentrations in the ocean are governed by the changes in the biological activity of the water column as well as physical properties. As a product of photosynthesis and substrate for respiration, the distribution of oxygen has been used to determine the locations of biological activity. The saturation oxygen content (SOC) is the amount of oxygen in one liter of sea water to which any addition of oxygen would result in outgassing, or to which any subtraction would result in gas solution, if the water parcel were at the ocean surface under 1 atm of pressure. Apparent oxygen utilization **(**AOU) on the other hand, provides an estimate

of the amount of oxygen used since the water parcel was at the surface and hypothetically fully saturated.

Equations used for the computation of solubility values of dissolved oxygen, in various units, was applied according to UNESCO recommendations (UNESCO, 1986) and (Weiss, 1970). Equations and constants used are as follows:

$$\ln(\mathbf{SOC}) = I + (J/T) + (K/T^2) + (L/T^3) + (M/T^4) - S^* (N + (P/T) + (Q/T^2))$$

$$\%\ SOC = [\ O_{2\,[m]} / O_{2[SOC]}) \times 100] - 100$$

$$AOU = O_{2\,[m]} - O_{2[SOC]}$$

where;

T= temperature in Kelvin

S = Salinity

$O_{2\,[m]}$ = measured oxygen concentration in uM

I = -135.90205

J = 1.575701×10^5

K= -6.642308×10^7

L = 1.243800×10^{10}

M = -8.621949×10^{11}

N = 0.017674

P = -10.754

Q = 2140.7

Plankton community sampling

Extra 100-ml of seawater was collected into borosilicate dark bottles from the same Niskin bottles that collects DNA samples for microbial community analysis. Samples were fixed with 2-ml 25% glutaraldehyde and stored at room temperature in a dark area until heterotrophic bacteria and *Synechococcus* spp. cell counts and light microscopy cell counts of eukaryotic phytoplankton species. Cell abundances are converted to biomass values for a better comparison. Furthermore, bio-volume and carbon content of the cells were used for abundance to biomass conversion. Biovolume of heterotrophic bacteria and *Synechococcus* spp. was

taken from (Boran, 2017) as an average for each depth and month and converted to biomass as suggested by Carlson and colleagues (1999) (Carlson et al., 1999) for heterotrophic bacteria (77 fgC/cell) and by Waterburry and colleagues (1986) (Waterbury et al., 1979) for cyanobacteria (123 fgC/cell). Phytoplankton cells' carbon and nitrogen quotas were estimated from cell volumes (Arin et al., 2002; Fanuko and Valcıc, 2009) using the equations given by the Menden-Deuer and Lessard (2000) (Menden-Deuer and Lessard, 2000, Table 3 and Table 4) .

Notes regarding the figures generated

Plots regarding the chemical profiles were started in August 2016 to better convey the dynamics in the patterns of overall biological and biogeochemical properties in September 2016. Minimum concentration values were set to the detection limits of the instrument corresponding to each parameter. For instance, detection limit of nitrate was set to 0.05 μM. Here it should be stated that data regarding heterotrophic bacteria, *Syneccococcus* spp., and phytoplankton abundances collected from 6 different depths throughout the water column whereas, chemical data was collected from 8 different depths.

Table 1: Bio-volume, carbon and nitrogen content of phytoplankton community for biomass calculation

	Volume (μm^3)	Ref.	PgC/cell	PgN/cell	Ref.
Nanoflagellates	93*	(Arin et al., 2002)	12.87939123	5.536585657	(Menden-Deuer and Lessard, 2000)
Coccolithophoride	265*	(Arin et al., 2002)	31.64361425	9.041032644	(Menden-Deuer and Lessard, 2000)
Diatoms	63942.5*	(Arin et al., 2002)	1999.404422	571.2584063 (3.5 C:N used for convertion)	(Menden-Deuer and Lessard, 2000)
Dinoflagellate	9404*	(Arin et al., 2002)	1365.03264	278.8318722	(Menden-Deuer and Lessard, 2000)
Cryptophyte	14	(Fanuko and Valcıc, 2009)	6.602050792	1.109334967	(Menden-Deuer and Lessard, 2000)
Dictyochale	13309**	(Fanuko and Valcıc, 2009)	1814.157979	374.4548551	(Menden-Deuer and Lessard, 2000)

* Average of given values in Arin et al., (2002) for their sampling stations C2 and C4.

** Average of given values for Dictyochale.

1.3. Results

1.3.1. Local physical features

General water column feature mainly represents Mediterranean characteristics: The water column is highly stratified in summer with relatively depleted nutrient concentrations in the surface layers whereas in winter water column is well mixed. The study area of this study, the Northeastern Levantine Basin, is under the influence of the westward-flowing Cilician Current and fresh water input from a small river called the Lamas River. Besides the Lamas River, larger Göksu River is to the west of the ETS stations and Seyhan and Ceyhan rivers to the east of the study area. Fresh water input from these rivers, especially in late winter to spring, may influence the abundances of planktons and nutrients in the study area. Still, the local Lamas river can be considered as the main freshwater input to the station locations. Lamas discharges higher volumes during early spring as observed for other rivers impacting the area (Koçak et al., 2010). Annual mean water discharge of the Lamas River (3 m^3s^-,) is lower than the Göksu (45 m^3s^-) and other rivers such as Seyhan, Ceyhan, Berdan. It has been shown that Lamas river has lower ammonium and phosphate concentrations (0.4 and 1 μM, respectively) and high nitrate concentrations (101 μM) compared to other rivers flowing into the Northeastern Levantine Basin (Koçak et al., 2010).

Seasonal variability of temperature and salinity during 2014-2017

In this section, seasonal variations in the water column in terms of salinity and temperature profiles for different stations (from shore to off-shore) will be discussed using the data collected during ETS monitoring studies between 2014 and August 2017.The water column along the ETS stations displays an annual cycle of cooling and warming. Warming of the water column begins around April and continues until approximately through end of October (Figure 7). The thermocline depth varied between 40-80 m during the summer at the deeper stations, with T= 27.5 C, and S= 39.3 - 39.5 (Figure 7 and Figure 8) in the observation period. Winter mixing was dominant in all stations from 20 m to 200 m and stratification was observed in the off-shore stations, in line with the general characteristic of Mediterranean Sea. In the summer period, whole water column in the 20 m station warms gradually starting from April-May and cools down from November on. Since the total water column depth is shallow, summer stratification could not be observed as could be in other observation stations. However, presence of summer stratification is apparent starting from the 100 m station (Figure 7). In all stations, deeper thermocline was observed in the summer of 2014 while a shallower one observed in 2015 due to extra heating compared to other years (Appendix B- SST comparison between 2014 and 2015). Thermocline depth varies between 40 to 80m in the ETS-200 throughout the years and the year 2014 has the deepest thermocline where SST averages in the summer were lower than those measured in the summer of 2015 (Appendix B).

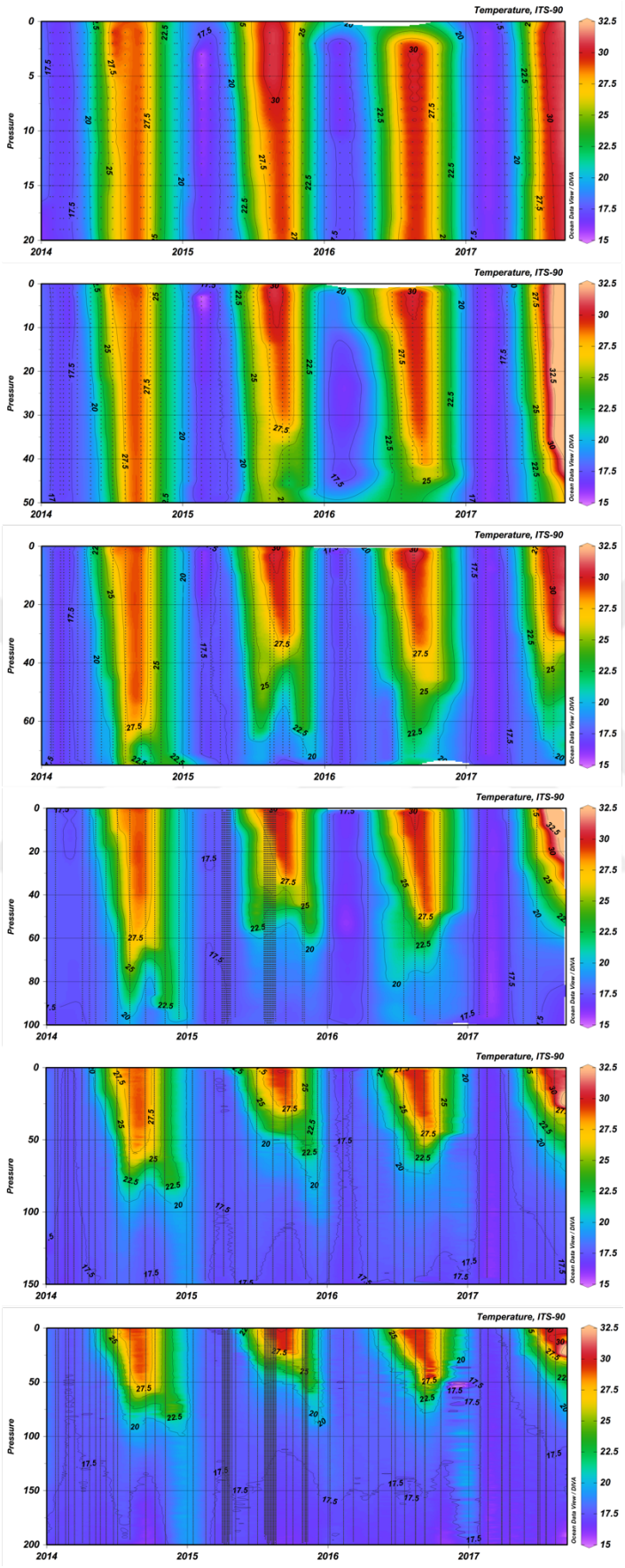

Figure 7. Temperature profiles of ETS stations located at depths of 20 m, 50 m, 75m, 100 m, 150 and 200 m.

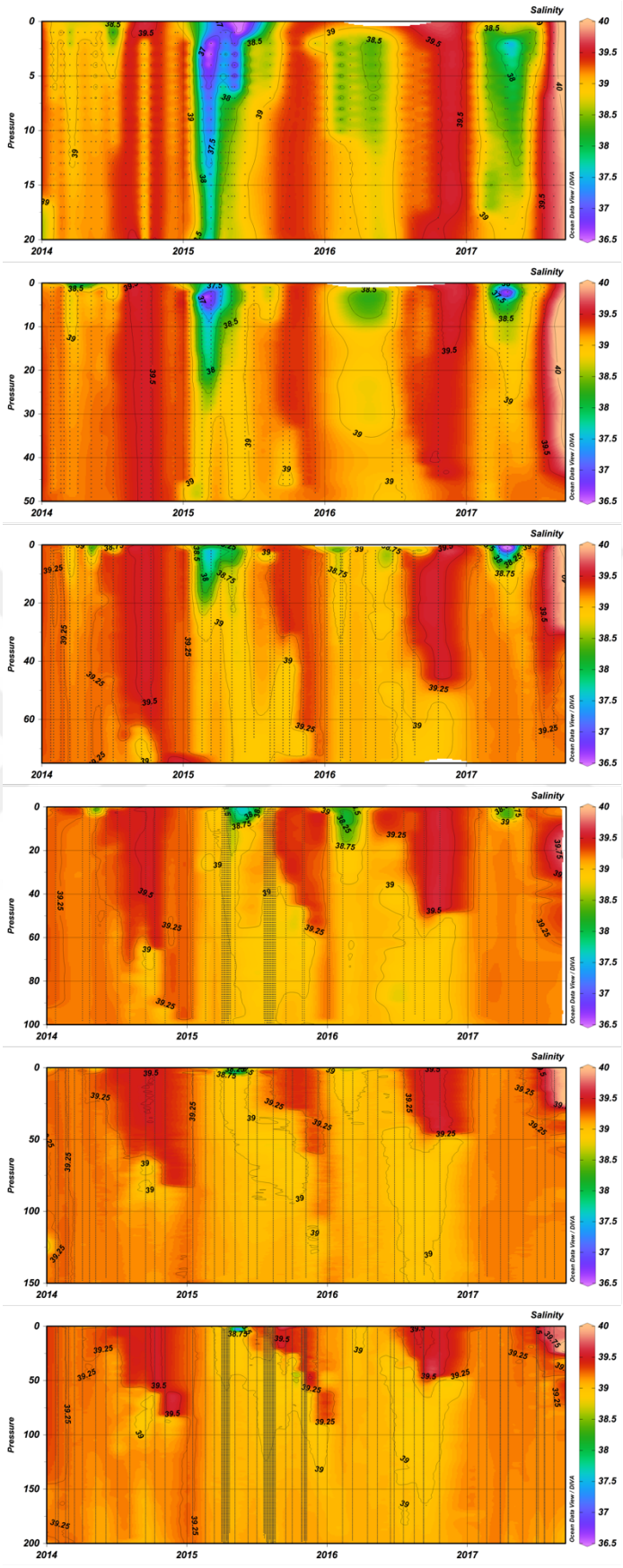

Figure 8. Salinity (PSU) profiles of ETS stations located at depths of 20 m, 50 m, 75m, 100 m, 150 and 200 m.

Among the ETS stations, the coastal one (ETS-20 m), is the closest one to the Lamas River in location. Fresh water intrusion might be the factor that affects the salinity and temperature profiles of ETS sampling stations (Figure 8). Fresh water entry that is present in the four of the stations are seen in February to April 2015 which is predominant in the coastal (20 m depth) station. Low salinity profile (35.6 in the surface of ETS-20 m) from April to May however was only observed in the first 6m of the water column. In the ETS-200 m station on the 6th of May 2015, low salinity water was observed in the surface with values of 37.2 psu. This water mass is possibly the intrusion of Lamas River as can be inferred from the turbidity profile (high in the surface) of the same date (Figure 9). One strong support that this low saline water is the plume from the Lamas River can be inferred also from the Figure 10, noting that during April and May precipitation was not dominant at the ETS-200 m station but rather high level of precipitation occurred over the continent. In May 2015, the dominant wind direction was from the N-NE sector, and the river water extended to ETS-200 (Figure 11 and Appendix C). In the March 2017 cruise, the extent of the Lamas river was limited to the nearshore stations only. While the dominant wind was again from the N-NE direction, winds from the SW were more frequent and stronger than in March 2015. It should be noted that the wind data are taken from Taşucu, which is about 60 km west of the ETS site. Wind-rose plots specifies how many times wind was blowing from specific directions. Wind direction and speed observed during February 2015 to May 2015, when fresh water plume reaches the ETS-200 m station, show that it was flowing from directions that supports the movement of the Lamas River plume to the ETS-200 m station (Figure 11 (a) and Appendix C). Dominant wind direction in March 2017 was coming from the opposite direction compared to 2015 March (Figure 11 (b)) which is why Lamas River could not reach to the relatively off-shore stations of ETS. To conclude, the dominant wind direction and speed is the determinative factor of the river plume to the off-shore stations in the study area together with heavy rain. Atlantic modified water is one of the sources of the less saline waters especially under the LSW during strong stratification periods in the Levantine Basin. However, available data is not sufficient enough to conclude AMW penetration to the study area. Furthermore, Cilician Current, which flows westward along the southern coast of Turkey, tends to follow its path without entering Mersin Bay (Dr. Mohammed Abdul Latif, personal communication).

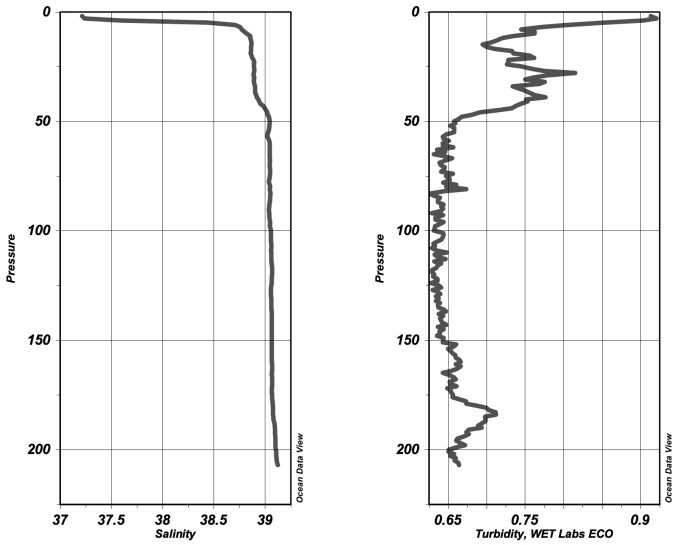

Figure 9. Salinity (a) and turbidity (b) profiles at the ETS-200 m station on 06 May 2015

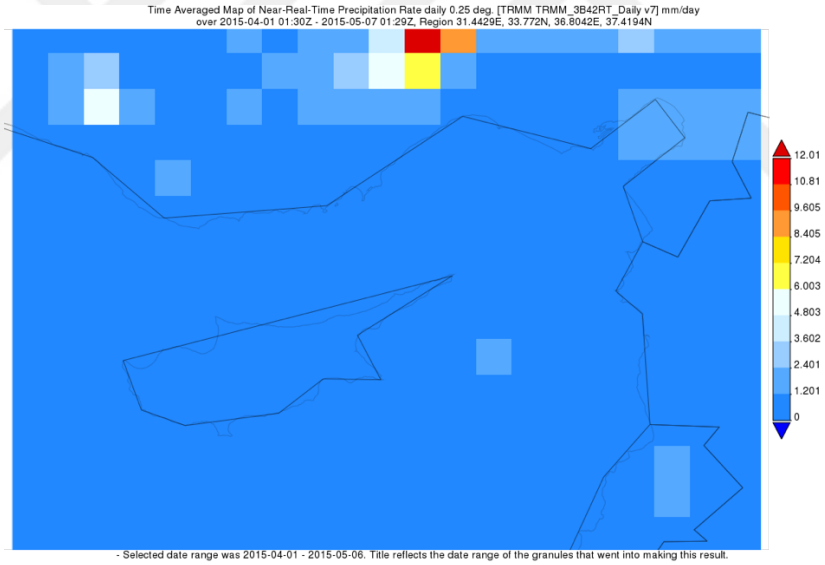

Figure 10. Time averaged map of precipitation in the region between 1st Apr 2015 and 5th May 2015. (https://giovanni.gsfc.nasa.gov/giovanni/).

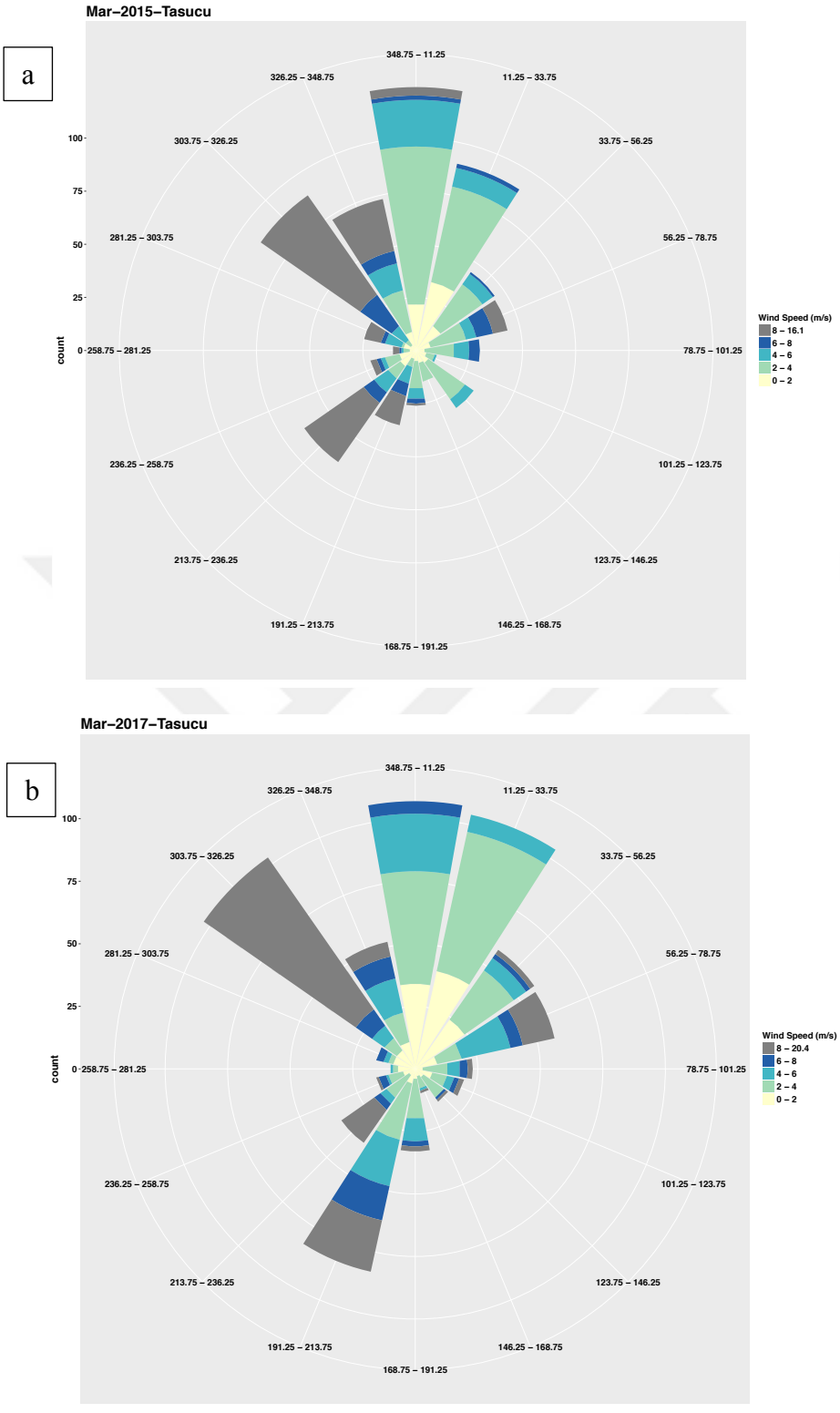

Figure 11. Dominant wind directions and speed measured in the Taşucu- Silifke on (a) March 2015 and (b) March 2017. Please note that wind directions are given as direction of origin (flowing from) and north is between 348.75 and 11.25 in direction degrees. Y-axis (counts) represents the number of the wind flowing from each direction.

1.3.2. Biogeochemical features of ETS-200 m station

One of the main aims in the present study is to understand the ecosystem dynamics of the ETS-200 m station and description of the main factors affecting the dynamics of the bacterial community structure. This part is devoted to the description of the ecosystem dynamics based on the data collected between September 2016 and August 2017 coinciding with the period of 16S rRNA microbial community sampling. Seasonal variations observed in each biogeochemical parameter were described.

Nitrate

Throughout the year nitrate concentrations (NO_3+NO_2) ranged between 0.04 and 1.81 µM (average, 0.37 µM ± 0.40) for all periods and all depths. First 100 m of the water column never exceeded concentrations of 0.65 µM. On the other hand, below 100 m, concentrations observed were relatively higher having a min of 0.21 and a max of 1.81 µM.

In the fall season, high concentrations of nitrate were observed under the 150 m depths (Figure 12). Especially at 200 m depth in October, maximum concentration of nitrate was noticed to be 1.81 µM. Such peak in the concentration noted in the deeper water column was contrary to lower concentrations observed in the upper water column. Low concentration of 0.08 µM was detected in October 2016 at 25 m depth. Concentration was relatively homogenous during winter mixing. From April to June 2017, upper water column (upper 75 m depth) appeared to have low concentrations while deeper water shows relatively high nitrate concentrations (Figure 12).

Overall, three different patterns were clear in the variation of nitrate concentrations. A sharp decrease of NO_3+NO_2 concentrations (that ranged between 0.08 and 0.16 µM) occurred from September 2016 to November 2016 in the upper 100 m. This was followed by a homogenized concentration along all water column (due to winter vertical mixing). Between January and March 2017 NO_2+NO_3 concentrations displayed higher values, ranging between 0.19 and 0.51 µM in the upper 100 m depths. A third different nutrient regime was clear in the surface waters starting from April and lasting until July 2017, when concentrations dropped dramatically (ranges: 0.04-0.65 µM, average:0.12) to values close to detection limit (0.05 for nitrate + nitrite).

In the deeper water column (150 and 200 m profiles), increase in the concentrations were well consistent with stratification during August to November 2016 and from April 2017 to August 2017. Between January and March 2017, concentrations were vertically homogeneous (0.21-0.45 µM, 0.32 ± 0.1) due to the vertical mixing.

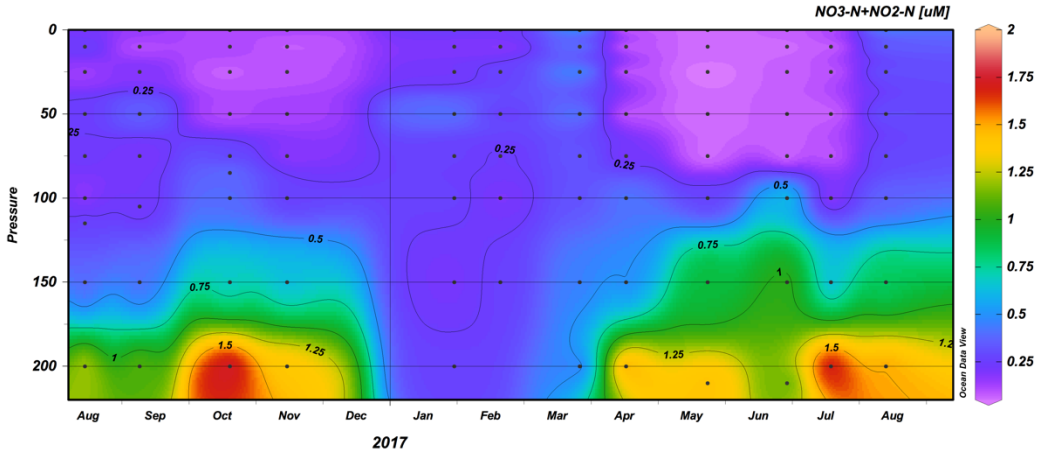

Figure 12. Nitrate concentrations between August 2016 and 2017 at the ETS-200 m station.

Nitrite

Seasonal variation in the nitrite (Figure 13) concentration was not as apparent as that in the nitrate. Throughout the year nitrite concentrations (NO_2) ranged between 0.02 and 0.81 µM (0.036 ± 0.025). Concentrations as high as 0.18 µM in June 2017 were observed at the depth of 150 m. Generally, in summer below the 100 m depth the concentrations of nitrite was increased compared to the other seasons.

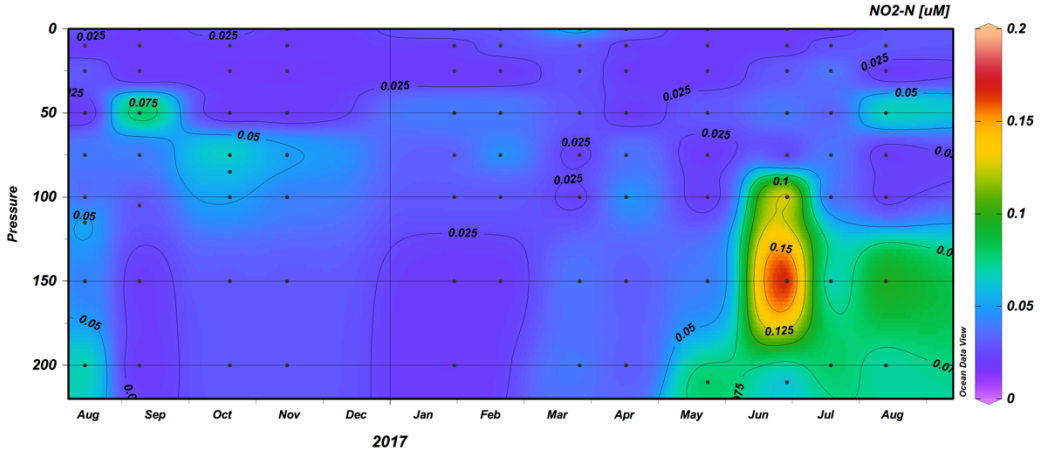

Figure 13. Nitrite (NO_2-) concentrations between August 2016 and August 2017 in ETS-200 m station.

Ammonium

Ammonium concentrations for all depths and season ranged between 0 and 0.45 uM (0.17 ± 0.075). In general, two different regimes were apparent apart from seasonality (Figure 14). November 2016 and January 2017 showed homogeneous profile. On the other hand, above 100 m depth, concentrations are observed to be higher than the below 100 m depth except for the period between November 2016 and Jan 2017.

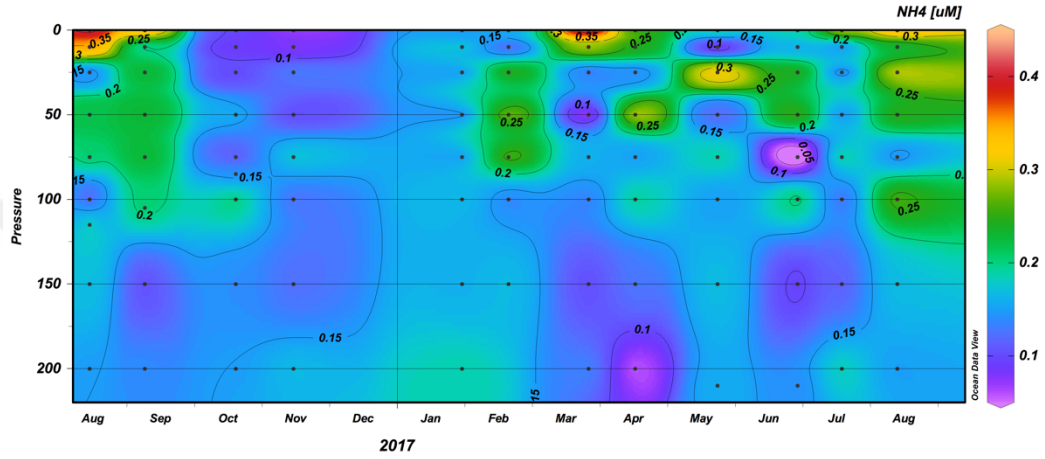

Figure 14. Ammonium (NH4+) concentrations between August 2016 and August 2017 in ETS-200 m station.

Phosphate

Throughout the year, concentrations of the phosphate (PO_4^+) were ranging between 0.02 µM (which is also detection limit) and 0.12 µM (0.044 ±0.021) (Figure 15). High variability was observed in the surface layer, as observed for NO_2+NO_3, with a slightly different pattern from NO_2+NO_3.

During fall season, upper water column shows lower concentrations of phosphate, except for the surface layer where concentrations observed in October 2016 were as high as 0.1 µM and 0.06 at a depth of 75m. Apart from that, concentrations were close to detection limit. In January and February 2017, high concentrations were observed in two different zones; one in the near surface depths of 25 m and 50 m, and the other one was at 200 m depth. There was a peak in the concentrations at 100 m depth in March while upper water column was depleted in bioavailable phosphate. January to March surface PO4 registered a general increase (0.03-0.07 µM) in respect to November 2016. Starting from April 2017, extremely low concentrations

were recorded till August 2017. The maximum value (0.12 µM) registered in May 2017 at 210m, can be also the result of interaction with the sediments, as the sample was collected very close to the bottom.

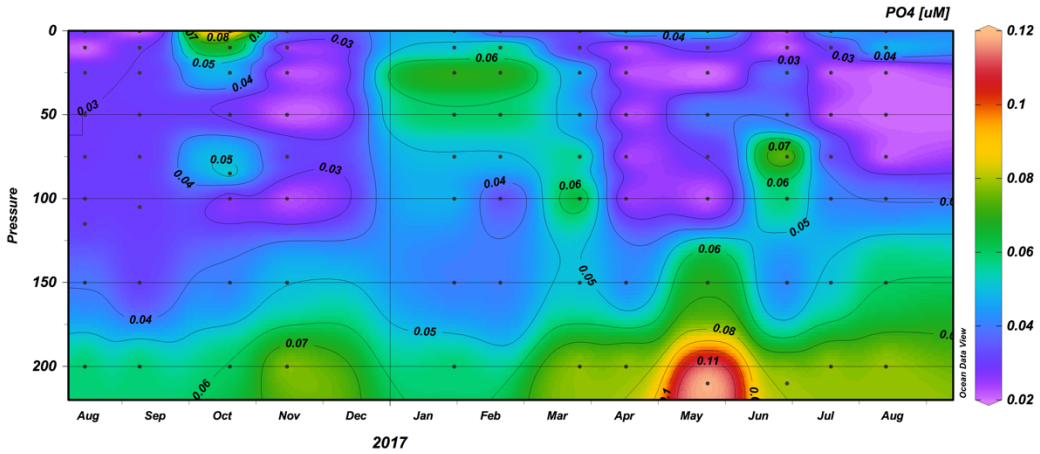

Figure 15. Phosphate (PO_4^-) concentrations between August 2016 and August 2017 at the ETS-200 m station.

N/P ratio

Within the upper 100 m, all throughout the year, N/P ratio (calculated ratio of NO_2+NO_3/PO_4) values were always below 16, indicating a N-limitation of algal production (Figure 16). The deep layer, apart from winter period, which displayed homogeneity of the water column (3.1-7.3), displayed an excess of N over P with respect to the canonical Redfield ratio of 16. Below 150 m N/P ratio varied between 9.5 and 30.2, coherently with the data reported in the literature for Mediterranean Sea.

While evaluating these values, it has to be considered that NH_4+ is not included in the DIN. However, as the values of NH_4 during all year were extremely low (~0.02 µM), not substantial difference is expected in the N/P ratios.

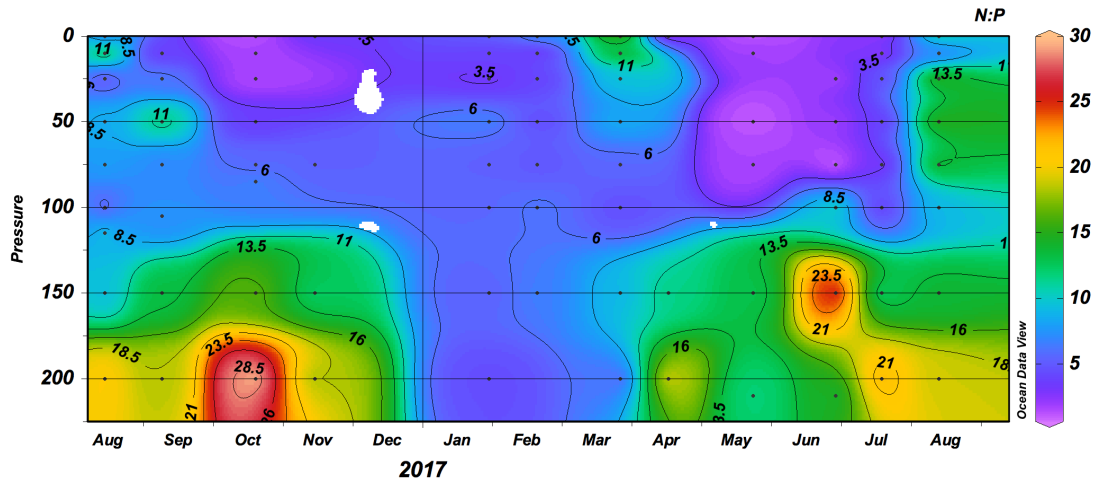

Figure 16. N to P ratio between August 2016 and August 2017 at the ETS-200 m station.

Particulate Organic Carbon (POC)

Throughout the year and all depths, concentrations of POC were ranging from 2.21 to 11.64 µM (4.81 ± 1.72). In August 2016, water column had high concentrations of 7 µM. Especially at 50 m depth, concentrations up to 9.55 µM were observed. Such high concentrations were not observed in 2017 August water column. However, in both years during August the variation in the vertical profile was detected to be the same. In September and October 2016, deeper water column (below 100 m depth) had relatively low concentration compared to 100 m depth, where concentrations were 5-6 µM. This might have reflecting the sinking particles observed in August 2016. The highest observed concentration was detected in January at 200 m depth. In March, depths between 25 m and 100 m possess high concentrations (~7 µM) compared to the very surface and the deeper water column. Starting from April, concentrations in the water column was generally between 2 to 5 with exception in June and July 150 and 200 m depths having slightly higher values (Figure 17).

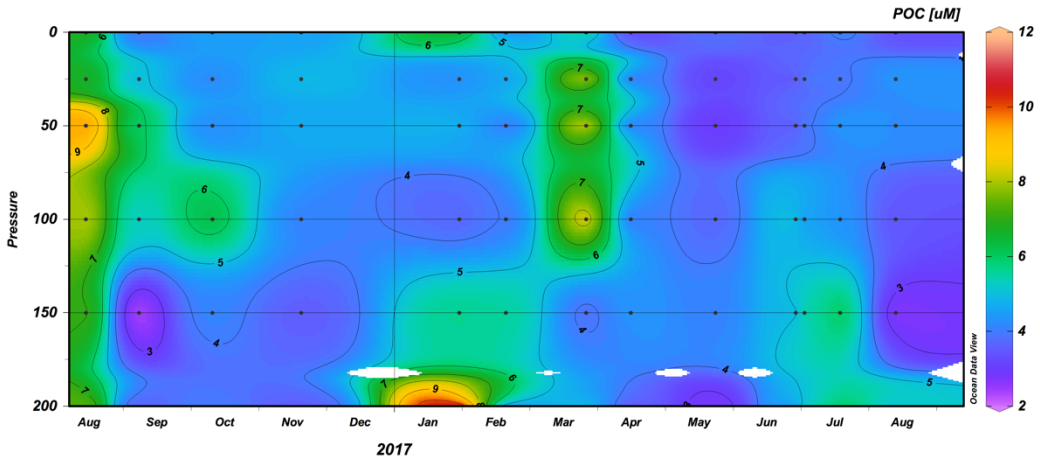

Figure 17. POC concentrations between August 2016 and August 2017 at the ETS-200 m station.

Particulate Organic Nitrogen (PON)

PON variation throughout the year showed mostly the same trend as POC concentrations. Concentration for PON has varied between 0.27 and 1.32 µM (0.60 µM ± 0.22), (Figure 18).

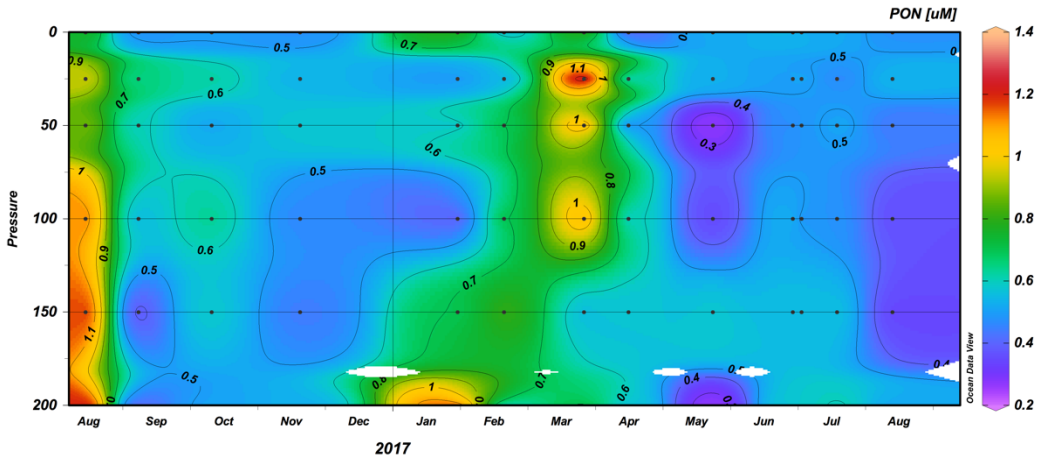

Figure 18. PON concentrations between August 2016 and August 2017 at the ETS-200 m station.

Total Phosphorus

Total phosphate variation in the ETS-200 m station was given in the Figure 20. Concentration values ranged between 0.09 and 0.20 µM (0.14 µM ± 0.02). There was no clear-cut seasonality in the variation of TP concentration as was observed in the inorganic phosphate trends (Figure

15). The highest value during the year-long sampling was observed in September 2016 at 50 m depth with a concentration of 0.2 μM. In October 2016, surface and 200 m depth had the values which are close to each other as 0.16 and 0.15 μM, respectively. In November 2016, below 50 m depth high values detected in the water column except for the 25 m depth which represented relatively lower values. During winter mixing conditions, inorganic nutrients exhibit relatively homogeneous profiles, and this was also the case in the TP measurements. In January 2017, concentration of TP showed lower values than in February 2017. In February, surface water shows lower TP concentrations than that of the water column below. At the beginning of the spring season, in April, 100 m depth shows peak values as high as in February and March 100 m depths. TP concentrations, from May to August 2017, were increased in the deeper water column, below 150 m. In July and August 2017, first 25 m depth has lower concentrations of TP, however high below 50 m depth.

Particulate Phosphorus

Variation in the particulate form of phosphate (PP) represented in the Figure 20, shows that the concentrations were ranging from 0.004 and 0.036 μM (0.014 ± 0.005). There were three seasonal patterns captured in the year-long profiles of PP. First pattern was observed during between September 2016 and December 2016 (transitional period), in which, above the 100 m depth, concentrations were higher than the deeper water column. The only exception was the concentration at 200 m depth in October, where also nitrate concentrations (Figure 12) exhibited a peak. Second pattern occurred during the winter mixing period where the homogeneous and relatively high concentrations were spread throughout the water column. In August 2016, 50 m depth showed the highest PP concentration observed throughout the year. April and May also showed the features of the transition period. The last predominant trend was observed in the summer, where relatively lower concentrations were present in the water column. In the fall season, first 100 m depths had concentrations higher than the surface except, October 2016 200 m depth sample, which had as high concentrations as the surface waters. October 2016, 200 m depth sample also shows high nitrate concentrations and high values of heterotrophic bacterial abundance. In winter, the water column had relatively homogeneous concentrations, and slightly higher concentrations were observed in the 200 m depths. March 2017measurements showed comparably lower concentrations at depths of 0m, 100 m and 150 m. In general, summer season can be summarized as having lower concentrations compared to the other seasons, except for the values 0.02 and 0.03 μM at the depths 0m and 25 m, respectively.

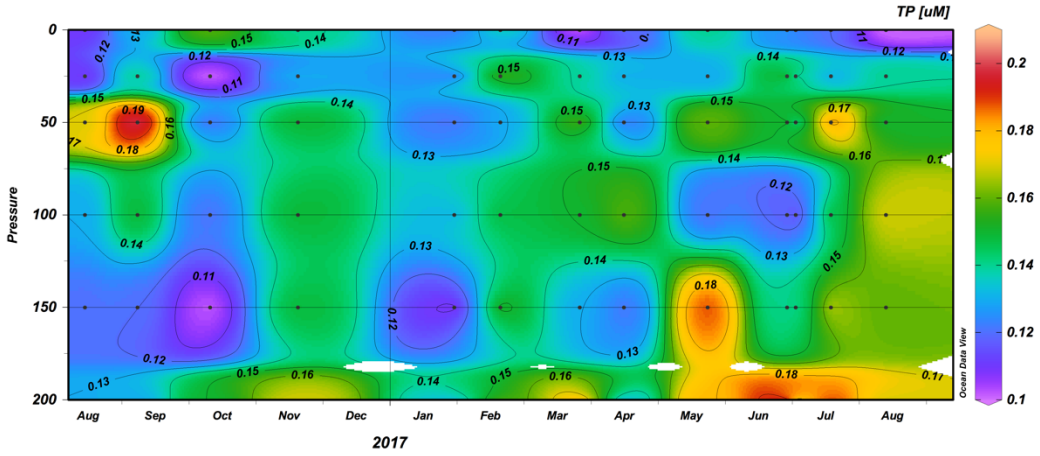

Figure 19. TP concentrations between August 2016 and August 2017 at the ETS-200 m station

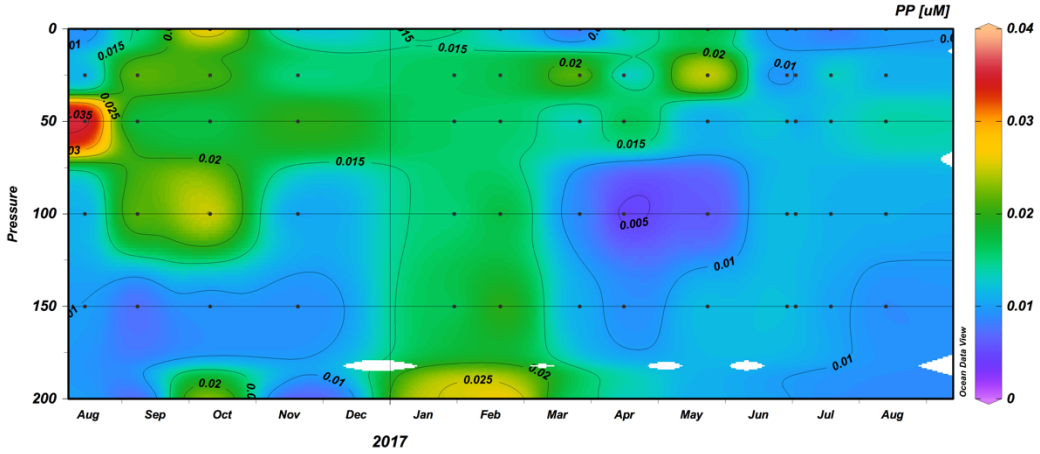

Figure 20. PP concentrations between August 2016 and August 2017 at the ETS-200 m station

Dissolved Organic Phosphorus (DOP)

Dissolved organic phosphate variations throughout the year are given in the Figure 21. Variation throughout the year did not possess any dominant pattern.

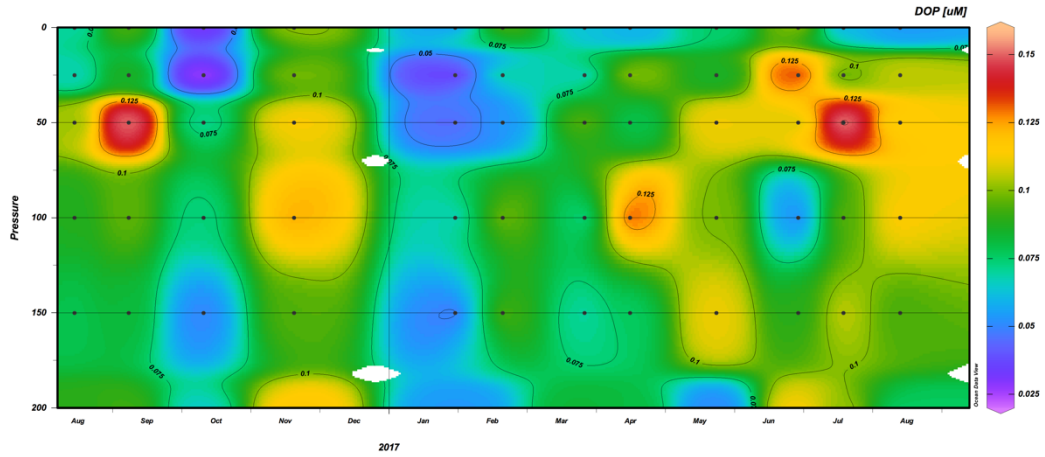

Figure 21. DOP concentrations between August 2016 and August 2017 at ETS-200 m station

Oxygen

Dissolved oxygen concentrations throughout the year were ranging from 196.8 µM to 251.14 µM (227.75 ± 13.68). Two different patterns were predominant in the data set (Figure 22). One period covers the seasons fall, spring and summer while, the other covers the winter season. In the period covering August-November and May-August, concentrations in the very upper layer (first 50 and 10 meters, respectively) are low. During the second period, which covers winter mixing, relatively homogenous concentrations were apparent in the vertical profiles. In the first 25 m of the sampled surface waters during fall season, oxygen concentrations were low compared to the waters below 25 m in the corresponding period. 200 m depth in October 2016 has the second lowest oxygen concentration after August 2017 surface waters. This sample was also detected to possess highest nitrate and silicate concentrations. During the winter period, where water column is well mixed, oxygen concentrations were relatively homogenous. From May to August 2017, 50 m depth zone presents increasing concentrations of oxygen and this high concentration zone extends down to 100 m depth in August 2017. In August 2017, 75m depth represented the highest observed oxygen concentration with values around 251.15 µM.

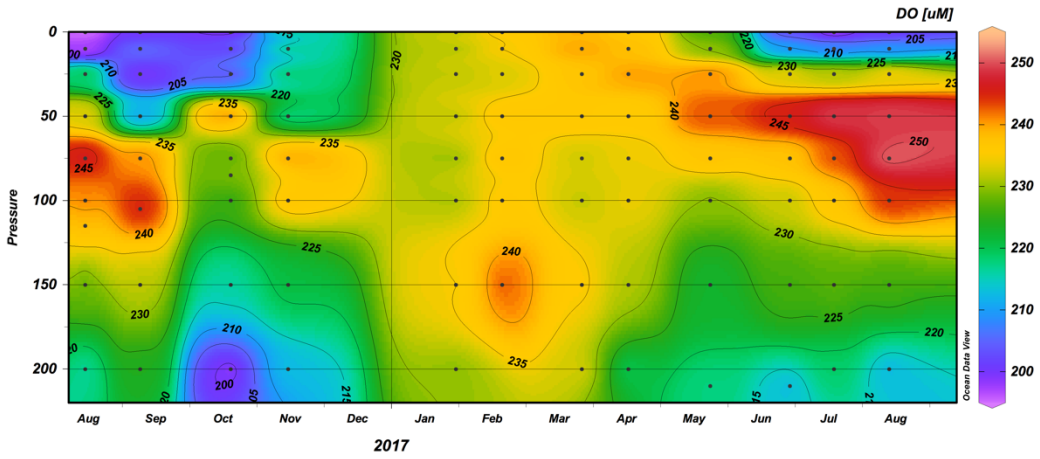

Figure 22. Dissolved oxygen concentrations between August 2016 and August 2017 at the ETS-200 m station

Total Chlorophyll

Total chlorophyll concentrations were very low for all periods (Figure 23) having minimum 0.01 µg/l and maximum 0.25 µg/l (0.066 ± 0.048), consistent with the oligotrophic conditions present at the region. Seasonal variation in the Chlorophyll-a concentrations show the DCM formation in fall and spring and in August. On the other hand, the highest concentration of total chl-a was observed during winter in all water column depths thus, deeper water has the highest concentration (Figure 23). As, the bioavailable nitrogen (NO_3^- + NO_2^-) was depleted in the upper water column except in winter, chl-a concentration was also low.

