NATURAL AND HUMAN INDUCED NUTRIENT IMPACTS ON PHYTOPLANKTON COMMUNITIES IN MERSIN BAY, NE MEDITERRANEAN

A thesis submitted to Graduate School of Marine Sciences of Middle East Technical University

By

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I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

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Abstract

NATURAL AND HUMAN INDUCED NUTRIENT IMPACTS ON PHYTOPLANKTON COMMUNITIES IN MERSIN BAY, NE MEDITERRANEAN

BORAN, Leona Julia Ph.D., Department of Marine Biology and Fisheries Supervisor: Prof. Dr. Zahit UYSAL METU-Institute of Marine Sciences October 2017, 302 pages

Monthly sampling (January 2014 to December 2015) of 8 Erdemli Times Series stations showed that heterotrophic bacteria and cyanobacteria dominate coastal and off-shore plankton communities and decrease with depth and distance to the coast. Cell-volume of *Synechococcus* was positively correlated with temperature and N:P-ratios while cell volume of heterotrophic bacteria cell was negatively correlated with temperature. Negative and positive significant correlations between species abundances and N:P-ratios, and the shifts from one to the other during the two years indicate that limitations are species-specific and switch among P-limitation, N-limitation and co-limitation. The often occurring positive significant correlations between cyanobacteria and N:P-ratios show their high need of nitrogen. Micro-phytoplankton species are higher abundant in the coastal ETS-20 station than the ETS-100 and ETS-200 stations which showed high Bray-Curtis-Similarities to each other and low similarities with the ETS-20 station. Nutrients carried by the Lamas River increased in nitrogen concentrations, and thus have higher in N:P-ratios and lower in Si:N-ratios than in the previous measurements conducted in 2003 and 2007. The effect of the seasonal river water addition to coastal and off-shore communities revealed that seasonality matters and especially nano- and micro-phytoplankton species are affected and effects are shown in an increase of chlorophyll-a. River water and sediment addition led to an increase in chlorophyll, triggered by an increase in diatom abundances. Chlorophyll in off-shore communities increased slightly less. Human induced nutrient addition led to a stronger effect on phytoplankton communities and altered the communities more severely.

Keywords: Phytoplankton abundance, picoplankton biomass, limiting nutrients, dredged material dumping, Northern Levantine Basin

DOĞAL VE İNSAN KAYNAKLI BESİN TUZLARININ KUZEYDOĞU AKDENİZ MERSİN KÖRFEZİNDEKİ FİTOPLANKTON TOPLULUKLARI ÜZERİNDEKİ ETKİSİ

BORAN, Leona Julia Doktora, Deniz Biyolojisi ve Balıkçılık Anabilim Dalı Tez Danışmanı: Prof. Dr. Zahit UYSAL ODTÜ-Deniz Bilimleri Enstitüsü Ekim 2017, 302 sayfa

Ocak 2014 ile Aralık 2015 tarihleri arasında 8 Erdemli Zaman Serisi (ETS) istasyonlarından alınan aylık örneklemler, heterotrofik bakteriler ve siyanobakterilerin kıyı ve açık sulardaki plankton topluluklarına baskın olduğunu ve bununla birlikte derinlik ve sahile olan mesafeyle azaldığını göstermiştir. *Synechococcus'*un hücre hacmi sıcaklık ve N:P oranları ile pozitif yönde ilişkili iken heterotrofik bakterilerin hücre hacmi negatif yönde ilişkilidir. Tür bollukları ve N:P oranları arasındaki istatistiksel olarak anlamlı negatif ve pozitif ilişkiler ve iki yıl süresince türler arasında gözlenen geçişler, sınırlamaların türe özgü olduğunu ve P-sınırlaması, N-sınırlaması ve eş-sınırlama arasında geçiş yaptığını göstermektedir. Siyanobakteri ve N:P oranları arasında ortaya çıkan istatistiksel olarak anlamlı pozitif ilişkiler, yüksek azot seviyelerine olan bağımlılıklarını göstermektedir. Mikro fitoplankton türleri, ETS-20 istasyonu ile düşük ancak birbirleri ile yüksek Bray-Curtis-Benzeşmeleri gösteren ETS-100 ve ETS-200 istasyonlarına kıyasla kıyıdaki ETS-20 istasyonunda daha fazladırlar. Lamas Nehri tarafından taşınan besin tuzları, azot açısından artış göstermekte ve dolayısı ile 2003 ve 2007 yıllarındaki son ölçümlere kıyasla N:P oranlarında artış ve Si:N oranlarında düşüş gözlenmiştir. Kıyı ve açık sulardaki topluluklara eklenen mevsimlik nehir suyunun oluşturduğu etki, mevsimselliğin önemli olduğunu, özellikle nano ve mikro fitoplankton türlerinin etkilendiğini ve etkilerin klorofil-a artışında gösterildiğini ortaya koymuştur. Nehir suyu ve sediman ilavesi, diyatom bolluklarının artmasıyla tetiklenen bir klorofil artışına neden olmuştur. Açık su topluluklarındaki klorofil kısmen daha az artmıştır. İnsan kaynaklı besin tuzlarının ilavesi, fitoplankton toplulukları üzerinde daha güçlü bir etki yaratmış ve toplulukları daha ciddi şekilde değiştirmiştir