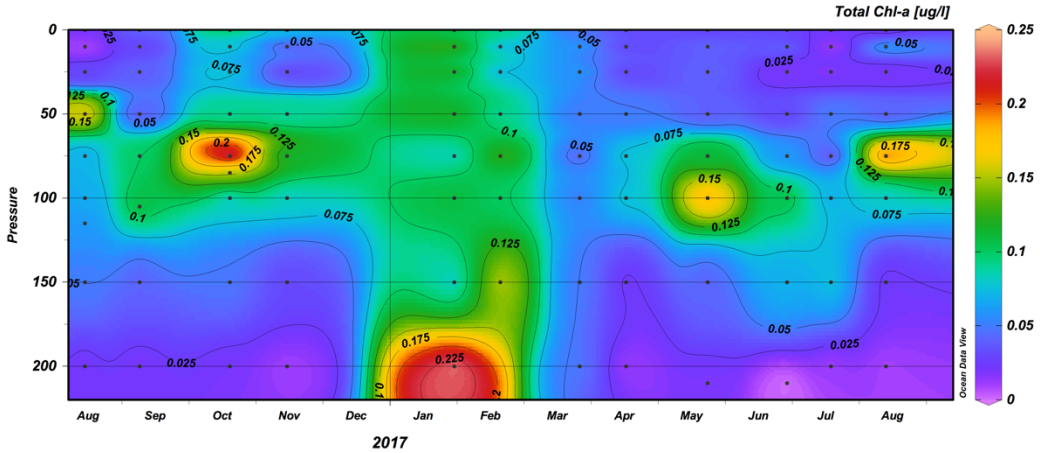

Figure 23. Total Chlorophyll concentrations between August 2016 and August 2017 at the ETS-200 m station

1.3.3. Biological features of ETS-200 m station

Physical and chemical features of ETS-200 m station were discussed in previous sections. This part is devoted to the description of the biological characteristics based on the data collected between September 2016 and August 2017 with the vertical resolution of 6 depths coinciding with the period and depths of the 16S rRNA microbial community sampling described in the following chapters. Seasonal variations observed in each biological parameter were described in the following sub-sections.

Heterotrophic bacteria and *Synechococcus* spp.

Heterotrophic bacteria abundance and biomass at the ETS-200 station for the one year of observation period are shown in Figure 24 and Figure 25. The abundance (number of cells per ml) ranged between 75,276 and 627,805 throughout all months and depths (334,613.3 ± 138,271.869). Heterotrophic bacterial abundance in cells/ml and in biomass possessed higher values during winter, especially in January 2017. Besides high abundance in the whole water column during January 2017, July surface and 25 m depths and August 2017 first 100 m depth of water column show peak in abundance as well. In general, except for winter period, bacterial abundance was higher in the surface and relatively less in the deeper water column. One important peak that was observed occurred in October 2016 at 200 m depth. This instance, as described in the previous section, coincides with the high N/P, nitrate and lower oxygen concentrations, which suggests activity with respect to remineralization. Thus, August 2016 apparently had less abundance in the surface (thus high concentration depths was observed relatively shallower) than in August 2017. POC concentrations between August 2016 and 2017 presented high in 2016 August (Figure 17).

Abundance values were lowest for the fall season at the 50 m depth. In the winter period, surface abundance was represented by the highest values observed throughout the sampling period. Abundance below the 50 m depth in January 2017, displays relatively lower values than surface. From May to August, abundance of *Synechococcus* was higher in the surface than the deeper water column. However, in May and especially in August 100 m depth, abundance was increased as much as the values observed in the surface. This also coincides with the increase in the total chl-a concentrations.

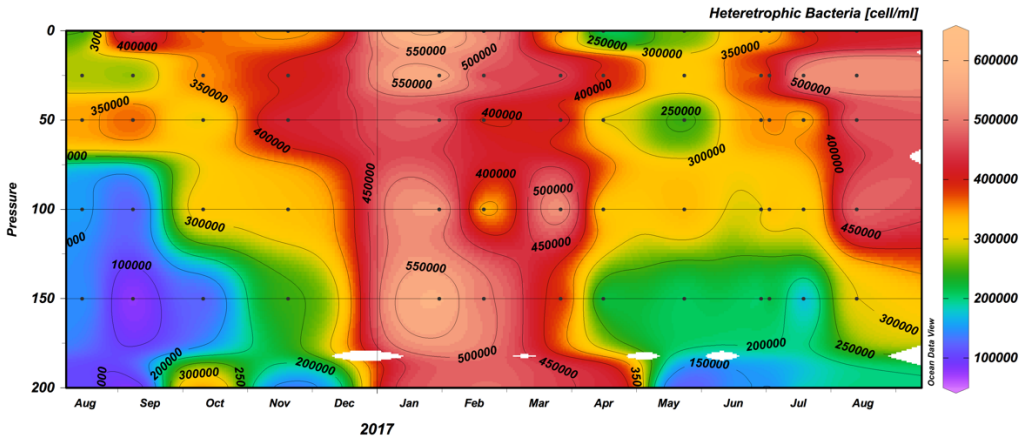

Figure 24. Heterotrophic bacteria abundance in cells/ml from August 2016 to August 2017 at the ETS-200 m station.

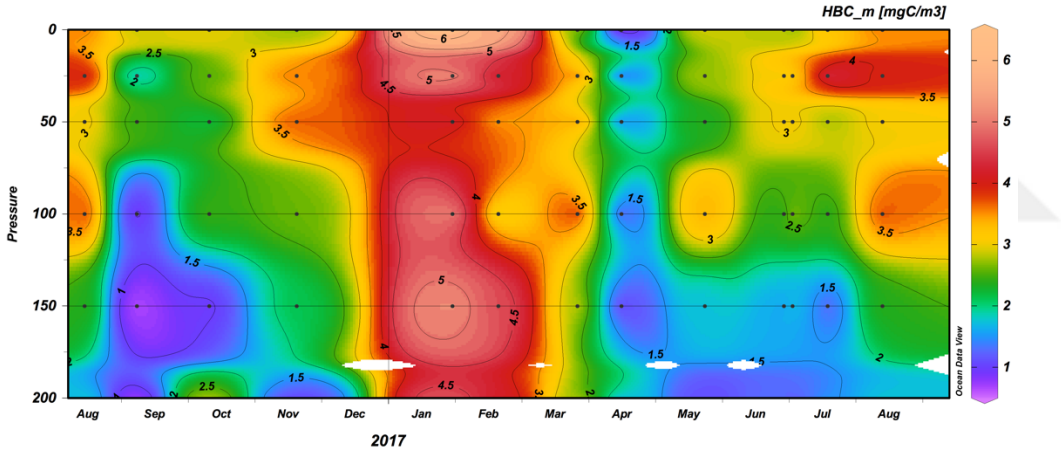

Figure 25. Heterotrophic bacteria biomass in mgC/m3 from August 2016 to August 2017 at the ETS-200 m station.

Seasonal variations in the abundance (cell/ml and mgC/m^3) of *Synechococcus* are shown in the Figure 26 and Figure 27. Variation observed in abundance ranged from a minimum of 481 to a maximum of 129,183 (24,244.2 ± 17,120.6). In the fall season, from September 2016 to November 2016, abundance of *Synechococcus* was comparably higher in the surface than in the deeper water column (below 50 m), with one exception at 50 m.

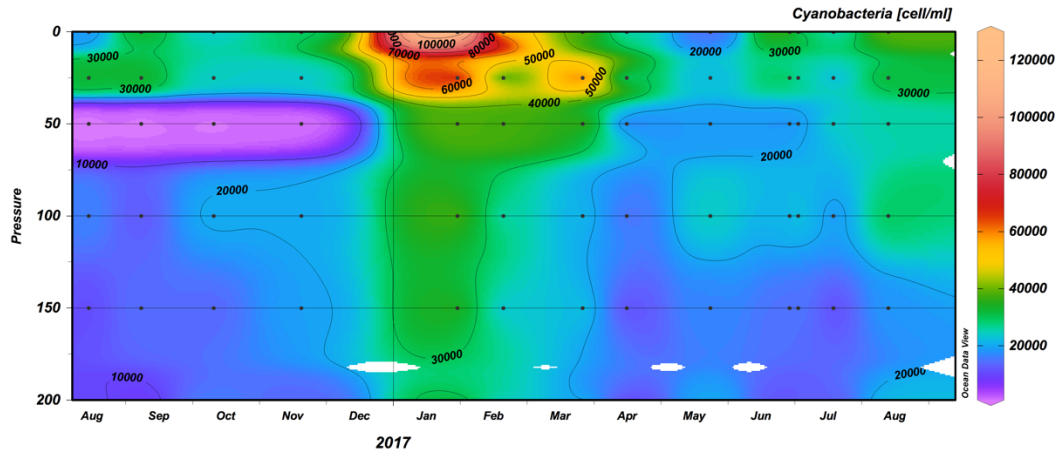

Figure 26. *Synechococcus* abundance in cells/ml between August 2016 to August 2017 at the ETS-200 m station.

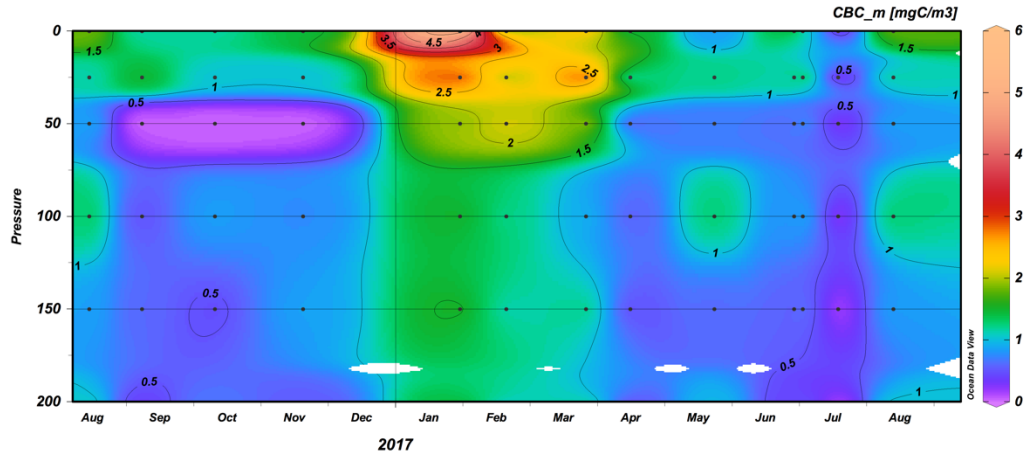

Figure 27. *Synechococcus* biomass in mgC/m3 between August 2016 and August 2017 at the ETS-200 m station.

Diatom

Diatom abundance in cells/l throughout the year ranged from 80 to 473,528 (17708,3 ± 76,698.4). It is apparent also from the Figure 28 that variation in abundance was high throughout the year. In August and September 2016, at 50 m depth there was high biomass values (33,200 and 4400 cells/l, respectively) compared to the water column above and below this depth. Below 100 m, biomass of diatom cells declined gradually. In October 2016, peak

in the diatom biomass was observed at two depths, surface and 100 m depth with 100 m depth having slightly higher abundance (3680 and 4720, respectively). November 2016 profile was vertically homogenous with maximum values that were recorded in the surface (1120 cells/ l and 2.2 mgC/m3) and minimum value was observed at 200 m depth as 440 cells/l. During winter mixing, January-March 2017, biomass values were generally high, ranging between 640 and 77152 cells/l, exception was on February 2017 below 100 m depth. Samples collected in February 2017 from depths of 100 m, 150 m and 200 m were acknowledge as the exceptionally high number of cells per liter (473,528; 449440; 207480, respectively). On the other hand, such high increase in the biomass was not detected in the total chlorophyll measurements (Figure 23), thus these results should be discussed with caution. April values exhibit a homogenous distribution of biomass but a slight increase in biomass was apparent in the 50 m depth sample (8720 cells/l). Surface waters of May 2017, was showing comparably higher biomass values vertically than the depths below 50 m. Summer season showed the lowest biomass values in general with exception of August 2017 surface values and values at 200 m depth.

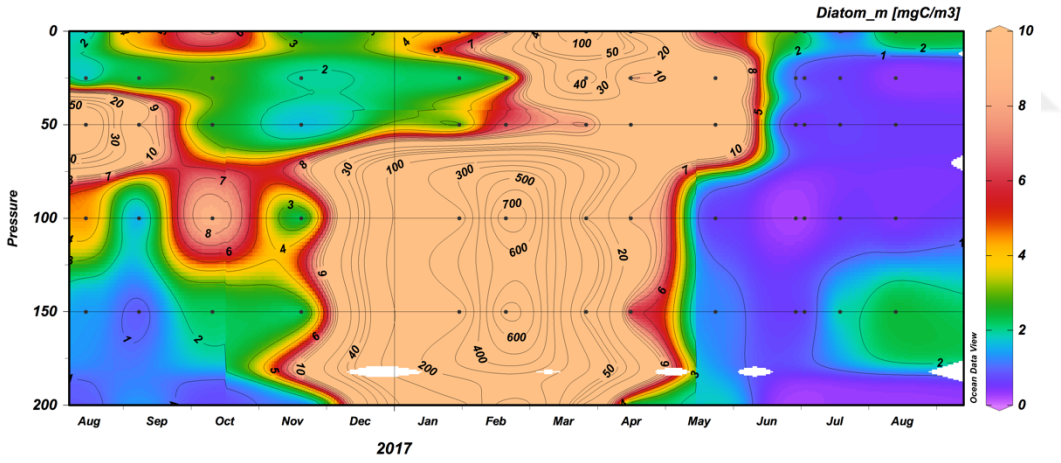

Figure 28. Diatom biomass in mgC/m3 between August 2016 and August 2017 at the ETS-200 m station. Since abundance values have high variation, isoclines were included for better representation of changes in the biomass.

Coccolithophoride

Coccolithophoride abundance in cells/l throughout the year and at all depths ranged from 59,392 to 460,288 (203,291.4 ± 89,869.2). It is apparent from the Figure 29 that variation in

biomass displays two different patterns. Period between August to November at above 50 m depth, abundance was comparably higher than the deeper water column, except for the 100 m depth in October 2016, where abundance was the highest. Winter period, observed abundance values were represented homogenously throughout the water column. This period was followed by the peak in the Coccolithophoride cells with minimum cell abundance observed at 100 m depth of July 2017 (117,856 cells/l) and maximum was in 25 m depth sample of April 2017 (460,288 cells/l). Between August 2016 and November 2017, abundance of Coccolithophorides decreases with increasing depth. However, from January on, there was no variation in the abundance observed with depth.

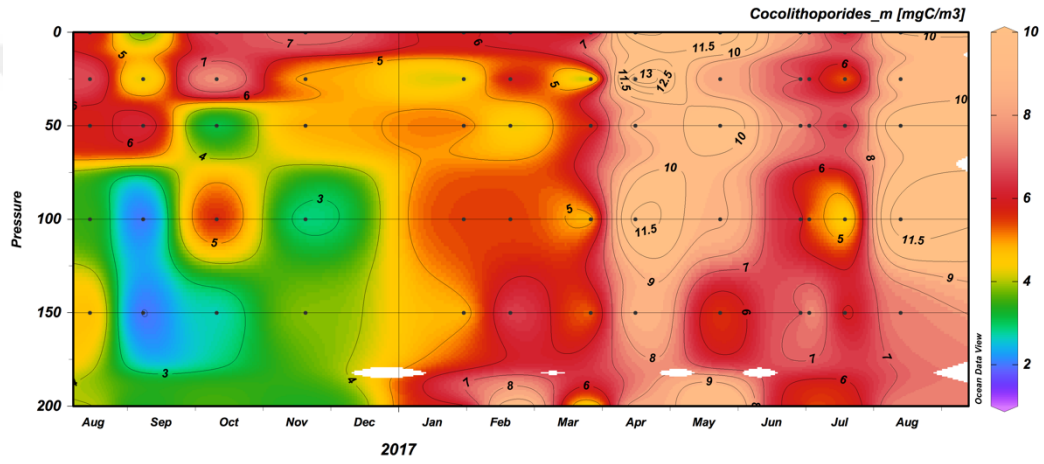

Figure 29. Cocolithoporides abundance in biomass in mgC/m3 between August 2016 and August 2017 at the ETS-200 m station.

Dinoflagellates

Variations in the abundance of Dinoflagellates are given in the

Figure 30. Minimum cell/l values as low as 40 were observed and the highest abundance was 31,440 (7210,6 ± 5876.9). Minimum abundance value detected at the 200 m depth sample of June 2017, while maximum abundance was observed at the 50 m depth sample taken in September 2016. In August 2016 in the upper 25 m, high abundance was observed and there was a decrease in abundance vertically with depth. In September, biomass values in terms of mgC/l were detected to be above 30 in the upper 50 m and they decreased to 3 mgC/l with depth. Surface peak values observed in September decreased in October. While the 100 m depth sample taken in October was below 2 mgC/l, 200 m depth sample was detected to have

a value >7 mgC/l. In November, abundance was decreased with increasing depth. February and March 2017 showed comparably homogenous profiles but January was an exception for the period of winter mixing. In January, first 100 m was high in abundance compared to 150 m and 200 m depths. While the highest abundance during January was 8224 cells/l in 25 m depth, minimum was observed in 200 m depth as 360 cells/l. April 2017 vertical profile also had the same trend, the lower abundance was observed in the 200 m depth. May 2017 was presented as another exception that 200 m depth sample (8672 cells/l) was more abundant than the 150 m depth sample (160 cells/l), but less than the samples above the 100 m depth. In June and July 2017, first 50 m was presenting the higher abundances than the deeper water column. But in August 2017, higher biomass values were observed down to 100 m depth and then the values decreased gradually with depth.

Cryptophyte

Cryptophyte cells were absent in some of the sampling depths such as in November at 25 m, 50 m and 200 m depths. Abundance values in cells/l ranged between zero and 59,392 (17,842 ± 14,735). Dominant patterns can be discussed under three different periods. In fall, abundances were measured to be low except for the sub-surface peak in October 2016. Between January and March 2017, abundances were high in the surface (up to 25 m) and below 100 m depth. After this period between April and June 2017, Cryptophyte abundances were decreasing with increasing depth. In August 2017, high abundances were observed starting from the 25 m depth down to 100 m depth and the values decreased with depth. As it was observed in August 2016, August 2017 surface waters were having comparably less abundances than 25 m depth (Figure 31).

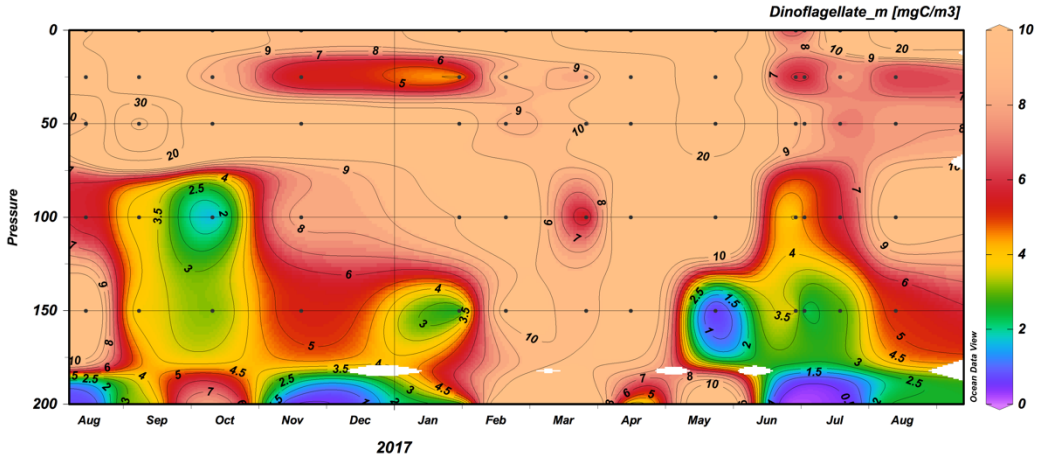

Figure 30. Dinoflagellate abundance in biomass in mgC/m3 between August 2016 and August 2017 at the ETS-200 m station.

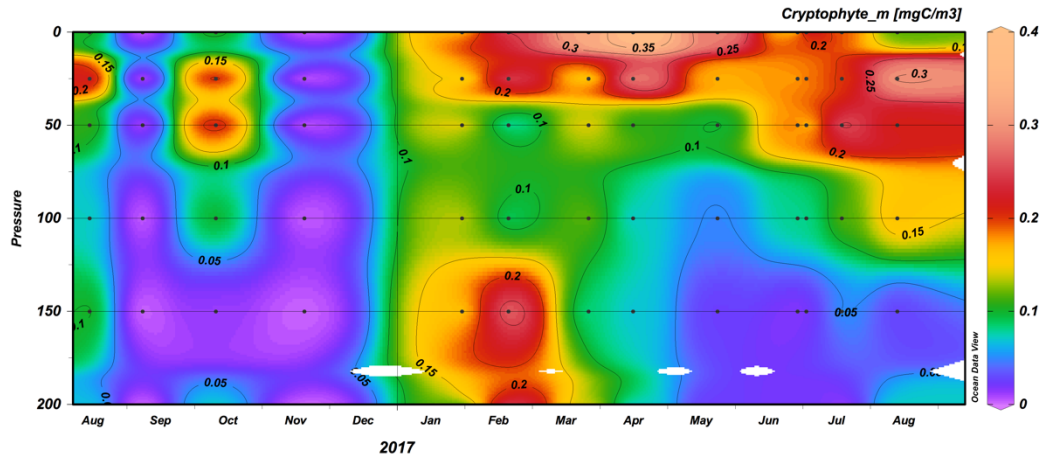

Figure 31. Cryptophyte abundance in biomass in mgC/m3 between August 2016 and August 2017 at the ETS-200 m station.

Dictyochale

Among the phytoplankton groups observed in ETS-200 m depth station, Dictyochale were the least abundant group in all sampling depths and periods. In 37 out of 66 samples there was no presence of Dictyochale cells Figure 32. Maximum abundance was detected in sample taken from 50 m depth of August 2016 (320 cells/l).

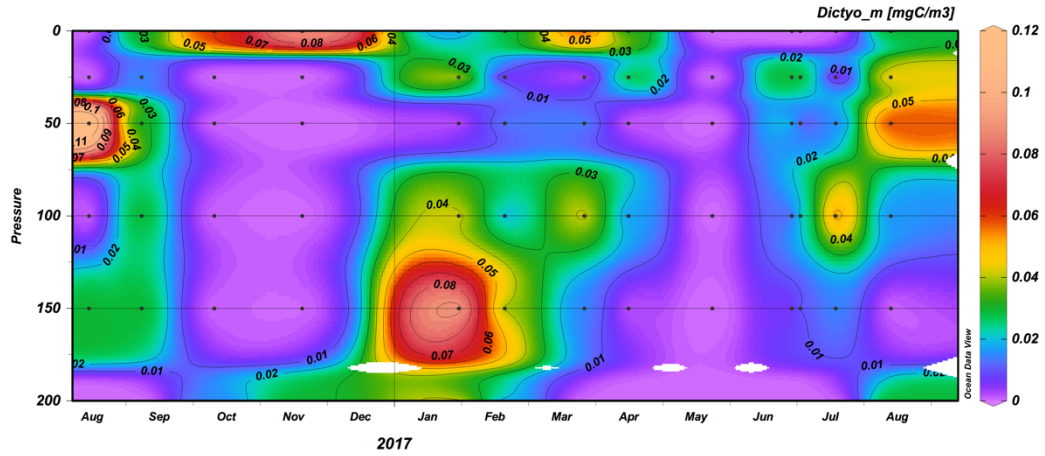

Figure 32. Dictyochale biomass in mgC/m3 betweenAugust 2016 and August 2017 at the ETS-200 m station.

1.4. Discussion

Physical conditions at the ETS-200 m station along the water column and throughout the year fit in with the Mediterranean characteristics which possess an annual cycle of cooling and warming. Stratification establishes around April with relatively depleted nutrient concentrations in the surface and extend until approximately the end of October. Winter season, on the other hand, shows a well-mixed water column (Figure 7). Depending on the prevailing wind direction and speed, sampling area is affected by the Lamas River freshwater input. Nonetheless, during the study period (August 2016 to September 2017) aforesaid freshwater intrusion was found to be minimal. Hence, the annual cycle of thermal alteration was apparent and surface waters of Mediterranean, especially in the summer stratification period, were fully nutrient depleted, consistent with other studies (e.g. Krom et al., 2005; Pujo-Pay et al., 2011). Characterization of ETS-200 m station as an open or coastal ocean is not straightforward. It is apparent that in some periods, freshwater flux influences the physical thus biochemical properties of the water column. On the other hand, while discussing the features of the water column, it should be kept in mind that lateral transport might also have implications to the biogeochemical properties. Pujo-Pay et al. (2011), while conducting survey along the east to west transect, divided Eastern and Western Mediterranean water column into three layers: “Biogenic Layer (BL)”, “Mineralization Layer (ML)” and “Deep Layer (DL)” during the BOUM cruise. Production of organic matter in the euphotic layer and its possible transfer to the deeper water for remineralization was considered for division of water column, thus this feature was defined using oxygen concentrations. Division according to oxygen concentration was as such: BL; ≥195 µmol kg oxygen (~200 µM), ML; 195-180 µmol kg oxygen and DL; <180 µmol kg (184 µM) oxygen. In the case of ETS-200 m station, the oxygen concentrations were between 196.8 and 251.14 µM. According to the oxygen concentrations, ETS-200 m station is located in the Biogenic Layer. On the other hand, observed nitrate (0.04 - 1.81 µM) and phosphate (0.02 - 0.12 µM) concentrations in the ETS-200 m station shows the characteristics of Eastern Mediterranean biogenic layer concentrations (0 - 3.45 and 0 - 0.11 µM). Besides, phytoplankton species composition was found to be different between coastal station (ETS-20) and ETS-200 earlier (Boran, 2017), indicating that ETS-200 m station is characterized more off-shore than coastal. Classification of ETS-200 m depth station as coastal or open sea was further questioned in the second chapter considering also heterotrophic bacterial community composition.

Nitrate concentrations, which were reported for the Eastern Mediterranean between surface waters and 200 m depth, were ranging between 0.01 and 3 µM (Krom et al., 2005). Nitrate

concentrations in ETS-200 m station (ranges between 0.04 and 1.81 µM) also show concentrations which falls in the concentration ranges reported (Figure 12).

Total chlorophyll concentrations throughout the year were low especially in the deeper water column. DCM formation was observed in fall, spring and summer. DCM (75m) in fall (October) was just observed below the Euphotic Zone Depth (EZD) (74). However, due to sampling depths' frequency, it's also possible that DCM might be above the EZD. Here, it should be stated that also calculation of calculation of EZD has uncertainty. It represents only one measurement from a certain time in a day. In May, DCM lied the bottom of EZD. On the other hand, in summer (both in August 2016 and 2017) DCM was observed above the EZD. DCM was formed in the 50 m depth in August 2016 (Figure 23) where concentration of total chlorophyll reaches up to 0.16 µM. This 50 m depth was also below the MLD (17m, Figure 33-a). This increase coincides with the high biomass values observed by the diatoms in the same period and depth.

Apparent oxygen utilization (AOU) is the indicator of either oxygen production (- values) or consumption (+ values) *in case of no lateral or vertical flux*. In August 2016, AOU value (Figure 34) was the lowest in the 25 m depth (in comparison with the other depths). At the depth of 25 meters, cyanobacteria abundance was comparably higher than the other depths (Figure 26 and Figure 27). The abundance of heterotrophic bacteria was slightly higher in 50 m depth where DCM and higher biomass of diatom were observed. These observations regarding the AOU values at the depths of 25 and, 50 meters conclude that the production in 25 m depth was higher than the consumption. Cyanobacteria play an important role in oxygen production even if carbon biomass of diatom over exceeds the cyanobacteria in the depth of 50 m. Additionally, concentrations of nitrate, ammonia and PO_4^+ were the lowest in 25 m depth, which indicates that these inorganic nutrients were taken up by cyanobacteria. At the depth of 200 meter in August 2016, AOU was positive and high, indicating heterotrophic activity coinciding also with the high concentrations of nitrate and phosphate as a result of degradation.

During September 2016, total chlorophyll observations showed slightly higher concentrations between the depths of 75m and 115m (green areas in Figure 23). This signal might have been contributed not only by live cells but also the dead phytoplankton cells. None of the phytoplankton groups' biomasses and cyanobacterial biomass were increased in the corresponding depths. Furthermore, AOU values were identified to be negative in the first 100 m depth from the surface. That strongly suggests the oxygen production due to photosynthesis exceeds the respiration. This is especially true in the depths below MLD (25 m) where the effect of oxygenation of the water column by atmospheric dissolution is limited. The lowest

AOU value was observed at 75 m depth (-16) and this was followed by the 100 m depth (-14). These depths correspond to the depths where also total chlorophyll measurements were relatively high. On the other hand, concentrations of ammonium, which is the first product of degradation, was high in the first 100 m depth. Even though AOU values indicate that production was the dominant process over heterotrophic activity, some level of degradation was still in process as heterotrophic bacterial abundance was also high in the upper 100 m depths. AOU shows high positive values indicating remineralization activity below 100 m depth during September. Abundances of eukaryotic phytoplankton were higher above the EZD (86m) and thus contribute to comparably high chl-a levels at 75 m depth. In addition, contribution of cyanobacteria to the high chl-a value at 100 m depth was predominant, since it has comparably high biomass at 100 m depth than 50 m depth.

In October 2016, the formation of DCM was also observed but predominantly at 75m depth. Contrarily, AOU generally showed positive values except for the 50 m depth and 25 m depth (-25 and -1, respectively). It can be confidently concluded that none of the photosynthetic organisms discussed in the previous section contributed to photosynthetic activity as inferred from negative AOU value. Abundances of any of the photosynthetic organisms did not correspond to the DCM depth. Negative AOU value could reflect that in 50 m depth heterotrophic activity might be low, however in the first 100 m depth heterotrophic bacteria abundance was higher than below the 100 m. Another significant instance during October was observed at 200 m depth where nitrate concentration (> 1.5 μM) and AOU value (>45) peaked to the highest values in the entire vertical profile and throughout the year. Additionally, the increase in the PP concentration and abundance of dinoflagellates (not the highest of the vertical distribution) coincide with this depth. High AOU value is enough to hypothesize, during October 2016, 200 m depth was the area where greater remineralization process is active. Total phosphate, particulate phosphate and especially inorganic phosphate concentration near the surface show comparably elevated values. This increase in phosphate sources did not appear in the nitrate sources, as a result, it cannot be concluded that there might be a degradation process. Possibility of the riverine input or rain impact also eliminated since, there were no evidence of low salinity in the surface. Hence, escalated phosphate concentrations might have been due to some contamination.

Winter period (sampling in December could not performed due to weather conditions) displays homogenous concentrations in terms of nitrate. Observed concentrations of nitrate were higher than surface values observed in fall and summer, and concentration of nitrate during winter were lower than the concentrations observed in the deeper depths. Total chlorophyll concentrations increased overall, especially the 200 m sample had the highest values throughout the water column. MLD in January was reaching down to 171m when we were

sampling. It is also possible that the depth of mixed layer can even be deeper when we were not taking any measurements. Diatom bloom was predominant below 100 m depth. Also, coccolithophorids were showing high abundance in the 200 m depth and in the surface waters. Peak in the chl-a concentrations at 200 m depth might be a result of the excretion of dead cells' chlorophyll content and might be due to the physical transfer of cells of phytoplankton from the euphotic layer to deeper layers. AOU values ranged between 1 and 8 and, significantly, these values were observed at depths of 150 m and 200 m, respectively. Interpretation of positive AOU values during winter should be made cautiously, especially in January when MLD corresponds to the depth of 171m. Saturated oxygenation of water column could be the result of strong vertical mixing. These positive values are not directly related with the consumption of oxygen.

In the surface, *Synechococcus* abundance was the highest in all depths and seasons. This reflects their preference for light when nutrients are available.

In February, AOU was negative in the depths of 150 and 200 meters. MLD in February moved up to 45m depth, indicating that below 150 m depth, negative AOU values might be due to primary production. This signal was also observed in the total chlorophyll concentrations which are high below the 150 m depth (Figure 23). Below 100 m depth, high abundances of diatoms were observed confirming photosynthetic activity in this part of the water column. While highest values of heterotrophic bacterial abundance were observed in winter, AOU showed that the remineralization process was not dominant over photosynthesis, especially in the deeper water column where transport of oxygen from the atmosphere is limited due to the shallower MLD (45m).

In May, DCM was observed at the depth of 100 meters. DCM depth in May also corresponded to the depth of high abundance of *Synechococcus* cells. In the first 75m, AOU values were negative. Dissolved oxygen from the atmosphere might have impact at the depths above the 25 meters since, MLD was around 25 meters. However, depths between the mixed layer and 75 meters negative AOU vales were indicating production of oxygen that exceeds consumption. Positive AOU values were observed between 100 m depth and the sea floor. Even DCM depth was in the 100 m, degeneration processes resulted in oxygen consumption were higher in this depth which was also apparent from the increased nitrate and phosphate concentrations.

During June and July, slightly higher chlorophyll concentrations were observed between 100 and 150 m depths. In June 2017, AOU values were positive above the 100 m depth, while the EZ depth was 102m. This suggests that, in the euphotic zone, photosynthetic activities were dominant. While below the EZ depth, processes consuming oxygen such as remineralization

were dominant. On the other hand, in July, positive AOU values were detected below the 150 m depth and thus 100 m depth has the slightly low negative value of AOU (-1). Changes in the vertical distribution of AOU values suggest that, remineralization was limited between the depths 150 and 200 meters.

MLD depth in August 2017 was slightly below the MLD (in 20 m depth) observed in previous months (11 and 12m for June and July, respectively). Also, remineralization process was stronger at 200 m compared to that observed in June and July. At 150 m diatom abundance increased and this might be the reason behind the lower AOU value (10) at the depth of 150 meters than the AOU value (30) at the depth of 200 meters. Oxygen consumption at 200 m during summer was predominant in August. However, higher negative AOU value was observed at 25 m, which specifically coincides with the increase in the abundance of Cryptophyte and *Synechococcus* cells during this period. Furthermore, the abundance of heterotrophic bacteria in the first 100 m of the water column was comparably higher than the abundance detected below 100 m depth. Oxygen production was competing with the consumption processes and this is further supported by the increased abundances of coccolithophorids and dinoflagellate cells.

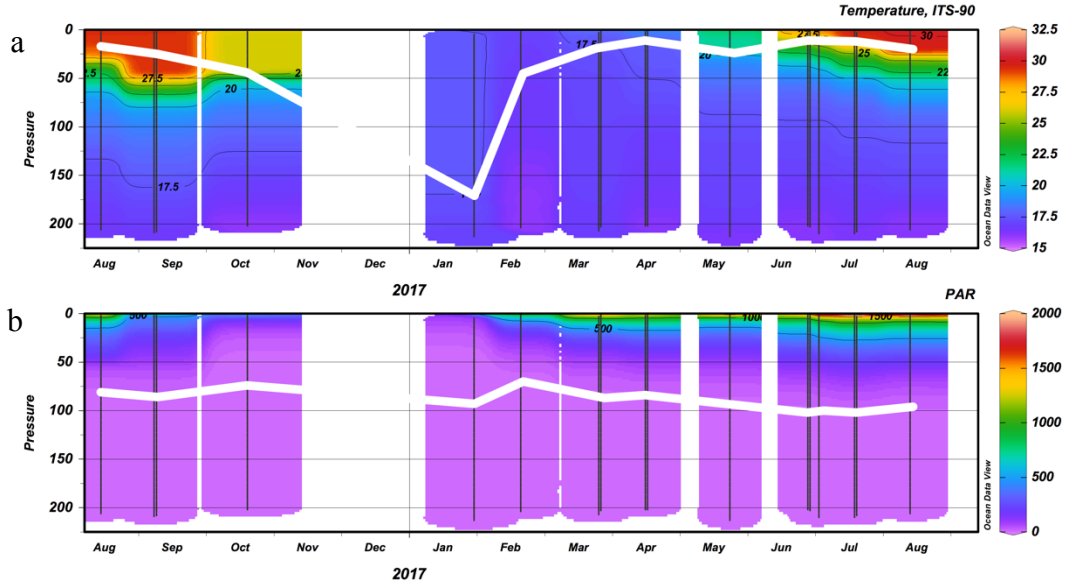

Figure 33. Mixed Layer Depth and Euphotic Zone Depth observed throughout the year at the ETS-200 m station. MLD (a) was plotted over the temperature profile as white line while EZD (b) was shown over the PAR data.

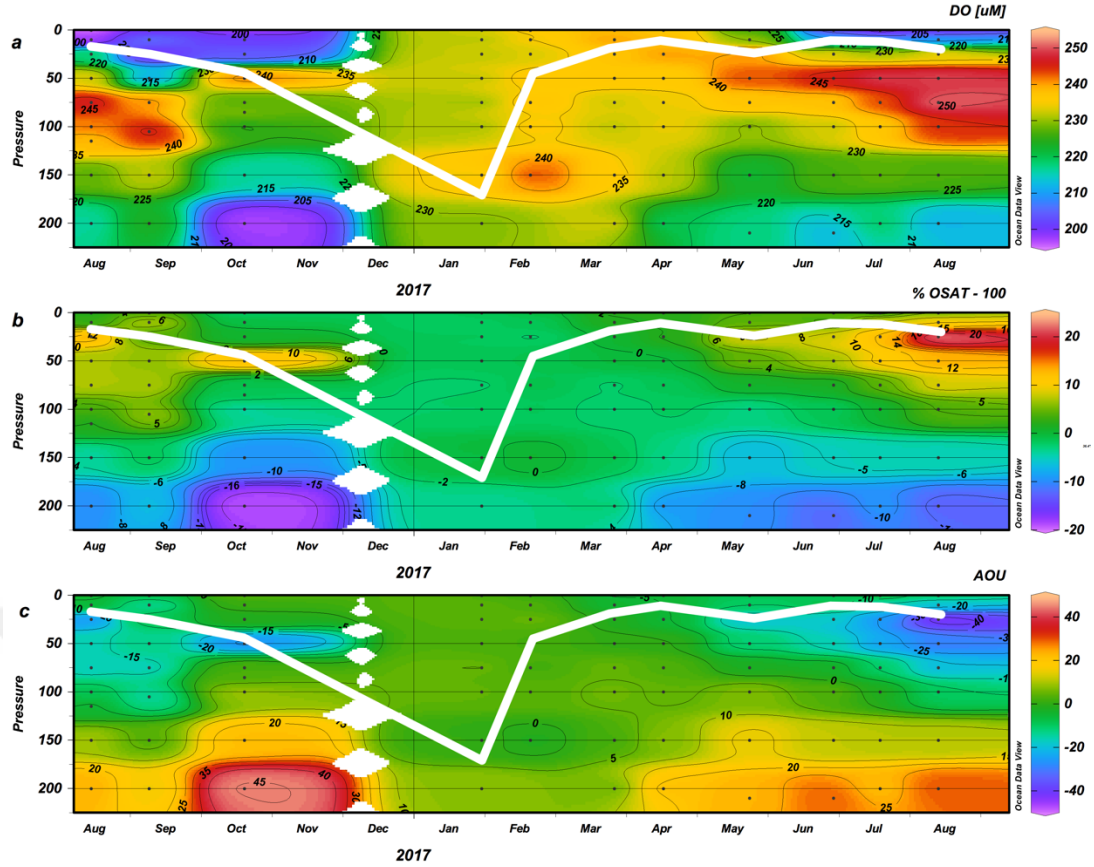

Figure 34. (a) Oxygen concentration, (b) percentage oxygen satutarion constant, and (c) apparent oxygen utilization at the ETS-200 m station . White lines in each figure represent the MLD location.

N to P ratio in the deep water of the Mediterranean Sea possesses a value that is higher than Redfield Ratio of 16 (Kress and Herut, 2001a; Krom et al., 2005). Deep waters of ETS-200 m station, especially below 150 m depth, has higher N/P ratio, which is compatible with these previous studies. This situation was only interrupted during winter mixing where the N to P ratio detected to be lower than the Redfield ratio. In the Eastern Mediterranean vertical mixing was reported to be the process which brings available nutrients from deep water to the to the surface waters (Powley et al., 2017). The decrease in the N/P ratio, increased inorganic phosphorus and total chlorophyll concentration observed during winter mixing periods supports this finding. If there is no lateral advection and no interaction with sediment, the N/P ratio in the deep layer of ETS-200 can be seen as the results of degradation of organic matter produced. Algae in the surface ocean typically have a C to N ratio (POC/PON) of 4-10 (Meyers, 1994). As the particulate organic matter sinks down, the C to N ratio increases due to preferential degradation of N sources over C. The ratio between POC and PON observed in

ETS-200 m station might indicate that the particulate organic matter is freshly produced (Figure 35) in our study area. However, POM input from riverine input cannot be ignored.

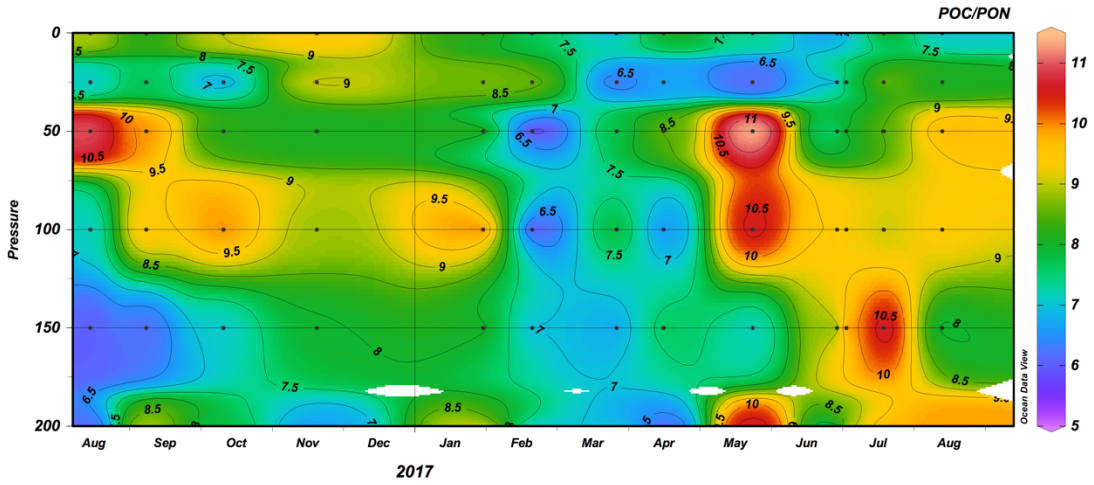

Figure 35. Ratio between particulate organic carbon and particulate organic nitrogen during the sampling period at the ETS-200 m station.

Earlier studies in the North Eastern Mediterranean showed that Seyhan, Ceyhan and Berdan rivers supply the nutrients to the Mersin Bay (Tuğrul et al., 2006; Doğan-Sağlamtimur 2007). They reported high N/P ratios (minimum 16.8 maximum 285) between 2001 and 2003 measured at these three rivers. In addition to these rivers, Lamas River, which is closer in location had high N/P ratios and POC/PON ratios in 2003 (Table 2) especially during rainy seasons. It was concluded that the POM and nutrient concentrations of surface waters in the North Eastern Mediterranean Sea (Mersin Bay) decline from the river-fed nearshore (bottom depth 20 m) to the shelfbreak station (bottom depth 200 m) and to the open stations (Doğan-Sağlamtimur 2007). Although, their sampling period is different than our sampling period, they showed that ETS-200 m depth station cannot be clearly classified as open-ocean since nutrient and POC/PON ratios are slightly higher than reference stations having open ocean characteristics. Variations of POC/PON ratios observed during our study might influenced by both biological POM production and coastal inputs.

Table 2. Lamas River POC and PON concentrations and N/P, POC/PON ratios during 2003. After Doğan-Sağlamtimur (2007).

Date	N/P	POC (µM)	PON (µM)	(POC/PON)
04/04/2003	71.4			
07/04/2003	119	270.6	33.4	8.1
28/05/2003	907	55.5	1.61	34.5
30/05/2003	1072	44.5	2.37	18.8
03/06/2003	300	124.9	13.8	9.1
14/07/2003	997	22	1.16	19
01/08/2003	717	19	0.83	22.9
02/09/2003	1226	19.9	1.71	11.7
22/09/2003	1734	13.3	1.16	11.5
28/10/2003	-	-	-	-
06/11/2003	-	-	-	-
01/12/2003	3100	6.83	1.04	6.6
11/12/2003	919	5.97	1.01	5.9
29/12/2003	546	-	-	-

P-limitation of the primary producers are well discussed in earlier studies (Krom et al., 2010; Thingstad et al., 2005; Cappellen et al., 2014). On the contrary, samples taken from the surface of ETS-200 m depth station show that this station is predominantly N-limited, with N:P-ratios lower than Redfield's ratio. During our sampling period (August 2016- August 2017), salinity profiles did not show any lateral riverine transport, however, it was also seen that some periods (February to April 2015) ETS-200 m station was under the influence of fresh water input. When considering N-limitation, it cannot be ignored that ETS-200 m station is close to coast-line. Lateral supply of P through rivers can be the possible reason of N-limitation observed from the N:P ratios.

Nanoplankton and picoplankton dominance was reported to be low where total chlorophyll and diatom biomasses increased in the North Atlantic (Li, 2002) and this was also true for the MS (Siokou-Frangou et al., 2010). When chl- a concentration in February and March is compared with diatom biomass in the same period, it is observed that, below 50 m depth, chl-a concentration is higher than the surface. This is most probably a result of diatom contribution

together with elevated coccolithophoride abundance. However, when considering the fact that the biomass value of diatoms reaches 900mgC/m^3, it was expected that the total chlorophyll concentration would have increase more drastically. In March, when the diatom (Moore et al., 1998) biomass increase observed near the surface, total chlorophyll peak is observed at 25 m depth, suggesting that the diatom chlorophyll is not really adding to total chlorophyll in our study area. Our findings support the study done in the basin that concludes the lesser importance of diatom contribution to the Levantine basin DCM (Ignatiades et al., 2009).

One important photoautotrophic group in the MS is *Prochlorococcus*, which has low light and high light adapted ecotypes (Moore et al., 1998). It was assumed that the total amount of divinyl-chlorophyll-*a* is correlated to the absorbance of the water sample at 665 nm wavelength using a UV-spectrophotometer and this is used for detection of divinyl-chlorophyll-*a* (Fernández-Pinos et al., 2015; Jeffrey and Humphrey, 1975). Total chl-a measurements in IMS-METU laboratories is performed using absorbance at 669nm wavelength. Therefore, total chlorophyll measurements in our lab may also interfered by the divinyl-chlorophyll-*a*. Spectrophotometric and flourometric methods are very simple and useful for quick estimation of the amount of pigments but do not always provide good results with the complex range of pigments and pigment degradation products found in natural samples. The absorption spectrums of chlorophyll b and c overlap with the chl-a spectrum, plus the spectrophotometric method provides data related only to the total and approximate amount of carotenoids (Sabina and Adam, 2011). It was showed in the open subtropical North Atlantic Ocean that measurements of total chl-a can be significantly biased by presence the of *Prochlorococcus* if traditional methods are used to determine total chl-a in samples when compared with the HPLC method (Goericke and Repeta, 1993). Goericke and Repeta (1993), concluded that total chl-a measurements might be underestimated in the samples that include *Prochlorococcus*. Comparison of flourometric chl-a measurement and HPLC chl-a showed underestimation of chl-a by flourometric method due to the presence of chl-b (Kumari, 2005). Inconsistency between total chlorophyll profiles and photosynthetic organisms' distribution might have been a result of this problem mentioned above, thus any conclusion should be drawn considering these issues. In January, Diatom bloom was predominant below 100 m depth and, coccolithophorids were showing high abundance in the 200 m depth and in the surface waters. But total chlorophyll measurements were homogeneous throughout the water column. High biomass observed in the diatom might have been sinking dead cells. On the other hand, even this biomass was a result of sinking cells of diatoms, total chlorophyll concentrations should show this signal since technique cannot distinguish between live and dead cells. Underestimation of total chlorophyll measurements in the samples containing different photoautotrophic groups might be the reason why there is a discrepancy between measurements of diatom and total chlorophyll.

Subsurface peak in chl-a concentration is known to be a permanent feature in the MS (Arin et al., 2002; Estrada et al., 1993) and this was shown to be true for the eastern Levantine basin (Ediger and Yilmaz, 1996). Our observations also support DMC formation which is especially well-developed in the depth of 75 m in autumn reaching up to 0.2 mg/l and in the deeper (100 m) depths in the spring.

Cocolithophores are known to possess high diversity in the Mediterranean Sea (Cros et al., 2002) and along an east to west transect study they were reported to be more abundant in the Eastern Mediterranean (Ignatiades et al., 2009). Previous study conducted in the central part of the Eastern Mediterranean Sea provided evidence that they dominate the sediment flux during winter and spring. *Emiliania huxleyi* is generally the most frequent and dominant group within Cocolithophores. In our study area, Cocolithophores were abundant especially in the 25 m depth sample in April 2017, below the MLD. *E. huxleyi* was overall the most dominant species by abundance in all sampling periods and depths with the exception of February 2017 at 100 m. In total, abundance of *E. huxleyi* was counted as 173,536 cells/l, whereas *Pseudo-nitzschia delicatissima* cells that belongs to diatoms had 297,888 cells/l. Species *E. huxleyi*, are well known by its success under P (Riegman et al., 2000) and N limited conditions (Tyrrell and Merico, 2004). Year-long observation in the ETS-200 m station reveals that variation in the *E. huxleyi* abundance supports the finding that low N and P concentrations did not show any impact on the abundance of cells. For instance, during August-November 2016, concentrations of nitrate and phosphate in the upper 100 m depths shows low values whereas, *E. huxleyi* possess high abundance. Especially in April 2017 surface waters, abundance of Cocolithophores were observed to be the highest and this coincides with phosphate concentrations close to the detection limit and low nitrate concentrations. Diatom species *Pseudo-nitzschia delicatissima* is described as a ubiquitous and physiologically variable species that form blooms in diverse environments (Kudela et al., 2005) and their growth can be positively affected by organic nutrients (Loureiro et al., 2009). Bloom of *Pseudo-nitzschia delicatissima* at the sampling station in February 2017, 100 m depth might be related with their physiological versality and availability of organic nutrients.

Synechococcus spp. is one of the main picoplankton groups in the Mediterranean (Siokou-Frangou et al., 2010; Uysal and Köksalan, 2006). A limited number of studies are available in the southern Levantine basin showing the dynamics of *Synechococcus* spp. (Uysal, 2006). It was shown that *Synechococcus* spp. was higher in terms of the abundance in the first 40 m and decreases with depth during September 2002. Our data related with the *Synechococcus* spp. abundance also shows that they are more dominant near the surface compared to the deeper water column in September 2016. On the other hand, contrary to the previous studies, the period in which they are abundant does not coincide with September but the winter period in

our study period. Additionally, in some periods such as January, August 2017 (200 m and 100 m depths, respectively), there were still slightly higher abundances were observed. These two instances coincide with elevated concentrations of ammonium in this part of the water column. Uptake of ammonia as a nitrogen source by *Synechococcus* spp. was shown earlier (Moore et al., 2002). May 2017 presents a different story in which the highest peak in the abundance of *Synechococcus* spp. was observed at 100 m, below the EZ and yet, none of the nutrient sources showed elevated concentrations at the same depth and month. On the other hand, abundance of heterotrophic bacteria increased and the AOU value was ~ 7, indicating consumption of oxygen. It can be speculated that *Synechococcus* spp. was using nutrients they need from the heterotrophic bacterial decomposition process. In general, elevated abundances of *Synechococcus* spp. were mainly observed above the euphotic layer, yet in some cases different preferences were apparent as ammonium utilization as a nitrogen source and opportunistic relationship with heterotrophic bacteria. In the Sargasso Sea, the *Synechococcus* uptake rate for P and ATP was reported to be 50-to-80 folds higher than other groups (Michelou et al., 2011) and, in the case of available inorganic phosphate, *Synechococcus* is able to outcompete bacteria and eukaryotic counterparts (Moutin et al., 2002; Tanaka et al., 2007). P-limitation over *Synechococcus* during our sampling period could not be detected.