Anahtar Kelimeler: Fitoplankton bolluğu, pikoplankton biyokütlesi, sınırlayıcı besin tuzları, taranmış madde, Kuzey Levant Baseni

To my husband, Murat

for his love, support and trust in me

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List of abbreviations

WW: Wet Weight

1. General Introduction

The Mediterranean Sea is a semi-enclosed basin positioned in mid-latitudes and surrounded in the north by Europe, in the south by Africa and in the east by Asia. Its area is approximately 1% of the world's surface area. The Mediterranean is connected to the Atlantic Ocean through the Strait of Gibraltar, to the Black Sea through the Turkish Strait and to the Red Sea through the Suez Canal. Since the only connection to the Atlantic Ocean is through the Strait of Gibraltar, the tidal amplitudes of the Mediterranean are mostly very small, i.e. in the size class of centimeters (McElderry, 1963). The Mediterranean is divided into the eastern basin and the western basin at the Sicily Strait. The western basin includes the Alboran Sea, the Balearic Sea, the Tyrrhenian Sea the Liguro-Provençal Basin, the Algerian Basin and the Algero-Provençal Basin. The eastern basin includes the Adriatic Sea, the Aegean Sea, the Ionian Basin, and the Levantine Basin (Figure 1.1). The maximum depth of 5267 m was found in the Calypso deep, located in the Ionian Sea. The eastern basin is bigger in volume and area and deeper than the western basin.

Figure 1.1: Geographical features of the Mediterranean Sea (Robinson *et al*., 2001).

Differences in topography, climate, connection to other water masses and thus differences in nutrient fluxes, and newly introduced species result in different environments and ecosystems within the Mediterranean and thus it can be considered as a scale model of the world's oceans (Lacombe *et al.*, 1981). Nevertheless, these basins are connected to each other and might influence one another despite the characteristic separation. The Mediterranean is characterized by an anti-estuarine circulation, a general large seasonal variability in hydrological structures and biological production, and strongly effected by global warming.

Since this study concentrates on the northern Levantine Basin (NLB) in the eastern Mediterranean, the main focus of attention will be on this area.

1.1 Physical properties of the Mediterranean

The Mediterranean is connected to the Atlantic Ocean through the Gibraltar Strait. A surface flow from the Atlantic Ocean enters the Mediterranean, travels to the eastern Levantine Basin and is transformed to the Levantine intermediate water (Malanotte-Rizoli *et al.*,2014; Alhammoud *et al.*,2005; Robinson *et al.*, 2001; Pinardi and Masetti, 2000; Lascaratos *et al.*, 1999; Millot, 1999; Özsoy *et al.*, 1989). As shown in Figure 1.2, this salty intermediate water mass crosses the basin in opposite direction below the surface flow and exits through the Gibraltar Strait into the Atlantic Ocean (Özsöy *et al.*, 1989). As Atlantic water flows in the surface layer from the west to the east, its salinity increases and its depth decreases due to high evaporation (Özsöy *et al.*, 1989). The salty intermediate water entering the Atlantic Ocean spreads throughout the Atlantic (Iorga and Lozier, 1999; Reid, 1979), and affects the Atlantic Ocean circulation and consequently the thermohaline conveyer belt.

Figure 1.2: Thermohaline circulation in the Mediterranean (Robinson *et al*., 2001).

The thermohaline circulation (Figure 1.2) consists of a single coherent convective cell connecting the Levantine and Ionian Basin (Roether and Schlitzer, 1991). The conveyer belt in the eastern Mediterranean is considered to have nearly constant characteristics (Robinson *et al.*, 1991). It was found that the engine behind the thermohaline circulation was the Aegean Sea, with Cretan deep water and transitional Cretan water in intermediate depths flowing from the southern Aegean to the basin interior (Theocharis *et al.*, 1999). The only connection between the western and eastern Mediterranean basins is via the flow of surface Atlantic waters through the Sicily Strait to the east and the flow of the intermediate Levantine water to the west. The Aegean became a more important contributor to the deep waters after 1987.