Heterotrophic bacteria dominate the whole water column with highest abundances observed during the winter period when water column is well mixed and N:P ratio is around 8 (Figure 16) also, DOP concentrations were depleted in this period. This period also shows elevated concentrations of total chlorophyll. Heterotrophic bacterial abundance was not reported to be the highest in the winter (Uysal et al., 2007). However, during our study period bacterial abundance peaked in the winter. This trend was also detected by Boran (2017) for the period between 2014 to 2015. Positive correlation between heterotrophic bacteria abundance and chlorophyll-a concentrations was previously shown at the same location of study area (Boran, 2017). Below the EZD (min 70m and max 102m), oxygen concentrations were observed to be below the saturation level and concentrations of nutrients were increasing with depth. If the possible impacts of lateral transport and interaction with sediment can be eliminated, remineralization was the dominant process in the deeper water column.

Bacterial activity and nitrogen cycle are closely coupled in marine ecosystems. Balance between recycled and exported organic matter has important implications on
new and regenerated production. New production in the marine ecosystems is fuelled by mainly NO_3^-, which is supplied from the nitracline. Regenerated production, on the other hand, is fuelled by mainly NH_4^+ and derived from biological processes (Dugdale and Goering 1967; Harrison et al., 1987). New production in the oligotrophic systems especially in summer

only occurs where there is a lateral nutrient supply (e.g. riverine fluxes) and where atmospheric deposition present (Krom et al., 2004; Koçak et al., 2010). During our study period, EZD observed between the depths of 75 and 100 meters. Concentrations of nitrate during summer stratification increased below the depth of 100 meters. Additionally, we did not observe riverine run-off impact in our sampling station during summer and nitrate concentrations are well depleted above the EZD. The prevailing conclusion of this study can be that the utilization of new nitrogen decreases and the regenerated production (the use of ammonium by phytoplankton) is favoured during summer above the EZD. During winter, vertical mixing provides nitrate to the euphotic zone and consequently primary production might be favoured by this nitrate supplied from deep waters.

1.5. Conclusion

In some periods 200 m depth station is affected by the fresh water input from Lamas River depending on the dominant wind direction and speed. However, such a condition was not observed during the year-long study period. It was clearly avoided here to define ETS-200 m station as an open ocean or coastal waters. The station has characteristics of both coastal waters and the open sea in different period. During the times of thermal stratification, system behaves more like open ocean. Some features such as N:P ratio during stratification presents open ocean behaviour.

Nutrient concentrations in the photic zone are well depleted except for the periods of vertical mixing. Hence, the lower N:P ratio in the photic zone supports the hypothesis that surface waters are N and P co-limited. Lower oxygen concentrations than the saturated oxygen content especially below the mixed layer depth, indicates the importance of degradation processes.
Heterotrophic bacteria and prokaryotic autotrophs are clearly dominating the study area by abundance. Both heterotrophic bacteria and *Synechococcus* spp. had elevated biomass during winter. This trend was also identified between the period January 2014 and December 2015 (Boran 2017). However, earlier studies conducted in the same location showed contradictory results. The period, when the increased biomasses were observed, was in September in 2007. It is clear that, increase in the heterotrophic bacteria and *Synechococcus* spp. biomass cannot be only associated with winter mixing. Occurrences of winter mixing were also present before 2014 when the first abundance shift detected. Additionally, one important lacking knowledge in the area was their community composition and metabolic activity in terms of nitrogen and phosphorus cycles. In Chapter II, it was aimed to describe the community composition of heterotrophic bacteria and how it responds to environmental parameters. Also, it was

investigated that if community composition is different in winter when elevated abundances were observed compared to other seasons. Can knowledge that will be gained in the second chapter help us to determine ETS-200 m station as open sea or coastal water? By using information regarding the species composition of heterotrophic bacteria, can we eliminate or provide information if there is an interaction with sediment in the deeper water column where we define the bacterial degradation processes are the main reasons of increased inorganic nutrient concentrations.

It is important to stress here that the observations discussed here provide a snap shot of a highly dynamic and variable system and only represent the conditions of the sampling day and time. For instance, MLD and EZD calculations were made based on the data collected at a specific time. Even if we gain information regarding to depths of euphotic zone and mixed layer, this does not provide information on the variation throughout the day and night and, on the whole month. These uncertainties were considered carefully during our discussion.

CHAPTER II

MICROBIAL DIVERSITY OF NORTH-EASTERN MEDITERRANEAN SEA DISCOVERED BY 16S rRNA AMPLICON SEQUENCING

General biogeochemical properties of the study area and the sampling station were discussed in the previous chapter. In this chapter, one important knowledge gap, which is the taxonomic resolution and community dynamics of heterotrophic bacteria is presented and discussed for the first time in the north-eastern Mediterranean Sea. Using Next Generation Sequencing technology, 16S rRNA amplificon sequencing was performed for one year, in monthly intervals and from 6 different depths. Taxonomic composition and how community dynamics change with respect to environmental variables are discussed.

Highlights

- It is the first time in the north of the Eastern Mediterranean Sea that bacterial taxonomic composition and community dynamics are investigated using NGS technology. Amplicon sequencing (16S rRNA) technique was used to discover taxonomic composition and diversity of prokaryotes.
- SAR11 clade was dominant in the ETS-200 m station throughout a year (min 22%, max 64%).
- Distribution and abundance of SAR 11 ecotypes differ throughout the water column, Clade Ia having the higher relative abundance in the surface, while Clade Ib and Clade II were mostly dominant in the deeper waters.
- Phylogenetic diversity was significantly different in all seasons. Higher phylogenetic diversity was captured during winter where also increased chlorophyll concentrations, higher heterotrophic bacterial and Cyanobacteria abundance (cell counts) and nutrient concentrations observed.
- Prochlorococcus MIT 9313 was abundant than *Synechococcus* CC9902 in the surface during fall. *Synechococcus* was found to be correlated with Euphotic Zone Depth and Prochlorococcus with depth.
- Patterns in bacterial communities were best explained by Temperature, AOU, Nitrate, Season and EZD, indicating seasonality as a main driver.
- Observed taxa in the ETS-200 m station were well in conformity with the indicator species of Atlantic Water.
- Elevated concentrations of nitrate, which were observed in the depth of 200 meters, found to be resulted by the activity of nitrite oxidizing phylum *Nitrospinae.*

2.1. Introduction

Oceans cover approximately 70 % of Earth's surface and they reach 4 km depth on average. Considering the enormous volume, they occupy, marine environments inhabit most of the life on Earth. This fact is particularly true due to economic importance and the protein source that fisheries sector provides. Interaction between human and marine environment is mostly limited to only coastal ecosystem and shallower continental shelves (10-200 km).

One-half of photosynthetic activity (primary production), which is responsible for organic carbon production that supports heterotrophic activity, occurs in the marine environment (Field et al., 1998). Understanding carbon fluxes, for instance how much has been transferred to support fish production (Pauly and Christensen, 1995), or how primary production has been changed under the global problem of climate change (Chavez et al., 2011) are important questions in marine sciences. Even if, society is interested in habitants of marine life which provides them job opportunity (fisheries economy) and food support from fish stocks, marine environment is dominated by microorganisms. Microorganisms include all three domains of life which are not visible to the naked eye, and deserved to be phrased as "**unseen majority**" (Whitman et al., 1998). So called unseen majority, dominate oceans in terms of abundance, biomass and most importantly metabolisms ranging from autotrophy to heterotrophy (Karl, 2007).

Prokaryotes were, for 3 billion years, the only forms of life on our planet. They largely shaped our environment and have formed that basis for the emergence and evolution of all other life forms on Earth (Falkowski et al., 2008). Discovery of microorganisms was delayed until the invention of the microscope by Robert Hooke in 1655. After him, Antoni van Leeuwenhoek, with a simple but higher magnification microscope, discovered tiny organisms from various environments in 1677 (Lane, 2015). After all, investigating and having a deep understanding of the roles of bacteria (also archaea) in the marine food webs has only been possible for the last few decades through the emergence of new technologies (Karl and Proctor, 2007). Karl and Proctor in 2007 pointed out that, at the beginning of the twentieth century, main question driving the sample collection in the marine waters was to answer if "marine" bacteria truly existed and whether they can be distinguished from terrestrial and freshwater originated counterparts.

Culture methods have been widely used to understand microorganisms and their interactions with their environment as well as their physiology. It was shown that culture depended quantification methods (e.g. viable plate count-culturing) in different ecosystems from the seawater to sediment underestimates the abundance of microbial cells when compared with direct microscopy counts (Amann et al., 1995). Metagenomics and 16S rRNA amplicon

sequencing are the emerging field that helps scientist discover uncultivable microorganisms, thus ending the discussion of 'great plate count anomaly' first discussed by Staley and Konopka, (1985) (Staley and Konopka, 1985). Great plate count anomaly is the disagreement between the sizes of populations estimated by culturing and by microscopy. These problems occurred due to technical difficulties and resulted in underestimation of role and great number of microorganisms in the environment. Construction of phylogenetic trees and classification of prokaryotes using small subunit ribosomal RNA (16S rRNA) is not a new approach. Carl Woese proposed the idea of using small subunit ribosomal RNA as a marker for classification (Woese and Fox, 1977). Furthermore, sequence library databases have increased after the study of Lane and colloquies in 1985 (Lane et al., 1985) that enables generating 16S rRNA sequence data without isolation of the 16S rRNA or cloning of its gene. Before Next Generation Sequencing Technology emerged, the polymerase chain reaction (PCR), rRNA genes cloning and sequencing, fluorescent in situ hybridization (FISH), denaturing gradient gel electrophoresis (DGGE and TGGE), restriction-fragment length polymorphism, and terminal restriction-fragment length polymorphism (T-RFLP) lead the studies of the uncultured world of diverse marine microbes (Rajendhran and Gunasekaran, 2011). However, Next Generation Sequencing (NGS) made it possible to study community dynamics directly after nucleic acid extraction from environmental samples such as seawater collected from the marine environment. Current estimates show that more than 99% of the microorganisms in different environments are not readily culturable and therefore understanding their role was not possible (Amann et al., 1995).

Recently, two important questions which are "Who is there?" and "What are they doing?" have become viable questions to as through NGS data analysis (Escobar-Zepeda et al., 2015). Two different approaches without requirement of culture methods are available for the microbial ecologists depending on their target question. One of them is amplicon sequencing (marker gene analysis) in which specific target regions of DNA such as 16S rRNA gene for prokaryotes and intergenic transcribed spacers (ITS) gene for eukaryotes can be amplified to determine taxonomic information (Sharpton, 2014). In this approach, rather than sequencing the whole genome of the organisms under study, only a portion of the genome is sequenced, therefore not giving detailed information about the genes each organism possesses. Second approach is called shot gun sequencing which is used to sequence the whole genome and provides more information than the previous approach such as characterization of a large number of coding and non-coding sequences. But the latter approach compared with the amplicon sequencing is more expensive.

2.1.1. Marine Biogeochemical Cycles and Role of Marine Prokaryotes

Carbon Cycle

Progressive increase of atmospheric CO_2 (carbon dioxide) has been observed after global agricultural and industrial development. Large quantities of CO_2 are emitted into the atmosphere mainly as a result of fossil-fuel burning but also because of land-use practices (Raupach et al., 2007). Only less than half of this emitted CO_2 remains in the atmosphere, the rest is taken up by the ocean, by the land biosphere, or by a combination of both (Sabine et al., 2004). Most significant uptake mechanism of atmospheric carbon dioxide in the ocean is the so-called "**biological carbon pump**". First step of the biological carbon pump is the fixation of solubilized CO_2 in the ocean by prokaryotic and eukaryotic microorganisms through photosynthesis. Photosynthesis converts inorganic carbon into organic carbon pool. Second main step is carbon export driven by sinking of particulate organic carbon (POC). Magnitude of biological carbon pump that is driven by photosynthesis and sinking of organic particles from the surface ocean to its interior is estimated to decline due to the global climate change, hence reducing the carbon storage capacity of oceans (Henson et al., 2011). Approximately 95 % of organic carbon in the ocean is present in the dissolved organic carbon (DOC) pool and only a small fraction of POC produced in the surface is buried in the sediment (Jiao and Zheng, 2011). It was also discussed that there might be stronger process that act as a sink for DOM in the ocean than biological carbon pump.

Marine prokaryotes have vital roles in the ocean carbon cycle in two ways: primary production and microbial carbon pump. Although both eukaryotic algae and their prokaryotic counterparts contribute to primary production globally, members of prokaryotic cyanobacterial genera are responsible for a significant proportion of photosynthesis in the open ocean (Goericke and Welschmeyer, 1993; Ting et al., 2002). *Prochlorococcus* and *Synechococcus* dominate by abundance the most phytoplankton assemblages in modern oceans (Flombaum et al., 2013; Johnson, 2006; Scanlan et al., 2009). Abundance of genus *Prochlorococcus* is dominant in the illuminated waters from 40^{o} N to 40^{o} S latitude, and can also be detected in the deeper water masses as 200 m (Partensky et al., 1999). In addition, *Prochlorococcus* can reach up to 21 to 43% of the photosynthetic biomass in oligotrophic oceans (Campbell et al., 1997; Durand et al., 2001; Goericke and Welschmeyer, 1993). Although, *Prochlorococcus* and *Synechococcus* are two close clades, they have two different light harvesting antenna. While *Prochlorococcus* uses chlorophyll a_2/b_2, *Synechococcus* possesses light-harvesting complex of phycobilisome (Urbach et al., 1998). Blue wavelength of light is available for Prochlorococcus genus which has unique pigments for light harvesting among photosynthetic organisms. This provides an advantage for Prochlorococcus and this is consistent with its dominance in the deeper water

(Moore et al., 1998; Partensky et al., 1999). Not only does their preferred niche change throughout the water column due to their light- harvesting complex, but also differential preferences in cellular nitrogen requirements between the two closely related genera could also be a factor in determining their distributions (Ting et al., 2002). Some strains of *Prochlorococcus* can be entirely dependent on ammonium, others could use nitrite and ammonium, and *Synechococcus* could use nitrate, nitrite, or ammonium (Ting et al., 2002; Zehr and Kudela, 2011). Discovering different ecotypes of cyanobacteria genera and their diversity is particularly important in the biological carbon pump of the oligotrophic marine environments due to their various light harvesting strategies and photosynthetic activity that converts inorganic carbon to organic carbon.

The nutrients required for primary production in the marine environments is supplied by bacterial degradation and remineralization of organic matter (OM) in addition to external sources such as river fluxes and atmospheric deposition. High heterotrophic bacterial production and thus dominance of bacterial secondary production over very low levels of primary production results in low vertical OM fluxes and an organic nutrient pool derived from heterotrophic activities instead of autotrophic activities (Allen et al., 2002). Dissolved organic carbon (DOC), produced from primary production by various mechanism in the food web, serves as food for heterotrophic bacteria. This pathway of organic matter into bacteria is an important part of the pelagic food web (Ducklow and Carlson, 1992). Oligotrophic areas where photosynthesis is lower compared to the respiration were considered previously as net sources of CO_2 (Gasol et al., 1997). However, Williams and colleagues debated the bias in the *in-vitro* measurements that was used previously. They stated depending on the ***in-situ*** observations that in the oligotrophic subtropical gyres open ocean are autotrophic (Williams, Quay, Westberry, and Behrenfeld, 2013). Microbial carbon pump (MCP) was proposed to understand the processes of long-term carbon storage by microorganisms. MCP operates by generating the Recalcitrant Dissolved Organic Matter (rDOM). The last product of MCP is unavailable for microbial degradation and serves as long-term carbon storage especially by sinking to the deep ocean (Jiao et al., 2010). Furthermore, in less productive ecosystems like the oligotrophic Mediterranean Sea, where prokaryotes are dominant, it is suggested that the microbial carbon pump can be more important than the biological carbon pump (Jiao et al., 2010). It shows how bacterial communities regulate primary production and carbon storage in the ocean and the importance to understand the complexity of these communities to gain insight into the biogeochemical cycling.

Nitrogen Cycle

The aforementioned conversion of inorganic carbon to organic carbon by photosynthesis and/or growth of organisms and productivity in the marine ecosystem mainly limited by the

essential nutrients such as nitrogen, phosphate, iron. Since, Nitrogen (N) is the fourth most abundant element after hydrogen, oxygen, and carbon in organic matter, and the atmosphere is composed of 80% N_2 gas, it was assumed that phosphate was the ultimately limiting nutrient (Krom et al., 1991). It was clearly stated by Zehr and Kudela that an important question that should drive future investigations is "*how does N limitation interact with the biogeochemical cycles to constrain productivity over long timescales, and how will it respond to global climate change over the ensuing decades*" (Zehr and Kudela, 2011).

Microbial processes are coupled with biogeochemical processes keeping nitrogen loss and input in the oceans balanced (Canfield et al., 2010). However, recently additional important processes of nitrogen cycle have been discovered. Such discoveries are the anaerobic ammonium oxidation (anammox) process (van de Graaf et al., 1995), the conversion of ammonia and nitrite to N_2 gas or denitrification by eukaryotic species such as foraminifera (Risgaard-Petersen et al., 2006). These two pathways basically act as the additional loss mechanism of N. These findings strongly suggest a significant imbalance of the oceans' nitrogen budget (Codispoti et al., 2001; Gruber and Galloway, 2008) rather than an equilibrium.

Nitrogen Fixation

Nitrogen fixation is one of the ways nitogen lost through denitrification can replenish the nitrogen in marine environments. Nitrogen fixation is the enzymatic conversion of atmospheric N_2 to ammonium, which is catalysed by the marine organisms. N_2 fixation is catalysed by the enzyme nitrogenase (Mehta et al., 2005; Zehr and McReynolds, 1989). Nitrogenase consists of two different proteins, a Fe-containing subunit and a Mo (molybdenum)-Fe-containing subunit (Bombar et al., 2016). The nifH, nifD, and nifK genes encode these subunit components of nitrogenase. Since the nitrogenase enzyme can be inhibited by oxygen, N_2 fixing phototrophs should have some strategies to conduct O_2 production and N_2 fixation in the same cell. The reaction (E1) requires high activation energy, approximately 16 ATP needed per N_2 reduced to $2NH_3$ (LaRoche and Breitbarth, 2005).

(E1) $$N_2 + 8\,H^+ + 16\,ATP + 8\,e^- \rightarrow 2\,NH_3 + H_2 + 16ADP + 16\,P_i$$

Diazotrophs are the organisms that can fix N_2 by converting it to ammonia. Among the known diazotrophs are the filamentous non-heterocyst-forming *Trichodesmium*, the filamentous heterocyst-forming symbionts with unicellular eukaryotic algae (Richelia, Calothrix and relatives), and single-celled or unicellular cyanobacteria (Crocosphaera and relatives of Cyanothece) (Zehr, 2011). Diazotroph *Trichodesmium* is known to be dominant in the tropical

and subtropical North Atlantic Ocean (Capone et al., 2005). Smaller sized (<10 μm) unicellular diazotrophs were only discovered recently by sequencing *nifH* genes and these groups belong to Crocosphaera watsonii, a cultured cyanobacterium that has many representative strains available; UCYN-A, an uncultured cyanobacterium; and UCYN-C, a distinct, uncultured clade that is related to Cyanothece sp. (Zehr et al., 2001). Their contribution to N_2 fixation was reported to be almost equally important as *Trichodesmium* in the Atlantic Ocean (Goebel et al., 2010; Langlois, Hümmer, and LaRoche, 2008) and the Pacific Ocean (Montoya et al., 2004). Another discovery regarding diazothrops is the discovery of the presence of *nifH* gene in gammaproteobacteria and alphaproteobacteria, and members of the anaerobic cluster III diazotrophs (Langlois et al., 2008; Riemann et al., 2010; Zehr et al., 2001).

Nitrogen fixation process is mainly limited by iron and phosphate. Iron is a cofactor in photosystem I and nitrogenase (as well as in other redox enzymes) and Fe requirement of nitrogen fixers is almost 5 times higher than that their requirement for ammonia (Berman-Frank et al., 2001). Besides, phosphate is an important building block of the cells, and is supplied to the oceans only by the geological processes. Even though most of the diazothrops are capable of substituting sulpholipids for phospholipids production during P deficiency (Van Mooy et al., 2009), it will only be sufficient enough for cell growth (Sohm et al., 2011).

In the Levantine Basin, which is considered to be P- limited (Krom et al., 2005), Phytoplankton communities are P limited in winter and N and P co-limited in the summer (Thingstad et al., 2005). Also, heterotrophic microorganisms are reported to be P limited (Thingstad et al., 2005; Zohary et al., 2005). Nitrogen fixation was thought to occur also in the Levantine Basin due to P and N co-limitation on the growth of phytoplankton. Only previous study including functional marker genes was conducted by Yogev and colleagues in the Israel to Crete off-shore waters, which reported that both *nifH* gene abundances and N_2 fixation rates were low. Contribution of N_2 fixation to new production was only 1% and 2% in the winter and spring respectively. NifH gene sequences revealed that diazotrops in the basin are from the nifH clusters I and II, and diatom-*Richelia* symbiosis (Yogev et al., 2011). Another important basin-wide study was conducted in the Atlantic, Eastern and Western Mediterranean investigating the presence of nitrogen fixation in the Mediterranean that might arise due to high N to P ratios in subsurface waters. Ibello and her colleagues in 2010 reported lower N_2 fixation rates throughout the different Mediterranean basins compared to the North Atlantic. Their findings suggest the dominance of other sources, such as atmospheric deposition, that fuel the Mediterranean ecosystem (Ibello et al., 2010).

Organic Matter Degradation and Regeneration of Nutrients

Basically, organic matter in the marine environment is divided into two operational groups based on size, particulate organic Matter (POM) and dissolved organic matter (DOM).

Distinction between these two classes of organic matter depends on the size classification. Particles that can pass through 0.45 μm pore sized filter are classified as DOM. Algal exudation and cell lysis (Azam et al., 1983), predation by viruses (Riemann and Middelboe, 2002; Rohwer and Thurber, 2009; Suttle, 2007) and the action of protists, which egest food vacuoles that contain DOM and regenerated N, P and Fe2+ (Barbeau et al., 2001), are the main processes driving the production of DOM. Motility and sensing ability of marine bacteria is an important adaptation strategy even if motility is limited to only a fraction (5 - 70%) of bacteria (Grossart et al., 2001). The bacterial cell surface is reactive for organic matter remineralization and uptake. Cell-surface-bound hydrolytic enzymes or ecto-hydrolases (protease, glucosidase, lipase, phosphatase, nuclease and chitinase) are used by bacteria to hydrolyse polymers and particles (Arnosti et al., 2005; Azam and Malfatti, 2007a; Martinez et al., 1996; Nagata et al., 2003). These enzymes are highly important for the degradation of organic matter into nutrients to support primary production in the marine ecosystems. The first breakdown product during the decomposition of organic matter, ammonium, is an energetically favourable Nitrogen source for most plants and algae. Thus, interaction of marine bacteria with DOM is an important process in marine food-webs.

Nitrification and nitrifying marine microorganisms

Ammonium available in the water column is subjected to oxidation by marine microorganism by a process called nitrification. Nitrification is a two-step reaction in which the first step is the oxidation of ammonium to nitrite while second step is nitrate production from nitrite. Ammonia oxidation is a chemolithoautotrophic process in which ammonia-oxidizing bacteria (AOB) gain energy via conversion of ammonia to nitrite. Therefore, these organisms are of considerable importance in the global nitrogen cycle. Almost all aerobic environments were reported to be possible habitats for AOB (Bock and Wagner, 2006). The first marine ammonia-oxidizing strain was isolated from seawater in 1965 (Watson, 1965) and was named *Nitrosocystis oceanus,* which is now known as *Nitrosococcus oceani*. Together with *Nitrosococcus halophilus*, *Nitrosococcus oceani* constitute the known species of ammonia-oxidizing bacteria in the γ-subdivision of the *Proteobacteria*. Besides AOB belonging to the γ-subdivision, the β-subdivision representatives, *Nitrosomonas marina*, are also present in the marine environment (Ward and Carlucci, 1985). Scientific knowledge on the ammonia oxidation organisms was restricted only to these two groups of AOB. However, Broda in 1977 suspected based on thermodynamic and evolutionary consideration that there should be one more group oxidizing ammonium to nitrogen with O_2 or nitrate as oxidant (Broda, 1977; Junier et al., 2010). Recent developments in sequencing technologies revealed the existence of genes that are playing role in ammonia oxidation. These genes were found in the genomic fragments derived from uncultivated Crenarchaeota in the Sargasso Sea (Venter et al., 2004).

Functional marker genes, such as genes encoding key enzymes involved in a specific metabolic pathway, have been used as an alternative method for ecological studies sometimes together with phylogenetic marker gene of 16S rRNA. In the nitrification reaction, ammonium is first oxidized by the membrane-bound enzyme ammonia monooxygenase. The oxidation of hydroxylamine to nitrite by hydroxylamine oxidoreductase -*hao*- (Arp et al., 2002) is the second step in the nitrification. Ammonia oxidizing archaea, however, use nitrite rather than oxygen as an electron acceptor. In anammox, a different metabolic pathway combines ammonia oxidation and denitrification (specifically nitrite reduction and N_2 production) in a single process (Ward, 2013). Anammox is a dominant process especially in the oxygen minimum zones as in Black Sea (Kirkpatrick et al., 2012; Lam and Kuypers, 2011).

2.1.2. Microbial Studies: Past and Present Approaches

Microbial ecology studies have been drastically changed since the first report of 'animalcules' made by Leeuwenhoek (1677) to characterization using the current molecular techniques such as NGS. In 1887, Julius Richard Petri, assistant of R. Koch, invented a box that became well known in all microbiological laboratories. Robert Koch, with his assistant and his colleagues thus developed methods for the isolation and maintenance of pure cultures of many bacteria on solid media. This method is still used today by researchers in laboratories and allows microbiologists to isolate microorganisms in pure cultures and to study them in detail. Culturing studies changed our perspective of the microbial world and provide a tool for understanding their metabolism. The discovery of the "**Great plate count anomaly**" (Staley and Konopka, 1985) which is the disagreement between the sizes of populations estimated by culturing and by microscopy, might be the turning point at which scientist started to question the presence and the dominance of uncultivable fraction of microorganisms. Understanding of the classification of both cultured and uncultured prokaryotes has been changed after the proposal of ribosomal small subunit (SSU) gene as a phylogenetic marker (Woese and Fox, 1977). Universality of the SSU gene makes it an ideal target for phylogenetic studies and taxonomic classification. The ribosome that is universal in all cells, are named according to their sizes in the ribosomal subunit:16S ribosomal RNA (rRNA) in bacteria and archaea, 18S rRNA in eukaryotes. The 16S rRNA is a structural part of the 30S ribosomal small subunit, whose functions are essential in the living cell and includes eight highly conserved regions, U1-U8, which are not variable across the bacterial domain (Gray et al., 1984). However, between these conserved regions, 16S rRNA has nine variable regions, V1-V9, which have less importance for ribosomal function. Different nucleotide substitution rates in the variable regions are promising for phylogenetic analysis and identification (Van de Peer et al., 1996). After the invention of Sanger sequencing in 1977 and the polymerase chain reaction in 1983

(PCR: Kary Banks Mullis, who received a Nobel Prize in chemistry in 1993 for his invention), which enable scientist to amplify specific target region in the genome with an appropriate primer pairs in 1985 studying SSU genes, became comparably much easier than Woese and Fox's study. Sanger sequencing was the primary approach while studying microorganisms and increased the repository of 16s rRNA sequences in the relevant data bases like GenBank (at the time of this chapter written, total 16S DNA segments' total hit in the GenBank was 23,754,441 for bacteria and 407,287 for archaea). Before application of sanger sequencing technology, each target sequence of bacteria was required to be cloned into separate bacterial culture. If the number of different bacterial species were considered, this method was uneasy to apply to community ecology studies. Additionally, it was still expensive to implement. Due to these circumstances, microbial ecology studies could not reach their climax (discussed in the elsewhere: (Handelsman, 2004; Walters and Knight, 2014)). First decade of the 21st century corresponds the time of availability of Next Generation Sequencing to the scientific community. High-throughput sequencing yielded orders of magnitude greater sequencing depth than it was previously available (Caporaso et al., 2011). Such huge data came with a problem of the handling of the sequences. Analysing a few hundred sequences by hand and comparing the target sequences with data base was possible, but was not affordable with tens of thousands or millions of sequences per sample and/or study. Automated pipelines available for microbial ecologist to overcome the problem of huge data processes include QIIME (Caporaso et al., 2010) used in this study, SILVAngs (Quast et al., 2013b) and Megan (Huson and Weber, 2013). Quantitative Insights Into Microbial Ecology (QIIME) toolkit is an especially promising pipeline for microbial ecologist since it can perform standard microbial community analysis techniques on projects that possess millions of sequences data. Standard community analysis in the area includes: quality filtering of reads, operational taxonomic unit (OTU) picking, taxonomy assignment, computation of α and β diversity measures (Figure 36) (Caporaso et al., 2011). Details of each step and its application to human microbiome data has been discussed in the Navas-Molina et al., 2013 (Navas-Molina et al., 2013).

Even if, sequence similarity does not correlate with the biological notion of 'species', defining species by sequence similarity from 16S rRNA partial sequences is an accepted standard in studies of microbes. Not to confuse these two concepts, in microbial studies, "Operational Taxonomic Units (OTUs)" are used to define differences in the sequences. Each OTU can be considered as a different species. Besides, implementation of multivariate statistical techniques is another important part of microbial ecology studies. Especially with such large data, statistical tools are needed to examine and interpret the results. Summary of such multivariate statistical techniques used in microbial ecology are given in the Section 2.1.4.

2.1.3. Global Distribution of Marine Prokaryotes and their diversity

Venter and colleagues' study (Venter et al., 2004), which employs whole-genome shot-gun sequencing to analyse marine microorganisms in the Sargasso Sea, is a highly important contribution to the understanding of the marine microbial populations. Study area- Sargasso Sea has long been monitored and availability of abiotic data goes back more than 60 years (Bermuda Atlantic Time-series Study site- BATS), which makes the study area unique since it is possible to correlate community composition differences with environmental factors (Venter et al., 2004). Sargasso Sea study is also important for the present study, because of similarities of the two regions in terms of nutrient limitation. They have reported, a total of 1412 OTUs, of which 148 are previously unknown. Phylotypes have been investigated by 6 different phylogenetic markers such as 16s rRNA and RNA polymerase B (RpoB). Most dominant group was reported as Alphaproteobacteria followed by Gammaproteobacteria and Cyanobacteria. Archaeal groups of Euryarchaeota and Crenarchaeota were the least represented groups. One important result from the study of Venter and colleagues' is the widespread "patchiness" in the bacterioplankton distribution. In the size fraction (0.2 – 0.8 μm) they studied, *Prochlorococcus* was more abundant than *Synechococcus* in the cyanobacterial group. Because of the observed size fractioning, they concluded that there is a chance that *Synechococcus* cells might have been excluded.

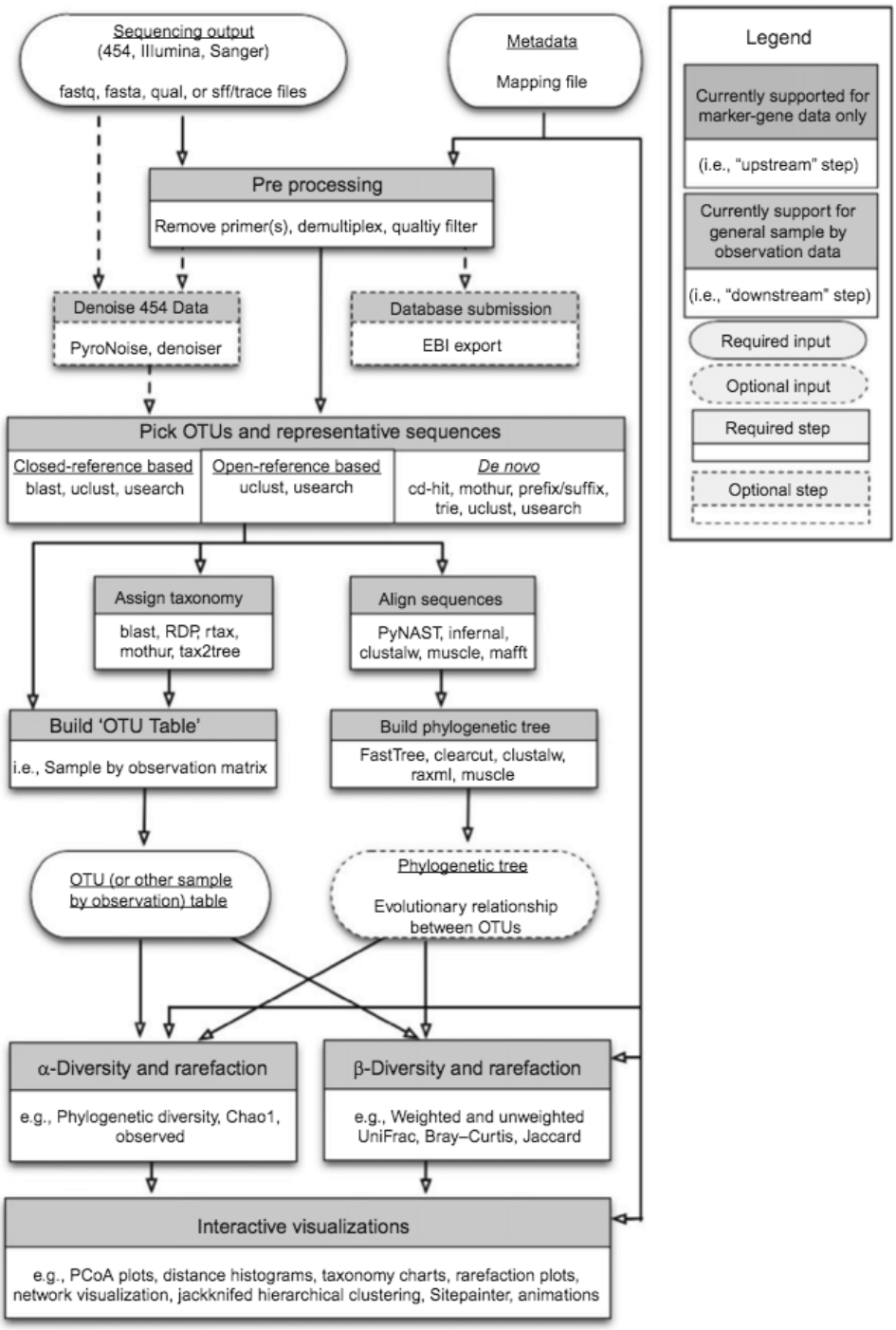

Figure 36. QIIME workflow overview. The upstream process (brown boxes) includes all the steps that generate the OTU table and the phylogenetic tree. This step starts by pre-processing the sequence reads and ends by building the OTU table and the phylogenetic tree. The downstream process (blue boxes) includes steps involved in the analysis and interpretation of the results, starting with the OTU table and the phylogenetic tree and ending with alpha- and beta-diversity analyses, visualizations, and statistics. After (Navas-Molina et al., 2013).

Global patterns of marine surface bacterioplankton communities were studied by 16s rRNA gene clone libraries 10 km off the shore at nine different locations: Arctic, Baffin Bay, Cape Town (South Africa), Chile, Fiji, Hawaii, San Diego, Sargasso Sea and Sydney during May and June. Only 2 of the observed OTUs (total 562) were found in all locations, and 386 (%69) of OTUs observed were endemic to one location. Alphaproteobacterium was reported to be the most cosmopolitan group and the second most was *Pelagibacter ubique* from SAR11 clade of Alphaproteobacterium (Pommier et al., 2006). Another study that provides insight into the distribution of bacterioplankton was conducted under the sampling framework of Global Ocean Sampling Expedition. Samples were collected along a transect from the North Atlantic through the Panama Canal and ending in the South Pacific. This metagenomics study reflected the diversity of microorganisms in the surface ocean and they showed that especially SAR 11 Surface 1 clade was widespread in tropical and temperate ecosystems (Rusch et al., 2007).

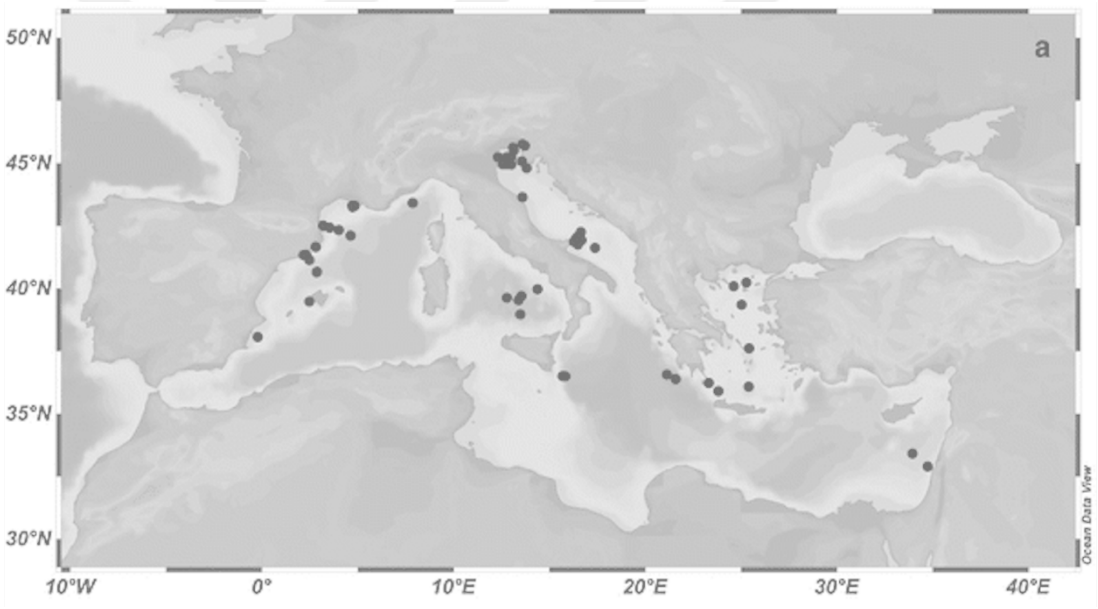

Figure 37. Map showing the study locations where bacterial and/or archaeal diversity has been described using molecular tools in the pelagic Mediterranean Waters. After (Luna, 2014).

Microbial diversity studies in the Mediterranean are still limited and mostly conducted in the North Western Mediterranean Sea and the Adriatic Sea (Luna, 2015). Seasonal successions of cultured fraction of the marine heterotrophic bacteria in the Adriatic Sea revealed that highest concentrations of heterotrophic bacteria were observed in the upper layers and, abundances were decreasing from coastal stations to the offshore station.

Most of the studies reported SAR11 clade to be the most abundant prokaryotic heterotrophs in surface waters (Alonso-Sáez et al., 2007; DeLong, 2009; Feingersch et al., 2010; Pham et al., 2008). Surface waters of the Southern Adriatic sub-basin (oligotrophic) were studied from coast to the offshore where the latter was characterized by higher temperature and salinity during March and April, 2012. Coastal waters were reported to be more diverse and difference between coastal and offshore communities explained by temperature. Dominant groups were identified as *Alphaproteobacteria* (SAR 11 cluster), uncultured *Gammaproteobacteria* (*Rhodobacteraceae*) and *Cyanobacteria* (*Synechococcus*) (Quero and Luna, 2014). Among *Cyanobacteria, Synechococcus* was found to be the more abundant than Prochlorococcus in the Adriatic Sea during March and April (Quero and Luna, 2014). *Bacteriodes,* which are known to be abundant in the coastal waters and can be observed attached to the organic particles (Alonso-Sáez et al., 2007), were also found to be more dominant at a location which has higher total suspended matter concentration (Quero and Luna, 2014a). Eastern basin of the Mediterranean Sea bacterial community studied very less both in the pelagic and sediment habitats. Culture based study in the south of Crete during winter showed that *Actinobacteria* and *Firmicutes* were dominantly represented and have clear advantage during nutrient starvation in the Eastern Mediterranean deep-sea sediments (Gärtner et al., 2011). Another important Culture independent amplicon sequencing study in the Levantine basin of the south-eastern Mediterranean Sea conducted during summer also reflects deep sea sediment microbial diversity. The ratios of abundances of bacteria and archaea were reported to be correlated with the TOC content in the sediments. Among archaeal reads, vast majority were assigned to the *Thaumarchaeota* and only ~2% were *Euryarchaeota.* Dominant bacterial OTUs belonged to *Proteobacteria* (with the most abundant classes being *Gammaproteobacteria*) *Deltaproteobacteria, Alphaproteobacteria,* and *Betaproteobacteria* in the sediment samples (Keuter and Rinkevich, 2016). Eastern Mediterranean Sea surface bacterial community were investigated using a culture independent method of large-insert DNA libraries from 12m deep water collected through a transect from Haifa to Cyprus and representing the summer period. Results showed that the abundant and cosmopolitan SAR11 group represented 45% of the *Alphaproteobacteria*, whereas the *Rhodobacterales* were the next most common *Alphaproteobacteria* group (26%). *Alphaproteobacteria* dominance was followed by *Cyanobacteria* and *Gammaproteobacteria*. Summer surface waters between Haifa and Cyprus were dominated mostly by Synechococcus (85%) and high light adapted *Prochlorococcus* sequences accounted for only 12% (Feingersch et al., 2010) by abundance. Low abundance of Prochlorococcus cells above the depth of 50 meter were reported also by Tanaka and colleagues in 2007 (Tanaka et al., 2007) by flow cytometric measurements. Low light adapted

ecotypes of *Prochlorococcus* were also reported in the summer surface waters of Eastern Mediterranean Sea by Feingersch and colleagues in 2010 (Feingersch et al., 2010). However, two different ecotypes of *Prochlorococcus* were shown to have clear-cut vertical partitioning associated with the strong vertical stratification typical for Mediterranean Sea waters in summer (Garczarek et al., 2007). Third most abundant surface water bacterial group belonged to *Gammaproteobacteria* and of mostly NOR5/OM60 plankton clade (Feingersch et al., 2010). Terminal-restriction fragment length polymorphism (T-RFLP) fingerprinting was used to discover the attached (filtered through Whatman GF/C filter to collect ‘‘particle-attached’’ bacteria) and free living (bacterial community passing the Whatman GF/C filters was considered to be the ‘‘free-living’’ bacteria and collected onto 0.2- μm pore sized filters) bacteria in the Aegean Sea from different water column depths. The communities were reported to be different from each other (Moeseneder et al., 2001). In the free-living bacterial community, distinct bacteria were observed particularly in the deeper water. And the deep-water community was found to be as complex as surface community (Moeseneder et al., 2001).

Archaeoplankton communities in the coastal surface waters of Blanes Bay in the Northwest Mediterranean were reported to be dominated by Euryarchaeota Marine Group II and followed by Crenarchaeota (now Thaumarchaeota) (Galand et al., 2010). Another study in the Northwest Mediterranean coastal waters reported the dominance of MGII.A sequences in the summer (Hugoni et al., 2013). Archaeal amoA gene copy numbers throughout the water column (surface waters and the meso- and bathypelagic layers (down to a maximum depth of 4350 m) of the Eastern Mediterranean Sea were investigated concluding Archaeal amoA copy numbers decreased from the 200–500m depth to 1000m and amoA copy numbers were found to be correlated with NO_2 concentrations (De Corte et al., 2009). One important study aiming to understand bacterial dynamics in the oligotrophic Eastern Mediterranean Sea was conducted by Sebastian and Gasol (2013). Response of some bacterial groups to the nutrient limitation in the Eastern Mediterranean was also investigated showing contradictory results between different bacterial groups. Heterotrophic bacterial activity was observed to be significantly stimulated in all the sample that contain the P. SAR11 clade of alphaproteobacteria, which was known by its dominance and cosmopolitan nature, was observed to be active regardless of the nutrient concentration. Besides the SAR 11 clade, Gammaproteobacteria abundance was also not affected strongly by the nutrient differences in the samples. However, Gammaproteobacteria was the group that more affected by P availability. The Roseobacter clade was likely co-limited by phosphorus and nitrogen, whereas Bacteroidetes activity requires organic carbon (Sebastián and Gasol, 2013).

2.1.4. Summary of multivariate statistical techniques

After the invention of next generation sequencing technologies and drop in the cost of sequencing, large amount of ecological data sets were produced. Large amount of data required powerful statistical techniques to examine and interpret the results. In order to accomplish these analysis multivariate statistical techniques are found to be well suitable (Paliy and Shankar 2017). Widely used statistical techniques include: exploratory, interpretive, and discriminatory procedures.

Exploratory methods: this method is used to understand relationship among objects (samples). This relationship is checked based on the values of variables (abundances of species). For instance, collected soil samples (object) in different location can be compared according to the abundances of microbial species in each sample. Output of these methods are the similarity visualization. Similar objects (samples) are located close to each other, whereas, dissimilar ones positioned apart from each other. Principal coordinates analysis (PCoA) is one of the exploratory methods applied in this study (Figure 54, Figure 55, and Figure 59).

Interpretive methods: These methods are also known as constrained techniques. In these techniques two different data sets were used. One data set includes measured variables (e.g. abundances of species in each sample), and other data set was explanatory variables such as known environmental gradients among samples. These techniques can be used to test specific hypotheses of how environmental variables determine response variable values. The analyses find the axes in the multidimensional space that maximize the correlation between the explanatory variables (environmental gradients) and the measured variables (called response variables). The "ordination axes" are constrained to be functions of the explanatory variables. It is also crucial here to stress that "ordination" is a term that is described as placing objects in order (Goodall, 1954). Ordination analysis generates a reduced number of new synthetic axes. These axes are used to display the distribution of samples along the main gradients (environmental gradients) in the dataset.

In order to find the relationship between two sets of variables abundances of species in each sample and environmental gradient, Canonical Correspondence Analysis was applied in this study (Details are given in the method section).

Discriminatory Methods- Discriminant Analysis: The goal of these analyses is to define discriminant functions that will maximize the separation of objects among different classes. In other words, analysis is used to classify samples into the predetermined groups. Examples of these methods are mostly seen in the human microbiome studies. This method was not applied in this study.

The main objective of this study is to enlighten the community composition of heterotrophic bacteria and how it varies throughout the year and depths using 16S rRNA amplicon sequencing approach. It was also aimed to relate physical, chemical and biological processes discussed in the first chapter with the taxonomic composition and diversity of bacterial community.

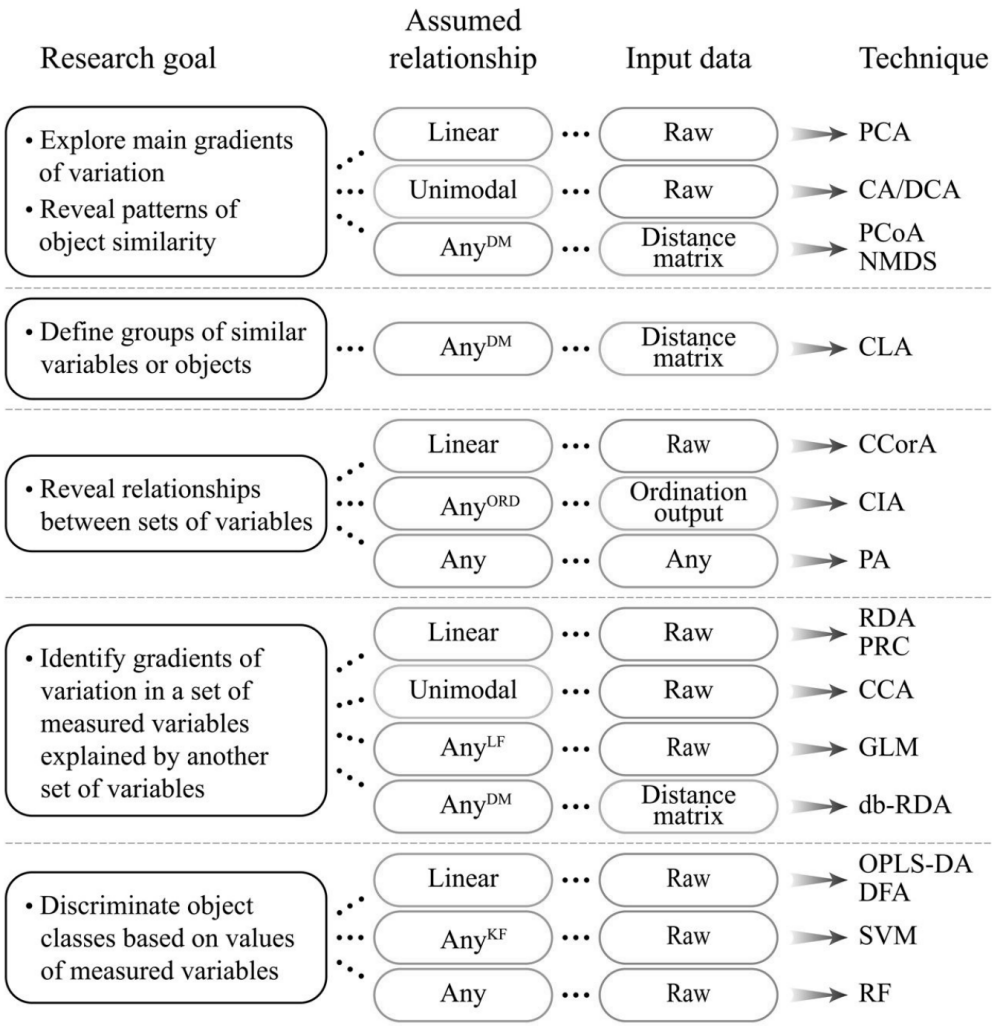

Figure 38. Network of potential choices of multivariate techniques based on the research goal, assumed relationship among variables, and input data structure. Assumed relationship depends on the chosen; DM (distance matric), ORD (ordination technique), LF (link function), KF (model can be linear or non-linear if a non-linear kernel function is used). Figure was taken from Paliy and Shankar, (2017) (Paliy and Shankar, 2017).

2.2. Material and Methods

Samples were collected between August, 15th 2016 and August 14th 2017 with a monthly interval for one year long from the ETS-200 m station of Erdemli Time Series (ETS), located at 36°26.17'North and 34°20.76' East. This station has a total water column depth of 200 m and is situated on the relatively narrow shelf area of the NLB. Seawater samples were collected during the ETS cruises mostly with the research vessel RV Bilim. When RV Bilim was not available, RV Lamas of METU-IMS was used. The seawater was collected from the surface, 25 m, 50 m 100 m 150 m and 200 m for 16S rRNA amplicon sequencing study by Niskin Bottles connected to the CTD Rosette. Two litres of the seawater were sampled into a borosilicate dark bottle from each depth and filtered on 0.22 µm pore sized filters (polyether sulfone membrane- MoBio) on board for the 16S rRNA amplicon sequencing study. The samples were then processed for DNA extraction in the genetics laboratory of the IMS-METU. Sampling could not be performed in December due to weather conditions. Sampling dates and depths and temperature measurements at the surface and 200 m depth are summarized in the Table 3.

Table 3. Sampling Dates and Water Temperature in the Surface and 200 m Depth

Month	**Date (DMY)**	**Depths (Meter)**	**Surface / 200 m Water Temperature (C°)**
September	08/09/2016	0, 25,50,100,150, 200	29.5/16.7
October	20/10/2016	0, 25,50,100,150, 200	26.2/16.0
November	15/11/2016	0, 25,50,100,150, 200	-
January	30/01/2017	0, 25,50,100,150, 200	17.6/17.1
February	20/02/2017	0, 25,50,100,150, 200	17.1/16.1
March	28/03/2017	0, 25,50,100,150, 200	17.9/16.9
April	17/04/2017	0, 25,50,100,150, 200	18.5/16.3
May	25/05/2017	0, 25,50,100,150, 200	21.7/16.4
June	30/06/2017	0, 25,50,100, 200	28.3/16.2
July	20/07/2017	0, 25,50,100,150, 200	29.6/16.5
August	14/08/2017	0, 25,50,100,150, 200	30.0/16.1

2.2.1. DNA extraction and Sequencing

DNA extractions were carried out with the methods given in Paz et al., (2003) (Paz et al., 2003). The amount of DNA from each sample was subsequently quantified by spectrophotometry. Sequencing was performed by Macrogen Inc. (Macrogen-Europe) on both forward and reverse directions. Amplicon sequencing was done by Illumina MiSeq platform (300bp paired-end sequencing) and 100.000 reads/30Mb data per sample was produced. The V3-V4 region of the 16S rRNA gene was amplified using 341F and 805R primer pairs (Herlemann et al., 2011). The sequencing library is prepared by random fragmentation of the DNA sample, followed by 5' and 3' adapter ligation. Scythe (v0.994) and Sickle programs were used to remove adapter sequences. After adapter trimming, reads shorter than 36bp are dropped in order to produce clean data. Total number of bases sequenced, total number of reads, GC content, AT content, ratio of reads that have phred quality score of over 20 and 30 per sample are given in the Appendix D (A phred quality score is a measure of the **quality** of the identification of the nucleobases generated by automated DNA sequencing). Experimental steps are given in the Figure 39.

Figure 39. Experiment overview (Taken from Macrogen Raw Data Report)

2.2.2. Bioinformatics analysis

Bioinformatics analysis are in the core of preparing sequence data for ecological interpretations. Bioinformatics analysis include different steps:

- Quality control of raw sequences and eliminating low quality sequence reads from analysis,
- Merging paired-end reads since, the sequence read are produced from forward and reverse directions
- Building OTUs (Operational Taxonomic Units) table (Feature Table). Each unique sequence is classified as a different OTU and then, similar OTUs are clustered together. Furthermore, each OTU is characterised by its abundance in the sample.

- Representative sequences of OTUs were then aligned against the reference data base for taxonomic classification.
- Representative sequences are used to calculate phylogenetic diversity metrics.
- For calculation of non-phylogenetic metrics' OTUs table is used.

Illumina trimmed paired-end reads were used for the analysis. For downstream analysis, QIIME 2 framework and its command line interface was used (https://qiime2.org), (Caporaso et al., 2010). Merging of paired-end reads, quality and primer filtering, chimera identification were performed by Divisive Amplicon Denoising Algorithm (DADA2) (Callahan et al., 2016). Feature table which is equivalent of *OTU/BIOM* table, and the "Feature data" which is the equivalent to *representative sequences* file were produced also with the same algorithm. Unlike some of the widely used algorithms by QIIME 1, DADA2 does not cluster similar sequences but rather groups unique sequences, which are the equivalent of 100% OTUs from QIIME 1, and are generally referred to as *sequence variants.* Consequently, DADA2 can identify unique strains within a species. Thus, output of DADA2 is an abundance table in which each unique sequence is characterized by its abundance in each sample (Stoll et al., 2018). Taxonomic classification accuracy was improved by training Naive Bayes classifier (https://github.com/qiime2/q2-sample-classifier) on the region of our target sequences (V3-V4 region) as suggested Werner and colleagues (2012) (Werner et al., 2012). Silva database version 132 (Quast et al., 2013) was used to assign taxonomy with a 99% cut-off. Sequence alignment were performed using QIIME 2 plug-in MAFFT (2017.12.0) (Katoh and Standley, 2013) before phylogenetic tree construction. Phylogenetic tree were constructed using FastTree 2 (Price et al., 2010). For statistical and diversity analysis, sequences that were assigned as chlorophyll were trashed out using q2 taxa plug-in (https://github.com/qiime2/q2-taxa).

2.2.3. Diversity Metrics calculation and statistical testing

Alpha and beta diversity metrics are important tools to investigate how diverse a given community is. Alpha diversity shows with-in sample diversity whereas beta diversity is used to compare diversity between samples in a given location.

Diversity within a given community (α diversity) is usually considered according to the total number of species (called species richness), the relative abundances of the species (species evenness), or combination of these two.

Similarly, the description of biological diversity among communities (samples) or along an environmental gradient (β diversity) is often characterized using the number of species shared between two communities.

For both alpha and beta diversity analysis quantitative metrics which uses abundance of features (phylogenetic and non-phylogenetic) was used. Qualitative diversity metrics (considers presence/absence of features) was not applied to the data produced in this study.

Alpha Diversity metrics used:

By measuring alpha diversity, it is questioned that whether any season have more diversity, whether increased nutrient concentrations coincides with increased diversity. We applied three different diversity metrics: observed OTUs, Faith Phylogenetic Diversity, Shannon's index. Observed OTUs is the total number of different OTUs (can be also considered as species) in the sample. This provides simply the species richness. Shannon's diversity index is commonly used to characterize species diversity in a community. The importance of Shannon's index is that it considers both abundance and evenness of the species present in the sample. For instance, if the sample have more OTUs but only small number of these OTUs dominate the sample, calculated Shannon index will be reported to be lower than the sample in which OTUs distributed evenly.

Additionally, in order to account for phylogenetic diversity in the sample, Faith's Phylogenetic Diversity (Faith's PD) was used. Faith's PD is a qualitative measure of community richness that incorporates phylogenetic relationships between the OTUs in the sample. Phylogenetic tree is in the base of the calculation of Faith's PD. It is the sum of all branch lengths. The phylogenetic tree represents the evolutionary relationship between each OTU in the sample. The sample having more distantly related OTUs will have higher phylogenetic diversity. The number of observed OTUs, Shannon index and, Faith's PD were calculated using QIIME 2.

Beta Diversity Metrics used:

Pairwise community similarity between 16S rRNA samples was computed using phylogenetic distance called weighted UniFrac distance (Chang et al., 2011; Lozupone et al., 2011) and Bray-Curtis (community composition) dissimilarity.

Bray-Curtis dissimilarity is the differences in species (OTUs) between two different samples. Dissimilarity is defined by the numbers between 0 and 1. Basically, the 0 dissimilarity means that these two samples share the same OTUs.

On the other hand, UniFrac distance is a phylogenetic distance to compare environmental samples (Lozupone et al., 2011). UniFrac measures the fraction of branch lengths in the phylogenetic tree that are unique to each sample and captures the differences between two samples as the evolutionary history. Additionally, weighted version of UniFrac accounts for the differences in relative abundances. In this study, unweighted UniFrac distance was used in order to only consider phylogenetic differences.

These beta diversity measures were coupled with Principle Coordinates Analysis to identify factors explaining differences among microbial communities sampled throughout the year from different depths.

As a widely used exploratory method, Principle Coordinates Analysis (PCoA) was used to explain the variance in the data set while trying to order the objects (samples) along the ordination axes (Paliy and Shankar, 2016). PCoA works with any dissimilarity or distance measure such as UniFrac distances and Bray- Curtis dissimilarity. Plots used in this study were generated using EMPeror (Vázquez-Baeza et al., 2013). Even though PCoA constitutes an important tool to discover characteristics of data sets, it does not provide a direct link between the components (environmental variables such as temperature) and the original variables (beta diversity measures or abundance of species).

Canonical correspondence analysis

To investigate species response to the environmental variation Canonical correspondence analysis (CCA), which uses the unimodal model to explain species response (Paliy and Shankar, 2016; Ramette, 2007; Wang et al., 2012), was used. CCA analysis was performed using *vegan* package of R (Oksanen et al., 2007).

To enlighten the relationship between biological assemblages and their environment a multivariate method which is called as CCA was used by high number of publications (Braak and Verdonschot, 1995; Ramette, 2007). Ecological observations by their nature involve large number of species and internal variability. Multivariate methods provide a means to structure the data by separating systematic variation from noise (Braak and Verdonschot, 1995). CCA is based on the unimodal species – environment relationship in which the axes are linear combinations of the environmental variables. Method operates on abundances of species (OTUs) and data on environmental variables at sampled locations. Synthetic gradients which are referred as ordination axes were extracted from the measured environmental variables in order to maximize the niche separation among species. It is based on Chi-squared distances. CCA primarily resulted in a graph with a coordinate system formed by ordination axes and consists elements of points for species (OTUs), sites (sampling units) and classes of qualitative environmental variables (like sampling season) and arrows that represents quantitative environmental variables such as temperature. Inertia is the measure of explained variation of the data in CCA. Since, CCA is sensitive to rare species sequence variants that were not observed in more than 20% of all samples were discarded from the analysis. Microbial communities were tested against the constrained set of environmental variables using CCA implemented in the vegan package (Oksanen et al., 2013). The constrained parameter set was determined by performing a stepwise selection (Akaike information criterion, 999 permutations per step) using the ‘step’ function in vegan.

Reading Ordination Plots:

- The ordination graphs show the main features of similarities and differences in the data
- If two sampling units (SUs) are close to each other, they have similar communities
- If two SUs are far away from each other, the communities differ
- If two species are close to each other, they have similar occurrence patterns, and occur most abundantly in SUs that are close to them
- It can be assumed that environmental conditions are behind these differences and we can identify the external variables that explain the ordination structure

(Jari Oksanen lecture notes:
http://cc.oulu.fi/~jarioksa/opetus/metodi/ordination101.html#1)

Terminology

- Canonical analysis: a general term for statistical technique that aims to find relationship(s) between sets of variables by searching for latent (hidden) gradients that associate these sets of variables.
- Constrained and unconstrained ordination – the constrained multivariate techniques attempt to 'explain' the variation in a set of response variables (species abundance) by the variation in a set of explanatory variables (environmental) measured in the same set of objects (e.g., samples or sites). The matrix of explanatory variables is said to 'constrain' the multivariate analysis of the dataset of response variables, and the output of constrained analysis typically displays only the variation that can be explained by constraining variables. In contrast, the unconstrained multivariate techniques only examine the dataset of response variables, and the output of unconstrained analysis reflects overall variance in the data.
- Distance – it quantifies the dissimilarity between objects in a specific coordinate system. Objects that are similar have small in-between distance; objects that are different have large distance between them.
- Eigenvector and eigenvalue – in ordination methods such as CCA, eigenvectors represent the gradients of dataset dispersion in ordination space and are used as ordination axes, and eigenvalues designate the 'strength' of each gradient.

- Ordination – a general term that can be described as ‘arranging objects in order’ (Goodall, 1954). The goal of ordination analysis is to generate a reduced number of new synthetic axes that are used to display the distribution of objects along the main gradients in the dataset.
- Triplot – a two-dimensional diagram of the multivariate analysis output that in addition to response variables and objects shown on biplot also displays explanatory variables.
- Variance, variability, and variation – the differences in the use of these three related terms can be described by the following statement: ‘Variance is a statistical measure of data variation and dispersion, which describe the amount of variability in the dataset’.

  Note: These terminologies are taken from Paliy and Shankar (2017) (Paliyand Shankar, 2017).

2.3. Results

Sampling has been conducted during ETS cruises and performed for one year with monthly intervals in ETS-200 m station. Each month same 6 depths sampled and in total 65 samples were collected. Following DNA isolation samples sequenced from both ends by Illumina MiSeq platform. In total, 31,969,956 raw reads were produced and total read bases are 9, 622 Mbp (Trimmed data statistics: Appendix D).

2.3.1. 16S rRNA Community Composition

In total, 2213 sequence variants (equals to OTUs) were observed and decreased to 1978 when chloroplast 16S rRNA sequences were trashed out. After filtering out chloroplast sequences analysis carried out. Maximum sequence count (13,652) was observed in the April 2017 surface sample and minimum was in September 2016 100 m sample. The mean frequency observed in 65 samples were 8,154.9 (Table 4. Data Statistics).

Table 4. Data Statistics showing minimum, maximum and mean frequencies in the samples.

	Frequency
Minimum frequency	2,060.0
1st quartile	5,556.0
Median frequency	8,039.0
3rd quartile	11,388.0
Maximum frequency	13,652.0
Mean frequency	8,154.923076923077

In total, 255 different taxa (Appendix E) were assigned to 1978 sequence variants, and 29 of them has been observed only once in 65 sample. In total, 38 of the taxa detected in more than 50% of the samples, 145 taxa however observed in less than 10 % of the samples. Moreover, 3 of the taxa detected in all the samples and all are belonging to SAR 11 clade: Clade Ia (frequencies: min = 2.2, max. 39.4, avr =23.3), Clade Ib (frequencies: min = 1.4, max. 23.5, avr =10.6) and Clade II (frequencies: min = 2.1, max. 19.34, avr =9.9). Sample taken in January from 100 m depth possesses the higher number of taxa with 85 different taxonomic assignment, 63 of them has abundance less than 1%. January surface sample was the second most rich sample with 81 different taxa. Sample taken from 200 m depth in July has only 21 different taxa and the highest abundance was belonging to the Clade Ib with 23.2%. In the samples taken there are 5 different taxa assigned under *Cyanobacteria,* 3 of which classified to genus *Cyanobium PCC-6307* (seen only in 7 sample, having max abundance of 0.75%), *Prochlorococcus MIT9313* (seen only in 48 sample, having max abundance of 14.9%) and *Synechococcus CC9902* (seen only in 40 sample, having max abundance of 3.8%). Other taxon could not be resolved down to genus level, but rather assigned to *Cyanobiaceae* family level (seen only in 7 sample, having max abundance of 2.5%). And the other two taxa just classified as *Cyanobacteria* (seen only in 7 sample, having max abundance of 2.5%) phylum and *Oxyphotobacteria* (seen only in 7 sample, having max abundance of 0.16 %) class.

September 2016

Sampling has started on September 2016 when first 50 m of water column was around 29 C° and, the temperature gradually decreased to 20 C° at 80 meters depth. Water temperature below 80m to 200 m depth further decreased to 16 C°. First two samples (0m and 25 m) were taken from the warmer water masses. Sample taken at the depth of 50 m was taken from the start of the thermocline and, the rest of the samples were collected from the depths where water temperatures were below 20 C°.

Clade Ia was the dominant taxon throughout the first three depth from the surface. The highest contribution was observed in the surface (31%), and the lesser in the 100 m depth.

Prochlorococcus MIT9313 was the most abundant group (12.5%) in 100 m depth and *Clade Ia* was second most abundant group in the 100 m depth contrary to the surface depths (Figure 40).

Samples from 50 m and 100 m depth possessed the lower number of different taxa, 28 and 36 taxa respectively. Other samples had comparable more taxa (0m: 57, 25 m:50, 150 m: 72 and 200 m: 66). Throughout the water column, 13 common taxa were present and 72 different taxa were observed and 59 of them detected only in one sample. Since, 150 m and 200 m depth had the highest number of taxa, relative abundance of "Other" taxa that had less than 1% share were higher than other samples. Prochlorococcus was second mostly observed genera in 50 m (%15). Other samples only have 2-5% of Prochlorococcus representation. Synechococcus CC9902 was only seen in the first 50 m samples with a really low representation (~1%). Cyanobiaceae that can be identified until family level was observed too but only in the sample 200 m with the abundance share 2.5 %.

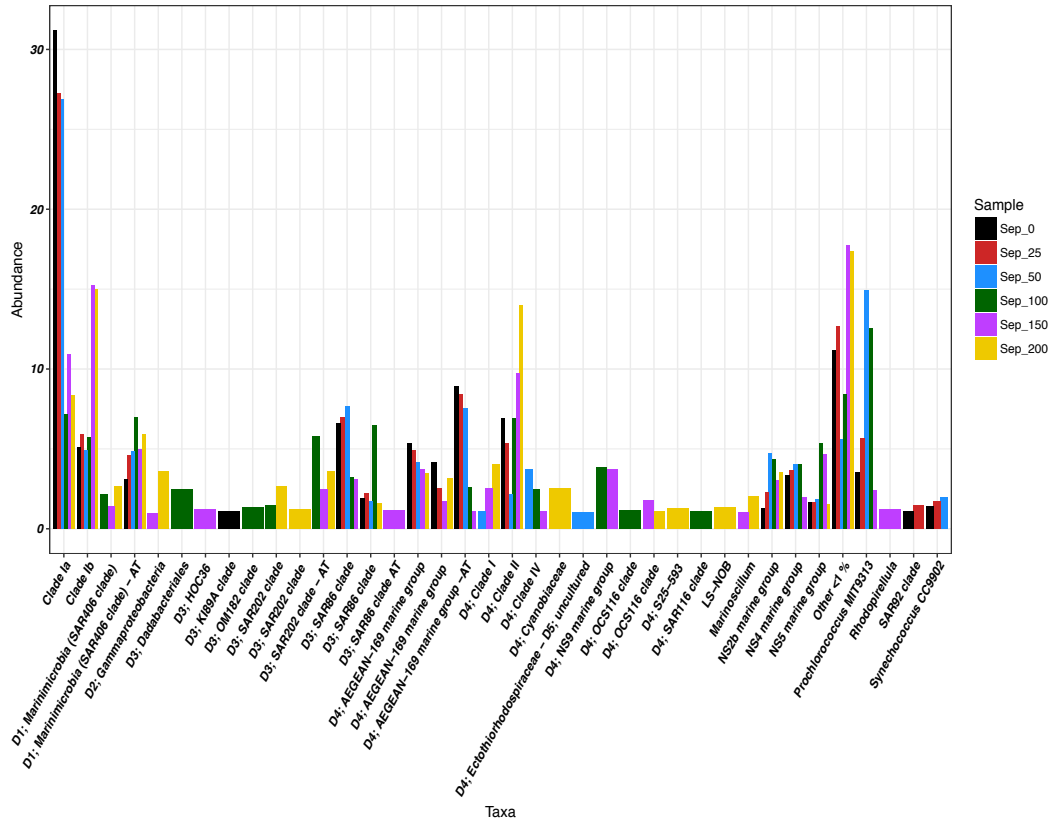

Figure 40. Bar plot of observed taxa in September 2016 sampling depths. "Other < 1%" shows taxa that have relative abundance of less than 1% in each sample. Taxa are listed in the alphabetical order. If the taxa cannot be resolved until level 7 available taxonomic information was given in corresponding level (e.g. D1). AT is the abbreviation for Ambiguous Taxa.

October 2016

In October, water column stratification slowly started to break down (around 50 m) but was still detectable (Figure 41). Surface water temperatures were lower than the previous month (0m: 26.2 C°, 25 m: 26 C°, 50 m: 22.6 C°, 100 m: 18 C°, 150 m: 17 C°, 200 m: 16 C°). Most dominant taxon observed in the first three sampling depths was the same with September samples being Clade Ia with the highest abundance share was in the surface and followed by 25 m and 50 m depth (29.2% and 23.6%, respectively). Dominance trend in the surface water was followed by AEGEAN-169 marine group, Clade II and SAR86 clade. AEGEAN-169 marine group was the second and third dominant taxa was NS5 marine group in the 25 m depth waters whereas in 50 m depth these ranks were as *Clade Ib* and *SAR86 clade*.

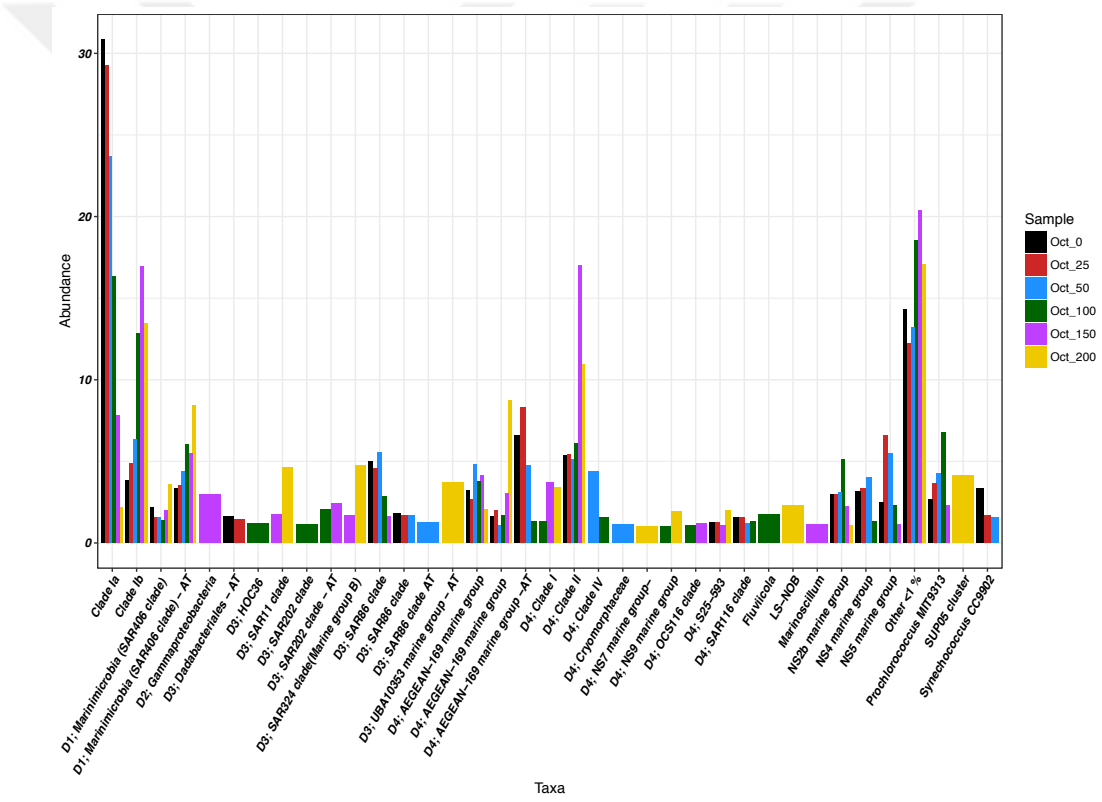

Figure 41. Bar plot of observed taxa in October 2016 sampling depths. “Other < 1%” shows taxa that have relative abundance of less than 1% in each sample. Taxa are listed in the alphabetical order. If the taxa cannot be resolved until level 7 available taxonomic information was given in corresponding level (e.g. D1). AT is the abbreviation for Ambiguous Taxa.

Prochlorococcus MIT9313 was the third dominant taxon in the 100 m depth (6%) and has the highest share in 100 m depth compared to other depths following Clade Ia and Clade Ib. *Prochlorococcus* genera was not observed in 200 m depth. Besides, its abundance was dropped to 4.2 and 6.7%, respectfully in 50 and 100 m depths where it was the second dominant taxon previous month. Also, *Synechococcus* genera were only observed in first 50 m depth (%1-3 abundant) but not in the deeper waters as it was the case in the September. Another classified taxon under *Cyanobiaceae* family was only detected in the surface and 150 m depth with less than 1% abundance. Among the archaea, only *Marine Group II* archaea observed in 200 m depth whereas *Cenarchaeaceae* family detected in the September 150 m depth both having low abundances (less that 1%). Clade II and Clade Ib was the most dominant in the 150 and 200 m depth than Clade Ia.

Among the six sampling depths in October, the community at 100 m depth had the highest number of different taxa (74), and the least diverse ones were the surface and 25 m depth (0m: 49, 25 m: 48). In total, 150 different taxa were observed and 62 of them was detected only in one sample and only 15 of observed taxa was common in all sampling depths. In total, 8 taxa were only observed in 150 m depth and had 49 different taxa having relative abundance less than 1% thus, relative abundance of "Other" taxa were higher than other samples.

November 2016

In November, only 4 taxon having relative abundance > 1% were common which are *Clade Ia, Clade Ib*, *Marinimicrobia (SAR406 clade)* and AEGEAN-169 marine group. Proportion of the taxa having relative abundance >1% accounts more than 20% in the sample taken from 150 m depth. Contrary to the previous months the abundance of the Clade Ia in 50 m has decreased but increased in the 100 m depth (up to 24%). Clade II and Clade Ib have relatively higher abundance in the 150 m and 200 m depth (Figure 42). While Clade Ib was more dominant than Clade II in the 200 m depth, Clade II represented higher relative abundance in the 150 m depth. The 150 m depth sample had the greatest number of taxa (67) which is followed by the surface depth (65). The sample taken at the depth of 25 m had the lesser taxonomic representation (51). In surface 25 m and 100 m depth samples, AEGEAN-169 marine group – AT were the second most abundant taxa, besides Clade Ib was the third in these depths. Clade II and Clade Ib had the same proportion (12%) in 50 m depth community, while being second dominant taxa. *Prochlorococcus MIT9313* was observed in all depths except for 200 m depth. *Prochlorococcus MIT9313* had the highest relative abundance in 100 m depth in October but in November 50 m depth was having the most of the proportion. AEGEAN-169 marine group – AT (6.8%) and Clade Ib (5.7%) were the following dominant taxa in 100 m depth after Clade Ia (31.5%) taxon. Within 150 m depth samples, Clade II taxon was the most abundant which is followed by Clade Ib, Clade Ia and SAR202 –AT. Taxon that

is classified as *Cyanobiaceae* was only observed in 100 m (<1%) and 150 m (1.3%) depth. Cyanobium PCC-6307 was detected only in the surface but constituting less than 1% of share. There were, in total, 139 different taxa found in all depths at least once and 51 of them were detected only once in 6 samples, with 12 taxa shared.

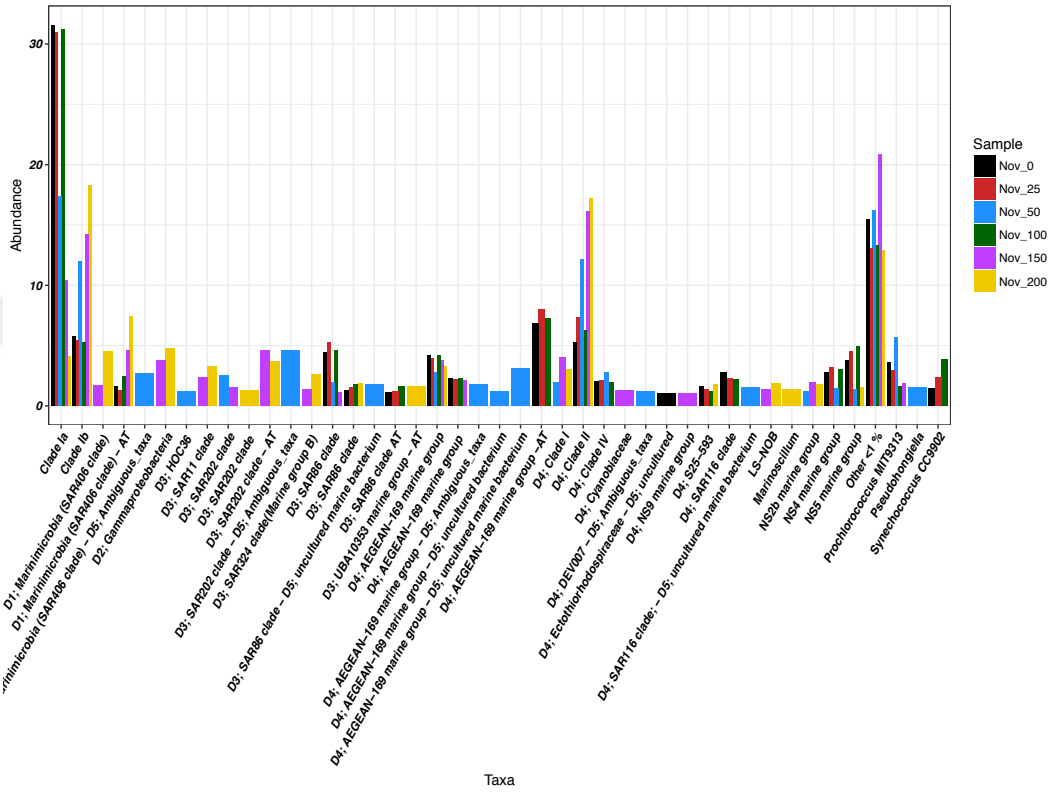

Figure 42**.** Bar plot of observed taxa in November 2016 sampling depths. "Other < 1%" shows taxa that have relative abundance of less than 1% in each sample. Taxa are listed in the alphabetical order. If the taxa cannot be resolved until level 7 available taxonomic information was given in corresponding level (e.g. D1). AT is the abbreviation for Ambiguous Taxa.

January 2017

January sampling has been conducted at the end of the month which corresponds to the time when water column was already well mixed (Figure 43). Water temperature difference between surface and 200 m depth was not more than 1 C°. In total, 80 different taxa were observed in all samples each depth having 44 to 54 taxa (0m: 51, 25 m: 45, 50 m: 47, 100 m: 54, 150 m: 48, 200 m: 44 taxa). Number of observed singletons were 32 and, 43 of the taxa

were common in all sampling depths. 100 m depth sample possess the greater number of taxa (85) while the 200 m depth community formed by only 69 taxa having the lowest grade.

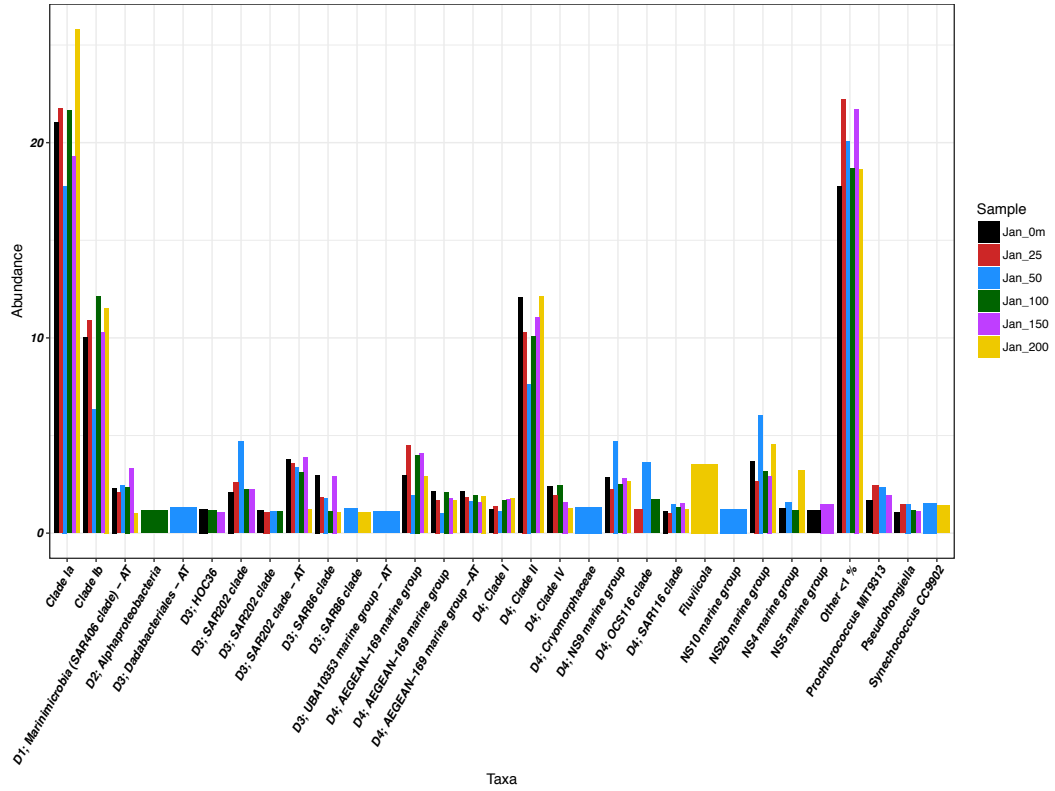

Figure 43. Bar plot of observed taxa in January 2017 sampling depths. “Other < 1%” shows taxa that have relative abundance of less than 1% in each sample. Taxa are listed in the alphabetical order. If the taxa cannot be resolved until level 7 available taxonomic information was given in corresponding level (e.g. D1). AT is the abbreviation for Ambiguous Taxa.

Relative abundance of Clade Ia, in November 200 m depth sample, was the lowest compared with the other samples but in January contribution percentage of taxon Clade Ia was the highest with 25.8%. In general, Clade Ia was dominant throughout the water column. Considering the taxa having >1% relative abundance in each of the samples, 14 taxa were observed in all sampling depths which are Clade Ia, Clade Ib, Marinimicrobia (SAR 406 clade) – AT, SAR 202 clade – AT, AEGEAN-169 marine group (3-different), Clade I, Clade II, Clade IV, NS9 marine group, SAR 116 clade and NS2b marine group.

In surface, Clade II (12.07%), Clade Ib (10%) and NS2b marine group, SAR202 clade – AT (each having 3% relative abundance) were the following dominance after Clade Ia. 59 of the

taxa observed in the surface were having relative abundance of less than 1%, and together account for 17.7 % of the total abundance. The 25 m depth sample also had the same dominant taxa as surface but Clade II, Clade Ib having the same relative abundance but followed by AEGEAN-169 marine group (4.4%) and SAR202 clade – AT (3.5%). The number of the taxa observed in the 25 m which had relative abundance of less than 1% was the same as surface sample. Clade Ia abundance was the least in 50 m depth among all samples and other dominant taxa had the relatively same abundance as 7-6% and they are: Clade II, Clade Ib, NSb2 marine group. The community present at 100 m had same two taxa as second and third being Clade Ib (12.1%) and Clade II (10.7%). AEGEAN-169 marine group and NS2b were following them with each presenting 3% of relative abundance. The number of taxa having less that 1% of relative abundance is 63 and all accompanies 18.6%, in total. Clade II (11%) and Clade Ib (10%) and AEGEAN-169 marine group (4%) were the following dominant taxa after Clade Ia in the 150 m depth community. In total, 60 of the taxa having less than 1% of abundance each account for 21% of the abundance in 150 m depth. Clade II (12.1%), Clade Ib (11.5%) and NS2b marine group (4.5%) were the dominant groups in the community from the deepest sample after *Pelagibacteraceae.* Interestingly *Fluviicola* had the 3.5 % relative abundance in 200 m depth but in others it possesses less than 1% of relative abundance. In total, 50 taxa had less than 1 % abundance were together representing 18.6% relative abundance.

Phylum Cyanobacteria were represented by 3 different taxa in the January sampling period. *Prochlorococcus* MIT9313 and *Synechococcus* genus and the other group that is classified as *Oxyphotobacteria. Prochlorococcus* MIT9313 were present in all sampling depths with relative abundance of 2.4 % in 25 m, 2.3% in 50 m, and 1.9 % in 150 m depths and 1.6% in surface but less than 1 % in the 200 m and 100 m depth. Genus *Synechococcus* were absent in 25 m but also in other depths it represents really less abundance than 1%. Other sequences that were classified under Cyanobacteria group as *Oxyphotobacteria* was present in only surface with 0.02%.

February 2017

Physical conditions in the water column were similar to those of January, indicating a well-mixed water column. Water temperature from surface to 200 m depth decreased from 17.1 to 16.1 C° (Figure 44). The number of different taxa observed in all samples at least once were 118 and each depth having 57 to 69 taxa (0m: 65, 25 m: 68, 50 m: 57, 100 m:73, 150 m: 69, 200 m: 69 taxa). Number of observed singletons in all samples and the common taxa seen all samples were 34 and 38, respectively. Similar to results presented so far, sequences that were classified under Clade Ia family again was the most dominant taxon in all depths having again higher relative abundance in 200 m as it was in January and lesser in 100 m depth. In surface

Clade Ib (11.9%), Clade II (9.1%) and AEGEAN-169 marine group (5.7%) were the following dominant taxa. *Prochlorococcus* MIT9313 was coming after NSb2 marine group (4.2%) by 3.8% of representation share. In total, 43 of the taxa observed in the surface had relative abundance of less than 1%, and together account for 14.2 % of the total abundance. 25 m depth sample also has the same dominant taxonomic groups as surface community in the first 3 dominant taxa.

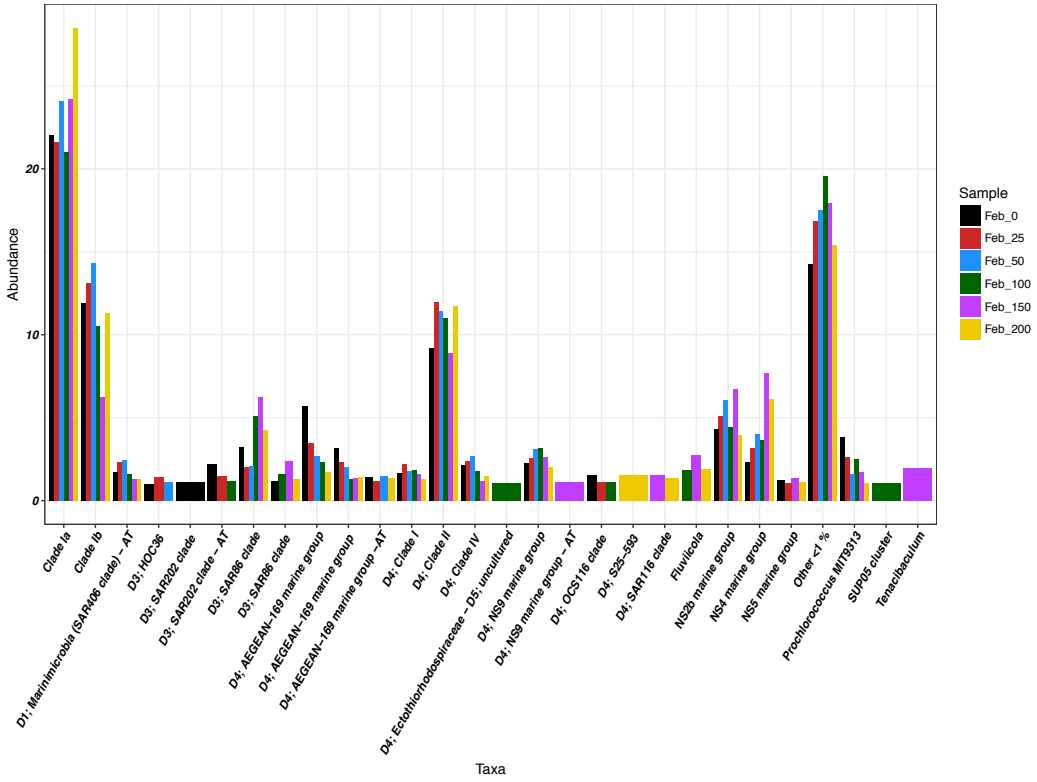

Figure 44. Bar plot of observed taxa in February 2017 sampling depths. "Other < 1%" shows taxa that have relative abundance of less than 1% in each sample. Taxa are listed in the alphabetical order. If the taxa cannot be resolved until level 7 available taxonomic information was given in corresponding level (e.g. D1). AT is the abbreviation for Ambiguous Taxa.

NS2b marine group was more abundant than AGEAN-169 marine group compared to surface in 25 m depth. The number of the taxa observed in the 25 m were having relative abundance of less than 1% was 49 but in total accounts for only 16.8%. Apart from Clade Ia (24%), other dominant taxa observed in 50 m depth community were having the relative abundances as follows: Clade Ib 14.2%, Clade II 11.4%, NS2b marine group (6%), NS4 marine group 4%.

41 taxa were having less than 1% each and accounts for only17.9% together. Clade II was the second dominant species in the 100 m depth community with relative abundance of 11%. Compared to shallower depths, in 100 m depth abundance of SAR86 clade was increased to 5%. The number of taxa having less than 1% of relative abundance is 52 and all sums up to 19.5 % in total in 100 m depth. Thus, 100 m depth community were the most diverse community having 73 different taxonomic annotations. Clade Ia dominance was most abundant in 150 m depth among all samples and the relative abundance of Clade II was 8.9%. Taxon NS4 marine group and NS2b marine group reached highest with 7% and 6.6%, respectively. However, that increase in the relative abundances of these taxa did not affect the number of taxa that were represented less that 1% as being in total 50 different taxa and in total sums up to 17.9%. Highly rated first three taxa in 200 m depth was also the same with 150 m, NS4 marine group and NS2b marine group with having relative abundances 11.7 % and 6.1 %, respectively. Taxa having less than 1 %, 50 taxa, was account for 15.4% of all taxa observed in 200 m community.

Phylum Cyanobacteria were represented by 4 different taxa in the February sampling period, *Prochlorococcus* MIT9313 and *Synechococcus* CC9902, Cyanobium PCC-6307 and another group that is classified as *Oxyphotobacteria*. *Prochlorococcus* MIT9313 and *Synechococcus* CC9902 were present at all depths. *Synechococcus* CC9902 was having again less than 1% abundance in all depths. Presence of *Prochlorococcus* MIT9313 was also low but accounting for 3.8% in surface, 2.6% in 25 m, 1.5% in 50 m, 2.5 % in 100 m, and decreasing from 1.7% to 1% in the last two depths of 150 and 200 meters.

March 2017

Another monthly sampling was conducted in the March 2017 which has the same physical conditions as previous months, January and February, when water column is well-mixed (Figure 45). As it was observed previous months, Clade Ia had the greatest abundance in all water column. However, relative abundance of Clade Ia increased this month in the surface 25 m and 50 m depths, and decreased in the sample taken at the depth 200 meters. This decrease continued at the 150 m depth in March compared to the February and January. In total, 117 distinct taxa were observed at least once in all depths, ranging between 50 to 70 taxa (0m: 55, 25 m: 53, 50 m: 50, 100 m: 60, 150 m: 70, 200 m: 69 taxa). In general, number of taxa observed in the first three depths stayed relatively same, but the numbers of different taxa observed were highest in the 150 m and 200 m depths. Singletons which are observed only in one sample accounts for in total 34 distinct taxa. And, the sum of common taxa detected in all depths were 37.

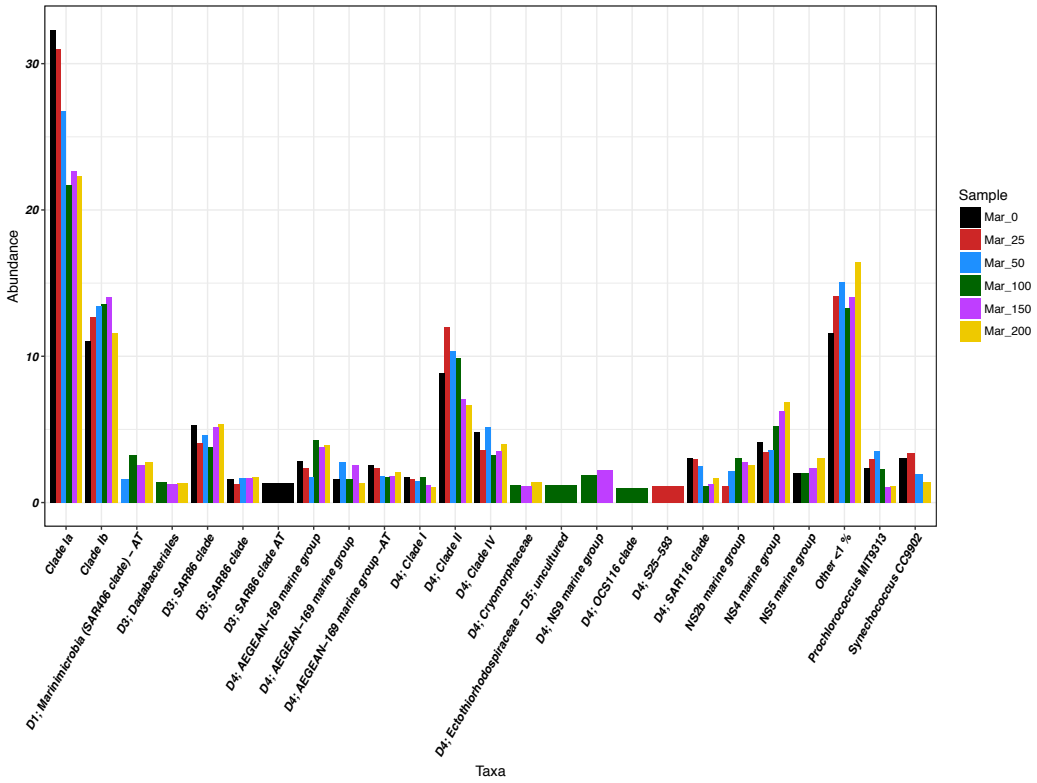

Figure 45. Bar plot of observed taxa in March 2017 sampling depths. “Other < 1%” shows taxa that have relative abundance of less than 1% in each sample. Taxa are listed in the alphabetical order. If the taxa cannot be resolved until level 7 available taxonomic information was given in corresponding level (e.g. D1). AT is the abbreviation for Ambiguous Taxa.

Clade Ib was the second dominant taxon (11%) observed in surface sample which was followed by Clade II (8.8%), SAR 86 (5.2%) and Clade IV (5%). In surface waters during March sampling, the number of distinct taxa accounts for 55 in which, 39 of them having less than 1% abundance. These 39 taxa sum up to 11.5% of all prokaryotic groups in the surface sample. Two important Cyanobacteria group which are *Synechococcus* and *Prochlorococcus* detected also in March and with slightly more share of *Synechococcus* CC9902 (3%) than *Prochlorococcus* MIT *9313* (2.3%) in the surface. The sum of diverse taxa discovered in the 25 m depth sample was 53. Among these 53 taxa 38 of them presented less than 1% and, these 38 taxa accounts for 14.1% of all observed taxa. Clade Ib and Clade II had approximately the same relative abundance as 12.6% and 11.9% respectively. And they are followed by SAR 86 (4%), Clade IV (3.5%), NS4 marine group (3.4%). The two Cyanobacteria genus *Prochlorococcus* MIT9313 (3.0%) and *Synechococcus* CC9902 (3.3%) were present in 25 m depth. Cyanobiaceae also detected with <1% of relative abundance. In the depth 50 m, community was mostly formed by (after Clade Ia) Clade Ib (13.4%), Clade II (10%), Clade

IV (5%). Among Cyanobacteria groups, P*rochlorococcus* MIT 9313 (3.4%) and *Synechococcus* CC9902 (1.9%) was also present. The taxon Cyanobium PCC-6307 detected also at the depth of 50 m. In total, 50 different taxa were discovered at the 50 m depth. Among these 50 taxa, in total 34 of them had less that 1% abundance with total abundance of 15%. Clade Ib represented 13% of the community in 100 m depth and also, the share of Clade II was 9.8%. Community observed in 100 m depth had 60 distinct taxa and 39 of them had relative abundance less than 1%. These 39 taxa sums up to 13% together. P*rochlorococcus* has the frequency of 2.2% but, *Synechococcus* abundance has decreased 0.5% in the 100 m depth. Dominant taxa observed in the 150 m and 200 m depth was having the same properties as 100 m depth. Community in the 150 m depth has 70 different taxa and, 50 of them had <1% of share with total 14% of abundance. Whereas in 200 m depth, the number of observed taxa was 69 and, among 69 taxa 49 of them had <1% of abundance but in total these taxa accounts for 16% of community.

April 2017

In April, surface water temperatures represent slight increase in the first 10m from 17 to 18.5 / 18.2 C degrees. However, below the depth of 100 m, we observe water temperatures around 16 C° as winter (Figure 46). In all sampling depths, as it was noticed earlier periods, Clade Ia was the most abundant taxon. The sum of distinct taxa observed at least in each sample were 117 and only 14 taxa were observed as common among which Clade Ia, Clade Ib, NS4 marine group, Clade II, SAR 86, NS5 marine group, AEGEAN-169 marine group – AT were having >1% relative abundance. Number of taxa present in each sample had increased from surface to 100 m and, 100 m depth sample had the highest number of taxa, 69 (0m: 49, 25 m: 53, 50 m: 57, 100 m: 69, 150 m: 34, 200 m: 29 taxa). Number taxa decreases from 34 to 29 taxa in the deeper samples (150 m, 200 m depths respectively).

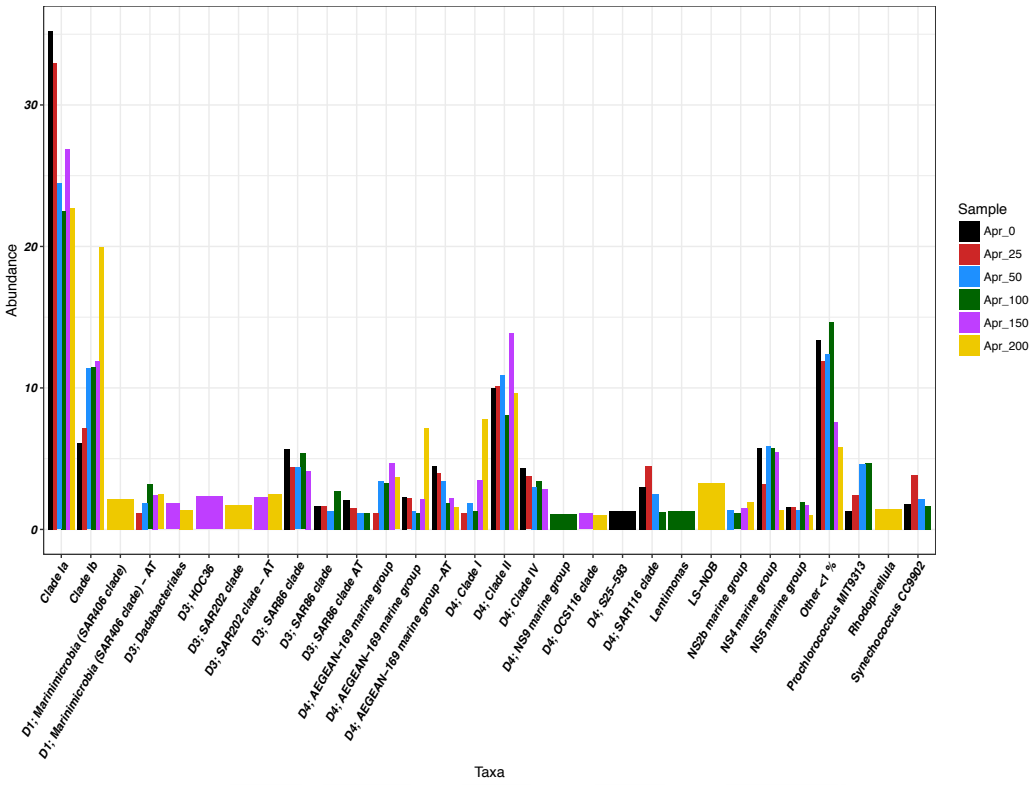

Figure 46. Bar plot of observed taxa in April 2017 sampling depths. "Other < 1%" shows taxa that have relative abundance of less than 1% in each sample. Taxa are listed in the alphabetical order. If the taxa cannot be resolved until level 7 available taxonomic information was given in corresponding level (e.g. D1). AT is the abbreviation for Ambiguous Taxa.