This newly built water mass is warmer and more saline than the previous Adriatic deep water (Robinson *et al.*, 1991). Dense, highly oxygenated and nutrient-rich deep water is found below the intermediate Levantine water, at approximately 500 m. Salinity and temperature are very homogenous in the deep water; 38.66 psu and 13.3 °C respectively (Schlitzer *et al.*, 1991).

Figure 1.3: Sub-basin scale and mesoscale circulations in the eastern Mediterranean (Robinson *et al.*, 2001).

The eastern Mediterranean circulation is linked to several sub-basin scale and mesoscale circulations. The sub-basin and mesoscale circulations of the eastern Mediterranean are shown in Figure 1.3. Atlantic water is believed to feed the Mid-Mediterranean-Jet, which bifurcates into a northward flow feeding the Asia Minor Current and a southward flow. The cyclonic Rhodes gyre, the anti-cyclonic Shikmona gyre, and the Asian Minor Current are important features for the water mass flow in the NLB and its ecosystem. Multiannual oscillations manifest themselves as the Ionian surface circulation and may have strong effects on the eastern basin ecosystems (Malanotte-Rizzoli *et al.*, 2014).

The SST (Sea Surface Temperature) of the Mediterranean increases from the west to the east and is the highest in the south-east. The lowest SST is found in the north-western parts of the western basin and in the northern part of the eastern basin (Figure 1.4). Sensitivity of the Mediterranean toward long- and short-term climatic changes in the North Atlantic modulates the SST in the Mediterranean (Cacho *et al.*, 2001). The surface temperature in the eastern basin increases faster than in the western basin. The upper layer temperature in the eastern basin increased at an average of 0.05 °C per year between 1985 and 2006 (Nykjaer, 2009).

Figure 1.4: Annual mean sea surface temperature [°C] of the Mediterranean (Inland and Marine Waters Unit, Institute for Environment and Sustainability, EU Joint Research Centre, Ispra, Italy – taken from Coll *et al*., 2010).

The warm climate and consequently the high SST in the eastern basin result in a saltier environment than the western basin. Limited freshwater input and high evaporation rates in the eastern basin lead to an increase of temperature and salinity in Atlantic surface waters (Özsoy *et al.*, 1989).

Figure 1.5: Temperature, salinity and density (sigma-theta) profiles on August, 2015 at the ETS-500 station.

The Levantine surface water is characterized with high temperature (16-25 \degree C) and salinity (38.8-39.9 psu) and the Levantine deep water is characterized with the lowest temperature (below 14 °C) and salinity (below 38.8 psu) while the Atlantic water mass is characterized by lower temperature (ca 17 °C) and salinity (38.5-39.0 psu) (Malanotte-Rizzoli *et al.*, 1999; Özsoy *et al.*, 1993; Schlitzer *et al.*, 1991).

Typical stratification features during summer months can be observed in the Levantine Basin (Figure 1.5). The stratified Levantine surface water is found within the first 100 m water depth whereas an intermediate water mass, the Atlantic Water, is found between 125 and 200 m water depth. Also, the Levantine deep water is found below 200 m water depth.