Apart from the Clade Ia, in the surface, Clade II (9.9%) pursued as the second dominant taxon and, Clade Ib (6%), NS4 marine group (5.7%), SAR 86 clade (5.6%) followed them. Additionally, Cyanobacterial sequences of *Synechococcus* CC9902 presented slightly higher abundance than *Prochlorococcus,* 1.7% and 1.3%, respectively in the surface waters. In total, surface community had 49 different taxa. In total, 34 out of 49 taxa had relative abundance of less than 1% and, their total abundance summed to 13.3% in surface. In the 25 m depth sample, highly rated Clade Ia (31%) chased by Clade II (10.1%), Clade Ib (7.1%) and SAR 116 clade (4.4%). *Synechococcus* CC9902 belonging to Cyanobacteria was increased as percentage to 3.8 whereas, Prochlorococcus MIT 9313 has relatively lesser share (2.4%) than the *Synechococcus* CC9902. In total 11.9 % of (35 taxa out of 53) all community has less than 1% of relative abundance. In the sample taken from 50 m depth, Clade Ib and Clade II has comparably same abundance as 11. 3% and 10.9%, respectively and NS 4 marine group had 5.9% abundance. In contrast with the *Synechococcus* CC9902 dominance over Prochlorococcus MIT 9313 in the upper water column, Prochlorococcus MIT 9313 was

leading the abundance share as 4.63% (*Synechococcus* had 2.1% share). In 50 m depth, 38 taxa had less than 1% relative abundance accounting for 12.4% of the community together. Cyanobium PCC-6307 belonging to Cyanobacteria, detected in the 25 m and 50 m depths but with <1% abundance. The community of 100 m depth, possessed the highest number of taxa compared to the other samples. Among these taxa observed, 48 taxa had less than 1% abundance for each, but in total they had 14.6% of abundance. Cyanobacterial genus, Prochlorococcus sums up to 4.67% and, followed the predominance of Clade Ib (11%), Clade II (8%), NS4 marine group and Sar 86 clade (5%). On the other hand, *Synechococcus* only presented by 1.6 percentage in the 100 m depth sample. In the deeper water column (150 m and 200 m) nan of the Cyanobacterial sequences were detected. Clade II (13.8%), Clade Ib (11.8%), NS4 marine group (5%) detected to be the dominant taxa after Clade Ia. Additionally, AEGEAN-169 marine group (4.6%) and SAR 86 clade (4.1%) followed them in the 150 m depth community. Less than half of the 150 m community (16 out of 34) had abundance of less than 1%. These taxa summed up only to 7.6 %. The 200 m depth community also presented the same dominant groups as 150 m depth. However, Clade Ib was as dominant as Clade Ia in 200 m community as a relative abundance of 19.9 %. Besides, Clade I having relative abundance of 7.8% became one of the dominant taxa. LS-NOB belonging to the Nitrospinaceae was observed only in 200 m depth community with 3.6% of community share. Only 10 out total 29 taxa had less than 1% of abundance and can only sums up to 5.7 %. Relative abundance of *NS4 marine group* (1.3%) was decreased noticeably compared to communities above 200 m.

May 2017

In May, water temperatures in the first 25 m starts to warm relatively to previous months (0m: 21.6, 25 m: 20.7, 50 m: 18.4, 100 m: 17.3, 150 m: 17, 200 m: 16.3 C degrees). Throughout the all water column, in total 74 different taxa detected which were observed at least in one sample. Only 8 taxa were cosmopolitan in all depths, whereas singletons account for up to 23 taxa. Common taxa were: Clade Ia, Clade Ib, SAR406 Clade – AT, SAR 86 clade, Clade II which were having >1% of relative abundance.

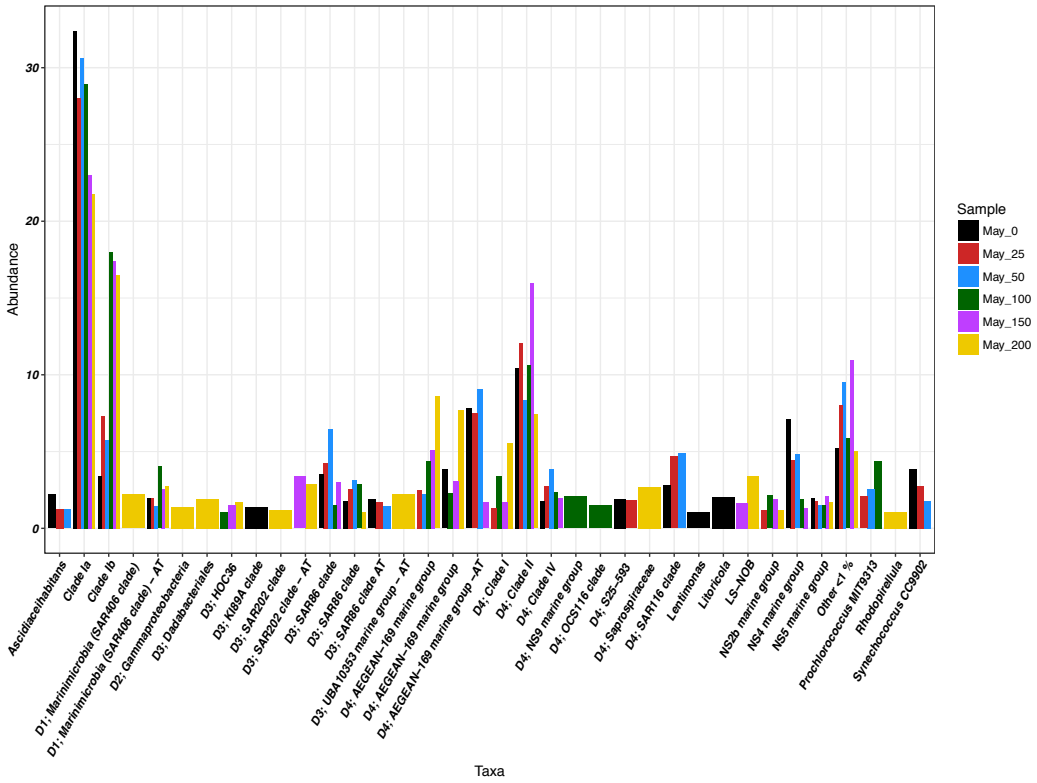

Figure 47. Bar plot of observed taxa in May 2017 sampling depths. “Other < 1%” shows taxa that have relative abundance of less than 1% in each sample. Taxa are listed in the alphabetical order. If the taxa cannot be resolved until level 7 available taxonomic information was given in corresponding level (e.g. D1). AT is the abbreviation for Ambiguous Taxa.

Contrary to the April community which showed the less number of taxa in the last two sampling depth (150 m and 200 m), in May minimum 29 different taxa observed. The minimum number of taxa observed in the surface sample and, maximum number of taxa was in samples taken from 25 and 150 m depths, 36 and 41, respectively. In each depth number of taxa observed were: 29 taxa in surface, 36 taxa in 25 m, 35 taxa in 50 m, 30 taxa in 100 m, 41 taxa in 150 m and 30 taxa in 200 m. In total, 74 different taxa were observed, and 23 of which was singleton and only 8 were common in all depths. Relative abundance of dominant Clade Ia reached its highest contribution in May 2018 in the surface (32%). In the surface, Clade II (10%), AEGEAN-169 marine group – AT (7.8%), NS4 marine group (7%) were the dominant groups following Clade Ia. Synechococcus CC 9902 (3.8 %) was the only species detected which belongs to the Cyanobacteria phylum. Genus Litoricola observed the first time having relative abundance 2% in the surface. There were only 9 taxa, out of 29 different taxa of each had less than 1 % of relative abundance in the surface sample. They hardly present 5.2 % of the total abundance. Community composition in 25 m depth sample was not distinct from the

surface. Clade II had relative abundance of 12%, Clade Ib had 17.9% abundance and, AEGEAN-169 marine group – AT had abundance of 7.4 %. However, there were 17 taxa each having less than 1% of relative abundance. Their abundance summed only 8% of the community. Furthermore, another distinct feature of 25 m depth community was that it had *Prochlorocuccus* MIT9313 sequences that sums up to 2% whereas, *Synechococcus* CC9902 had slight dominance with 2.7%. In the sample taken from 50 m depth, community was represented more by *Prochlorocuccus* (2.5%) than *Synechococcus* (1.7%). As observed before, Clade Ia was the dominant taxon in the 50 m depth sample. The contribution of AEGEAN-169 marine group – AT and Clade II to the total community were 14%, 9 and 8 percentage of each, respectively. Taxa represented by less than 1 % abundance (18 different taxa) summed up to 9.5% in total. Clade 1b and Clade II were the following dominant taxa in the 100 m depth sample in which Clade Ia represented by the highest abundance as 28 %. *Prochlorocuccus* MIT 9313 sequences were the only sequences among Cyanobacteria representing the 4.4% of the community in the 100 m depth sample. Additionally, *Prochlorocuccus* MIT 9313 was only present at the depths of 25, 50 and 100 meters. AEGEAN-169 marine group had relatively lesser abundance in 100 m depth than *Prochlorocuccus* MIT 9313. Only 12 of the 30 taxa observed in 100 m depth in May, had relatively lower abundance than 1% and accounts for only the 5.8% of all community. The number of discovered taxa which were the highest among the samples detected in 150 m depth. More than half of these taxa (24) had less than 1% of abundance summing up to 10% of all sample. Cyanobacteria group had no representative taxa in 150 m depth and 8 of the taxa detected in only this depth but each having less than 1% of abundance. Clade Ib and AEGEAN-169 marine group were the second and third dominant group in 200 m depth, having 16% and 8% of relative abundance, respectively. One third of the taxa observed in 200 m community had relative abundance of less than 1% each, their share sums up to 5% of all community.

June 2017

Between September 2016 and August 2017 when sampling had been conducted, June sampling corresponds to the stronger stratification observed. Water temperature, in the surface was 28.394 C°, in 25 m was 22.2776 C°, in 50 m 18.84 C°, in 100 m 17.47 C °. In 150 m and 200 m water temperatures were 16.9 and 16.1 C °, respectively. In total, 72 different taxa obtained in June 2018, and the number of taxa discovered in 200 m depth were the highest as 35 and followed by surface community having 31 taxa (25 m having 30, 50 m having 29 and 100 m having only 27 taxa). Only 7 taxa were common in all sampling depths and 35 of the taxa seen only in one sample. Contrary to the previous months, Clade Ia was not the most

dominant taxon in all depths. In the first 50 m of the water column, Clade Ia still was the most dominant taxon, however the dominance of the Clade Ib detected in the samples taken from 100- and 200-meters depths. Compared to fall and winter communities, June samples had the least number of taxa in each depth. Contribution of taxa having relative abundance of <1% to the whole community was only 10%. The number of taxa detected in all sampling depths were 29 in surface, 36 in 25 m, 35 in 50 m, 30 in 100 m, 41 in 150 m and 30 in 200 meters depth (Figure 48). In the surface, AGEAN marine group had the 16% of proportion of the community whereas Clade II had the third rank in dominance as 9.8%. Synechococcus CC9902 was one of the two Cyanobacterial species represented with the relative abundance of only 2.2 %. Another taxon assigned as Oxyphotobacteria had only less than 1% in the surface community. Besides, 15 out of the 31 taxa represented less than 1% but, summing to 7.9% of the community all together. In the 25 m depth, relative abundance of AEGEAN-169 marine group – AT was 12.8% and Clade II had 10% of share. The only representative of *Cyanobacteria, Synechococcus* CC9902 had only 1%. Clade III (1.2%) and SAR92 clade (<1%) were the taxa observed in 25 m depth community only. Among all taxa observed, 12 out of 30, in total 12 of them were represented by less than 1% in the community accounting for 6 percentage together.

Clade II obtained as the high abundance as 14% and, followed Clade Ia (30%) and AEGEAN-169 marine group – AT (11%) in the 50 m depth community. Uncultured SAR 116 had 4.6% of abundance and it had 4.4% in the surface. Prochlorococcus MIT 9313 which was not present in the first 25 m samples were appeared in 50 m but representing only 0.8% of the community. The 13 taxa out of total 29 taxa in the 50 m depth had in total only 7% of the whole community, these 13 taxa each having less than 1% of abundance. Genus Prochlorococcus was the 4th dominant taxon by abundance of 8.6% in the 100 m depth and its close relative *Synechococcus* CC 9902 had only 0.6% of abundance. Among the taxa discovered in 100 m community, 12 of the all taxa (27) had less than 1% and they summed to 7 % altogether. In the 200 m depth community, 22 taxa observed and none of the cyanobacterial sequences were detected. Clade Ib (22%) was more dominant than Clade Ia (10%) as opposed to first 100 m depth samples. Other dominant taxa with proportion of 8% and 6.5% were AEGEAN-169 marine group and Clade I, respectively.

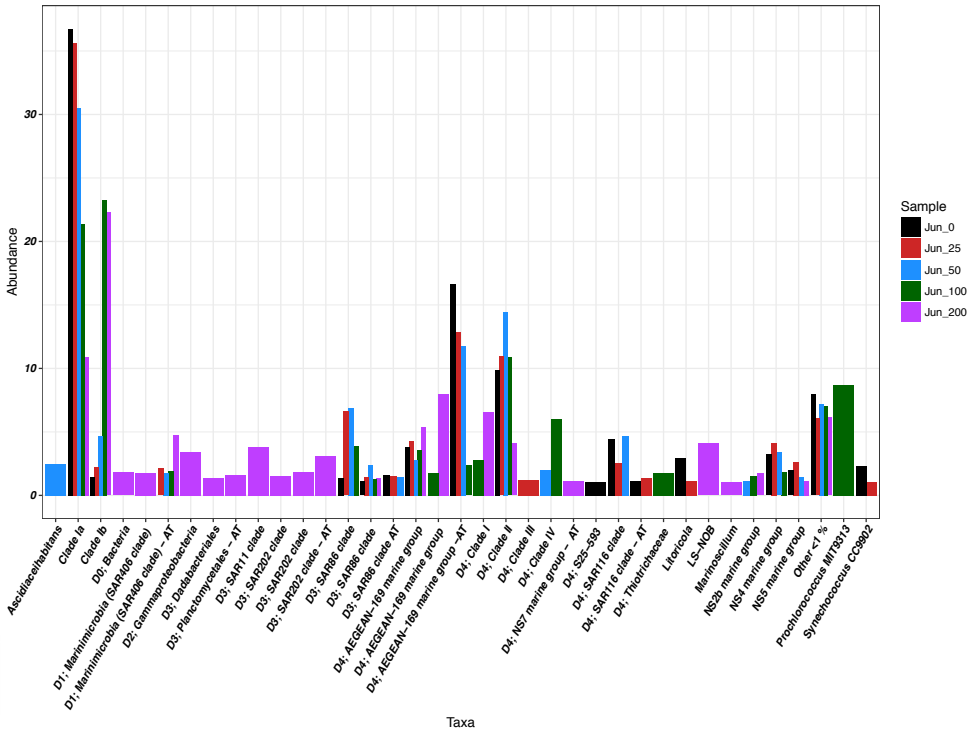

Figure 48. Bar plot of observed taxa in June 2017 sampling depths. "Other < 1%" shows taxa that have relative abundance of less than 1% in each sample. Taxa are listed in the alphabetical order. If the taxa cannot be resolved until level 7 available taxonomic information was given in corresponding level (e.g. D1). AT is the abbreviation for Ambiguous Taxa. The 150 m depth sample was lost in the laboratory processes.

July 2017

Another sampling has been conducted in July 2017, which has surface water temperature of 29.6 C degrees and from 10 m depth to 25 m depth water temperature dropped almost 4 C degrees.

When stratification prolongs, total number of common taxa between samples decreased to 5 taxa. Clade Ia, Clade Ib, Clade II, SAR 86 and AEGEAN -169 marine group were the common taxa in all depths. Thus, number of observed different taxa increased to 75.

In the surface, Clade Ia was the most dominant taxa (38 %) reaching its highest abundance, followed by AEGEAN-169 marine group – AT (13%) and AEGEAN-169 marine group (7.9%). Synechococcus CC 9902 abundance accounted only for 1.9% of the surface community and it was the only Cyanobacteria sequence detected. In total, 16 taxa out of 33 covering only 6% of the community and each were represented by less than 1%. Community in the 25 m depth represented by Clade Ia which had relative abundance of 32%, Clade II (10%), SAR 86 clade (8%), AEGEAN-169 marine group – AT (7%) and NS4 marine group

(6%). In total 33 taxa discovered in 25 m depth and 17 of which had relative abundance of less than 1% (accounts for 9.5% of all abundance). Cyanobacterial sequence belonging to *Synechococcus* CC9902 was not present. In the 25 m depth sample only *Prochlorococcus* MIT 9313 observed with 2.3% relative abundance. S25-593 belonging to Rickettsiales, NS9 marine group and *Coxiella* only observed in 25 m depth. In 50 m depth, in total 34 different taxa discovered. Uncultured marine bacterium belonging to Alphaproteobacteria and Fluviicola observed only in this sample in July. AEGEAN-169 marine group – AT (9.9%) and Clade II (9.4%) were the following dominant taxa after Clade Ia (29%). In the 100 m depth community, only 28 different taxa detected. Out of 28 taxa, 10 of them had less than 1% of relative abundance. Their total accounted for only 5% in the community. First three dominant taxa were; Clade Ia (22%), Clade Ib (14%) and Clade II (12%). Prochlorococcus MIT9313 was the 4th abundant taxon in the 100 m depth with the highest contribution (7%) in all sampling depths (Figure 49).

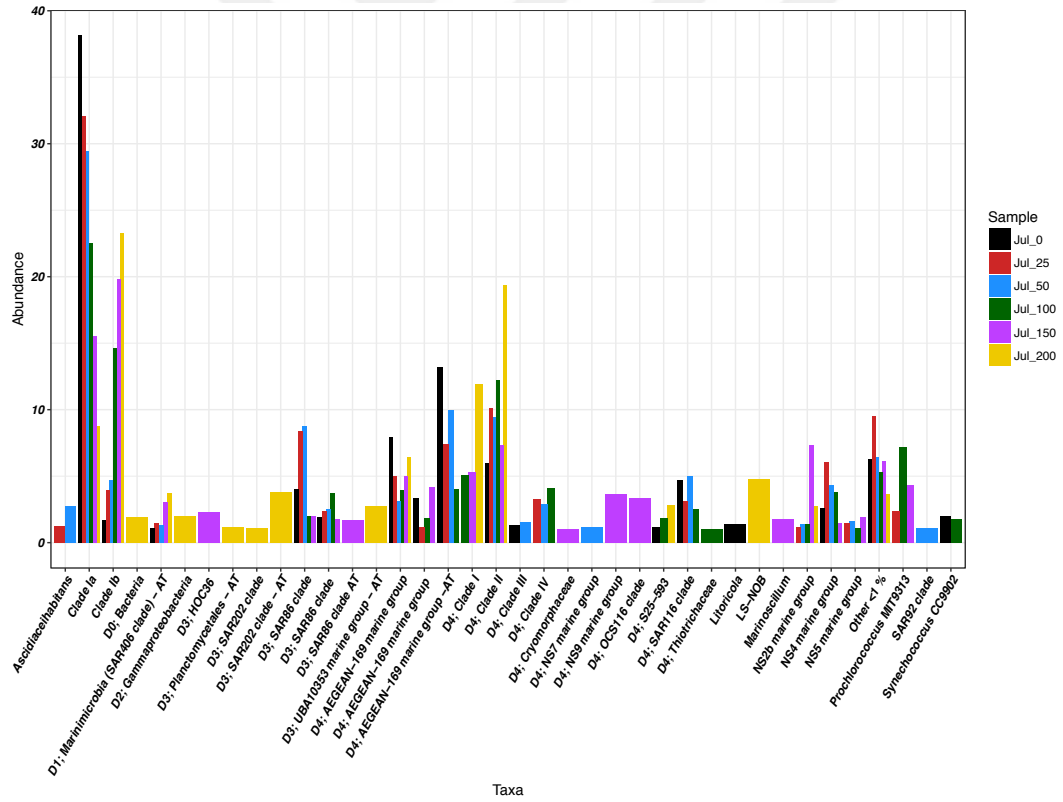

Figure 49. Bar plot of observed taxa in July 2017 sampling depths. "Other < 1%" shows taxa that have relative abundance of less than 1% in each sample. Taxa are listed in the alphabetical order. If the taxa cannot be resolved until level 7 available taxonomic information was given in corresponding level (e.g. D1). AT is the abbreviation for Ambiguous Taxa.

Rather than Clade Ia, Clade Ib was the dominant taxon having 19% of the community while Clade Ia having 15.5% in the 150 m depth. The proportion of *Prochlorococcus* in the 150 m depth community however was not high as 100 m depth sample but 4%. In total, 35 different taxa identified and 15 of which had less than 1% of abundance, representing in total 6% of the 150 m depth community. Frequency of Clade Ia, in 200 m depth was not the highest proportion rather it was in the 4th order by dominance. In 200 m depth the dominance was as follows: Clade Ib (23%), Clade II (19%), Clade I (11.8%) and AEGEAN-169 marine group (6%). There haven't any Cyanobacterial sequences presented in the 200 m depth community.

August 2017

August 2017 represents the well stratified water column conditions. Measured water temperatures were the highest recorded among all months at the surface which was 30.0 C°.

In total 78 different taxa discovered. Among which 29 of them observed in only one sample and only 6 taxa were common in all sampling depths during August 2017. Common taxa were as follow: Clade Ia, Clade Ib, Clade II, AGEAN-169 marine group, NS2b marine group and Marinimicrobia (SAR406 clade – AT).

Clade Ia was not overall the most dominant taxa discovered in each depth. The 200 m depth community composed of Clade Ib as relative abundance of 23% but Clade Ia had only 4.6%. Clade I (10%) and AEGEAN-169 marine group (9.9%) were the secondary abundant taxa in the 200 m depth community. 11 out of 31 taxa observed in 200 m was represented only by less than 1% of abundance accounts for up to 5.5% of the community in total. None of the Cyanobacterial sequences observed in the 200 m depth. The number of different taxa observed in the 150 m depth sample was 30 and presented Clade Ia (17%), Clade II and Clade Ib (16%) and Clade I (6%) as taxa among the dominant groups. Relative abundance of Clade I which had the second rank by abundance in 200 m depth had only 6% of abundance in 150 m depth. As it was the case in 200 m depth there were no sequences representing Cyanobacteria in 150 m depth. Clade II, NS4 marine group (highest abundance detected in 100 m depth) and SAR 86 clade were following the Clade Ia by dominance in 100 m depth. *Prochlorococcus* MIT 9313 detected with the relative abundance of 3.9 percentage and, *Synechococcus* CC9902 had only 0.39 % of share in 100 m depth community. In total, 43 different taxa observed and 24 of them represented by less than 1% of abundance in 100 m depth.

Clade Ia (24%), Clade II (11%), SAR 86 clade (7%) and AEGEAN-169 marine group (7%), Clade Ib (5.9%) represented also in the 50 m depth community. *Prochlorococcus* MIT9313 was the only group observed belonging to the Cyanobacteria in the 50 m with 4.4% relative abundance. In the surface, *Synechococcus* CC 9902 detected with relatively higher abundance of 3.2%. Clade II was the second dominant group in 25 m depth community but the fourth in

the surface. In the surface AEGEAN-169 marine group –AT was the second one rather than Clade II or Clade Ib. Among 28 taxa, in the surface, 11 of them had less than 1% of relative abundance (Figure 50).

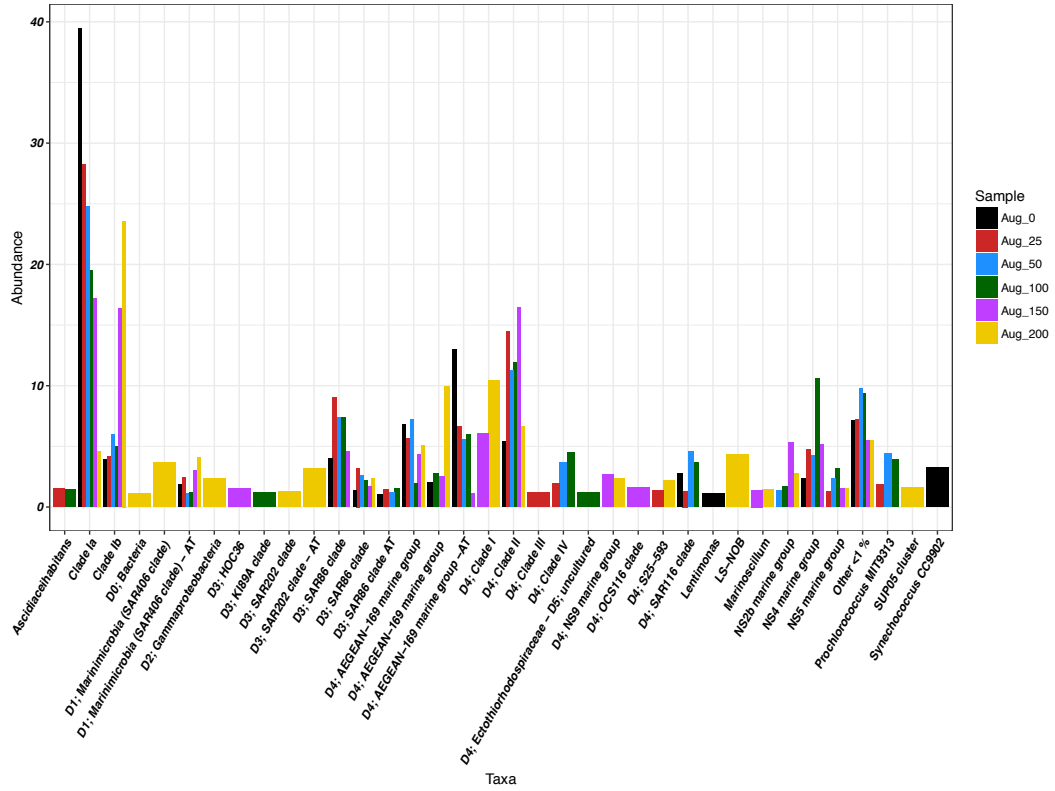

Figure 50. Bar plot of observed taxa in August 2017 sampling depths. "Other < 1%" shows taxa that have relative abundance of less than 1% in each sample. Taxa are listed in the alphabetical order. If the taxa cannot be resolved until level 7 available taxonomic information was given in corresponding level (e.g. D1). AT is the abbreviation for Ambiguous Taxa.

2.2.3. Diversity within and between samples (alpha and beta diversity)

One of the important questions ecological studies try to answer is how diverse the observed community is and how this richness/diversity varies between samples. Alpha and beta diversity metrics are particularly important to infer impact of environmental gradients on the diversity of samples.

To determine if the richness of the samples has been fully covered and sequenced, alpha diversity (Observed OTUs) rarefaction plots were generated (Figure 51). The lines belonging to each sample in the plot appear to approach a slope of zero at some sampling depth along the x-axis. This suggests that collecting additional sequences or sampling more sea water

beyond that sampling depth would not be likely to result in the observation of additional features.

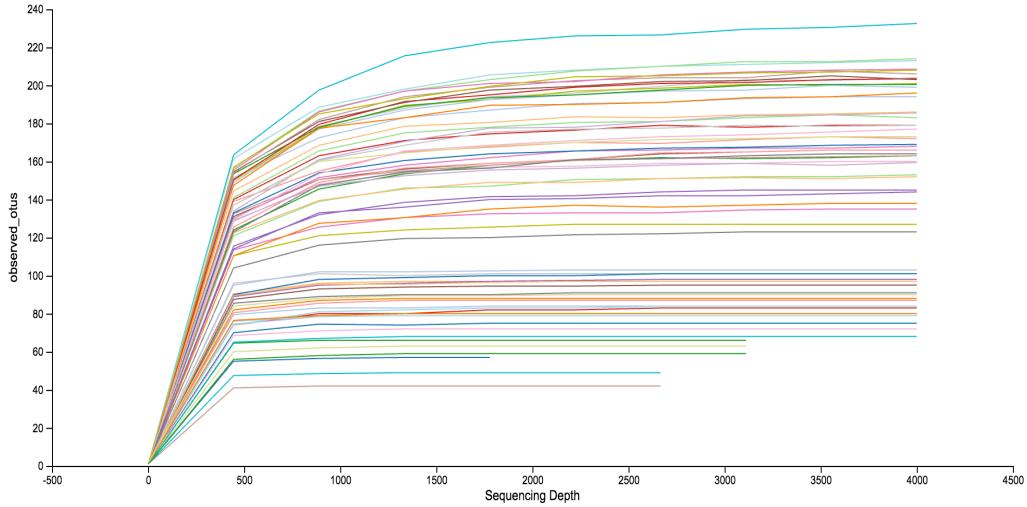

Figure 51. Alpha diversity (Observed OTUs) rarefaction plots of 59 samples.

Since diversity metrics are sensitive to different sampling depths, each sample was subsampled to 4000 sequences. In total, 59 samples, which had more than 4000 sequences out of 65 samples, were further analyzed. For instance, surface community sampled during April had the highest number of sequences, 13,652, however 100 m depth sample taken in September had the total 2,060 sequences. Thus, September sample was excluded from the analysis.

Depending on the Faiths PD measure, which takes in to account diversity that incorporates phylogenetic difference between species in the sample, samples collected between May and August had the lower scores. Besides, Shannon index (a measure of diversity considers richness and evenness in the same samples) were also low in the same period. The samples collected from 100 m depth in January and 150 m depth in September had the highest phylogenetic diversity with observed OTU number 230 and 194, respectively. The samples collected from 100 m in January also had the highest Shannon index which considers OTU numbers and evenness. Community sampled in the depth of 150 meters in September however having relatively lower Shannon index (Figure 52).

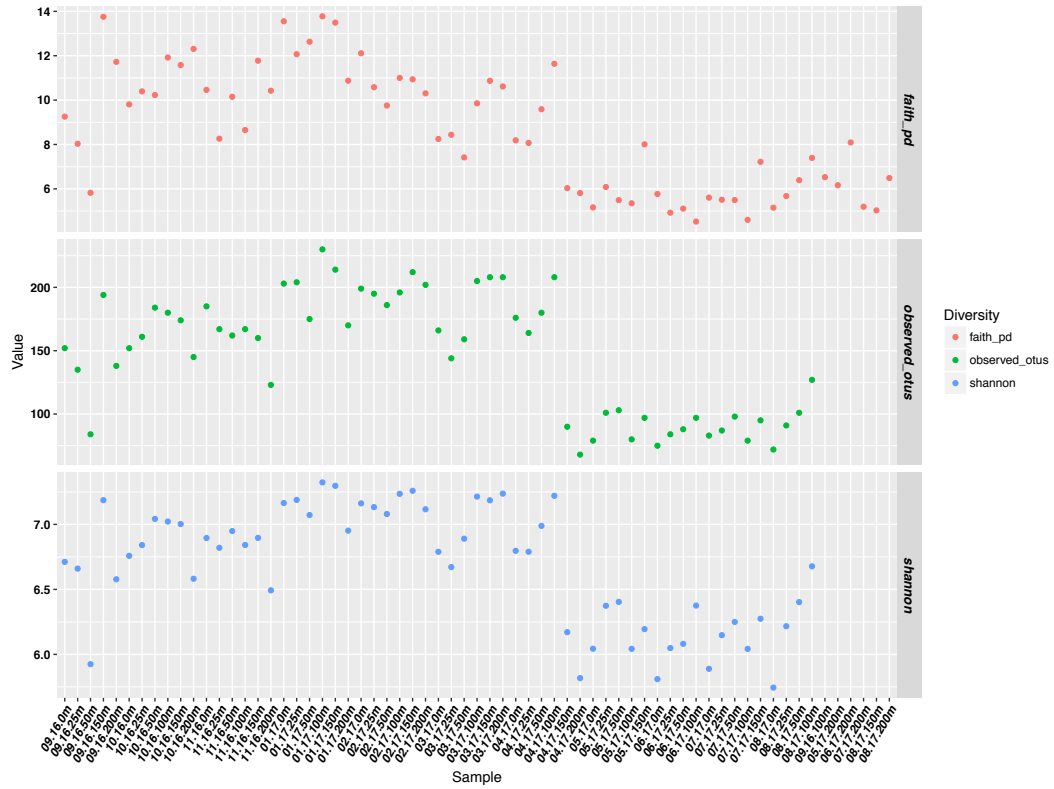

Figure 52. Three different alpha diversity metrics, Faiths PD (Calculates faith's phylogenetic diversity) and non-phylogenetic measure of Shannon index and observed number of OTUs for 59 sample. Sample names were coded as month.year.depth and given in the order of sampling period.

Faiths PD index was further questioned by Spearman correlation to see if any of the physico-chemical features were correlated. Faiths PD index was found to be negatively correlated with salinity (r_s = -0.2667, n= 59, p = 0.0411), nitrite (r_s = -0.2466, n= 65, p = 0.0477), total dissolved phosphate (r_s = -0.3509, n= 65, p = 0.0042), total phosphate (r_s = -0.3145, n= 65, p = 0.0107), total number of phytoplankton cells/l (r_s = -0.3549, n= 65, p = 0.0037), dissolved organic phosphate (r_s = -0.3741, n= 65, p = 0.0021) and, positively correlated with apparent oxygen utilization (r_s = 0.3397, n= 59, p = 0.0085), total chlorophyll (r_s = 0.3403, n= 65, p = 0.0055) (Figure 53).

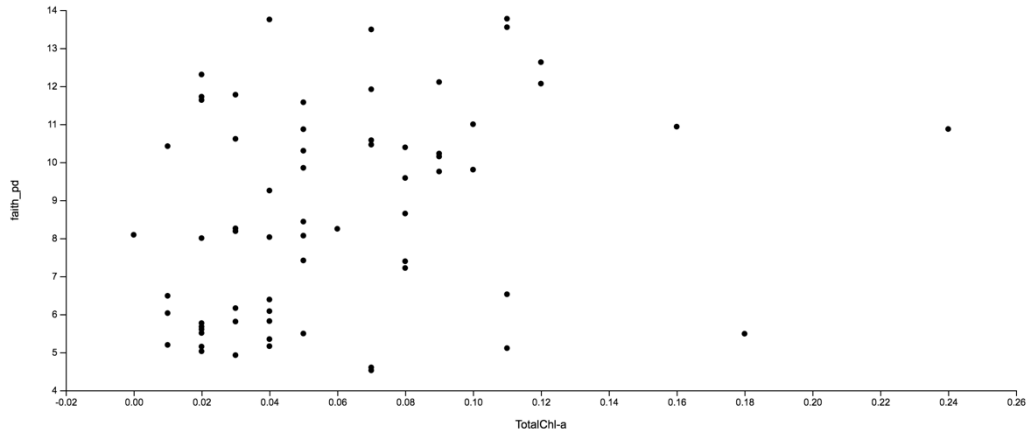

Figure 53. Faiths PD index and Total Chl-a plot of 65 samples.

Diversity (non-phylogenetic-Shannon index) in each sample was significantly different in each season (Kruskal- Wallis pairwise comparison- Appendix G). However, the diversity between spring and fall was found insignificant. In summer, diversity was lowest and winter, the diversity maximized. On the other hand, Faiths PD index (phylogenetic) in each sample was significantly different in each season (Kruskal- Wallis pairwise comparison- Appendix G).

Similarity between samples (Bray- Curtis dissimilarity, Figure 54) explained by 39.8% variance by first two axes. PCoA results showed that especially in winter (January) when water column is well mixed samples are more similar to each other. January samples correspond to 4th column in the month axis- first ones after red dots). Especially in September (Figure 54, first right) similarity between surface samples (high temperature) were closer than the deeper water column samples. Such similarity cannot be distinguished in August samples clearly since, there were only 4 samples that had higher than 4000 sequences.

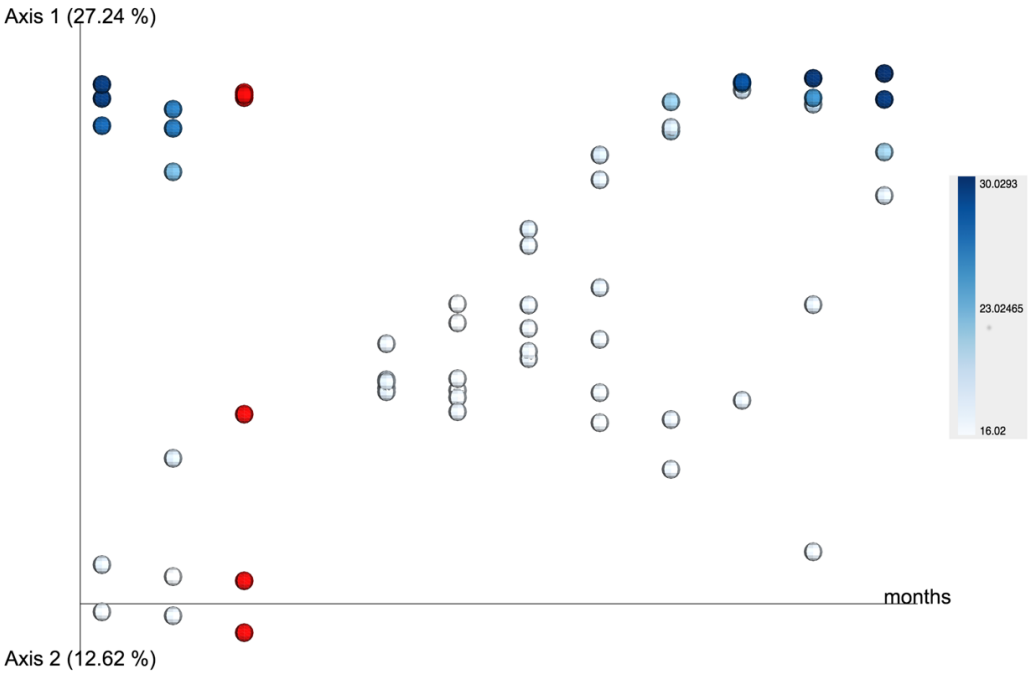

Figure 54. PCoA results of 59 samples. Measure of Bray- Curtis Dissimilarity (compositional dissimilarity between two different sites, based on counts at each site) was used to perform analysis. Colouring was done according to the water temperature that sample was taken (red: no temperature data available). To visualize the time-series data custom axis (months) were created, starting from the first sampling month September (left) to August (right).

Unweighted Unifrac Distances between samples (Figure 55) explained by 38.07% variance by two axes. When considering phylogenetic differences among all samples which were captured more similar to each other with Bray-Curtis dissimilarity found to be distant as seen in January (Figure 54) samples that were grouped distantly. In September and October (first two from left in the Figure 54) and August (first from right) samples were observed to be grouped according to the ambient temperature.

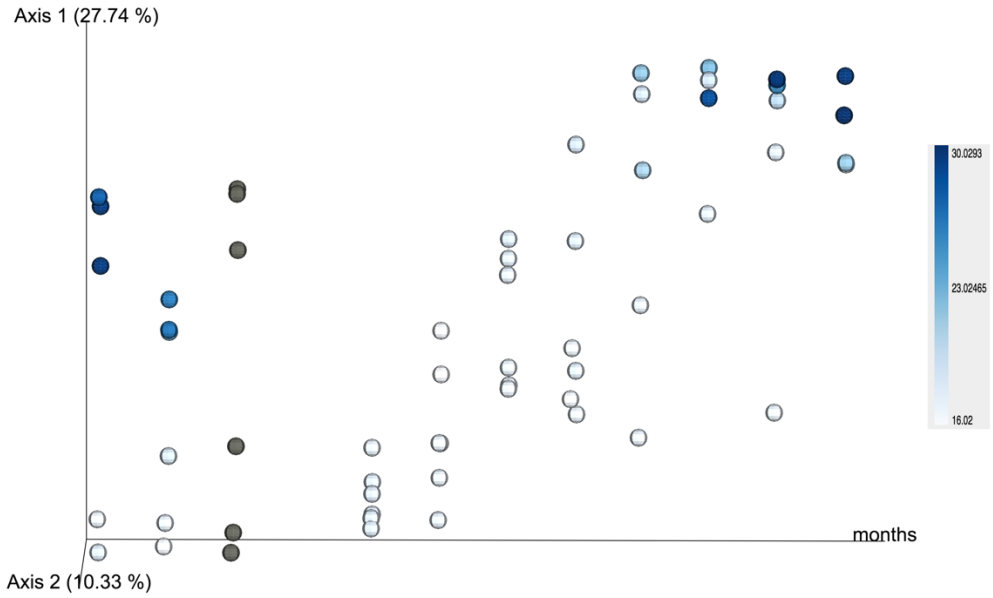

Figure 55. PCoA plot of 59 samples. Measure of Unweighted UniFrac Distance (Phylogenetic tree branch lengths are weighted according to relative abundance) was used to perform analysis. Colouring was done according to the water temperature that sample was taken (grey: no temperature data available). To visualize the time-series data custom axis (months) were created, starting from the first sampling month September (left) to August (right).

2.2.4. Understanding microbial dynamics in relation to environmental factors

The biggest challenge of microbial environmental studies is to understand which abiotic factors are controlling the microbial community dynamics. In an attempt to understand these factors in this study, raw data (OTUs table) were tested against environmental parameters by Canonical Correspondence Analysis (CCA). CCA is one of the ordinations techniques that incorporates graphical tools to display multivariate data. Multivariate data can be explained as that they do not have one obvious dependent variable. CCA is an interpretive technique to understand the major features of such data.

Additionally, the most abundant 2 taxa (Clade Ia and Clade Ib), plus Prochlorococcus and Synechococcus was investigated using non-parametric tests which do not rely on any null distribution (

Table 5. Statistical tests summary for four major groups 5). Environmental parameters considered are as follows: depth, EZD, MLD, season, dissolved oxygen, temperature, salinity, ammonium, nitrite, nitrate, phosphate, silicate, total chlorophyll, N/P, POC, PON, POC/PON, TP, PP, TDP, DIP, DOP, AOU.

Clade Ia family which was the mostly dominant taxa in all samples found to be correlated with depth and EZD. Thus, water column physical conditions, temperature and salinity, was also positively correlated with Clade Ia. Only, nitrate was detected to be negatively correlated, with the most abundant taxon observed, Clade Ia. PO_4^+ was the other nutrient which shows negative correlation. Additionally, AOU was negatively correlated with the taxa Clade Ia.

Another mostly observed taxon Clade Ib was significantly correlated with the depth, MLD and EZD. Besides, abundance fluctuations were found to be correlated with the temperature and salinity profiles but negatively. Nitrite and nitrate were positively correlated with the taxon Clade Ib, especially nitrate was more significant and for nitrite, p-value was slightly smaller than 0.05 being 0.023. Ammonium, on the other hand, was negatively correlated. Another important environmental parameter that was found to be positively correlated is inorganic phosphate with Clade Ib. Particulate phosphate and TDP was also showed positive correlation.

Two important Cyanobacteria genus's, *Synechococcus* CC9902 and *Prochlorococcus* MIT9313 was also investigated in relation to environmental factors. *Prochlorococcus* showed strong association with depth. *Synechococcus,* however, showed correlation between MLD, EZD and depth. *Synechococcus* was significantly correlated with salinity p-value being 2.055e-06 and positively correlated with temperature (p-value = 0.023). Besides, *Synechococcus* abundance was found to be negatively correlated with nitrate concentrations. *Synechococcus* was also found negatively associated with AOU value. Prochlorococcus, on the other hand, was the only taxon that had significant relationship with particulate organic carbon sources and total chlorophyll. Inorganic phosphate was also having significant negative relationship with Prochlorococcus. Prochlorococcus abundance, also showed significant negative correlation with AOU value.

Table 5. Statistical tests summary for four major groups

		Kruskal-Wallis			Spearman rank correlation (none parametric)		
		chi-squared	df	p-value	S	rho	p-value
Clade-Ia							
	Depth	30.857	5	9.998E-06	-	-	-
	EZD	20.863	2	0.00002949	-	-	-
	Temperature	-	-	-	16750	0.5105202	3.61E-05
	Salinity	--	--	-	10138	0.7037405	5.07E-10
	NO3NNO2N	-	-	-	68856	-0.5047173	1.81E-05
	PO4	-	-	-	61360	-0.3408987	0.005455
	AOU	-	-	-	54546	-0.5939801	7.08E-07
Clade-Ib							
	Depth	31.624	5	7.05E-06	-	-	-
	MLD	9.9062	3	0.01938	-	-	-
	EZD	18.965	2	7.62E-05	-	-	-
	Temperature	-	-	-	57162	-0.6704267	6.30E-09
	Salinity	-	-	-	51708	-0.5110462	3.54E-05
	NH4	-	-	-	58196	-0.2717583	0.02853
	NO2	-	-	-	32890	0.2812454	0.02324
	NO3NNO2N	-	-	-	19917	0.5647569	9.56E-07
	PO4	-	-	-	24144	0.4723851	7.10E-05
	PP	-	-	-	57374	-0.253805	0.04134
	TDP	-	-	-	33573	0.2663257	0.032
	AOU	-	-	-	8588	0.7490357	8.93E-12
Prochlorococcus							
	Depth	26.998	5	5.71E-05	-	-	-
	PO4	-	-	-	62482	-0.3654363	0.002759
	Total Chl	-	-	-	23620	0.4838222	4.45E-05
	POC	-	-	-	30343	0.336908	0.006065
	AOU	-	-	-	44544	-0.3016976	0.02022
Synechococcus							
	Depth	21.861	5	0.0005564	-	-	-
	MLD	9.0412	3	0.02875	-	-	-
	EZD	16.671	2	0.0002398	-	-	-
	Temperature	-	-	-	24105	0.295594	0.02303
	Salinity	-	-	-	16891	0.506411	4.27E-05
	NO3NNO2N	-	-	-	65406	-0.4293289	0.0003587
	AOU	-	-	-	47258	-0.3810011	0.00291

To discern the possible relationship between microbial community structure and environmental parameters, CCA was performed (Figure 56) using all measurable environmental variables. Among 23 measurable environmental factors 9 of them were selected. Significance of overall CCA model and first three axes tested and found significant (Appendix F). According to the CCA, total variation that can be explained by our environmental variables (constrains: Temperature, salinity, AOU, nitrate, nitrite, PO_4^+, TP, DOP, EZD and season) was 44%, whereas 55% of total inertia (variation) remained unexplained. First three CCA axes' proportion that explains proportion of total inertia in our data set was 21%, 0.085% and 0.038%, respectively. The amount of inertia explained as a fraction of total **explainable** inertia were 47%, 19% and 8.7% for CCA1, CCA2 and CCA3 axes in which first two CCA axes explained 75.2% of total explainable inertia. The length of an environmental parameter arrow in the ordination plot indicates the strength of the relationship of that parameter with the community composition.

Temperature, AOU, Nitrate (NO3NNO2N) and dummy variables Season and EZD (above and below) appear to be the most important environmental parameters (p = 0.001 for each) that explain community composition and this can be inferred from the length of arrows. Variables nitrite (NO2N), PO4 and TP was selected for the model, however their p-values were found to be higher than 0.05. If, variance inflation factors (VIF) is > 10, it indicates that a variable is strongly dependent on other variables and does not provide independent information. VIF of TP and DOP were bigger than 10, indicating that these variables might have been dependent on other factors and should be interpreted cautiously.

Samples that are collected above (purple) and below (green) the EZD grouped according to the different environmental factors. Samples that correspond to the depths above the EZ mainly related with the high to mid temperatures, except for the ones that are collected in the winter. Winter samples (samples that are close located to dummy variable SeasonWINTER) both for EZD below and above were responding to the low temperature environments. It is also apparent that samples below the EZD responds to high to mid nitrate concentrations and high to mid AOU values. CCA analysis results revealed that patterns in bacterial communities were best explained by Temperature, AOU, Nitrate (NO3NNO2N), Season and EZD.

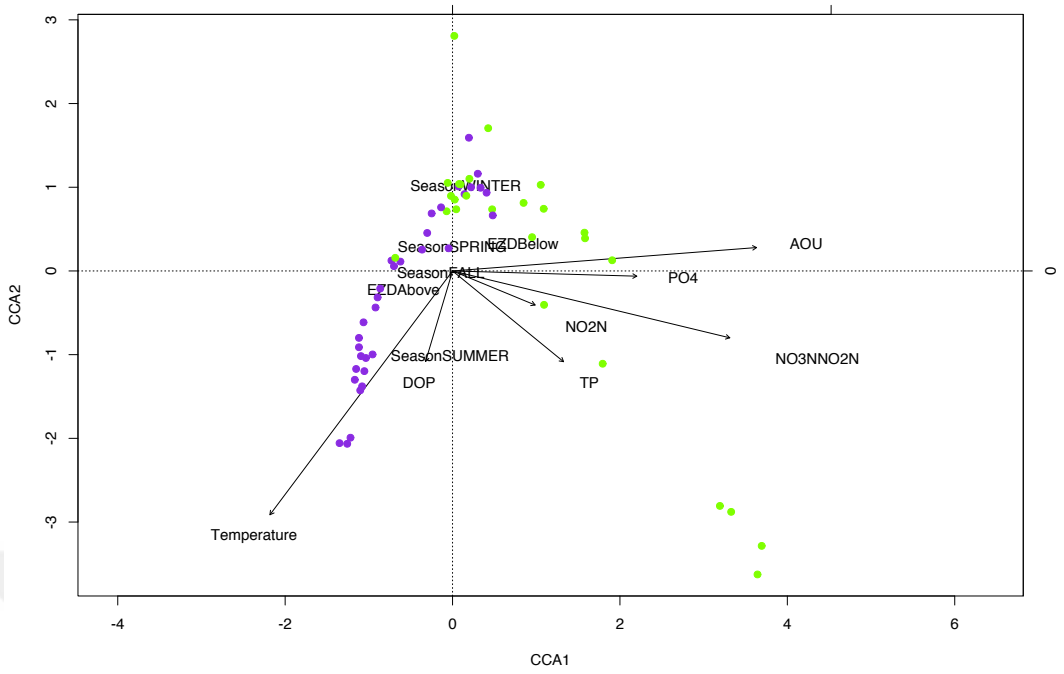

Figure 56. Triplot based on a canonical correspondence analysis. Quantitative environmental variables are indicated by arrows. The class variables (Season and EZD) are indicated by text corresponding to that variable. Purple coloured samples were taken from the depths which are above the EZD, greens are from below the EZD. Species were not shown for simplicity. Eigen values and their contribution to the mean squared contingency coefficients of CCA1 and CCA2 axis are 0.3412 (21.94%) and 0.1465 (%0.942) correspondingly.

2.3. Discussion

In order to understand how microbial community composition changes over a year and identify the key factors affecting microbial community, sampling had been conducted for one year at the ETS-200 m depth station and from six depths with monthly intervals. In total, 2213 sequence variants (OTUs) were observed from all samples but this number reduced to 1978 after filtering our contaminant chloroplast sequences.

The *Alphaproteobacterial* SAR11 clade, is reported to be one of the ubiquitous bacterial clades in the world's oceans and dominant by abundance (Brown et al., 2012; Morris et al., 2002). Widespread distribution of SAR11 clade, after its first discovery in the Sargasso Sea (Giovannoni et al., 1990), is supported by numerous studies in marine and freshwater environments. Although, in most of the studies their ecological role remained uncertain, their ubiquity and success is clear (Brown et al., 2012; Giovannoni et al., 1990; Morris et al., 2002).

Consistent with other studies, SAR11 clade was dominant in the ETS-200 m station throughout a year (min 22%, max 64%). However, distribution and abundance of SAR 11 ecotypes differ throughout the water column, Clade Ia having the higher relative abundance in the surface, while Clade Ib and Clade II were mostly dominant in the deeper waters (depth of 150 and 200 meters) except for winter mixing conditions.

Considering different seasons, in winter, taxa having less than 1% of abundance accounted the highest proportion in each sampling depth but this share gradually decreased from January to April in all depths. In the summer, number of taxa having <1% of relative abundance was contributing less than approximately 10% of the community. Moreover, diversity in each sample (phylogenetic and non- phylogenetic) showed that summer periods, when water column stratification is strong, were less diverse compared to other seasons. Winter was observed to be highly diverse, on the other hand. However, no significant differences were found in spring and fall if non-phylogenetic diversity matrix considered. On the other hand, phylogenetic diversity values were significantly different in all seasons. Diversity maxima in winter and minima in summer captured in our data set was also consistent with the findings of Gilbert et al., (2011). Throughout seven years of time series study which was conducted in the near-surface waters of north-western Mediterranean showed the positive relationship between phylogenetic diversity and bacterial production (Galand et al., 2015). In our data set, higher phylogenetic diversity was detected during winter where also increased chlorophyll concentrations, higher heterotrophic bacterial and Cyanobacteria abundance (cell counts) and nutrient concentrations observed. Uniform distribution of the nutrients appears to be the supporting factor of such high phylogenetic diversity observed in winter. According to the spearman correlation results, elevated concentrations of TP, DOP, nitrite, salinity and number of phytoplankton cells limit the phylogenetic diversity. It can be suggested that increase in these above-mentioned factors might limit the diversity while benefiting the abundance of other groups which are not bacteria. For instance, during summer coccolithophoride abundance were observed to be the highest compared to the other seasons.

In the first chapter, it was revealed that abundances of heterotrophic bacteria and Cyanobacteria (acridine orange cell counts) were elevated in the winter. Possible reasons were left unanswered in the first chapter. According to the community composition determined in this chapter, it is suggested here that increased phylogenetic diversity might play an important role. However, one question is still remained unanswered. What is the main reason of shift in the period of dominance in the heterotrophic bacteria and Cyanobacteria. Our sampling strategy cannot distinguish between active and dormant cells, and the phylogenetic diversity was not standardized according to the number of taxa in a sample as calculated by Galand et al., 2015. Keeping these factors in mind, higher phylogenetic diversity, chlorophyll

concentration in winter, also higher relative abundance of *amoA* genes (Chapter 4) in winter can indicate that winter was the period where bacterial production was high.

According to the chemical data presented in the first chapter, elevated concentrations of nitrate and inorganic phosphate in the deeper water column (between the depths 150
and 200 meters) were observed except for the winter. These observations were suggested as a result of bacterial degradation according to AOU values discussed in the first chapter. However, increased concentrations of ammonium were not observed in these depths. These elevated concentrations were further questioned under the light of observed taxa. Sequences belonging to the phylum *Planctomycetes* (Pirellulales family) and *Nitrospinae* were found to be present in the depths of 150 m and 200 m during fall and summer (Figure 57). Additionally, Planctomycetes presence was detected in the whole water column in January and in the first 100 meters in February. Some species of Planctomycetes phylum are known to be the so-called anammox bacteria (Boedeker et al., 2017) and, *Pirellulale*s family was especially attributed with this function (Lawler et al., 2016). Anammox is known to be an anaerobic process and, intracytoplasmic compartment, anammoxosome might be providing anaerobic environment for this reaction. It can be speculated that the reason why we haven't observed ammonium accumulation in the deeper water column might be the result of ammonium oxidation mediated by *Pirellulale*s family. This conclusion should be further supported by metatranscriptomics studies. However, no annamox genes were detected in metatranscriptomics study (Chapter 4). Phylum *Nitrospinae* observed between April and August 2017 at the depth of 200 meters (Figure 57-b, pink bars). These chemoautotrophic bacteria rely on nitrite oxidation and, there was no evidence found to imply other energy-generating pathways (Hofer 2018; Pachiadaki et al., 2017). Phylum *Nitrospinae* were reported to be abundant in the open-ocean depths below 200 m (Hofer 2018). Furthermore, it was estimated that carbon fixation by marine Nirospinae cells are 10 times more than organisms which are depend on ammonium oxidation (Hofer 2018). As a conclusion, elevated concentrations of nitrate observed in the depth of 200 meters might be due to the activity of nitrite oxidizing phylum *Nitrospinae.*

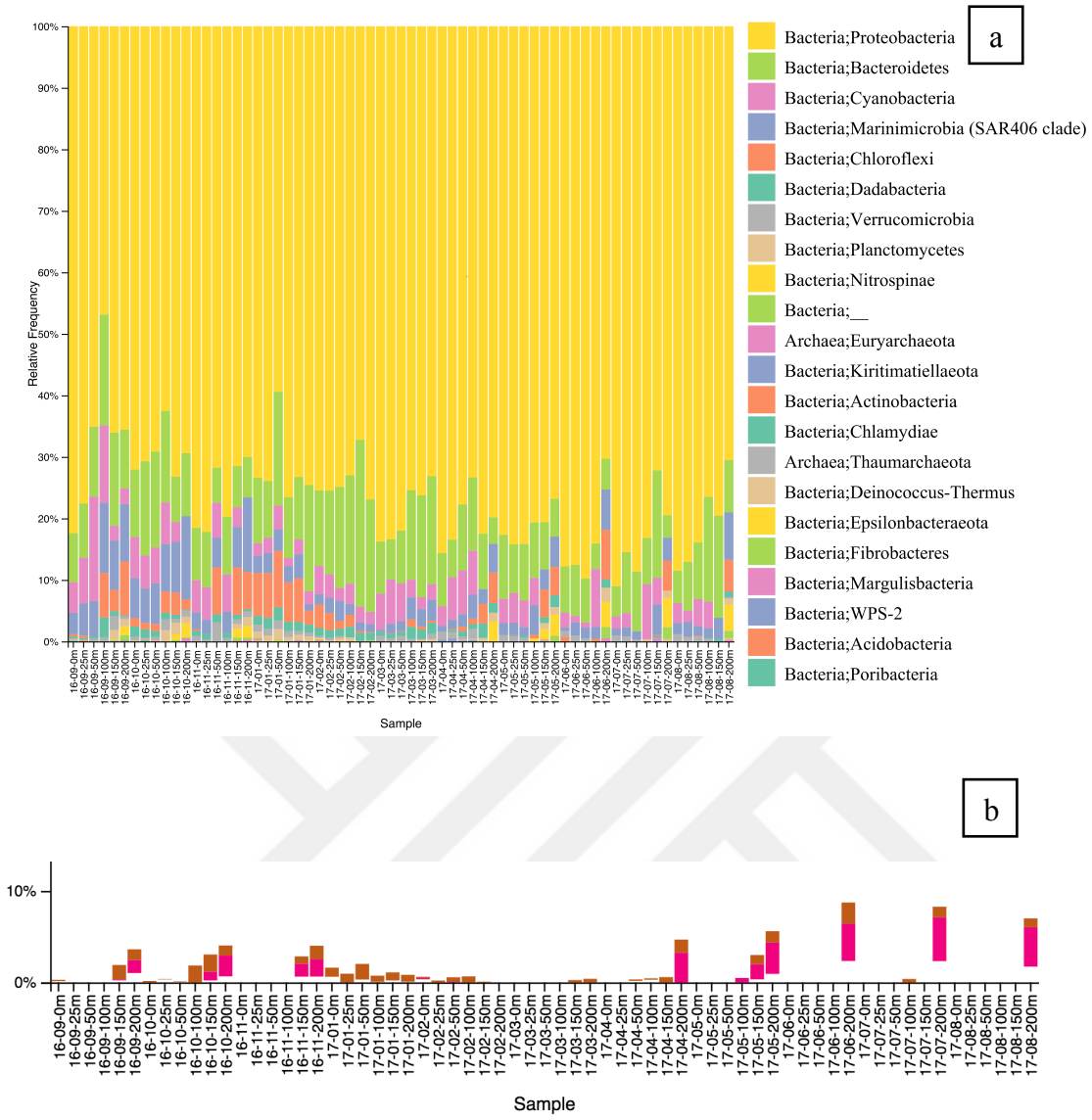

Figure 57. Relative frequencies of (a) observed phylum at the ETS-200 m station and; (b) phylum *Planctomycetes* (brown) and *Nitrospinae* (pink*)*.

Distinct microbial taxa were found in the AW, LIW and EMDW in the south of Eastern Mediterranean, and indicator species for these three water masses were captured (Techtmann et al., 2015). *Alphaproteobacteria* showed the highest abundance in the AW. In addition to this taxon Cyanobacteria, Verrucomicrobia, Gammaproteobacteria and Bacteriodetes were reported to be the indicator species of AW mass. Among Bacteriodetes, Flavobacteria class were the predominant one (Techtmann et al., 2015). On the other hand, in the study of Techtmann et al., (2015) *Thaumarchaeota* were observed as the most abundant taxa in the LIW and EMDW masses. In the ETS-200 m station samples, *Thaumarchaeota* were detected

in only four samples, thus abundances were found to be less than 1%. Additionally, physical data revealed that LIW was not present in the study area. Observed taxa in the ETS-200 m station were well in conformity with the indicator species of AW reported by Techtmann and colleques (2015).

CCA analysis results revealed that patterns in bacterial communities were best explained by Temperature, AOU, Nitrate, Season and EZD. AOU should not be considered as a driving factor of community composition. But rather bacterial activities should be considered as the factor explaining AOU values. Temperature, EZD and Season are associated with community changes. This result consistent with the previous surveys that found seasonality as a main driver (Gilbert et al., 2012; Sunagawa et al., 2015, Ward et al., 2017). Light (EZD) and water temperature are the resulting factors of dynamic seasonality which best explain bacterial communities in ETS-200 m station. Additionally, nitrate was found to be among the important factors. Except for the *Prochlorococcus*, taxa that were tested statistically (Table 5) showed correlation with nitrate sources. Community of samples taken below the EZD and especially from the depth of 200 meters show correlation with increased nitrate concentrations. Statistical techniques used in this study cannot be used to conclude whether nitrate is consumed or produced by bacteria. However, it is clear from the CCA analysis that nitrate is one of the factors that explains patterns in bacterial community.

In this study only physical and chemical properties were considered in order to reveal the patterns in bacterial communities. However, there are different environmental factors that were not considered in this study such as the presence of antibiotics, oil hydrocarbons, pesticides and ship ballast waters that shape the bacterial community composition. *Enterobacteriaceae* family which are considered to be the indicator of bacteriological contamination (Türetken and Altuğ, 2016) were not observed in the samples taken at ETS-200 m station. This might indicate that our sampling area is not contaminated by domestic or industrial waste and maritime transport. On the other hand, we observed *Phenylobacterium* belonging to *Caulobacteraceae* family in our samples (Chapter 3) that having relative abundance ranging between 3.9 to 6.6 %. Type species of *Phenylobacterium* are known as degrade chloridazon which is the active ingredient of the herbicide Pyramin. Their presence in our samples indicate some sort of contamination via rivers. Türetken and Altuğ (2016) showed the presence of faecal bacteria dominance indicating that human induced factors may disturb the community composition of bacteria in the Northern Aegean Sea (Türetken and Altuğ, 2016). Possible increases of the human-induced pollution can also disturb the biochemical capacities of the indigenous bacteria of the study regions.

SAR11 Clade and ecotypes

SAR 11 clade belonging to *Pelagibacteriaceae* was highly dominant in our sampling area throughout the year and depths. However, dominance of different ecotypes (defined as similar phylogenetic sub-groups of bacteria that differ in physiological details that was a signature of niche specificity), were observed at different depths.

During fall season, from surface to 100 m depths dominant ecotype was Clade Ia. In deeper water column (150 m and 200 m) dominant ecotype was Clade Ib and Clade II. In contrast to season fall, in winter period, Clade Ia belonging to SAR11 clade was overall the most abundant taxon throughout the water column. Clade Ib and Clade II were the next dominant taxa, showing different trends at different depths. In March, April and May compared to the January and February surface waters, Clade Ia showed an increase in relative abundance in the first 25 m depth. Clade Ib was the second dominant ecotype especially at the depths of 100 m and below. However, Clade II was over abundant than Clade Ib in the first 50 m community, after Clade Ia. With the formation of summer stratification, in summer, Clade Ia was dominant in the first 50 m and Clade Ib was having lesser representation as 1-6%. Clade Ib was abundant in 150 and 200 m depths except for August when Clade Ia, Clade Ib and Clade II having relatively same proportion in 200 m depth.

Our findings support previous studies that Clade Ia is a surface ecotype and, abundant in the stratified and nutrient replete summer surface waters. Clade Ia was found to be positively correlated with temperature whereas Clade Ib was negatively correlated. Additionally, their correlation with nitrate was also opposite when Clade Ia was negatively correlated Clade Ib was found positively correlated. The surface ecotype, Clade Ia was found to be negatively correlated with PO_4^+ which also supports that Clade Ia can succeed in nutrient depleted surface waters during summer stratification. On the other hand, Clade Ib showed positive correlation with PO_4^+. To conclude, Clade Ia was inhabiting surface waters whereas, in deeper water column Clade Ib and Clade II were abundant. It is proposed here, according to the available data studied, that main driver of the changes in the ecotype composition is seasonal cycles such as stratification and winter mixing which impacts temperature and availability of nutrients also. Such periodic reset was also shown in the oligotrophic Northwest Mediterranean Sea by Salter et al.,2015 and others (Brown et al., 2012; Field et al., 1997).

Cell- specific substrate uptake experiments on the numerically dominant SAR11, shed light on their importance in organic carbon oxidation (Mou et al., 2007). Observed ecotypes of SAR 11 clade did not show any correlation with POC or PON. This might indicate their dependencies on dissolved form of organic matter. Additionally, both ecotypes showed correlation with AOU value. Clade Ia, which is more abundant in the surface waters during stratified periods, showed negative correlation. This result will not be speculated since the

surface waters are intact with atmosphere. On the other hand, during stratification, atmospheric input of oxygen to the deeper water (below the MLD) is limited. Deep-water ecotype of SAR 11 clade (Clade Ib) showed positive correlation with AOU. This suggests that, Clade Ib plays role in the degradation of organic matter especially during the stratified water column conditions.

Cyanobacteria

Prochlorococcus MIT 9313 was more abundant than *Synechococcus* CC9902 in the surface during fall. Especially in September, relative abundance of Prochlorococcus MIT 9313 reached 15% and 12% in 50 m and 100 m depths, respectively. In the depth of 100 meters, *Prochlorococcus* MIT 9313 was the most dominant taxon by relative abundance and, the second dominant taxon in the 50 m depth in September. The highest observed concentrations of total chlorophyll were between the depths 75 meters and 115 meters. These increased concentrations of total chlorophyll are most probably due to the dominance of *Prochlorococcus* MIT 9313. Some strains of *Prochlorococcus* are entirely depending on the ammonium whereas, others are on the nitrite and ammonium. Ammonium concentrations were observed to be high in the first 100 meters depths. Nitrate concentrations present the peak in the 50 m depth only. Additionally, spearman rank correlation test did not show any correlation between *Prochlorococcus* MIT 9313 neither with ammonium nor nitrite. Our data suggest that the *Prochlorococcus* MIT 9313 can use both nitrogen sources. However, no information was found in the literature to support our hypothesis.

In the previous chapter, DCM formation was discussed. In January 2017 at the depth of 200 meters, it was observed that there was elevated concentration of chlorophyll- a. According to the sequences of Prochlorococcus MIT 9313 and *Synechococcus* CC9902, this increase in the total chlorophyll is not the result of presence of any of these taxa. This further supports that this is the result of the physical transfer of cells of phytoplankton from the euphotic layer to deeper layers.

DCM formation was observed at the depth of 100 meters in May. Formation of DCM at this depth coincides with the presence of *Prochlorococcus* MIT 9313. Relative abundance of *Prochlorococcus* MIT 9313 was the highest observed abundance for this taxon. This suggests relative importance of *Prochlorococcus* MIT 9313 to the measured total chlorophyll concentration.

From October to November *Prochlorococcus* MIT 9313, decreased in 100 m and *Synechococcus* CC9902 was over abundant. Two of the Cyanobacterial genus presented less than 5% of relative abundance during winter and spring. In May, when water stratification has started, *Synechococcus* CC9902 was slightly more dominant at the surface and 25 meters

depth, while at 50 m and 100 m communities *Prochlorococcus* MIT 9313 was abundant. In June, Prochlorococcus MIT 9313 was observed in the 100 m depth (~9%) while in other depths their contribution to the community was <1%. *Synechococcus* CC9902 was observed in the surface and 25 m depths. Relative abundance of Prochlorococcus MIT 9313 in 100 m was also high in July as 7%, such high relative abundance was followed by the depth 150 m (4%) and 25 m (2%). Sequence variants belonging to the *Synechococcus* CC9902 was only present in surface and 100 m depth with ~1% of relative abundance. In August, except for surface *Prochlorococcus* MIT 9313 was dominant but *Synechococcus* CC9902 was only present in the surface (3%) and 100 m depth (<1%) while *Prochlorococcus* MIT 9313 was not present in 150 m and 200 m depths.

Since nutrient uptake capacity can be impacted by the size of the cell, *Synechococcus* which are larger than *Prochlorococcus* cells (0.9 μm and 0.6 μm, respectively) are more abundant in high nutrient coastal zones but Prochlorococcus is more abundant in oligotrophic waters (Partensky, Blanchot, and Vaulot, 1999). However, recent findings on the global distribution of picocyanobacteria showed that nutrients were not the significant factor impacting the distribution at local scale but they were rather modulated by PAR in a positive and non-linear fashion (Flombaum et al., 2013). In our study, neither *Synechococcus* nor *Prochlorococcus* sequences showed correlation with variable PAR. However, *Synechococcus* was found to be correlated with EZD and Prochlorococcus with depth. To better elucidate, which environmental variable is the driving factor, more detailed study should be conducted using cyanobacteria specific primers.

2.4. Conclusion

After the development of next generation sequencing technologies and the drop of the cost of sequencing (still not really applicable to developing countries), study of microbial dynamics was concentrated on molecular approaches which does not require culturing prior to any sequencing studies. In Erdemli Time Series stations, bacterial abundance data have been already present. This data was generated using acridine orange cell counting. But this technique was inadequate to identify taxonomic composition and phylogeny-based analysis since it only presents the abundance by total cell numbers per ml. It was the first-time that bacterial community composition was highlighted in the north of Eastern Mediterranean Sea. Even there is a growing attention to usage of NGS technologies in the microbial ecology field, the number of studies is still not enough to picture their physiology in the environment. Throughout a year monthly sampling of ETS-200 m station was conducted from six different depths to study effects of environmental factors on community composition and diversity.

Temperature, apparent oxygen utilization and nitrate were revealed to explain the patterns in the community. Seasonal variation in the phylogenetic diversity was apparent. Bray-Curtis dissimilarity among samples showed that during winter, samples were more similar to each other indicating that winter mixing and seasonality might have been the driving physical factor. Throughout the year, SAR11 clade was the most dominant taxon. Whereas, Clade Ia was more dominant in the surface waters, Clade Ib and Clade II were having the higher abundance in the deeper depths especially during stratified conditions. Presence of *Pirellulale*s family which is responsible for anammox and nitrite oxidizing phylum *Nitrospinae* were detected at the depths of 150 and 200 meters. Possibility of anammox reaction mediated by *Pirellulale*s family is further evaluated in the Chapter 4. On the other hand, presence of *Nitrospinae* explains nitrate accumulation in these depths especially during stratified periods.

It is suggested to include total DNA samplings in the long-term monitoring station, Erdemli Time Series. This study should be further advanced to cover coastal communities.

CHAPTER III

USING 16S rRNA AMPLICON SEQUENCING TO ASSESS FREE-LIVING AND MICRO-PLASTIC ATTACHED MICROORGANISM COMMUNITIES ALONG THE STRATIFIED WATER COLUMN IN NORTH-EASTERN LEVANTINE BASIN

Highlights

- It is hypothesized that communities that were collected on 25 μm sized filter are Micro-plastic (MP) attached communities.
- The differences between free-living (FL) and micro-plastic (MP) attached communities were revealed using high-throughput amplicon sequencing technology in the 4 different water column depths during the highly stratified water column conditions in the Erdemli Time Series Station.
- The distribution and relative abundances of free- living community throughout the water column was consistent with the earlier studies that diversity increases with increasing depth.
- MP community composition was found significantly different than the FL community. Furthermore, dominant groups observed in MP community did not match with studies from other locations.
- The family *Caulobacteraceae* was the most dominant group in MP samples. Frequency of *Caulobacteraceae* family did not show any variation with depth.

3.1. Introduction

Mediterranean Sea, as a semi-enclosed basin, is surrounded by highly populated land and thus reported to face human activity driven pressures to the greater extent in the future (Durrieu de Madron et al., 2011). It is highly critical to assess the dynamics of Mediterranean marine ecosystem and interactions to be able to mitigate anthropogenic pressures. Most of the primary production in the ultra-oligotrophic Eastern Mediterranean Sea is consumed by the bacteria (Turley et al., 2000) and their role and importance in the biogeochemical cycles have been discussed earlier (Farooq Azam and Malfatti, 2007c). So called "unseen majority", dominate oceans in terms of abundance, biomass and most importantly metabolisms ranging from autotrophy to heterotrophy (D. M. Karl, 2007). Under the changing climatic conditions, interaction of marine bacteria with organic carbon throughout the water column has attracted the attention of microbial ecology studies to understand carbon export dynamics to deeper ocean for long-term storage. Conventionally, the organic matter that produced by photosynthesis is classified as particulate organic matter (POM). Additionally, the sizes of organic matter in the marine ecosystems was defined to be continuous range, from a single macromolecule to particles that are bigger than 100 μm (Verdugo et al., 2004). The fate of the POM is mainly driven by the bacteria, and degradation of particulate matter is an important process for the carbon and nutrient cycling in the oligotrophic ocean. The POM pool now is expanding due to the increasing input of plastics from land to seas.

Since plastic usage has entered the consumption area, it became a major environmental concern (Quero and Luna, 2017). Gregory and Ryan (1997) reported that plastic accounted for a significant proportion (from 60 to 80%) of the total debris encountered in the seas of the Southern Hemisphere (Gregory and Ryan, 1997). Most of the previous studies conducted to identify the community differentiations between the Free-Living (FL) and Particle Attached (PA) communities (Acinas, Antón, and Rodríguez-Valera, 1999; Moeseneder et al., 2001). Virtually, most of the bacterial communities inhabit micro-environments such as marine snow and aggregates. This brings another question; can marine plastic debris also be a possible niche for marine bacteria, and is a similar niche differentiation occurring in the North-western Levantine Basin waters. Studies that provided evidence for microplastic transportation from the surface water to the sediment by phytoplankton aggregates (Long et al., 2015) and zooplankton faecal pellets (Cole et al., 2016), also showed the incorporation of microplastic with organic matter. Interactions between marine aggregates, microplastic and microbial communities and how they change as they migrate in the water column has become an emerging field of research (Galloway, Cole, and Lewis, 2017a). Mostly found microplastic

particles were studies in the laboratory conditions. These microplastic particles are shown to incorporate with marine snow and impacted the sinking rate from the surface to the bottom (Porter, Lyons, Galloway, and Lewis, 2018).

Characteristic of the microbial community that are colonizing different types of plastic are distinguishable from the surrounding free-living community (Amaral-Zettler et al., 2015; Bryant et al., 2016; Carson, Nerheim, Carroll, and Eriksen, 2013; Zettler, Mincer, and Amaral-Zettler, 2013). A prominent study conducted by Amaral-Zettler and colleagues (2015) at two different locations, the North Pacific and North Atlantic subtropical gyres. According to study, the bacterial communities of plastic samples from these locations were different from each other and they pointed out the importance of the regional management strategies (Amaral-Zettler et al., 2015). The magnitude of plastic particles ranged from approximately 16,000 to 520,000 particles per km^2 of the surface waters at the Turkish Mediterranean Sea coasts and 58% of stomach/intestine of sampled fishes were contaminated by microplastic particles (Güven, Gökdağ, Jovanović, and Kıdeyş, 2017). According to this study (and studies referenced there) Mediterranean Sea is classified as a medium contaminated basin. Additionally, investigation of the synthetic plastic -BPA - degradation in the same region of the present study was reported to occur only in the seawater due to the presence of biological activity since, artificial seawater was used to eliminate organisms as a control group (Kocaman, 2018). Their results indicate the presence of bacterial degradation of plastics in the study region.

As pointed out, Mediterranean is classified as medium contaminated basin by micro-plastic, in the present study we investigated the bacterial composition of free-living and microplastic attached communities using high-throughput amplicon sequencing technology in 4 different water column depths and during the occurrence of strong stratification throughout the water-column in the Erdemli Time Series 200 m depth station. The aim of the study was to determine micro-plastic attached and free-living bacterial communities by using Siva database (v. 132) and to compare them to understand specific and common taxa between those two samplings.

3.2. Material and Method

Sample collection and microplastic quantification

Samples were collected from ETS-200-Erdemli Time Series (ETS) station, located at 36°26.17'North and 34°20.76' East, on August 14th 2017 cruise. The total water column depth of the station is 200 m and is situated on the relative narrow shelf area of the Northern Levantine Basin. The seawater samples were collected with the IMS-METU's research vessel

RV Bilim-II from the surface water and 25 m, 150 m, 200 m depths by using Niskin bottles connected to the CTD rosette system.

Seawater were collected during two different casts. One cast's samples were dedicated to micro plastic attached (MP) community and the other cast's samples were used for the study of the free-living (FL) community. Two litres of the seawater were kept into a borosilicate dark bottle from each depth and filtered from 0.22 µm pore sized filters (polyether sulfone membrane- MoBio) on board to investigate FL community. Twelve liters of seawater was filtered from 25 µm pore sized filter that attached to the bottom of a homemade sampling bottle for the MP community analysis. Twelve liters of seawater were collected as 4 replicates, three filters were used to classify microplastic amount and composition. The other one was used for 16S rRNA amplicon sequencing analysis. Filters collected for plastic particle counting and classification were processed with 30% of hydrogen peroxide to eliminate organic matters. Hydrogen peroxide did not applied to samples taken for 16S rRNA amplicon sequencing analysis. Plastic particles were counted and classified using Olympus SZX16 Stereomicroscope following the Güven et al., (2017) protocol.

DNA extraction and sequencing

Total DNA extractions of eight samples were processed in the genetic laboratory of the IMS-METU. DNA extractions were carried out using the methods in Paz et al., (2003) (Paz et al., 2003). DNA amount of each sample was subsequently quantified by spectrophotometry. Sequencing was performed by Macrogen Inc. (Macrogen-Europe) for both direction (forward and reverse). Amplicon sequencing was done by Illumina MiSeq platform (300bp paired-end sequencing) and the V3-V4 region of the 16S rRNA gene was amplified using 341F and 805R primer pairs (Herlemann et al., 2011).

Bioinformatics analysis, taxonomic classification and diversity measures

Details of bioinformatics analysis steps were given in the section 2.2.2. Briefly, trimmed Illumina paired- end reads were imported to the Qiime 2 analysis pipeline. Representative sequences of each Operational Taxonomic Units were aligned against the Silva data base for taxonomic assignments.

Pairwise community similarity between 16S rRNA samples was computed using Bray-Curtis similarity index. As a widely used exploratory method Principle Coordinates Analysis (PCoA) was used to explain the variance in the data set (Paliy and Shankar, 2016) and plots were generated using EMPeror (Vázquez-Baeza et al., 2013).

3.3. Results

3.3.1. General features of the sampling location and data statistics

Samplings had been carried out in the north-eastern Mediterranean Sea. During the sampling, the water column stratification was strong and mixed layer depth was around 20 m (Figure 58). Total trimmed read (after quality control and chimera check) was counted as 3, 126,346 and 65,457 sequence. In total of eight samples, 664 features (sequence variants) were captured after filtering out chloroplast sequences.

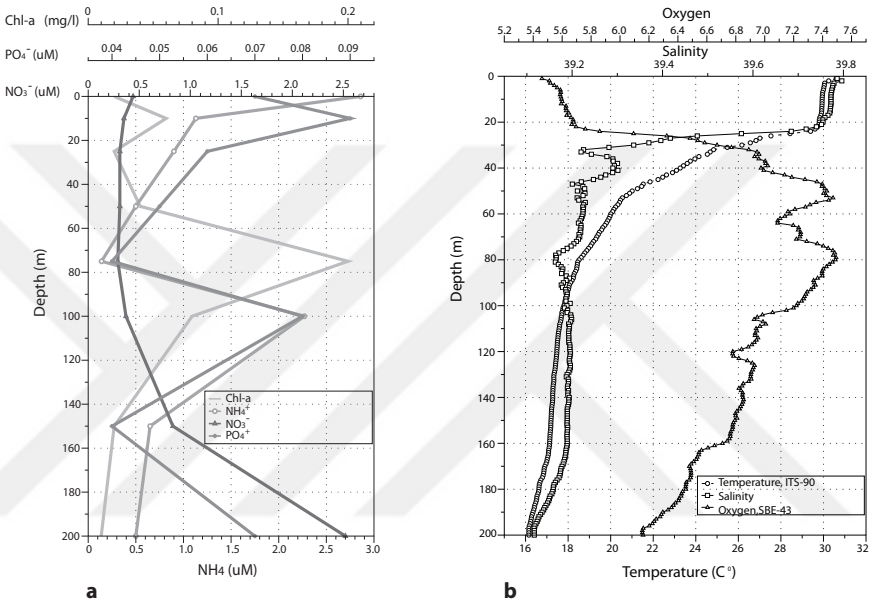

Figure 58. Profiles of inorganic nutrients and total Chl-a, b-) Temperature, Salinity and Oxygen profiles of sampling station in August. DNA samples were taken from the surface, 25 m, 150 m and 200 m depths.

3.3.2. Plastic type and amount in the water column

Microplastic amounts were counted as 8.16, 16.80, 10.41 and 7 pieces for the per liter of the surface, 25 m, 150 m and 200 m samples respectively. Fibres were the mostly counted microplastic particles in all depths and their dominant size fell between 101-500 μm size classes. Highest particle amount (in 25 m sample) was detected just below the mixed layer depth (20 m). Below the mixed layer depth, numbers of plastic particles were decreasing.

3.3.3. Alpha and Beta Diversity

Among the all samples the highest OTUs number were observed in the FL community that was collected from 200 m depth. Thus, elevated nitrate concentrations were observed in the corresponding depth. The least number of OTUs (even phylogenetic diversity index) were observed in surface sample of the FL community. On the other hand, the least diversity observed in the free-living community was higher than the highest diversity observed in the samples of MP attached community (Figure 59). According to Kruskal-Wallis pairwise comparison test, Faith's Phylogenetic Diversity measurement differences between FL and MP attached samples were found to be significant (H =5.33, p-value =0.021, q-value=0.021). Regardless of the diversity index used, alpha diversity in the FL community increases from surface to deeper water column. On the other hand, the highest diversity of MP attached community among the all MP samples was observed at 150 m depth. Diversity between each sample (beta-diversity) was measured by using Bray-Curtis dissimilatory and the first three components of Principle Coordinate Analysis (PCoA) explained 89.99 % of total variance (Axis 1=54.8%, Axis 2 =22.7%, Axis 3 =12.4%).

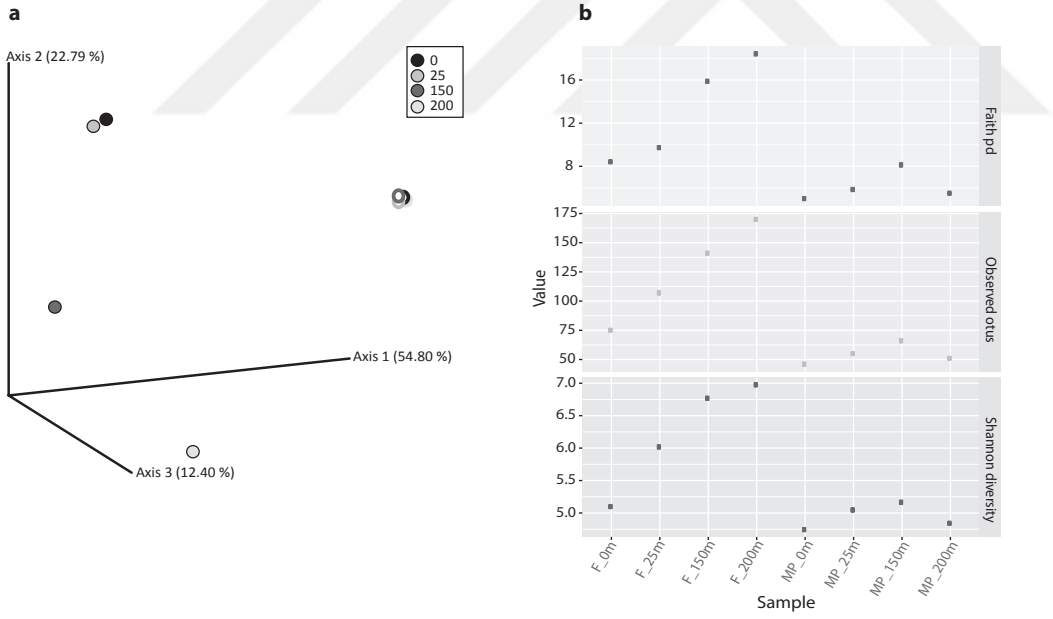

Figure 59. a-) PCoA on Bray-Curtis distance matrix. Proportion explained by each axis was given in the figure. Samples coded by coloured fill circles are representing FL community whereas coloured open circles are samples of MP community. Each sample coloured according to the depth they are taken, b-) Three different alpha diversity measures for each sample collected

3.3.4. Community Composition

Common taxa observed in FL and MP samples

Out of 32 different class presented in the all samples only four of which were observed common in all samples; *Alphaproteobacteria, Gammaproteobacteria, Bacteroidia* and *Verrucomicrobiae* classes. While these four classes were accounting up to 99% of relative abundance in each MP samples, relative contribution of these differs between 95.6% and 60.4% in the FL fraction of the samples. *Alphaproteobacteria* was overall the most abundant class in all samples and depth. Relative abundance in the FL community was decreasing by the increasing depth. Highest relative abundance was observed in the surface with 76 % in all samples and the least contribution was in the FL-200 m depth sample (% 40.1). On the other hand, in the MP samples variation in the relative abundance was between 50.1 and 56.1 % and these min and max values were observed in the MP-25 m and MP-200 m samples, respectively. Higher contribution of *Gammaproteobacteria* was observed in the MP samples when compared with the FL community. While in the MP samples, distribution was between 35.3% and 40.5%, it was between 11.2 and 19.7% in the FL samples.

The third mostly observed class was the *Bacteroidia* in all samples except for the FL-200 m sample that *Dehalococcoidia* having 11.2% relative abundance while *Bacteroidia* has the 7.3 % of contribution in the FL-200 m sample. On one hand, contribution of *Bacteroidia* within the FL community samples was 7.0, 10.2, 17.2 and 7.3 % in the depths 0m, 25 m, 150 m and 200 m respectively. On the other hand, relative abundance in the MP samples from surface to 200 m depth was as follows: 8.6, 8.2, 7.7 and 7.0 percentages.

In total, 664 different sequence variants (OTUs) were captured from all the samples. The number of OTUs that observed in the FL community (542) was higher than the MP attached community, (129). Among 664 OTUs, 529 of them were only observed in one sample. In the highest taxonomic level, FL and MP samples (when considered as a group) share 13 common taxa. Among the common taxa none of them was present in all 8 samples. Each of these taxas' relative abundances in the MP samples were accounted 1 to 2% than 1% of the community. However, relative abundances of these taxa were summing up to 65% in the FL-0m sample and decreases with depth.

Community composition of FL

SAR11 clade was overall the mostly observed taxon in the free-living fractions of the samples. Relative abundance was decreasing with as sampling depth increases. In the surface (sample F_0m), SAR11 clade's abundance (59.9%) was followed by the Rhodospirillales with relative abundance of 9.1%. SAR86 and Flavobacteriales were the third and fourth mostly annotated

taxa with relative abundances of 8.8% and 6.4%, respectively. In the sample taken from 25 m depth (sample F_25 m), SAR11 clade showed relative abundance of 46.2 percent and following taxa were SAR86 clade, Flavobacteriales and Rhodospirillales with relative abundances of 12.2%, 9.3% and 8.9%, respectively. While in the surface Rhodospirillales was the second dominant taxon, second rank in respect to abundance was taken by SAR86 clade. Flavobacteriales was observed to be the second dominant group (15.3%) in the 150 m depth sample following SAR11 clade (32.3%) which was followed by SAR86 (11.1%) and Rhodospirillales (5.3%). The sample representing the deepest water column was present SAR11 clade as the abundance value of 30.3 percent. Abundance of the above-mentioned taxa for surface, 25 m and 150 m depth after SAR11 clade was the case for the sample F_200 m. SAR202 was the second dominant taxa (11%) and Marinimicrobia (SAR406 clade) was the third one in regard to abundance with the 6.7 percent. Rhodospirillales, Flavobacteriales and SAR86 clade were not as dominant as other samples of free-living community and represented by the abundance values of 6.1%, 4.8% and 3.9%, respectively.

Community composition of MP

Abundance of Caulobacterales in the MP samples was ranging between 43.9 and 50.5 %. The highest share was observed in the surface sample (50.5%) while the lowest was in the 25 m sample. Samples taken from the depths 150 and 200 m depth showed the relative abundance of Caulobacterales as 46.8 and 49.9%, respectively. The most abundant five taxa in all samples were recorded as follows: Caulobacterales, Pseudomonadales, Betaproteobacteriales, Xanthomonadales and Sphingobacteriales. The highest relative abundance of Pseudomonadales was detected in the 25 m depth sample and in the 200 m depth share of this taxa in the community was the lowest with 15.3% abundance. In the surface and 150 m depth the relative abundances were observed as 17.4% and 15.8%, respectively. Betaproteobacteriales abundances from surface to 200 m depth was as follows: 10.5%, 9.6%, 8.3% and 9.4%. In the surface and 150 m depth, share of Xanthomonadales in each sample was the same as 6.7%. the highest share was observed in the 200 m depth sample (9.2%) and the lowest was in the 25 m depth sample as having 5.9%. Sphingobacteriales abundance was same as the Xanthomonadales abundance in the 25 m depth sample (5.9%). Sphingobacteriales was presenting the 6.2% of the surface sample whereas, in the 150 and 200 m depth samples relative abundance values were observed as 4.5% and 4.6% respectively.

3.4. Discussion

Free-living microbial communities in our samples turned out to be more diverse than microplastic-attached communities (Figure 59-b). PCoA plot result shows that MP samples grouped very closely to each other, whereas FL community samples are located away and their distribution is representing the stratification of water column (Figure 59-a). Our findings supports earlier studies that FL community is more diverse than the particle attached ones (Acinas et al., 1999).

Community composition and dominant taxa of FL and MP samples

Dominant bacteria types of two sampling fractions (FL and MP) were observed clearly different (Figure 60). SAR11 clade, belonging to the *Alphaprotebacteria,* was the most dominant taxon among the all FL community throughout the depths. More than half of the surface community was represented by SAR11 clade and its relative abundance was decreasing with depth, they represent only with 13% in the 200 m-depth community. Presence and distribution of SAR11 clade in the marine environment has already well documented (Morris et al., 2002; Rappé et al., 2002; Salter et al., 2015). In the present study at the higher taxonomic classification level, Clade-Ia showed dominance over Clade-II and Clade-Ib in the surface water and 25 m depth by 52.3% and 33.03% relative abundance, respectively. However, in the deeper water, 150 m and 200 m depths, Clade-II and Clade-Ib ecotypes were dominant. Relative abundance of Clade-Ib was peaked to 10.88% in the 150 m depth while in 200 m depth it was represented only by 6.5% of abundance. Results of the study on SAR11 clade from 7 years' time series analysis in the Northwest Mediterranean Sea surface water showed that SAR11-Ia ecotypes were the dominant SAR11 clade in all of the samples (Salter et al., 2015). Ecotype SAR11-Ia was also dominant in our surface water samples but not in the deeper water column. Clade-Ia also captured in the MP samples. It was observed only in the samples taken from the 25 m and 150 m depths, with lesser relative abundances (0.41% and 0.75% respectively). High relative abundance was observed for Clade-II in the deeper water FL samples, and this is observed only in 150 m depth MP samples with only 0.1% of relative abundance.

SAR86 clade belonging to the *Gammaproteobacteria* was observed in all the depths in the FL fraction of the samples with the relative abundances of 8.8, 12.2, 11.1 and 3.9% from the surface to 200 m depth respectively. Presence of SAR86 clade during summer period in the surface waters of BATS site was shown by Treusch et al. (2009), relative abundance reported as 3% and according to study they were not recorded in the waters below the 100 m depth. Sampling in our study was also corresponds summer stratification period but relative abundance of SAR86 Clade was higher than the surface (8.8%) record of Treusch et al., (2009). Thus, higher relative frequency was observed in the 25 m depth. On the other hand,

contrary to the findings of Treusch et al. (2009), in this study the relative abundance of SAR86 clade at 200 m was almost 4%. In the highest taxonomic rank, SAR86 clade in our dataset revealed eight different sub-clades (having different OTUs and assigned different sub-clades). The one that is ranked as 'SAR86 clade- metagenome' according to Silva database was not present in the 200 m depth and has relatively less abundance in the above depths, 2.8% (150 m), 1.7% (25 m) and 1.2% (surface). Other clade as given taxonomic name by Silva database as 'D_3__SAR86 clade; Ambiguous_ taxa' was present in all depths but higher abundance was in 150 m depth. Sub-clade that is classified as uncultured marine bacterium observed in the depths 25 m (0.85%) and 150 m (2.54%). Two of the SAR86 sub-clade was only seen in the 25 m depth. SAR86 clade is known to possess phylogenetically distinct clade and metabolic streamlining as SAR11 clade (Dupont et al., 2012). Their light and nutrient preferences might play a role in their distribution in the water column.

SAR324 was represented by three different members, the first was abundant in the 200 m depth (4.8%) and 150 m depth (< 1%), second in only 150 m depth (<1%), and the third one was observed in the surface water (1.3%) and 25 m (< 1%) depth. When all SAR324 clades (Marine group B) sequences were investigated together, in the 4th taxonomic rank samples taken from the depths of 150 m and 200 m constituting relatively higher abundances than above depths. This finding is consistent with the previous study (Treusch et al., 2009) that SAR324 clade niche was recorded as deeper water column.

On the other hand, most dominant taxa were assigned as *Caulobacteraceae* family for the MP samples. Depth and relative abundance relationship that observed in the SAR 11 clade, were not recorded for *Caulobacteraceae*. In the present study MP's *Caulobacteraceae* samples comprised 2 different genera; *Brevundimonas* and *Phenylobacterium*. *Brevundimonas* genera were represented by two different species, one was classified in only genus level "*Brevundimonas"*, and the other was assigned as *Brevundimonas diminuta.* Whereas *B. diminuta* represented less than 1% in the all samples, *Brevundimonas sp.* comprised 36-45% of the MP community. Presence of the genus *Brevundimonas* in marine environment was shown previously (Abraham et al., 2005), however their relatively high abundance in the particle- attached micro-environments was not reported (Acinas et al., 1999; Crespo et al., 2013). Main reason of these differences might be due to different size fractionation technique and method. While previous studies used conventional methods e.g. 16S rDNA clones (Acinas et al., 1999; Moeseneder et al., 2001), our study applied 16S rRNA amplicon sequencing approach and generated min 5542 sequences per sample and resolved in total 664 sequence variants (OTUs). Furthermore, this taxon was not reported to be dominant also in studies that investigate plastic attached communities previously (Oberbeckmann et al., 2016; Zettler et al., 2013).

Communities attached to polypropylene and polyethylene particles can be positively and were highly represented by phototrophs and *Pelagibacter* (Zettler et al., 2013). Polyethylene terephthalate (PET) drinking bottles were reported to be colonised by Bacteroidetes and diatoms dominantly in the North Sea. However, PET community were not significantly different from the particle attached and glass surface colonizers (Oberbeckmann et al., 2016). *Caulobacteraceae* family that dominates MP attached community in our samples, are usually known to be biofilm colonizer and production of exopolysaccharides contribute to their stability in the environment (Cavanaugh et al., 2006). Oberbeckmann et al. (2016) showed that PET colonizing community and particle attached communities are not different from each other, it is suggested that plastic surface is using as a media for attachment and this driven by the biofilm formation (Oberbeckmann et al., 2016). Here it should be stated that our sampling procedure did not provide enough evidence to conclude that our MP- attached community is micro plastic-specific colonizers but rather, should be considered as bacterial cells aggregated or attached to particles of more than about 25 μm particle size.

Another member of *Caulobacteraceae* that were observed in our MP samples were *Phenylobacterium* that having relative abundance from 3.9 to 6.6 %. Type species of *Phenylobacterium* are known as degrade chloridazon which is the active ingredient of the herbicide Pyramin and a synthetic molecule which is not present normally in the nature (Lingens et al., 1985). Their presence in our samples might be due to the chloridazon containing herbicide" use in the catchment area of our sampling station or their ability to use other substrates in the nature rather than chloridazon.

Acinetobacter junii belonging to the Pseudomonadales was another taxon that represent high relative abundances in the MP samples from surface to the 200 m deeps with 10.4%, 5.8%, 2.2% and 4.5% values respectively. *A. junii* species that isolated from soil was shown to have ability to degrade Poly ε-caprolactone (PCL), polytetramethylene succinate (PTS) and polyhexamethylene carbonate (PHC) products (Suyama et al., 1998). PHC is known to be a carbon storage material in bacteria whereas PCL and PTS are chemically synthesized materials.

Members of genus *Pseudomonas* are drawing attention due to their ability to degrade and metabolize synthetic plastics (Wilkes and Aristilde, 2017) and they were observed only in the MP samples of present study. *Pseudomonas* members' proportions were represented as highest in the surface sample with 8.5% share and followed by the 200 m depth (6.9%) and 150 m depth samples (6.5%). Minimum relative frequency was found to be present at the 25 m depth with 5% value where the numbers of fibre particles were observed to be highest. It is critical to stress here that amplicon sequencing results were giving only the relative abundance in the sample, not the absolute abundance of specific genus. Even though, low proportion that reported in 25 m depth does not mean that the absolute abundance is less also. *Pseudomonas*

genus was represented by three different taxa, 2 of which can be classified in the species level as *Pseudomonas stutzeri* and *Pseudomonas alcaligenes*. Among other taxa that only observed in MP samples were the *Nubsella* which belongs to the *Sphingobacteriaceae* family. So far presence of *Nubsella* has not been reported in any of the study that conducted in the marine ecosystem looking for either particle attached or microplastic attached community. Relative frequencies of *Nubsella* genus were observed higher in the surface samples (surface: 6.2% and 25 m: 5.9%) than the 150 m and 200 m depth (4.5 and 4. 9 %, respectively) samples of present study. According to a study that took place in the Lake Erie (North America), *Nubsella* abundance were recorded as relatively low (<1%) in the summer period (Wilhelm et al., 2014). Hence, *Nubsella* genus stated to produce biosurfactants in the palm oil contaminated soils (Saisa-ard et al., 2014). Their presence in the present study's MP samples that represents bacteria attached to particles of more than 25 μm particle size but not in the samples that representing FL-community might be explained by their niche requirements.

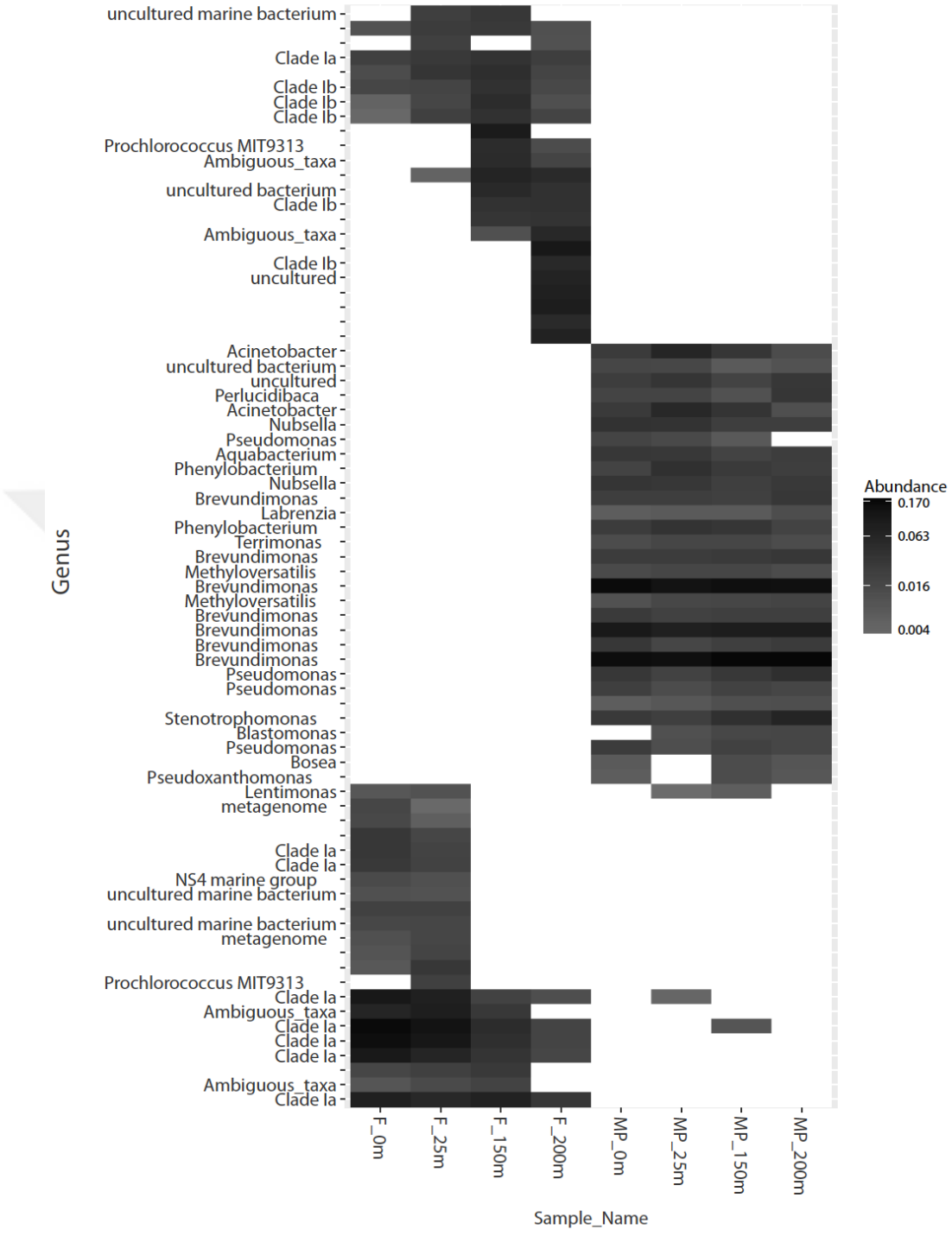

Figure 60. Heat map of most abundant seventy-five sequence variants (OTUs). Sequence variants were annotated in the genus level, empty annotations indicate that sequence variant could not classified down to genus level. Heatmap is generated using R version 3.3.2., phyloseq package with unconstrained redundancy analysis (Principle Components Analysis, PCA) method following Rajaram and Oono (2010).

Another taxon that observed in only MP samples was assigned as genus *Methyloversatilis*. Minimum and maximum frequencies of this taxon were recorded as 2.2 and 3.8 %. Type strain of this genus is known as methylotrophic (utilises one carbon compound) (Oren, 2014) and stains that isolated from active sludge is presented to degrade herbicide benazolin-ethyl (4-chloro-2-oxobenzothiazolin-3-yl-acetic acid) as sole carbon source (Cai et al., 2011).

Here we presented differences between the bacterial community composition of FL and MP particles attached (bigger than 25 µm) communities during the summer period of 2017 when the water column stratification is high (Figure 58). To avoid misleading results, it should be stated here that our sampling and analysis framework does not provide certain prove that the community we sampled by 25 µm mesh size are only microplastic attached. Hence, so called MP attached community we shed light on were different by the observed taxa from the previous studies which were considering particle- attached (Acinas et al., 1999; Crespo et al., 2013) and microplastic attached (Oberbeckmann et al., 2016; Zettler et al., 2013) communities. However, bacteria that are known to form biofilm (which is the first step of plastic degradation) were discovered. Beyond that, taxa which are responsible for bio-degradable plastic decomposition and herbicide degradation were presented. Another study that conducted in the same location (NE Mediterranean Sea- ETS sampling stations) also supports our MP samples classification. Their results provide evidence on the BPA presence and degradation in the seawater (Kocaman, 2018). Xenobiotic enzyme activities that are reported to be responsible for the plastic particles degradation was also detected in the study area by a metatranscriptomics study (unpublished work of the Arzu Karahan and Selin Deliceırmak).