1.2 Chemical properties of the Mediterranean Sea

Nutrient concentrations within the straits of Gibraltar and Sicily, atmospheric and terrestrial nutrient input regulate the nutrient budgets in the Mediterranean basins. The inflowing Atlantic water contains mainly organic nutrients and is depleted in inorganic nutrients (Béthoux *et al.*, 1998). The deep outflow water carries high concentrations of nutrients from the Mediterranean into the Atlantic Ocean (Béthoux *et al.*, 2002). This circulation is called anti-estuarine or reverse thermohaline circulation (Redfield *et al.*, 1963). The increase of nutrients in the depth is a consequence of longer residence time of the deep water (Lacombe *et al.*, 1981) and vertical transportation of particulate organic matter (POM) and its utilization by bacterial communities (Pujo-Pay *et al.*, 2011; Moutin and Raimbault, 2002). Pujo-Pay *et al.* (2011) measured POM in the western and eastern Mediterranean basins and observed a fast reduction of POM below the 100 m water depth where the degradation depth deepens to the east (Figure 1.6). Simultaneously, they observed an increase in the dissolved organic nutrients with depth, which supported the assumption that in deep water high nutrient concentrations are mainly due to remineralization processes by bacteria. As a consequence of this flux of nutrients to the deep and the anti-estuarine circulation, the Mediterranean Sea is one of the most oligotrophic seas with low nutrient low chlorophyll (LNLC) characteristics (Ignatiades *et al.*, 2009; Krom *et al.*, 1991, 2004; Pitta *et al.*, 2005). Besides the input of nutrients through the Gibraltar and Sicily straits, nutrients are supplied mainly by terrestrial, such as river and coastal run-off, and atmospheric inputs (Koçak *et al.*, 2010). Regional surface nutrient concentrations can additionally be enhanced by upwelling of deep water and winter water mixing (Kress and Herut, 2001; Yılmaz and Tuğrul, 1998). A strong nutricline with low nutrient concentrations in surface waters and high concentrations below the euphotic zone (EZ) is characteristic for both basins whereas the nutricline deepens from the west to the east (Pujo-Pay *et al.*, 2011). During summer months the stratification of the water column appears and shallows the euphotic zone (EZ) and thereby the volume of water for primary production (Figure 1.7). Consequently, nutrients are rapidly diminished and production within the EZ is dependent on bacterial degradation (Kress and Herut, 2001).

Figure 1.6: Particulate organic matter, carbon (up), nitrogen (middle), and phosphorus (down), concentrations [µM] in the Mediterranean Western (A), Ionian (B) and Levantine (C) basins (Pujo-Pay et al., 2011).

Under stratified conditions, silicate concentrations are the lowest in the stratified surface water and increase with depth (Figure 1.7). Deep chlorophyll maxima (DCM) between 100 and 200 m water depths and directly above the nutricline are well known for off-shore waters in the Mediterranean (Yücel, 2013; Puji-Pay *et al.*, 2011; Ediger and Yılmaz, 1996; Estrada *et al.*, 1993; Cullen, 1982). Increasing nutrient concentrations with decreasing oxygen were reported in the northern (Yılmaz and Tuğrul, 1998) as well as the southern Levantine Basins (Kress and Herut, 2001).

Pujo-Pay *et al.* (2011) recorded an increase of chlorophyll maximum depth from the west to the east in the Mediterranean and reported that the zone of remineralization deepens simultaneously. New production during summer months is only possible either at coastal areas where the coastal run-off and river influx supply new nutrients throughout the year or if atmospheric deposition occurs (Koçak *et al.*, 2010; Krom *et al.*, 2004).

The Mediterranean, particularly the eastern basin, is exposed to high amount of regular atmospheric nutrient input, especially by dust originating from Saharan, Middle Eastern and Arabian Deserts (Koçak *et al.*, 2010; Markaki *et al.*, 2010; Krom *et al.*, 2004; Kubilay *et al.*, 2000; Herut *et al.*, 1999; Kubilay and Saydam, 1995).

Figure 1.7: Nitrogen, ammonium, phosphorus and silicate profiles (left) and oxygen and chlorophylla profiles (right) on August, 2015 at the ETS-500 station.

Rivers supply nutrients to coastal areas and their surroundings, and even sometimes far offshore due to the relatively narrow shelf zones leading to direct interactions between the coastal and off-shore nutrient budgets and the biological communities. However, there is a sharp contrast between productive coastal areas and oligotrophic off-shore areas in the NLB regarding nutrient concentrations and production (Yücel, 2013; Uysal *et al.*, 2004, 2008; Eker-Develi, 2004). Despite the atmospheric input, nutrients in the off-shore water increase due to sub-basin and mesoscale circulations. The cyclonic Rhodes Gyre supplies nutrients to surrounding waters by vertical mixing during winter and spring, and a nutricline reaching the bottom of the EZ (Kress and Herut, 2001; Yılmaz and Tuğrul, 1998; Ediger and Yılmaz, 1996).

Seasonal observations of off-shore nutrient concentrations show varying concentrations for nitrogen and phosphorus but similar concentrations for silicate (Kress and Herut, 2001). NLB coastal concentrations, on the other hand, vary for all nutrients as a consequence of riverine input and biological uptake (Yücel, 2013; Uysal and Köksalan, 2006; Uysal *et al.*, 2002, 2004). 90% of bioavailable silicate is supplied by rivers into the NLB. More than 85% of nutrients supplied by freshwater sources are coming from the Seyhan and Ceyhan Rivers, followed by Göksu as the third most important nutrient importing river (Koçak *et al.*, 2010). Trace metal profiles are homogenous and fluxes show that the straits support the main amount of metals to the western basin (Elbaz-Poulichet *et al.*, 2001).