Since, incorporation of plastic particles to organic matter is evident (Galloway et al., 2017b; Porter et al., 2018) and marine waters are well polluted by plastics, clear distinction between particle attached or micro plastic attached community by our study design is not straight forward. Clear distinction between FL and PA samples were apparent even if prefiltration was not applied to the FL samples. The reason of the differences between communities might be due to the volume of filtered water (2L for FL and 12L for MP). However, capturing MP community on the 25 µm pore sized filter was possible only using with higher volumes of seawater.

3.5. Conclusion

Plastic and their fate in the marine ecosystems are attracting the attention of not only the scientist but also the public authorities. Regional differences in the plastic type, seasonal variations at their accumulation level throughout the water-columns and community structures are needed to be evaluated at regional scale. On the other hand, describing MP community and plastic composition is also crucial to understand the impact of the end products on the ecosystem and human health that comes from the degradation processes of plastics.

Direct link between MP attached community observed in our study and nitrogen cycle cannot be found in the literature. However, according to the results revealed in this chapter, there are some important aspects to be considered in the future studies to shape our understanding on nitrogen and carbon cycle coupling and how these micro-habitats can impact these interactions.

- Clear distinction between particle attached or micro plastic attached community by our study design is not straight forward. Classification of community collected on 25 µm pore sized filter was done based on the observed taxa and their possible role in plastic degradation. However, using different pore sized filters revealed the colonization of particles by different taxa clearly.
- Our understanding of particulate matter is mostly limited by operational classifications such as classification of particles according to their size classes. But, each size classes of particulate matter are a potential habitat for microorganisms.
- It can be stated that these particles provide a microhabitat for variety of functions from aerobic to anaerobic processes. But, how these small-scale processes can impact large scale ecosystem dynamics should be further questioned in the future studies.
- Microplastic particles are shown to incorporate with marine snow and impacted the sinking rate from the surface to the bottom. It should be further questioned how this change in sinking rates will shape organic matter degradation by marine bacteria.

In this study, differences between FL and MP attached communities were revealed. However, above mentioned points could not be addressed due to limitations of methodological approach followed. Functional differences between FL and MP attached communities should be addressed in the future works by using metatrascriptomics approach.

CHAPTER IV

METARANSCRIPTOMIC ANALYSIS OF THE BACTERIAL COMMUNITY

Highlights

- Total RNA sampling was performed seasonally from four different depths in order to apply the metatranscriptomics approach. It was the first time that this technique is applied in order to understand nitrogen cycle in the region.
- Expressions of genes that are responsible for ammonia oxidation indicate that the heterotrophic bacteria use the produced organic material.
- Only expression of genes that are responsible for the first step of nitrification were detected in the samples. It is hypothesized that the oxidation of ammonia to nitrite was not complete and only gene expressions of oxidation of ammonia was present at the sampling station. However, 16S rRNA results might implicate the presence of nitrite oxidation.
- There was no expression of N_2 – fixation genes.
- PhoX gene expressions showed that bacteria adapt to different sources of phosphate at periods of low PO_4^- concentrations in the sampling stations.

4.1. Introduction

Metatranscriptomics approaches take advantage of the fact that enzymes responsible for metabolisms such as phosphorus and nitrogen metabolisms are all proteins. Proteins are encoded in the genes (DNA) and manufactured by two steps: transcription and translation (Figure 61). In order to synthesis a protein first messenger RNAs (mRNAs) should be transcribed. Therefore, messenger RNAs are the indicators of active metabolism and their sequences give information on possible active functions within the microbial ecosystem under study.

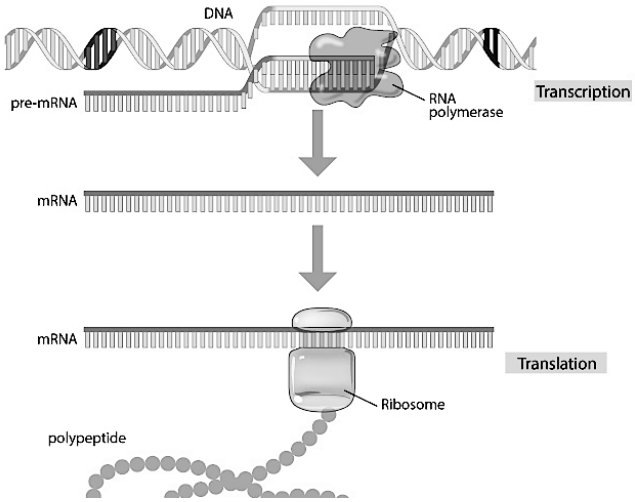

Figure 61. Expression of a gene through transcription and translation. Figure was taken from: https://www.nature.com/scitable/topicpage/translation-dna-to-mrna-to-protein-393

Next-generation sequencing has triggered a rapid increase in the number of microbiome targeted studies (Frias-Lopez et al., 2008; Gilbert et al., 2008). Metagenomics and amplicon sequencing provide information on the taxonomic composition and metabolic potential of a microbial community. It answers the question of "who is there?". On the other hand, metatranscriptomics unveils the actual metabolic activities of the community exactly during sampling and place. The question that can be answered with metatranscriptomics is "What are they doing there?". Therefore, metatranscriptomics is an important tool for investigating marine microbial reactions.

The metatranscriptomic study of this chapter will particularly focus on an exploration of nitrogen and phosporus-related metabolic processes. Nitrogen is cycled through four major biologically mediated reaction: (1) N_2-fixation, (2) nitrification, (3) denitrification and (4) anammox, detailed scheme indicating marker genes for each reaction was given in the Figure 62. Inorganic phosphate (PO_4^-) is an important inorganic nutrient that causes limitation related stress on bacteria as well as primary production in oligotrophic seas such as the open Mediterranean Sea. Therefore, to meet the requirements of phosphate (P), bacteria have developed different strategies to adapt to low or variable P concentrations. Alkaline phosphatase (Pho) activity is one of the way that bacteria adapts to low P- conditions by using of alternative phosphate sources (e.g. phosphate esters and phosphonates) (Sebastian and Ammerman, 2009).

In order to understand variations in the active (expressions) nitrogen cycle genes and P-limitation on bacteria, seasonal sampling of total RNA was conducted at the ETS-200 m station.

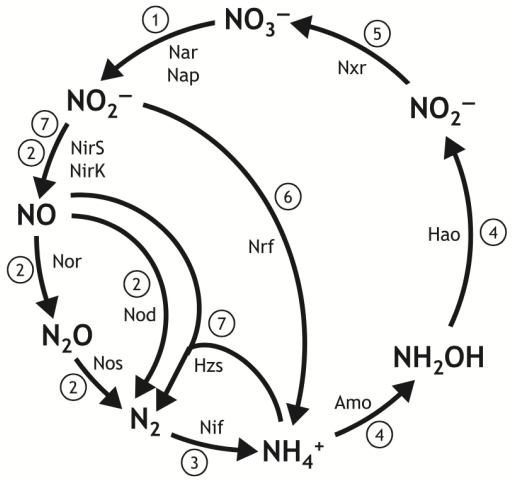

Figure 62. Key genes involved in the biological nitrogen cycle are: 1, nitrate reduction; 2, denitrification; 3, nitrogen fixation; 4, aerobic ammonia oxidation; 5, aerobic nitrite oxidation; 4+5, comammox; 6, dissimilatory nitrite reduction to ammonium (DNRA); 7, anaerobic ammonia oxidation. Nar/Nap, dissimilatory nitrate reductase; NirK/NirS, dissimilatory NO- forming nitrite reductase; Nor, nitric oxide reductase; Nod; nitric oxide dismutase; Nos, nitrous oxide reductase; Nif, nitrogenase; Amo, ammonia monooxygenase; Hao, hydroxylamine oxidoreductase; Nxr, nitrite:nitrate oxidoreductase; Nrf, dissimilatory ammonia- forming nitrite reductase; Hzs, hydrazine synthase Figure was taken from Rasigraf et al., (2017).

4.2. Material and Method

In order to gain insights into active metabolism of bacterial community, total RNA samples were collected seasonally. In addition, a sampling of 4 different depths (0, 25, 150 and 200 meters) was planned to be sequenced from each season. The sampling was performed in parallel with the other samplings on 15 November 2016, 20 February 2017, 30 June 2017 and 04 July 2017. For the total RNA isolation, 2 liters of sea water taken from the same Niskin bottle with 16S rRNA amplicon sequencing samples and immediately fixed with carnoy fixative (Feike et al., 2012). Seawater samples were filtered through a 0.2 µm pore sized filter in the laboratory and the filters were stored at -20 °C. Final volume that can be filtered for each sample due to clogging of filters were given in the Table 6. After the sampling of all seasons was completed, total RNA isolation was completed using the EZ-RNA Total RNA Isolation Kit (Biological Industries). Samples were sent to Zeydanlı Life Sciences Ltd. for mRNA purification, library preparation and sequencing.

Table 6. Final volumes of each sample

Sample	Date	Depth	Final volume (ml)
R1	15.11.2016	0	1000
R2	15.11.2016	50	1000
R3	15.11.2016	150	1500
R4	15.11.2016	200	1200
R5	20.02.2017	0	2000
R6	20.02.2017	50	1700
R7	20.02.2017	150	1000
R8	20.02.2017	200	1000
R9	30.06.2017	0	2000
R10	30.06.2017	50	2000
R11	30.06.2017	150	2000
R12	30.06.2017	200	2000
R13	04.07.2017	0	2000
R14	04.07.2017	50	2000
R15	04.07.2017	150	2000
R16	04.07.2017	200	2000

4.2.1. Sequencing and data statistics

Trueseq stranded total RNA kit (with ribosomal depletion (microbe)) was used in the library preparation step. RIN (RNA integration number) and RNA quantities were detected to be quite low during the quality controls of the samples prior to the library preparation process (Table 7. RNA samples quality and quantity values). Considering these, it was decided to combine samples from different depths of the same month and samples were renamed accordingly: R1-R4, R5-R8, R9-R12, R13-R16. Thus, sequencing was carried out in four samples and the water column integrated results reflected. The Illumina MiSeq platform (100bp paired-end - sequencing) was used as a sequencing platform.

Table 7. RNA samples quality and quantity values

Sample	Concentration (ng/µl)	Final Volume (µl)	Total quantity (µg)	RIN
R1	1.052	63	0.066	1
R2	1.367	30	0.041	1.9
R3	1.406	30	0.042	3.5
R4	1.633	30	0.049	1
R5	5.316	30	0.159	3.5
R6	3.856	30	0.116	4.3
R7	1.135	65	0.074	2.5
R8	0.831	65	0.054	3.2
R9	1.771	65	0.115	2.7
R10	2.175	65	0.141	1.1
R11	1.522	65	0.099	1
R12	0.975	65	0.063	1
R13	2.518	63	0.159	4.2
R14	2.44	30	0.073	4.6
R15	1.854	28	0.052	3.8
R16	0.848	30	0.025	1

4.2.2. Bioinformatics analysis

The reads were analysed using the online metatranscriptomics data analysis program COMAN (a web server for comprehensive metatranscriptomics analysis). The program has been developed to analyse sequence data automatically and comprehensively (Ni et al., 2016). The

primary reason why COMAN was preferred is because this online tool is free of charge. The other reasons are (i) the COMAN analysis pipe-line includes quality control of raw readings, (ii) performs removal of non-coding RNA-derived readings from analysis and, (iii) provides functional annotations. Based on mapping of the readings and the results of the functional annotations of the reference genomes, COMAN performs functional profiling assignments and calculates the relative abundance of each functional group and enzyme (Ni et al., 2016). The KEGG pathway result file was obtained from the COMAN server. The target genes for the nitrogen and phosphate cycles were exported from this file using home-made script based on the KEGG numbers obtained from KEGG reference pathway (https://www.kegg.jp/kegg-bin/show_pathway?ko00910). KEGG numbers belonging to the nitrogen cycle and the role of the enzymes were given in the Table 8.

Table 8. KEGG definition and numbers of nitrogen cycle related genes.

KEGG-definition	**KEGG number**	**Gene**
K00370; nitrate reductase 1, alpha subunit [EC:1.7.99.4]	K00370	NarG
K00371; nitrate reductase 1, beta subunit [EC:1.7.99.4]	K00371	NarH
K00374; nitrate reductase 1, gamma subunit [EC:1.7.99.4]	K00374	NarI
K02567; periplasmic nitrate reductase NapA [EC:1.7.99.4]	K02567	NapA
K02568; cytochrome c-type protein NapB	K02568	NapB
K03385; cytochrome c-552 [EC:1.7.2.2]	K03385	NrfA
K15876; cytochrome c-type protein	K15876	NrfH
K00367; ferredoxin-nitrate reductase [EC:1.7.7.2]	K00367	NarB
K00372; nitrate reductase catalytic subunit [EC:1.7.99.4]	K00372	NasA
K00360; nitrate reductase (NADH) [EC:1.7.1.1]	K00360	NasB
K00366; ferredoxin-nitrite reductase [EC:1.7.7.1]	K00366	NirA
K00368; nitrite reductase (NO-forming) [EC:1.7.2.1]	K00368	NirK
K15864; nitrite reductase (NO-forming) / hydroxylamine reductase [EC:1.7.2.1 1.7.99.1]	K15864	NirS
K04561; nitric oxide reductase subunit B [EC:1.7.2.5]	K04561	NorB
K02305; nitric oxide reductase subunit C	K02305	NorC
K00376; nitrous-oxide reductase [EC:1.7.2.4]	K00376	NosZ
K02586; nitrogenase molybdenum-iron protein alpha chain [EC:1.18.6.1]	K02586	NifD
K02591; nitrogenase molybdenum-iron protein beta chain [EC:1.18.6.1]	K02591	NifK
K02588; nitrogenase iron protein NifH [EC:1.18.6.1]	K02588	NifH
K00531; nitrogenase [EC:1.18.6.1]	K00531	AnfG
K10944; ammonia monooxygenase subunit A [EC:1.14.99.39]	K10944	AmoA
K10945; ammonia monooxygenase subunit B	K10945	AmoB

K10946; ammonia monooxygenase subunit C	K10946;	AmoC
K10535; hydroxylamine oxidase [EC:1.7.3.4]	K10535;	Hao
K00370; nitrate reductase 1, alpha subunit [EC:1.7.99.4]	K00370	NxrA
K00371; nitrate reductase 1, beta subunit [EC:1.7.99.4]	K00371	NxrB
K10534; nitrate reductase (NAD(P)H) [EC:1.7.1.1 1.7.1.2 1.7.1.3]	K10534	NR
K00362; nitrite reductase (NADH) large subunit [EC:1.7.1.15]	K00362	NirB
K00363; nitrite reductase (NADH) small subunit [EC:1.7.1.15]	K00363	NirD
K17877; nitrite reductase (NAD(P)H) [EC:1.7.1.4]	K17877	NIT-6
K20932; hydrazine synthase subunit [EC:1.7.2.7]	K20932	Hzs
K20933; hydrazine synthase subunit [EC:1.7.2.7]	K20933	Hzs
K20934; hydrazine synthase subunit [EC:1.7.2.7]	K20934	Hzs

4.3. Results

The genes involved in the nitrogen cycle and the activity of the alkaline phosphatase enzyme in 4 metatranscriptomics samples from the period of November 2016 (R1-R4), June 2017 (R5-R12), and July 04, 2017 (R13-R16) were investigated. It should be noted here again that the samples represent the entire water column, not a particular depth.

4.3.1. Analysis of higher-level metabolisms

The most expressed genes among all samples were determined to be related to energy metabolism (KEGG identifiers and energy metabolisms that are included were given in the Table 9). The highest relative abundance of energy metabolism genes was defined in the sample of June (samples R9-R12) with 41,1 %. November 2016 (R1-R4) and February 2017 (R5-R8) samples, also, found to have energy metabolism activity quite high with relative abundances of 38.8% and 39.3%, respectively. On the other hand, the expression of genes of energy metabolism in samples of 04 July (25.6%) was observed at the lowest levels among samples. The carbohydrate metabolism activity was almost the same in all samples. Sample taken in July 04 (R13-R16), for instance, "replication and repair" enzymes associated with metabolic activity possess relative abundance of 2.8% while in other samples, the ratio was observed below the 1% (Figure 63).

Table 9. KEGG identifiers and energy metabolism

Energy metabolism	
KEGG identifiers	**Explanation**
00190	Oxidative phosphorylation
00195	Photosynthesis

00196	Photosynthesis - antenna proteins
00710	Carbon fixation in photosynthetic organisms
00720	Carbon fixation pathways in prokaryotes
00680	Methane metabolism
00910	Nitrogen metabolism
00920	Sulphur metabolism

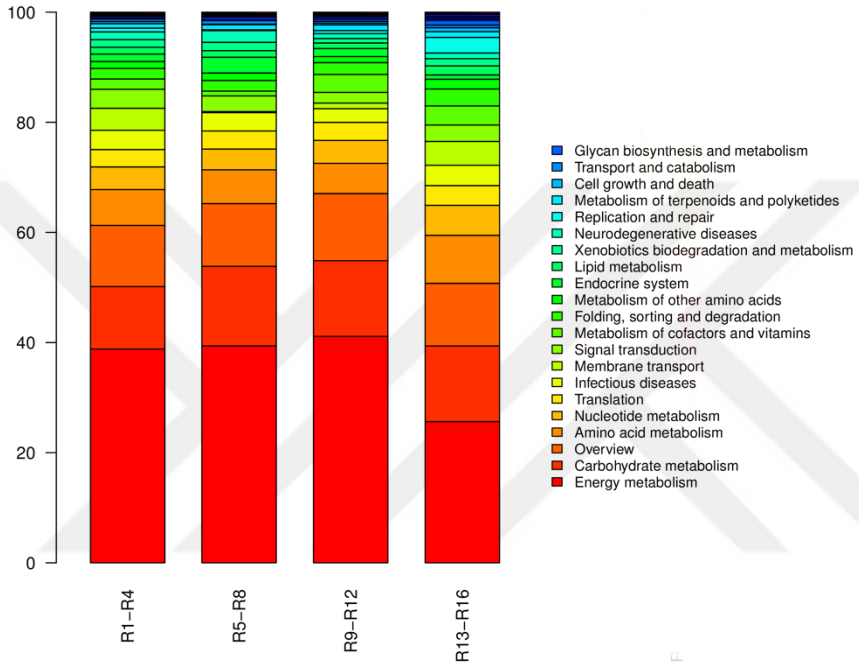

Figure 63. KEGG pathway classes in the samples; R1-R4 = November 2016, R5-R8 = February 2017, R9-R12 = June 2017 and R13-R16 = July 2017.

4.3.2. Nitrogen Metabolism

Changes in the expression of genes involved in the nitrogen cycle have been investigated. All of the genes involved in this cycle are given in Figure 62 and Table 8. The genes involved in the nitrogen cycle in the ETS-200 m station were examined and evaluated, whereas the gene products (e.g. anammox genes, Figure 62, step 7) that were not detected at this station is not discussed.

Nitrification

Marker genes that specify the first step in nitrification are; amoA (encodes for a subunit of membrane bound ammonium monooxygenase) and the hao gene (encodes for hydroxylamine oxidoreductase) which converts ammonia to nitrite. Ammonia oxidation takes place in two steps: first ammonia (NH_4^+) is converted to hydroxylamine (NH_2OH) via the amoA gene. The highest expressions of amoA mRNA reads was recorded in November 2016. A significant drop in amoA expression was observed in February, also the decrease still maintained in June (R9-R12). However, it is observed as an increase in July (R13-R16). It was observed that water column ammonia concentrations were quite low in November.

The second step in the oxidation of ammonia after the formation of the hydroxylamine is the nitrite formation step from the hydroxylamine where the hao gene plays a key role. There were none hao gene expression was recorded in any of the samples, which is in line with the below detection levels of nitrite in most samples.

Another step of nitrification is the one in which nitrite (NO_2) is oxidized to nitrate (NO_3) and this is known as the step that controls the nitrification process. The mRNA expressions of nitrite oxidoreductase (nxrA) which is the marker gene for this step was evaluated in the samples. However, nxrA mRNA expression was not detected in any of the samples.

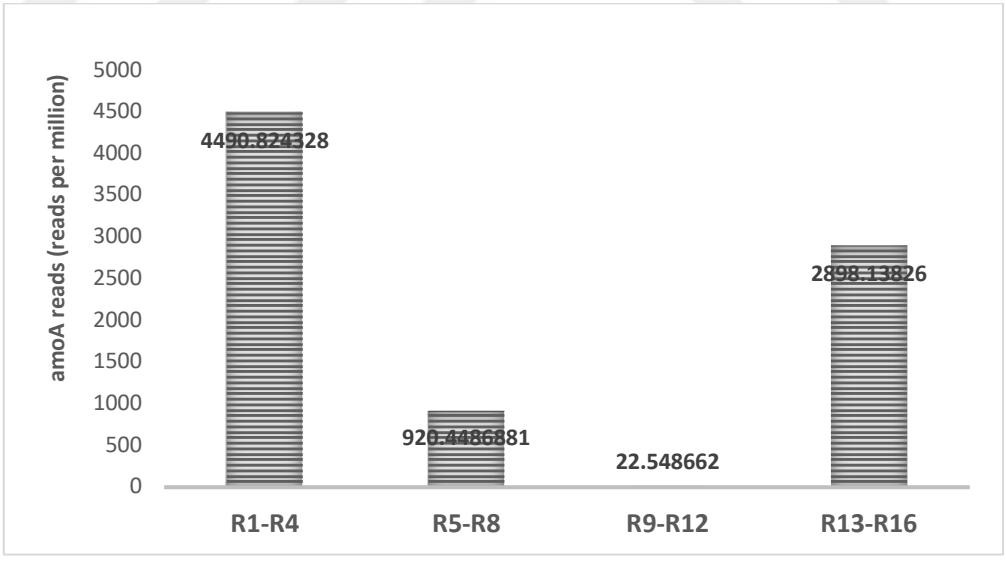

Figure 64. Relative abundances of *amoA* genes (reads per million)

Denitrification

Denitrification is an important mechanism on a global scale as it causes nitrogen to be released from the system by biological processes. It also maintains the continuity of facultative anaerobic bacteria respiration in the absence of oxygen, resulting in decomposition of organic matter. Therefore, it is a biological process which is important for the carbon and nitrogen cycle. Although this process takes place under anaerobic conditions, the presence of denitrification process in oxygenated environments is now known (Omnes et al., 1996). Marker genes and their expression, which are known to play a role in denitrification steps, were discussed in this section.

Dissimilatory nitrate reduction: narG/napA

Dissimilatory nitrate reductase enzymes which are encoded by narG and napA indicator genes that control the nitrite reduction step of nitrate. The mRNA expression levels of these genes are given in Figure 65.

Particularly noteworthy situation was the lack of expression of these enzymes in the samples taken in June (R9-R12), however, the highest expression was observed in the sample taken in July (R13-R16). There were really low expression levels of narG during February sampling (R5-R13).

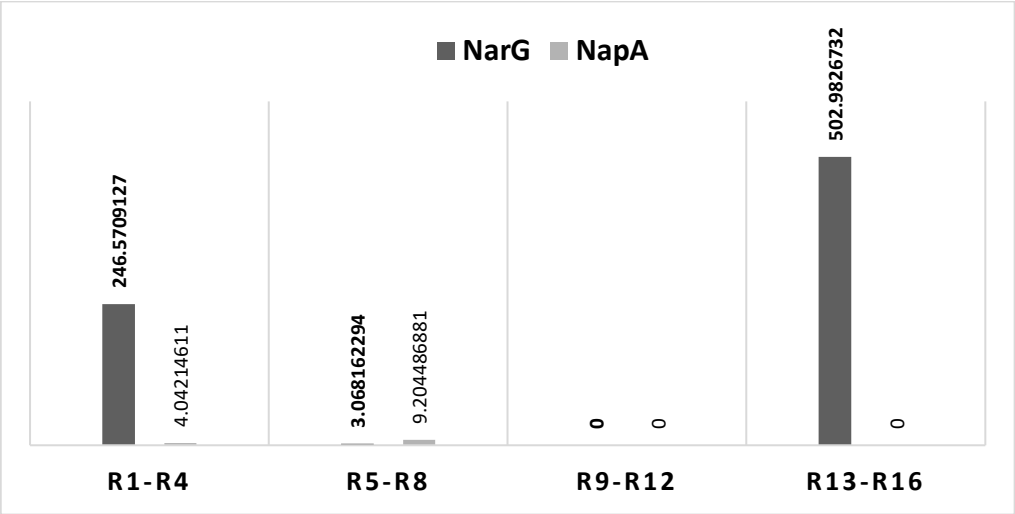

Figure 65. Relative abundances of narG and napA genes (reads per million)

NO-forming nitrite reductase: nirK / nirS

The other steps of denitrification were investigated by checking expression of nitrite reductase genes known as nirK and nirS, which couses the reduction of nitrite to nitric oxide. As shown in, no activity was found in the nirS gene. While the highest expression of nirK was observed in November, as in the narG gene, expression of nirK was not observed in the R9-R12 (June) samples.

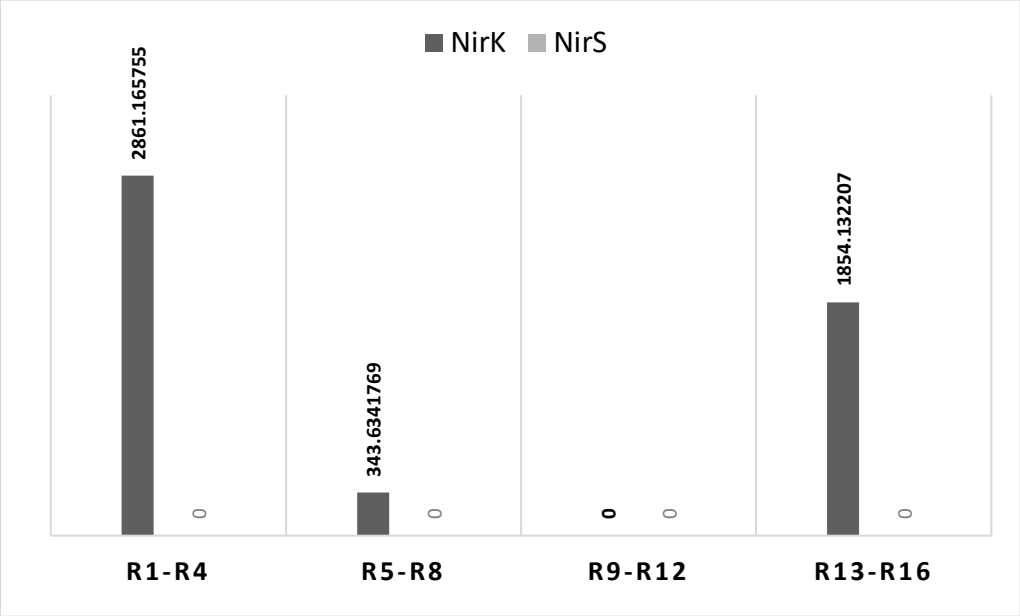

Figure 66. Relative abundances of nirK and nirS genes (reads per million)

Dissimilatory nitric oxide reductase (norB) and nitric oxide dismutase (NosZ)

In contrast to the high reads/million of NirK observed in November, the mRNA reads of the nitric oxide reductase (Figure 67) and nitric oxide dismutase (Figure 68) gene were quite low. While NirK expression was highest in November, norB and nosZ gene expressions were high in the sample taken in July. NosZ gene reads were presented as lower quantities than norB in July.

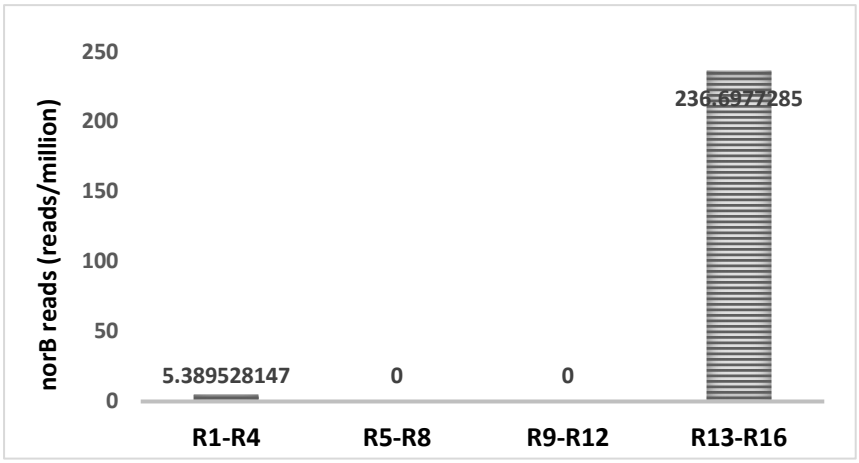

Figure 67. Relative abundances of nosZ genes (reads per million)

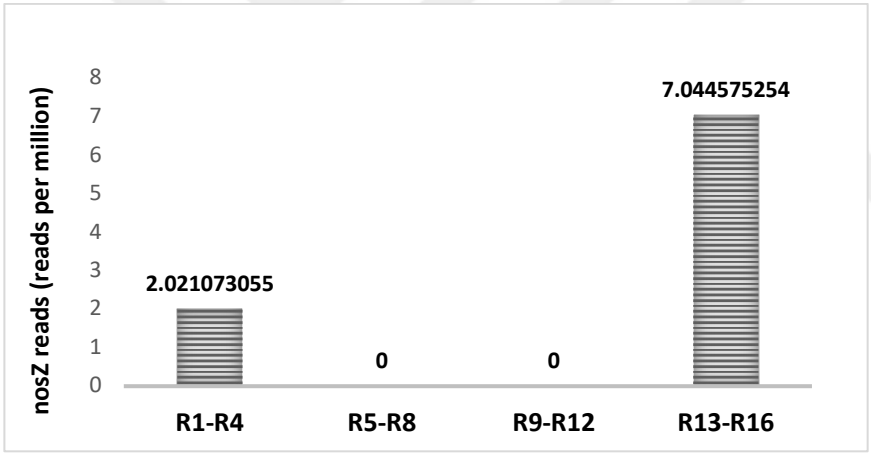

Figure 68. Relative abundances of norB genes (reads per million)

Nitrogen fixation

The mRNA expressions of 4 different genes (NifD, NifK, NifH, AnfG), which are the nitrogen fixation marker genes, were examined but no expression of these genes was observed in any of the samples. These findings coincide with the low N_2-fixation in the Eastern Mediterranean as shown in previous studies.

4.3.3. Alkaline phosphatase activity

Alkaline phosphatase (Pho) is the protein that provides the usage of alternative sources of phosphate (e.g. phosphate esters and phosphonates) at low P concentrations. For this reason, alkaline phosphatase activity in RNA samples was investigated by reference to the expression of PhoX mRNA which is known as the marker gene. PhoX expression was observed to be quite high during the sampling periods where water column PO_4^- concentrations were comparably low (Figure 69). In November, water column integrated PO_4^- values were calculated to be low. The level of mRNA expression in RNA samples taken during this period (R1-R4) was considerably higher than in other periods. The lowest expression level was observed in February samples (R5-R8) during water column PO_4^- concentrations show a homogeneously distributed profile due to the vertical mixing.

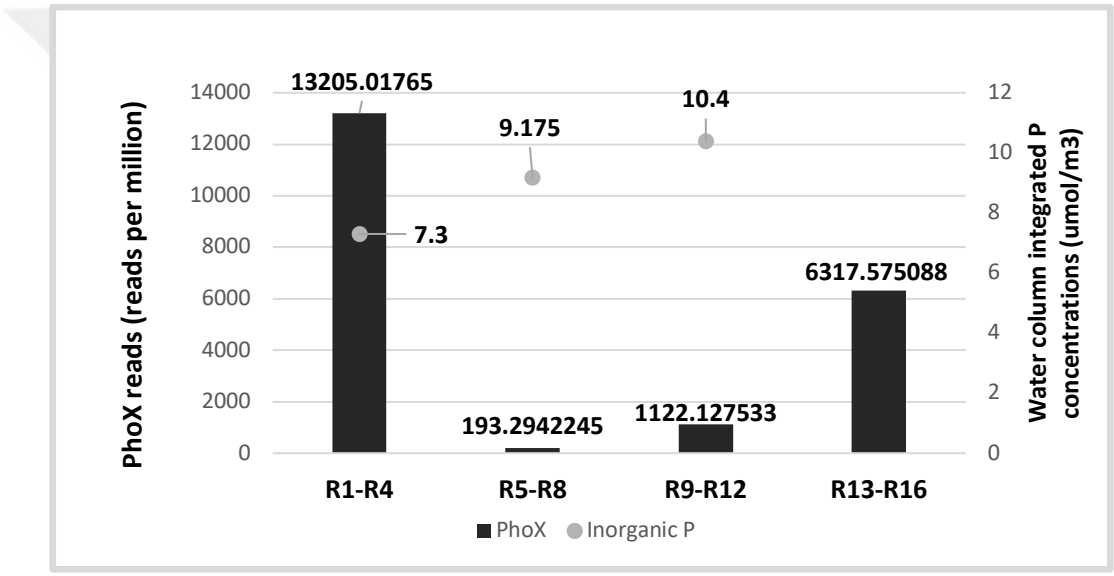

Figure 69. PhoX gene reads (reads/million) in samples and water column integrated inorganic phosphate concentrations. Water column integrated calculations were made by calculating the area between each successive depth of sampling (Moutin and Raimbault, 2002). Since the measurement of inorganic phosphate was not available in February 200 m depth, the measured value at 150 m was used during the calculation.

4.4. Discussion

The energy metabolism in the sample taken in July (R13-R16) is relatively lower compared to November and February. However, it is not possible to make any conclusion why R13-R16 sample has lower relative expressions of energy metabolism. This category includes variety of different energy metabolisms; therefore, it should be considered separately.

There were low concentrations observed in the ammonium profiles in November 2016. This decrease in the concentrations was thought to be due to the high relative expression of the mRNA sequences of the amoA gene. The increased ammonia concentrations observed in February 2017, especially at 25 and 75 m depths, were observed to be consistent with the decrease in the expression of genes responsible for ammonia oxidation. The decrease in ammonia concentrations observed in November was resulted due to ammonia oxidation. Phytoplankton blooms were observed especially in January and March; thus, nitrifying bacteria might have been competing with phytoplankton in terms of resource utilization in November. Previous studies have shown that bacteria responsible for nitrification are readily adaptable to low temperatures (Ward, 2000). It was also discussed that there were seasonal changes in the nitrification in the oceans and higher nitrification in November than in June (Schweiger et al., 2000). Ammonium monooxygenase mRNA expression was higher in the water column in November as consistent with previous studies. Expressions of hao mRNA reads were detected to be zero in all samples. As a conclusion, it is hypothesized that the oxidation of ammonia to nitrite was not completed under the sampling conditions. Hydroxylamine is the intermediate product of two important steps in the nitrogen cycle. Occurs during nitrification, after ammonia oxidation ($NH_4^+ \rightarrow NH_2OH \rightarrow NO_2^- \rightarrow NO^-_3$) and during anaerobic ammonia oxidation ($NO_2^- \rightarrow NH_2OH + NH_4^+ \rightarrow N_2$). Anaerobic ammonia oxidation is not possible at the ETS-200 m station where the sampling was carried out since, the water column is well oxygenated. Also, the mRNA sequences (hzs) of the enzymes performing this reaction were not observed in any of the samples. Based on the results obtained from the mRNA sequences, NH_2OH accumulation was expected in the environment. In addition, water column NH_2OH measurements should be carried out to support this view. Measurements of NH_2OH concentration in the marine environment were carried out only in the Baltic Sea (Schweiger et al., 2007). Study conducted in the Eckernförde Bay (Baltic Sea) and, samples were collected from the depths of 1, 5, 10, 15, 20 and 25 meters where the total water column depth is 28 meters. Anoxic conditions were reported for this site during summer stratification. In the Baltic Sea they reported that ammonia oxidation was much faster than nitrite oxidation and therefore NH_2OH accumulation in the all sampling depths were observed between September and January. The physical and chemical properties of the water column in the Baltic sea and ETS are totally different.

Nxr (nitrite oxidoreductase) was identified in two different groups that were phylogenetically distinct; anammox Nitrospira-Nitrospina and Nitrobacter-Nitrococccus-Nitrolancea groups (Mogollón et al., 2016). According to the results of 16S rRNA amplicon sequencing (Chapter 2), only Nitrospina (Nitrospinaceae; D_5__LS-NOB) from the groups given above was observed in the samples of the present study. The highest observed abundance was 4.6% in July 2017 and in 200 m depth. In addition, only samples taken from 200,150 and 100 m presented this group and especially in the summer months the relative abundance was between 3-4%. The number of samples where the abundance observed above 1% was only 11. In the mRNA samples R9-R12 and R13-R16, the mRNA expression of the nxrA gene was not found even though the relative abundance of the group was around 4%. In the Erdemli Time Series 200 m station where sampling was conducted, it is clear that nitrite concentrations are close to detection limits most of the year. Nitrospina rely on nitrite oxidation and, there was no evidence found to imply other energy-generating pathways for this phylum (Hofer 2018; Pachiadaki et al., 2017). Even if no nitrite oxidation genes were detected, presence of nitrite oxidizing bacteria and their dependence on nitrite oxidation are evident. Expression levels of nxr genes and hao genes might be too low to be detected.

The water column is homogeneously containing low concentrations of nitrate in the samples taken in February (R5-R8). In addition, the concentrations of particulate organic matter, which provide anoxic conditions to bacteria that play a role in nitrate reduction, are also quite low in February. It is suggested here that these are the reasons of low expressions levels of nitrate reductases. There were differences in the relative expressions of nitrate reductases between June (R9-R12) and July (R13-R16) samples. Since there were no measurements of inorganic nutrients during July, the reason of this variation could not be concluded. nirK gene read/million was found to be highest in the November (R1-R4) compared to other samples. Nitrite concentrations in November were close to detection limits along the water column. These low concentrations observed in the nitrite in November might have been due to the high nirK gene mRNA expression. However, since nitric oxide measurements cannot be made within the scope of this study, a more detailed evaluation cannot be made. Regarding the presence of expressions of nitric oxide reductase and nitric oxide dismutase, since nitric oxide (NO) and nitrous oxide (N_2O) measurements were not available, an accurate assessment cannot be made. However, NO is a highly reactive molecule and its solubility in water is quite low (Capone et al., 2008). It has been reported that it reacts very rapidly with O_2, O_2^- and NO_2 in aquatic environments (Zafiriou et al., 1990). At this point, the last two steps of denitrification have been shown to be present but not a dominant process at the sampling station.

PhoX gene activity showed that bacteria adapt to different sources of phosphate at periods of low PO_4^- concentrations in the sampling stations. Since the alkaline phosphatase enzyme

activity allows the use of organic P sources by bacteria, it allows if there was any P-limitation over bacteria. At this point, it can be said that avoiding any speculation, prokaryotes are adapting to this stress by other sources of P in response to low inorganic PO_4-concentrations. It should be noted that since there were a quality and RIN issue during library preparation, samples were combined and as a result they represent water column integrated expressions. It is not possible to make any conclusion if P-limitation present above or below the EZD.

4.5. Conclusion

Messenger RNA expressions of nitrogen cycle marker genes were investigated first time in the north eastern Mediterranean Sea. In order to gain insight into nitrogen cycling in this region power of high-throughput sequencing technology was applied. Metatranscriptomics provides an unprecedented amount of data of gene expression in natural microbial communities. Among this huge amount of data, only nitrogen cycle genes and alkaline phosphatase activity were investigated. Other metabolic pathways such as carbon metabolism cannot be investigated due to time limitations. However, it is highly important to integrate carbon metabolism related gene expressions into the results of this study.

In the first and second chapter, it was aimed to classify ETS-200 m station as open or coastal Mediterranean Sea. Results obtained in this chapter used to answer this question. There were no active N_2 – fixation genes detected. This result indicates that ETS-200 m station cannot be classified as an open sea.

Alkaline phosphatase (Pho) which is expressed under the P limited conditions enabling bacteria to use alternative sources of phosphate. PhoX gene activity showed that some bacteria can adapt to different sources of phosphate at periods of low PO_4^- concentrations. This further supports that the patterns in bacterial communities were best explained by Temperature, Nitrate, Season and EZD but not by the any phosphate sources.

According to the results of mRNA expressions, only genes that are responsible for the first step of nitrification were detected in the samples. This hypothesized the oxidation of ammonia to nitrite is incomplete. However, presence of phylum Nitrospina which has only nitrite oxidation as energy- generating pathway was revealed in the second chapter. This suggests the presence of nitrite oxidation especially at the depth of 200 meters.

GENERAL CONCLUSION

The role of bacteria in the marine biogeochemical cycles has been mostly ignored until recent developments in molecular techniques. Development of high-throughput sequencing technologies revolutionized the microbial ecology studies since it enabled researchers to study dynamic nature of microbes without the requirement of culture dependent methods. In this study power of 16S rRNA amplicon sequencing and metatranscriptomics approaches were integrated with the observed physico-chemical data to investigate community composition and active metabolism of marine bacteria. It was the first-time 16S rRNA amplicon sequencing and metatranscriptomics approaches performed in the north eastern Mediterranean Sea. Species composition, community variation in respect to environmental factors were revealed. Additionally, expression of nitrogen cycle marker genes provided information on how bacteria shape its environment.

Heterotrophic bacteria and prokaryotic autotrophs were clearly dominant the study area by abundance. SAR11 clade which is known to be ubiquous in the marine environment, was also the most dominant taxon in the study region. SAR 11 ecotypes did not show any correlation with particulate organic carbon. However, organic matter oxidation by SAR 11 clade was presented in the other studies. This suggests that heterotrophic SAR 11 clade is the main taxon responsible for dissolved organic matter degradation in the study region. Organic matter produced by primary production becomes dissolved by various mechanisms such as cell lysis and viral shunt. Dissolved organic matter concentrations cannot be measured in our study and, only available data regarding the dissolved form of organic matter is dissolved organic phosphorus. Additionally, this data is not a direct measurement, rather derived from the measurements of total phosphorus, particulate phosphorus and inorganic phosphorus. Due to aforementioned limitations, direct link between particulate organic matter and heterotrophic activities cannot explained.

16S rRNA results showed that during winter, whole water column dominated by one ecotype, Clade Ia. On the other hand, when water column presents stratified conditions Clade Ib observed to be more dominant in the deeper water. Main reason of this ecotype speciation is still unclear. And, possible explanations for the shift in the dominant season cannot be drawn in this study. However, biofilm production and adhesion function were reported for Clade Ib in the study of Jimenez-Infante et al., (2017). This might provide a benefit to this deep-water ecotype to bind to and use aggregated organic matters that sink to the deep water.

Changes in the community composition detected to be determined by temperature, apparent oxygen utilization, nitrate, season and euphotic zone depth. AOU value should not be considered as a driving factor but rather a resulting factor from heterotrophic activities. Other factors suggest that seasonality is the main driving mechanism for community structure.

16S rRNA amplicon sequencing data and metatranscriptomics data showed that remineralization, not nitrogen fixation, is the dominant process. The expression of the genes that are responsible for the first step of nitrification were detected in the samples. It is hypothesized that the oxidation of ammonia to nitrate was not complete and only oxidation of ammonia was present at the sampling station. This also explains why ammonium concentrations were low especially in the deeper water where nitrate concentrations increased. However, metatranscriptomics data cannot explain the elevated concentrations of nitrate in the deeper water during stratified period. 16S rRNA results showed the presence of taxa (*Nitrospinae* phylum) which depends on nitrite oxidation for energy-production. The abundance of this taxa is especially coinciding with the depths where we observed increased concentrations of nitrate. Although, nitrite oxidation genes are not evident in the transcriptomics library, it can be confidently suggested that nitrate accumulation in deeper water is the result of the *Nitrospinae* phylum's activity. This result is particularly important to highlight the importance of integrating genetic studies into chemical measurements in order to explain dynamic interactions in the marine ecosystems.

It has been elaborated throughout this study whether ETS-200 m station presents open ocean or coastal ocean features. Since the expression of nitrogen fixation marker genes were not detected, ETS-200 m station should be classified as more coastal than the open ocean.

Different types of bacteria can inhabit different micro habitats. This study further showed that particle attached community was highly distinct taxa than the free-living counterparts. Impacts of this micro-scale habitats on larger scale ecosystem processes should be addressed in the future studies.

Future study suggestions:

- Molecular studies are now in the centre of most of the disciplines. Even if it is costly and uneasy to perform -omics studies in the oceanographic cruises, integrating samplings of DNA, RNA and proteins to physico-chemical monitoring activities are essential.
- Metatranscriptomics data produced in this study should be further investigated to explain carbon cycle dynamics.

- Due to time limitations, energy metabolism gene expression can be introduced briefly. These genes can be used to highlight all possible energy metabolisms dynamics occurring in this region.
- Usage of molecular markers such as 16S rRNA and marker genes for specific metabolisms should be extended for other components of ecosystems such as eukaryotic phytoplankton.
- The global importance of micro-habitats and small-scale processes on ecosystem level interactions should be addressed.

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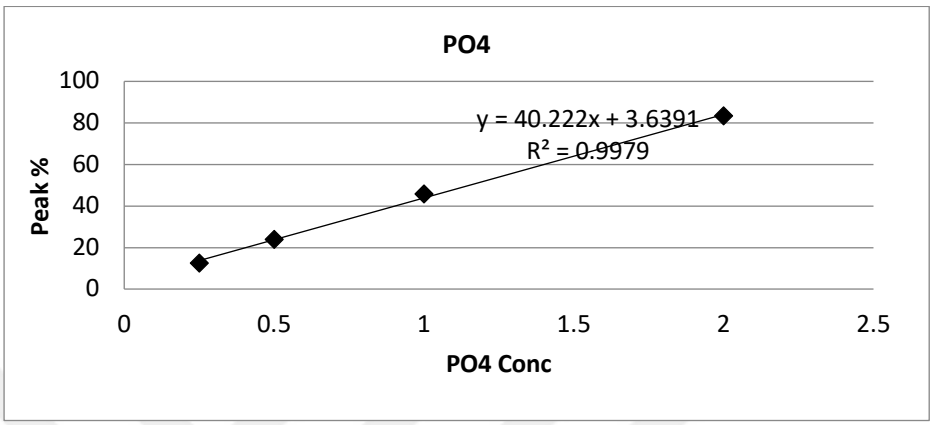
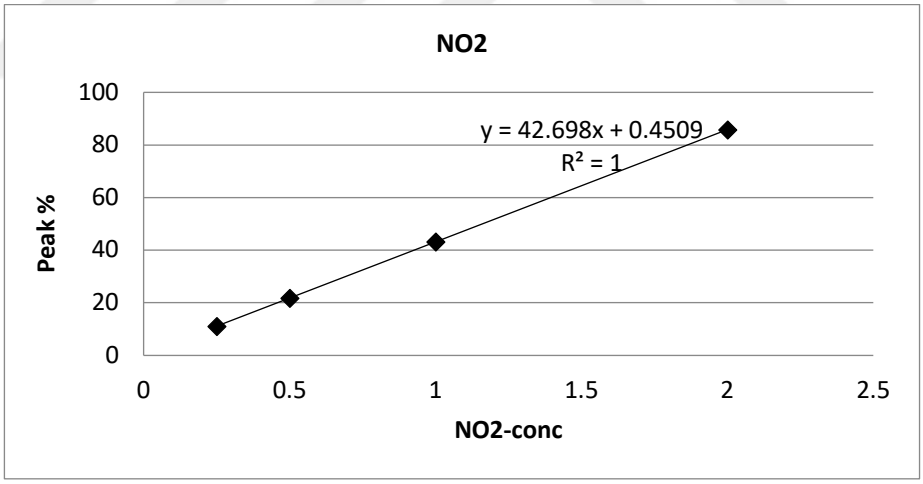
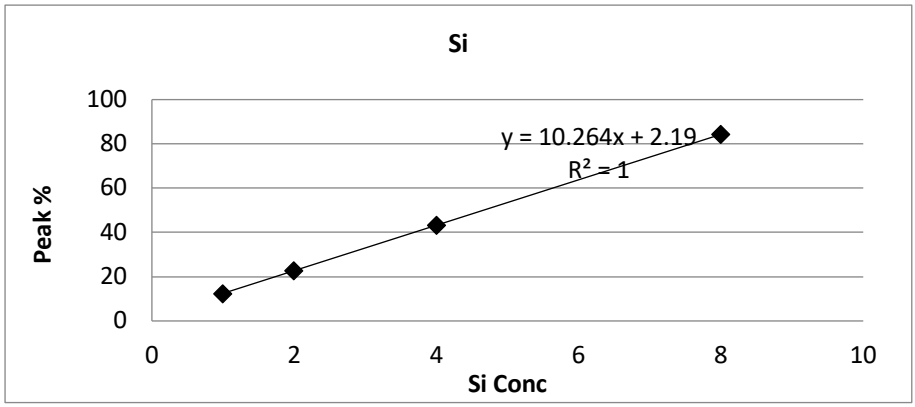
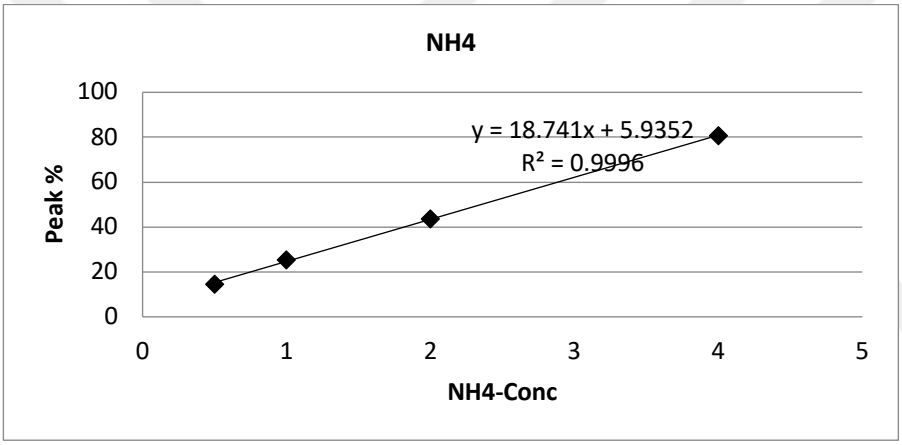
Appendix A: Standard Curves of Inorganic Nutrient Measurements

Standard curves of inorganic nutrient concentrations were given below figures. Below figures represents nutrient concentrations versus Peak % - Baseline.



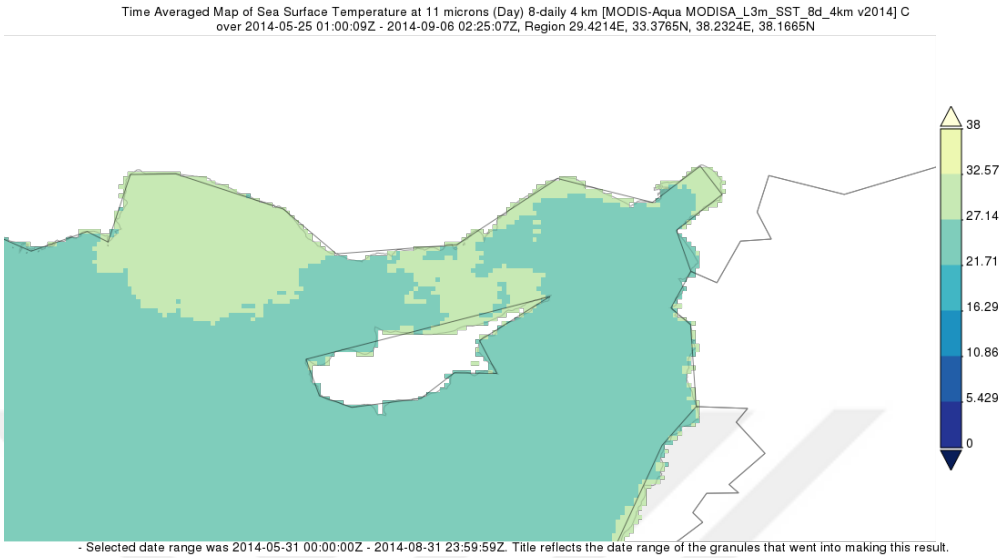



Si
100
80
60
40
20
0
Peak %
y = 10.264x + 2.19
R² = 1
0
2
4
6
8
10
Si Conc

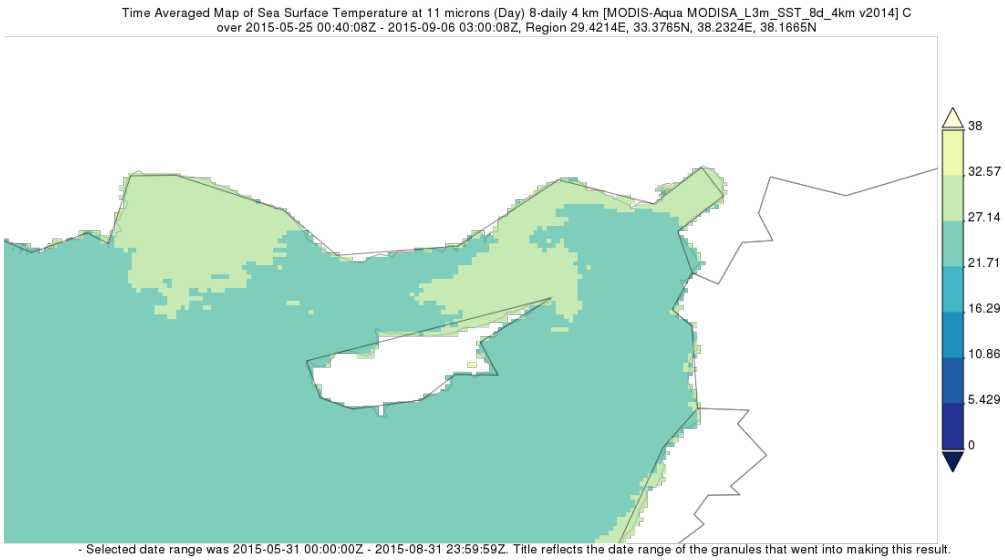

NH4
100
80
60
40
20
0
Peak %
y = 18.741x + 5.9352
R² = 0.9996
0
1
2
3
4
5
NH4-Conc

Appendix B: SST comparison between 2014 and 2015

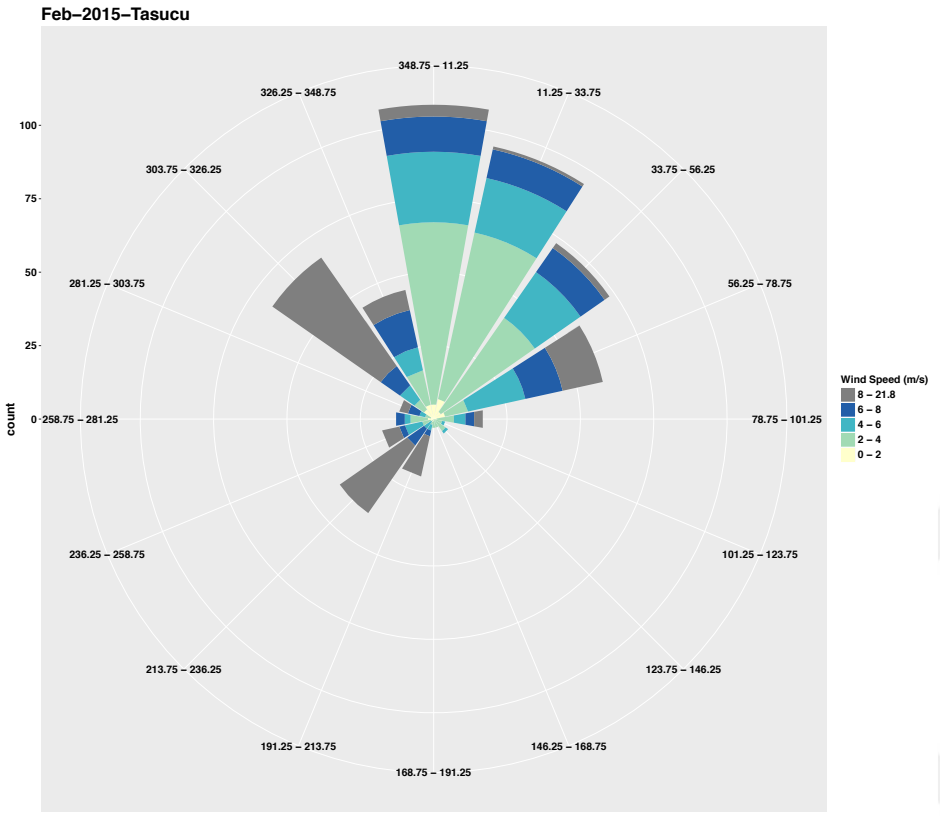
SST map of the region in 2014

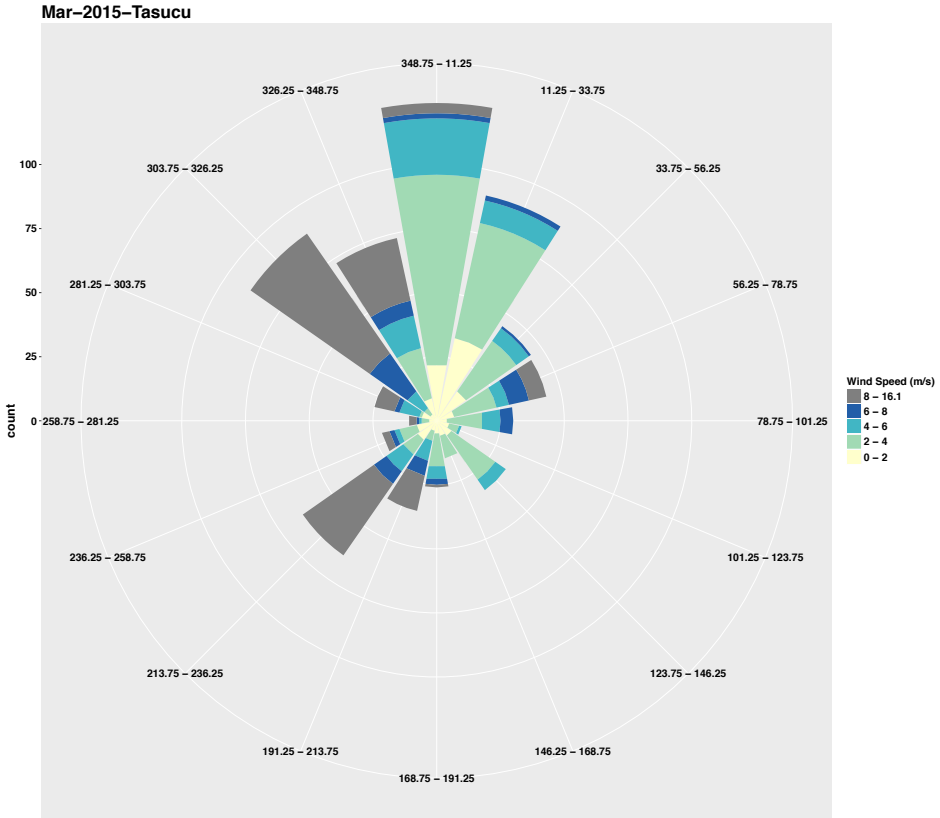


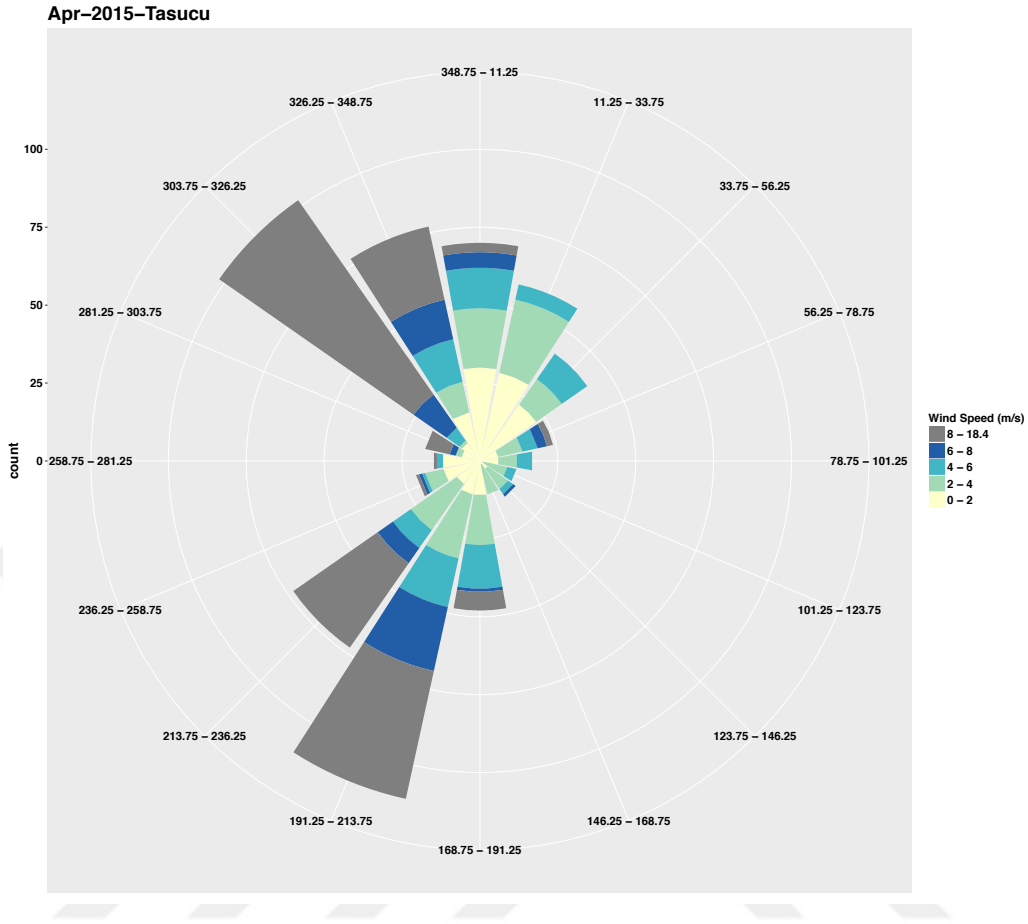
SST map of the region in 2015



Appendix C: Dominant wind directions and speed in the region






Apr–2015–Tasucu
348.75 – 11.25
11.25 – 33.75
33.75 – 56.25
56.25 – 78.75
78.75 – 101.25
101.25 – 123.75
123.75 – 146.25
146.25 – 168.75
168.75 – 191.25
191.25 – 213.75
213.75 – 236.25
236.25 – 258.75
258.75 – 281.25
281.25 – 303.75
303.75 – 326.25
326.25 – 348.75
count
100
75
50
25
0
Wind Speed (m/s)
8 – 18.4
6 – 8
4 – 6
2 – 4
0 – 2

Appendix D: Trimmed Data Statistics

Software: Illumina package bcl2fastq v1.8.4

Fastq Quality Encoding: Sanger Quality

(ASCII Character Code = Phred Quality Value + 33)

Sample	Total Bases	Read Count	GC (%)	AT (%)	Q20 (%)	Q30 (%)
11	176,889,477	589,624	50.79	49.21	86.10	76.15
12	151,888,409	506,404	51.05	48.96	85.55	75.49
13	103,575,909	345,266	51.50	48.50	85.62	75.66
14	132,985,882	444,830	52.83	47.17	79.56	67.73
15	183,201,222	611,558	52.52	47.48	84.38	73.95
16	167,437,112	559,128	53.49	46.51	84.14	73.74
21	150,946,925	503,392	51.29	48.71	85.65	75.55
22	150,338,270	501,226	51.02	48.98	85.85	75.84
23	160,229,372	534,192	51.51	48.49	85.56	75.47
24	169,536,444	565,474	51.88	48.12	85.25	75.06
25	160,138,513	534,410	52.63	47.37	85.05	74.89
26	174,988,055	584,366	53.47	46.53	84.22	73.85
31	168,192,996	560,578	50.86	49.14	86.49	76.65
32	155,589,262	518,466	50.80	49.20	86.64	76.83
33	142,753,668	476,104	51.78	48.22	85.33	75.15
34	170,484,209	568,132	50.80	49.20	86.53	76.72
35	144,680,315	482,882	52.41	47.59	84.90	74.70
36	151,246,652	504,822	52.79	47.21	84.76	74.53
51	173,732,544	579,232	51.35	48.65	86.30	76.37
52	178,745,009	595,990	51.59	48.41	85.71	75.65
53	189,117,942	631,140	52.14	47.86	84.38	74.02
54	199,435,778	665,032	51.55	48.45	85.85	75.86
55	170,187,524	567,544	51.54	48.46	85.93	75.94
56	181,769,230	606,158	51.12	48.88	85.57	75.48
61	178,377,832	594,740	50.93	49.07	85.82	75.72
62	181,123,189	603,892	50.95	49.05	85.78	75.75
63	170,113,474	567,094	50.70	49.30	86.14	76.12
64	146,411,579	488,038	50.72	49.28	86.33	76.27
65	170,806,741	569,222	50.30	49.70	87.06	77.15
66	178,168,577	593,942	50.33	49.68	86.26	76.14
71	161,071,551	536,674	50.55	49.45	86.31	76.34
72	157,761,737	525,616	50.76	49.24	86.11	76.10
73	146,162,037	486,970	50.65	49.35	86.28	76.28
74	158,339,377	527,762	50.43	49.57	86.48	76.45
75	156,983,741	523,290	50.45	49.55	86.34	76.22

76	161,688,887	539,034	50.45	49.55	86.44	76.38
81	171,173,011	570,334	50.17	49.83	86.92	77.06
82	164,223,635	547,114	50.89	49.11	86.41	76.48
83	173,049,976	576,792	51.07	48.93	85.42	75.10
84	179,259,617	597,474	51.11	48.90	86.13	76.10
85	121,166,667	403,618	51.48	48.52	88.27	81.03
86	126,285,676	420,886	52.56	47.44	87.77	80.49
91	113,146,113	376,728	51.34	48.66	88.54	81.46
92	120,895,459	402,412	50.69	49.31	89.15	82.13
93	107,611,188	358,138	50.49	49.51	89.68	82.88
94	100,559,276	334,830	50.77	49.24	88.62	81.42
95	130,922,767	436,232	51.87	48.13	88.15	80.92
96	118,518,430	395,072	52.76	47.24	87.60	80.22
101	110,749,625	368,782	51.21	48.79	88.62	81.49
102	129,675,361	431,678	50.46	49.54	89.06	82.06
103	135,570,175	451,278	50.49	49.51	89.22	82.29
104	119,489,023	397,782	50.90	49.10	88.44	81.34
106	119,196,201	397,218	53.33	46.67	87.70	80.35
111	110,071,872	366,376	50.48	49.52	89.18	82.22
112	117,389,326	390,736	50.67	49.33	89.15	82.21
113	110,140,238	366,658	50.58	49.42	89.35	82.40
114	117,351,242	390,680	51.07	48.93	88.35	81.20
115	112,730,771	375,464	51.50	48.50	88.51	81.34
116	112,682,264	375,708	53.33	46.67	87.29	79.86
121	141,141,039	470,088	50.85	49.15	88.10	80.94
122	123,197,469	410,162	50.84	49.16	88.69	81.63
123	128,281,862	427,084	51.05	48.95	88.80	81.72
124	148,505,397	494,372	51.06	48.94	88.99	81.96
125	111,437,934	371,438	51.86	48.14	87.0	78.94
126	127,208,608	424,108	53.27	46.74	87.28	79.78

Sample ID : Sample name.

Total read bases : Total number of bases sequenced.

Total reads : Total number of reads. In illumina paired-end sequencing, read1 and read2 are added.

GC(%) : GC content.

AT(%) : AT content.

Q20(%) : Ratio of reads that have phred quality score of over 20.

Q30(%) : Ratio of reads that have phred quality score of over 30.

Appendix E: Whole List of Assigned Taxonomies at Taxonomic Level 6

D_0__Archaea;D_1__Euryarchaeota;D_2__Thermoplasmata;D_3__Marine Group II;D_4__uncultured marine archaeon;D_5__uncultured marine archaeon

D_0__Archaea;D_1__Euryarchaeota;D_2__Thermoplasmata;D_3__Marine Group II;__;__

D_0__Archaea;D_1__Euryarchaeota;D_2__Thermoplasmata;D_3__Marine Group III;Ambiguous_taxa;Ambiguous_taxa

D_0__Archaea;D_1__Thaumarchaeota;D_2__Nitrososphaeria;D_3__Nitrosopumilales;D_4__Nitrosopumilaceae;D_5__Candidatus Nitrosopelagicus

D_0__Bacteria;D_1__Acidobacteria;D_2__Acidobacteriia;D_3__Subgroup 2;Ambiguous_taxa;Ambiguous_taxa

D_0__Bacteria;D_1__Actinobacteria;D_2__Acidimicrobiia;D_3__Actinomarinales;D_4__Actinomarinaceae;D_5__Candidatus Actinomarina

D_0__Bacteria;D_1__Actinobacteria;D_2__Acidimicrobiia;D_3__Microtrichales;D_4__Microtrichaceae;D_5__Sva0996 marine group

D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Micrococcales;D_4__Microbacteriaceae;__

D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__PeM15;__;__

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Chitinophagales;D_4__Chitinophagaceae;D_5__Asinibacterium

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Chitinophagales;D_4__Saprospiraceae;D_5__Aureispira

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Chitinophagales;D_4__Saprospiraceae;D_5__uncultured

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Chitinophagales;D_4__uncultured;Ambiguous_taxa

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Cytophagales;D_4__Cyclobacteriaceae;D_5__Fabibacter

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Cytophagales;D_4__Cyclobacteriaceae;D_5__Marinoscillum

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Cytophagales;D_4__Cyclobacteriaceae;D_5__uncultured

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__Crocinitomicaceae;D_5__Crocinitomix

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__Crocinitomicaceae;D_5__Fluviicola

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__Crocinitomicaceae;D_5__Salinirepens

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__Crocinitomicaceae;__

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__Cryomorphaceae;D_5__NS10 marine group

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__Cryomorphaceae;D_5__uncultured

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__Flavobacteriaceae;D_5__Flavobacterium

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__Flavobacteriaceae;D_5__Formosa

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__Flavobacteriaceae;D_5__Leeuwenhoekiella

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__Flavobacteriaceae;D_5__NS2b marine group

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__Flavobacteriaceae;D_5__NS3a marine group

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__Flavobacteriaceae;D_5__NS4 marine group

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__Flavobacteriaceae;D_5__NS5 marine group

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__Flavobacteriaceae;D_5__Tenacibaculum

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__Flavobacteriaceae;D_5__Ulvibacter

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__Flavobacteriaceae;D_5__uncultured

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__Flavobacteriaceae;__

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__NS7 marine group;Ambiguous_taxa

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__NS7 marine group;D_5__uncultured bacterium

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__NS7 marine group;__

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__NS9 marine group;Ambiguous_taxa

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__NS9 marine group;D_5__hydrothermal vent metagenome

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__NS9 marine group;D_5__uncultured marine bacterium

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__NS9 marine group;__

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;__;__

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Sphingobacteriales;D_4__Lentimicrobiaceae;D_5__uncultured deep-sea bacterium

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Sphingobacteriales;D_4__Lentimicrobiaceae;__

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Sphingobacteriales;D_4__NS11-12 marine group;Ambiguous_taxa

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Sphingobacteriales;D_4__NS11-12 marine group;D_5__unidentified marine bacterioplankton

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Sphingobacteriales;D_4__NS11-12 marine group;__

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Sphingobacteriales;__;__

D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;__;__;__

D_0__Bacteria;D_1__Bacteroidetes;D_2__Rhodothermia;D_3__Balneolales;D_4__Balneolaceae;D_5__Balneola

D_0__Bacteria;D_1__Bacteroidetes;D_2__Rhodothermia;D_3__Balneolales;D_4__Balneolaceae;D_5__uncultured

D_0__Bacteria;D_1__Chlamydiae;D_2__Chlamydiae;D_3__Chlamydiales;D_4__Chlamydiaceae;D_5__uncultured

D_0__Bacteria;D_1__Chlamydiae;D_2__Chlamydiae;D_3__Chlamydiales;D_4__Simkaniaceae;D_5__Candidatus Fritschea

D_0__Bacteria;D_1__Chlamydiae;D_2__Chlamydiae;D_3__Chlamydiales;D_4__Simkaniaceae;D_5__uncultured

D_0__Bacteria;D_1__Chloroflexi;D_2__Dehalococcoidia;D_3__SAR202 clade;Ambiguous_taxa;Ambiguous_taxa

D_0__Bacteria;D_1__Chloroflexi;D_2__Dehalococcoidia;D_3__SAR202 clade;D_4__marine metagenome;D_5__marine metagenome

D_0__Bacteria;D_1__Chloroflexi;D_2__Dehalococcoidia;D_3__SAR202 clade;D_4__uncultured Chloroflexi bacterium;D_5__uncultured Chloroflexi bacterium

D_0__Bacteria;D_1__Chloroflexi;D_2__Dehalococcoidia;D_3__SAR202 clade;D_4__uncultured bacterium;D_5__uncultured bacterium

D_0__Bacteria;D_1__Chloroflexi;D_2__Dehalococcoidia;D_3__SAR202 clade;D_4__uncultured deep-sea bacterium;D_5__uncultured deep-sea bacterium

D_0__Bacteria;D_1__Chloroflexi;D_2__Dehalococcoidia;D_3__SAR202 clade;D_4__uncultured marine microorganism;D_5__uncultured marine microorganism

D_0__Bacteria;D_1__Chloroflexi;D_2__Dehalococcoidia;D_3__SAR202 clade;__;__

D_0__Bacteria;D_1__Chloroflexi;__;__;__;__

D_0__Bacteria;D_1__Cyanobacteria;D_2__Oxyphotobacteria;D_3__Synechococcales;D_4__Cyanobiaceae;D_5__Cyanobium PCC-6307

D_0__Bacteria;D_1__Cyanobacteria;D_2__Oxyphotobacteria;D_3__Synechococcales;D_4__Cyanobiaceae;D_5__Prochlorococcus MIT9313

D_0__Bacteria;D_1__Cyanobacteria;D_2__Oxyphotobacteria;D_3__Synechococcales;D_4__Cyanobiaceae;D_5__Synechococcus CC9902

D_0__Bacteria;D_1__Cyanobacteria;D_2__Oxyphotobacteria;D_3__Synechococcales;D_4__Cyanobiaceae;__

D_0__Bacteria;D_1__Cyanobacteria;D_2__Oxyphotobacteria;__;__;__

D_0__Bacteria;D_1__Dadabacteria;D_2__Dadabacteriia;D_3__Dadabacteriales;Ambiguous_taxa;Ambiguous_taxa

D_0__Bacteria;D_1__Dadabacteria;D_2__Dadabacteriia;D_3__Dadabacteriales;D_4__uncultured marine bacterium;D_5__uncultured marine bacterium

D_0__Bacteria;D_1__Dadabacteria;D_2__Dadabacteriia;D_3__Dadabacteriales;__;__

D_0__Bacteria;D_1__Deinococcus-Thermus;D_2__Deinococci;D_3__Deinococcales;D_4__Trueperaceae;D_5__Truepera

D_0__Bacteria;D_1__Epsilonbacteraeota;D_2__Campylobacteria;D_3__Campylobacterales;D_4__Arcobacteraceae;D_5__Arcobacter

D_0__Bacteria;D_1__Fibrobacteres;D_2__Fibrobacteria;D_3__Fibrobacterales;D_4__Fibrobacteraceae;D_5__uncultured

D_0__Bacteria;D_1__Kiritimatiellaeota;D_2__Kiritimatiellae;D_3__Kiritimatiellales;D_4__Kiritimatiellaceae;D_5__R76-B128

D_0__Bacteria;D_1__Margulisbacteria;D_2__metagenome;D_3__metagenome;D_4__metagenome;D_5__metagenome

D_0__Bacteria;D_1__Margulisbacteria;D_2__uncultured bacterium;D_3__uncultured bacterium;D_4__uncultured bacterium;D_5__uncultured bacterium

D_0__Bacteria;D_1__Marinimicrobia (SAR406 clade);Ambiguous_taxa;Ambiguous_taxa;Ambiguous_taxa;Ambiguous_taxa

D_0__Bacteria;D_1__Marinimicrobia (SAR406 clade);D_2__Alteromonas macleodii;D_3__Alteromonas macleodii;D_4__Alteromonas macleodii;D_5__Alteromonas macleodii

D_0__Bacteria;D_1__Marinimicrobia (SAR406 clade);D_2__marine metagenome;D_3__marine metagenome;D_4__marine metagenome;D_5__marine metagenome

D_0__Bacteria;D_1__Marinimicrobia (SAR406 clade);D_2__metagenome;D_3__metagenome;D_4__metagenome;D_5__metagenome

D_0__Bacteria;D_1__Marinimicrobia (SAR406 clade);D_2__uncultured Candidatus Marinimicrobia bacterium;D_3__uncultured Candidatus Marinimicrobia bacterium;D_4__uncultured Candidatus Marinimicrobia bacterium;D_5__uncultured Candidatus Marinimicrobia bacterium

D_0__Bacteria;D_1__Marinimicrobia (SAR406 clade);D_2__uncultured Fibrobacteres/Acidobacteria group bacterium;D_3__uncultured Fibrobacteres/Acidobacteria group bacterium;D_4__uncultured Fibrobacteres/Acidobacteria group bacterium;D_5__uncultured Fibrobacteres/Acidobacteria group bacterium

D_0__Bacteria;D_1__Marinimicrobia (SAR406 clade);D_2__uncultured bacterium;D_3__uncultured bacterium;D_4__uncultured bacterium;D_5__uncultured bacterium

D_0__Bacteria;D_1__Marinimicrobia (SAR406 clade);D_2__uncultured marine bacterium;D_3__uncultured marine bacterium;D_4__uncultured marine bacterium;D_5__uncultured marine bacterium

D_0__Bacteria;D_1__Marinimicrobia (SAR406 clade);__;__;__;__

D_0__Bacteria;D_1__Nitrospinae;D_2__Nitrospinia;D_3__Nitrospinales;D_4__Nitrospinaceae;D_5__LS-NOB

D_0__Bacteria;D_1__Nitrospinae;D_2__P9X2b3D02;D_3__uncultured bacterium;D_4__uncultured bacterium;D_5__uncultured bacterium

D_0__Bacteria;D_1__Planctomycetes;D_2__OM190;Ambiguous_taxa;Ambiguous_taxa;Ambiguous_taxa

D_0__Bacteria;D_1__Planctomycetes;D_2__Phycisphaerae;D_3__Phycisphaerales;D_4__Phycisphaeraceae;D_5__CL500-3

D_0__Bacteria;D_1__Planctomycetes;D_2__Phycisphaerae;D_3__Phycisphaerales;D_4__Phycisphaeraceae;D_5__FS140-16B-02 marine group

D_0__Bacteria;D_1__Planctomycetes;D_2__Phycisphaerae;D_3__Phycisphaerales;D_4__Phycisphaeraceae;D_5__JL-ETNP-F27

D_0__Bacteria;D_1__Planctomycetes;D_2__Phycisphaerae;D_3__Phycisphaerales;D_4__Phycisphaeraceae;D_5__Urania-1B-19 marine sediment group

D_0__Bacteria;D_1__Planctomycetes;D_2__Pla3 lineage;Ambiguous_taxa;Ambiguous_taxa;Ambiguous_taxa

D_0__Bacteria;D_1__Planctomycetes;D_2__Planctomycetacia;D_3__Pirellulales;D_4__Pirellulaceae;D_5__Blastopirellula

D_0__Bacteria;D_1__Planctomycetes;D_2__Planctomycetacia;D_3__Pirellulales;D_4__Pirellulaceae;D_5__Pir4 lineage

D_0__Bacteria;D_1__Planctomycetes;D_2__Planctomycetacia;D_3__Pirellulales;D_4__Pirellulaceae;D_5__Pirellula

D_0__Bacteria;D_1__Planctomycetes;D_2__Planctomycetacia;D_3__Pirellulales;D_4__Pirellulaceae;D_5__Rhodopirellula

D_0__Bacteria;D_1__Planctomycetes;D_2__Planctomycetacia;D_3__Pirellulales;D_4__Pirellulaceae;D_5__Rubripirellula

D_0__Bacteria;D_1__Planctomycetes;D_2__Planctomycetacia;D_3__Pirellulales;D_4__Pirellulaceae;D_5__uncultured

D_0__Bacteria;D_1__Planctomycetes;D_2__Planctomycetacia;D_3__Pirellulales;D_4__Pirellulaceae;__

D_0__Bacteria;D_1__Planctomycetes;D_2__Planctomycetacia;D_3__Planctomycetales;D_4__Gimesiaceae;D_5__uncultured

D_0__Bacteria;D_1__Planctomycetes;D_2__Planctomycetacia;D_3__Planctomycetales;D_4__Rubinisphaeraceae;D_5__uncultured

D_0__Bacteria;D_1__Planctomycetes;D_2__Planctomycetacia;D_3__Planctomycetales;D_4__Rubinisphaeraceae;__

D_0__Bacteria;D_1__Planctomycetes;D_2__Planctomycetacia;D_3__Planctomycetales;D_4__uncultured;Ambiguous_taxa

D_0__Bacteria;D_1__Planctomycetes;D_2__Planctomycetacia;D_3__Planctomycetales;D_4__uncultured;__

D_0__Bacteria;D_1__Poribacteria;Ambiguous_taxa;Ambiguous_taxa;Ambiguous_taxa;Ambiguous_taxa

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Caulobacterales;D_4__Caulobacteraceae;D_5__Brevundimonas

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Caulobacterales;D_4__Hyphomonadaceae;D_5__Hyphomonas

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Caulobacterales;D_4__Hyphomonadaceae;D_5__Maricaulis

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Caulobacterales;D_4__Hyphomonadaceae;D_5__uncultured

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Micavibrionales;D_4__Micavibrionaceae;D_5__uncultured

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Parvibaculales;D_4__OCS116 clade;D_5__uncultured marine bacterium

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Parvibaculales;D_4__OCS116 clade;__

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Parvibaculales;D_4__PS1 clade;__

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Parvibaculales;D_4__Parvibaculaceae;D_5__uncultured

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Puniceispirillales;D_4__SAR116 clade;Ambiguous_taxa

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Puniceispirillales;D_4__SAR116 clade;D_5__Candidatus Puniceispirillum

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Puniceispirillales;D_4__SAR116 clade;D_5__uncultured Oceanibaculum sp.

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Puniceispirillales;D_4__SAR116 clade;D_5__uncultured alpha proteobacterium

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Puniceispirillales;D_4__SAR116 clade;D_5__uncultured marine bacterium

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Puniceispirillales;D_4__SAR116 clade;__

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Puniceispirillales;D_4__uncultured;__

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhizobiales;D_4__Xanthobacteraceae;D_5__Bradyrhizobium

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhodobacterales;D_4__Rhodobacteraceae;D_5__Ascidiaceihabitans

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhodobacterales;D_4__Rhodobacteraceae;D_5__Planktomarina

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhodobacterales;D_4__Rhodobacteraceae;D_5__Rhodovulum

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhodobacterales;D_4__Rhodobacteraceae;D_5__uncultured

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhodobacterales;D_4__Rhodobacteraceae;__

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhodospirillales;D_4__AEGEAN-169 marine group;Ambiguous_taxa

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhodospirillales;D_4__AEGEAN-169 marine group;D_5__uncultured bacterium

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhodospirillales;D_4__AEGEAN-169 marine group;D_5__uncultured marine bacterium

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhodospirillales;D_4__AEGEAN-169 marine group;__

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhodospirillales;D_4__Magnetospiraceae;D_5__Magnetospira

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhodospirillales;D_4__Magnetospiraceae;D_5__uncultured

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhodospirillales;D_4__Magnetospiraceae;__

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhodospirillales;D_4__Rhodospirillaceae;D_5__uncultured

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhodospirillales;D_4__Terasakiellaceae;D_5__uncultured

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhodovibrionales;D_4__Kiloniellaceae;D_5__uncultured

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rickettsiales;D_4__Midichloriaceae;D_5__MD3-55

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rickettsiales;D_4__Rickettsiaceae;D_5__uncultured

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rickettsiales;D_4__Rickettsiaceae;__

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rickettsiales;D_4__S25-593;D_5__marine metagenome

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rickettsiales;D_4__S25-593;D_5__uncultured bacterium

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rickettsiales;D_4__S25-593;D_5__uncultured marine bacterium

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rickettsiales;D_4__S25-593;__

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rickettsiales;D_4__uncultured bacterium;D_5__uncultured bacterium

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__SAR11 clade;D_4__Clade I;D_5__Clade Ia

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__SAR11 clade;D_4__Clade I;D_5__Clade Ib

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__SAR11 clade;D_4__Clade I;D_5__uncultured

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__SAR11 clade;D_4__Clade I;__

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__SAR11 clade;D_4__Clade II;D_5__unidentified marine bacterioplankton

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__SAR11 clade;D_4__Clade II;__

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__SAR11 clade;D_4__Clade III;Ambiguous_taxa

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__SAR11 clade;D_4__Clade III;__

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__SAR11 clade;D_4__Clade IV;Ambiguous_taxa

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__SAR11 clade;D_4__Clade IV;D_5__uncultured bacterium

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__SAR11 clade;D_4__Clade IV;__

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__SAR11 clade;D_4__uncultured;D_5__uncultured bacterium

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__SAR11 clade;__;__

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Sneathiellales;D_4__Sneathiellaceae;D_5__AT-s3-44

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Sphingomonadales;D_4__Sphingomonadaceae;D_5__Erythrobacter

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Thalassobaculales;D_4__Nisaeaceae;D_5__OM75 clade

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__uncultured;Ambiguous_taxa;Ambiguous_taxa

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__uncultured;D_4__uncultured bacterium;D_5__uncultured bacterium

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__uncultured;D_4__uncultured marine bacterium;D_5__uncultured marine bacterium

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__uncultured;__;__

D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;__;__;__

D_0__Bacteria;D_1__Proteobacteria;D_2__Deltaproteobacteria;D_3__Bdellovibrionales;D_4__Bacteriovoracaceae;D_5__uncultured

D_0__Bacteria;D_1__Proteobacteria;D_2__Deltaproteobacteria;D_3__Bdellovibrionales;D_4__Bdellovibrionaceae;D_5__OM27 clade

D_0__Bacteria;D_1__Proteobacteria;D_2__Deltaproteobacteria;D_3__Myxococcales;D_4__P3OB-42;D_5__uncultured bacterium

D_0__Bacteria;D_1__Proteobacteria;D_2__Deltaproteobacteria;D_3__NB1-j;__;__

D_0__Bacteria;D_1__Proteobacteria;D_2__Deltaproteobacteria;D_3__Oligoflexales;D_4__Oligoflexaceae;D_5__uncultured

D_0__Bacteria;D_1__Proteobacteria;D_2__Deltaproteobacteria;D_3__PB19;Ambiguous_taxa;Ambiguous_taxa

D_0__Bacteria;D_1__Proteobacteria;D_2__Deltaproteobacteria;D_3__PB19;D_4__uncultured delta proteobacterium;D_5__uncultured delta proteobacterium

D_0__Bacteria;D_1__Proteobacteria;D_2__Deltaproteobacteria;D_3__SAR324 clade(Marine group B);Ambiguous_taxa;Ambiguous_taxa

D_0__Bacteria;D_1__Proteobacteria;D_2__Deltaproteobacteria;D_3__SAR324 clade(Marine group B);D_4__uncultured marine bacterium;D_5__uncultured marine bacterium

D_0__Bacteria;D_1__Proteobacteria;D_2__Deltaproteobacteria;D_3__SAR324 clade(Marine group B);__;__

D_0__Bacteria;D_1__Proteobacteria;D_2__Deltaproteobacteria;__;__;__

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Alteromonadales;D_4__Alteromonadaceae;D_5__Alteromonas

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Alteromonadales;D_4__Alteromonadaceae;D_5__Glaciecola

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Alteromonadales;D_4__Idiomarinaceae;D_5__Idiomarina

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Alteromonadales;D_4__Pseudoalteromonadaceae;D_5__Algicola

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Alteromonadales;D_4__Pseudoalteromonadaceae;D_5__uncultured

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Alteromonadales;D_4__Shewanellaceae;D_5__Shewanella

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Alteromonadales;__;__

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Arenicellales;D_4__Arenicellaceae;D_5__uncultured

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales;D_4__Burkholderiaceae;D_5__MWH-UniP1 aquatic group

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales;D_4__Methylophilaceae;D_5__OM43 clade

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales;D_4__Nitrosomonadaceae;D_5__IS-44

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales;D_4__Nitrosomonadaceae;D_5__mle1-7

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales;D_4__Nitrosomonadaceae;__

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Cellvibrionales;D_4__Halieaceae;D_5__OM60(NOR5) clade

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Cellvibrionales;D_4__Porticoccaceae;D_5__SAR92 clade

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Cellvibrionales;D_4__Spongiibacteraceae;D_5__BD1-7 clade

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Cellvibrionales;D_4__Spongiibacteraceae;__

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Coxiellales;D_4__Coxiellaceae;D_5__Coxiella

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__EPR3968-O8a-Bc78;D_4__uncultured bacterium;D_5__uncultured bacterium

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__EPR3968-O8a-Bc78;D_4__uncultured gamma proteobacterium;D_5__uncultured gamma proteobacterium

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Ectothiorhodospirales;D_4__Ectothiorhodospiraceae;D_5__uncultured

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Francisellales;D_4__Francisellaceae;D_5__uncultured

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Ga0077536;D_4__uncultured bacterium;D_5__uncultured bacterium

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Ga0077536;__;__

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Gammaproteobacteria Incertae Sedis;D_4__Unknown Family;D_5__uncultured marine bacterium

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__HOC36;Ambiguous_taxa;Ambiguous_taxa

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__HOC36;D_4__uncultured marine bacterium;D_5__uncultured marine bacterium

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__HOC36;__;__

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__KI89A clade;Ambiguous_taxa;Ambiguous_taxa

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__KI89A clade;D_4__uncultured bacterium;D_5__uncultured bacterium

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__KI89A clade;__;__

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Nitrosococcales;D_4__Nitrosococcaceae;D_5__Cm1-21

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__OM182 clade;Ambiguous_taxa;Ambiguous_taxa

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__OM182 clade;D_4__uncultured bacterium;D_5__uncultured bacterium

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__OM182 clade;D_4__uncultured gamma proteobacterium;D_5__uncultured gamma proteobacterium

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__OM182 clade;D_4__uncultured marine microorganism;D_5__uncultured marine microorganism

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__OM182 clade;__;__

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Oceanospirillales;D_4__Alcanivoracaceae;D_5__Alcanivorax

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Oceanospirillales;D_4__Litoricolaceae;D_5__Litoricola

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Oceanospirillales;D_4__Nitrincolaceae;D_5__uncultured

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Oceanospirillales;D_4__Pseudohongiellaceae;D_5__Pseudohongiella

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Piscirickettsiales;D_4__Piscirickettsiaceae;D_5__Candidatus Endoecteinascidia

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Pseudomonadales;D_4__Pseudomonadaceae;D_5__Pseudomonas

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__SAR86 clade;Ambiguous_taxa;Ambiguous_taxa

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__SAR86 clade;D_4__marine metagenome;D_5__marine metagenome

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__SAR86 clade;D_4__metagenome;D_5__metagenome

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__SAR86 clade;D_4__uncultured SAR86 cluster bacterium;D_5__uncultured SAR86 cluster bacterium

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__SAR86 clade;D_4__uncultured bacterium;D_5__uncultured bacterium

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__SAR86 clade;D_4__uncultured gamma proteobacterium;D_5__uncultured gamma proteobacterium

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__SAR86 clade;D_4__uncultured marine bacterium;D_5__uncultured marine bacterium

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__SAR86 clade;__;__

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Steroidobacterales;D_4__Woeseiaceae;D_5__Woeseia

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Tenderiales;D_4__Tenderiaceae;D_5__Candidatus Tenderia

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Thiomicrospirales;D_4__Thioglobaceae;D_5__SUP05 cluster

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Thiotrichales;D_4__Thiotrichaceae;D_5__uncultured

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__UBA10353 marine group;Ambiguous_taxa;Ambiguous_taxa

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__UBA10353 marine group;__;__

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Vibrionales;D_4__Vibrionaceae;D_5__Photobacterium

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Vibrionales;D_4__Vibrionaceae;D_5__Vibrio

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Vibrionales;D_4__Vibrionaceae;__

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Xanthomonadales;D_4__Xanthomonadaceae;D_5__Stenotrophomonas

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;__;__;__

D_0__Bacteria;D_1__Proteobacteria;__;__;__;__

D_0__Bacteria;D_1__Verrucomicrobia;D_2__Verrucomicrobiae;D_3__Arctic97B-4 marine group;Ambiguous_taxa;Ambiguous_taxa

D_0__Bacteria;D_1__Verrucomicrobia;D_2__Verrucomicrobiae;D_3__Arctic97B-4 marine group;D_4__metagenome;D_5__metagenome

D_0__Bacteria;D_1__Verrucomicrobia;D_2__Verrucomicrobiae;D_3__Arctic97B-4 marine group;__;__

D_0__Bacteria;D_1__Verrucomicrobia;D_2__Verrucomicrobiae;D_3__Opitutales;D_4__Puniceicoccaceae;D_5__Cerasicoccus

D_0__Bacteria;D_1__Verrucomicrobia;D_2__Verrucomicrobiae;D_3__Opitutales;D_4__Puniceicoccaceae;D_5__Coraliomargarita

D_0__Bacteria;D_1__Verrucomicrobia;D_2__Verrucomicrobiae;D_3__Opitutales;D_4__Puniceicoccaceae;D_5__Lentimonas

D_0__Bacteria;D_1__Verrucomicrobia;D_2__Verrucomicrobiae;D_3__Opitutales;D_4__Puniceicoccaceae;D_5__MB11C04 marine group

D_0__Bacteria;D_1__Verrucomicrobia;D_2__Verrucomicrobiae;D_3__Opitutales;D_4__Puniceicoccaceae;D_5__Pelagicoccus

D_0__Bacteria;D_1__Verrucomicrobia;D_2__Verrucomicrobiae;D_3__Opitutales;D_4__Puniceicoccaceae;__

D_0__Bacteria;D_1__Verrucomicrobia;D_2__Verrucomicrobiae;D_3__Pedosphaerales;D_4__Pedosphaeraceae;D_5__SCGC AAA164-E04

D_0__Bacteria;D_1__Verrucomicrobia;D_2__Verrucomicrobiae;D_3__Pedosphaerales;D_4__Pedosphaeraceae;__

D_0__Bacteria;D_1__Verrucomicrobia;D_2__Verrucomicrobiae;D_3__Verrucomicrobiales;D_4__DEV007;Ambiguous_taxa

D_0__Bacteria;D_1__Verrucomicrobia;D_2__Verrucomicrobiae;D_3__Verrucomicrobiales;D_4__Rubritaleaceae;D_5__Roseibacillus

D_0__Bacteria;D_1__WPS-2;Ambiguous_taxa;Ambiguous_taxa;Ambiguous_taxa;Ambiguous_taxa

D_0__Bacteria;__;__;__;__;__

Appendix F: Summary statistics result for CCA

Call: cca(formula = temp.otu ~ Temperature + Salinity + Season + DOW + NO3NNO2N + NO2N, data = temp.map)

	Inertia	Proportion	Rank
Total	1.5551	1.0000	
Constrained	0.6745	0.4337	8
Unconstrained	0.8806	0.5663	50

Inertia is mean squared contingency coefficient

Eigenvalues for constrained axes:

CCA1	CCA2	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8
0.3412	0.1466	0.0652	0.0353	0.0311	0.0225	0.0186	0.0139

Eigenvalues for unconstrained axes:

CA1	CA2	CA3	CA4	CA5	CA6	CA7	CA8
0.11833	0.08526	0.07103	0.05239	0.04760	0.03599	0.03444	0.03111

(Showed only 8 of all 50 unconstrained eigenvalues)

Permutation test for cca under reduced model

Permutation: free

Number of permutations: 999

Model: cca(formula = temp.otu ~ Temperature + Salinity + Season + DOW + NO3NNO2N + NO2N, data = temp.map)

	Df	ChiSquare	F	Pr(>F)
Model	8	0.67448	4.7871	0.001 ***
Residual	50	0.88060		

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Permutation test for cca under reduced model

Forward tests for axes

Permutation: free

Number of permutations: 999

Model: cca(formula = temp.otu ~ Temperature + Salinity + Season + DOW + NO3NNO2N + NO2N, data = temp.map)

	Df	ChiSquare	F	Pr(>F)	
CCA1	1	0.34116	19.3707	0.001	***
CCA2	1	0.14657	8.3223	0.001	***
CCA3	1	0.06524	3.7042	0.001	***
CCA4	1	0.03535	2.0069	0.157	
CCA5	1	0.03112	1.7670	0.201	
CCA6	1	0.02252	1.2788	0.568	
CCA7	1	0.01863	1.0576	0.677	
CCA8	1	0.01390	0.7895	0.741	
Residual	50	0.88060			

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Permutation test for cca under reduced model

Terms added sequentially (first to last)

Permutation: free

Number of permutations: 999

Model: cca(formula = temp.otu ~ Temperature + Salinity + Season + DOW + NO3NNO2N + NO2N, data = temp.map)

	Df	ChiSquare	F	Pr(>F)	
Temperature	1	0.22203	12.6068	0.001	***
Salinity	1	0.03574	2.0293	0.030	*
Season	3	0.16964	3.2107	0.001	***
DOW	1	0.16310	9.2607	0.001	***
NO3NNO2N	1	0.05250	2.9810	0.004	**
NO2N	1	0.03147	1.7869	0.059	.
Residual	50	0.88060			

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Appendix G: Kruskal- Wallis pairwise comparison test results

Shannon index

Group 1	Group 2	H	p-value	q-value
FALL (n=18)	SPRING (n=18)	0.4004004	0.526882546	0.526882546
FALL (n=18)	SUMMER (n=17)	16.74945534	4.27E-05	8.53E-05
FALL (n=18)	WINTER (n=12)	16.86200717	4.02E-05	8.53E-05
SPRING (n=18)	SUMMER (n=17)	9.421568627	0.00214448	0.002573376
SPRING (n=18)	WINTER (n=12)	9.813620072	0.001732242	0.002573376
SUMMER (n=17)	WINTER (n=12)	20.4	6.28E-06	3.77E-05

Faith's PD index

Group 1	Group 2	H	p-value	q-value
FALL (n=18)	SPRING (n=18)	8.47247247	0.00360561	0.00432673
FALL (n=18)	SUMMER (n=17)	22.5882353	2.01E-06	1.20E-05
FALL (n=18)	WINTER (n=12)	5.62007168	0.01775592	0.01775592
SPRING (n=18)	SUMMER (n=17)	10.6764706	0.00108507	0.0016276
SPRING (n=18)	WINTER (n=12)	16.1738351	5.78E-05	0.00011557
SUMMER (n=17)	WINTER (n=12)	20.4	6.28E-06	1.88E-05

Curriculum Vitae

PERSONAL INFORMATION

Name and Surname: Selin DELİCEIRMAK

Birth Date and Place: 1984, Istanbul- Turkey

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Marital status: Married

Maiden Surname: KÜÇÜKAVŞAR

EDUCATION

PhD (2013- continues): Middle East Technical University, Institute of Marine Sciences Thesis Title: Investigating marine microbial reactions using novel approaches: genetics and biogeochemistry

MSc (2011- 2013): Middle East Technical University, Graduate School of Natural and Applied Sciences Earth System Science Programme

BS: Hacettepe University, Science Fac., Biology Department (2004-2009)

WORK EXPERIENCE

September 2012- January 2013: Project Assistant - SAD (Underwater Research Society), Development of Responsible Recreational Fishery in the MPA's in Turkey with special reference to KAŞ MPA

January 2011- May 2012: Project Engineer - T&T Environmental Technologies Co., Ltd. Ankara Turkey

April- December 2010: Project Assistant- Mediterranean Coastal Foundation (MEDCOAST), PEGASO Project- FP7

January 2010: Biologist and diver in WWF Kaş- Kekova Environmental Protected Area, Planning and Performing the Marine Management Methods.

June- August 2009: Worked as a Dive Master in Kaş- Arşipel Diving Centre, Antalya / TURKEY

VOLUNTARY STUDIES

August 2009: Volunteer diver- WWF Kaş- Kekova Environmental Protected Area, Planning and Performing the Marine Management Methods

June 2007: Participated in Sand-bar Sharks (*Carcharinus plumbeus*) Conservation and Research Project as a researcher in the Boncuk Bay, Gökova, Turkey- Underwater Research Society.

COURSES, CONFERENCES PARTICIPATED

12-16 May 2015: 4th Course in Next Generation Sequencing, Hibrid Kurs, Istanbul, by European Scholl of Genetic Medicine, Istanbul University and Medical Genetics Association

28 October-1 November 2013: 40th CIESM Congress, Marseille France (poster and oral presentation)

July 23-28 2012: ClimECO3 Summer School, A View Towards Integrated Earth System Models Human-Nature Interactions in the Marine World, held in Ankara-Turkey

June 27- July 1, 2011: Integrated Coastal Zone Management Certificate Program, Gazi University, Ankara

November 2010 – April 2011: MedOpen Advanced Course on ICZM prepared by UNEP-MAP Priority Actions Programme Regional Activity Center

September 2010: Participated in the 8th International Training Workshop on Integrated Coastal Management in the Mediterranean and the Black Sea held in Dalyan- Aegean Coast, Turkey, between 14-30 September 2010

April 27- May 1 2010: Turkey's National Congress of Turkeys Coasts and Seas

June 2008: Participated in Prof. Dr. ERDOĞAN OKUŞ Science Camp on Sandbar Sharks (*Carcharinus plumbeus*) in the Boncuk Bay, Gökova, Turkey

LICENSES

CMAS 3* Diver Certificate: Course taken in Kaş-Arşipel diving Centre, Antalya / TURKEY during July- October 2008.

PROFESSIONAL SKILLS

R

QIIME2-for bioinformatics analysis

LANGUAGES

Turkish (Native), English: (Advance), German and French (Beginner)

STUDY INTERESTS

Biotechnological applications of marine resources, marine nitrogen cycle, integrating genetics to marine biogeochemical cycle modeling, metagenomics, metatranscriptomics, biogeochemical cycle modeling, marine ecosystems, integrated coastal zone management, climate change and impacts on marine environments, microbial carbon pump

PUBLICATIONS

(conference papers are not included)

- Karahan A., **Kucukavsar** S., Gökdağ M., Kıdeyş A. E., Temiz B., Öztürk E., "Metagenomics of the northeastern Mediterranean; cave and marine habitats" (2018). ISJ-Invertebrate Survival Journal. Volume:15, Pages: 109-109

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