High N:P ratios are common in the Mediterranean; N:P ratios are slightly higher in the eastern basin than the western basin in surface water and vice versa in deep waters (Pujo-Pay *et al.*, 2011; Krom *et al.*, 2004; Moutin and Raimbault, 2002; Kress and Herut, 2001; Béthoux *et al.*, 1998; Yılmaz and Tuğrul, 1998).

Nutrient ratio descriptions within this study follow the commonly used definition of Redfield with C:Si:N:P of 106:15:16:1. Stoichiometric ratios are often used as a tool to describe biogeochemical cycles and the functioning of complex food-webs, and to trace water masses (Schroeder *et al.*, 2010; Deutsch *et al.*, 2007; Kress and Herut, 2001). While deep waters in the Levantine Basin are almost stable throughout the year, variations within the upper 500 m water column were observed by Kress and Herut (2001).

Variations of N:P ratios in summer months are either due to biological activities and the fast take-up rate of phosphorus by primary producers (Thingstad and Rassoulzadegan, 1999) or atmospheric input of nutrients (Koçak *et al.*, 2010; Krom *et al.*, 2004). Recent studies have shown that river and atmospheric nutrient sources have already higher N:P ratios than Redfield's 16 (Koçak *et al.*, 2010; Ludwig *et al.*, 2009; Krom *et al.*, 2004, 2005, 2010) and thus support the non-Redfield ratio of nitrogen to phosphorus.

Phosphorus limitation was observed regularly (Koçak *et al.*, 2010; Krom *et al.*, 1991, 2004, 2005; Pitta *et al.*, 2005; Thingstad and Rassoulzadegan, 1995; Thingstad *et al.*, 1998, 2005) but recently, there have been observations of nitrogen limitation in the western (Alcoverro *et al.*, 1997; Estrada, 1996) and eastern (Kress *et al.*, 2005) basins. Nutrient limitation might change with the season and area. Co-limitation of nitrogen and phosphorus was recorded (Yücel, 2013; Kress *et al.*, 2005; Zohary *et al.*, 2005) and coastal waters are debated to become silicate limited in the future (Koçak *et al.*, 2010).

The Mediterranean however is a very variable system regarding nutrient concentrations and stoichiometry (Millot *et al.*, 2006; Béthoux *et al.*, 1998; Yılmaz and Tuğrul, 1998) and the seawide-generalized assumptions due to stoichiometry have only limited significance. Nutrient concentrations are often measured at the detection limit and N:P ratios are therefore not exact. Furthermore, stoichiometric ratios need to be handled with care when estimations of limiting nutrients for primary producers are suggested. Primary producers might vary in their uptake-rate due to not only the species-specific preferences but also the abiotic and biotic conditions (Pujo-Pay *et al.*, 2011; Bertilsson *et al.*, 2003; Van Wambeke *et al.*, 2002; Goldman *et al.*, 1979). A faster turnover rate for phosphorus than nitrogen in coastal waters was discussed by Benitez-Nelson and Buesseler (1999) and shown to be true in the Mediterranean water, as well (Pujo-Pay *et al.*, 2011). This shows that biological activities and chemical properties of the Mediterranean are strongly connected with and control each other tightly, as stated by Arrigo (2005).

1.3 Biological properties of the Mediterranean Sea

The nutrient limitation leads to an oligotrophic system in the Mediterranean with an increase in oligotrophy from the west to the east. As a result of this, the primary production decreases from the west to the east. Moutin and Raimbault (2002) reported a decrease in primary production from 350-450 mgC/m²day (the west) to 150 mgC/m²day (the east). Less than 70% of the global oceans are defined as oligotrophic and might contribute approximately 40% of the total production (Berger, 1989).

Indeed, Patara *et al.* (2009) estimated a very fast sinking rate and thus an efficient biological pump transporting organic matter from the surface to deeper water in the eastern Mediterranean. This sinking organic matter is, however, remineralized in the intermediate waters (Pujo-Pay *et al.*, 2011) and subsequently, the accumulation and sinking of the dissolved organic carbon becomes more significant in the Mediterranean (Thingstad *et al.*, 1997; Carlson *et al.*, 1993). The sedimentation fluxes are lower in the eastern basin (Moutin and Raimbault, 2002).

Figure 1.8: Annual mean primary production 2002 (Inland and Marine Waters Unit, Institute for Environment and Sustainability, EU Joint Research Centre, Ispra, Italy–taken from Coll *et al*., 2010).

Regardless the oligotrophic state, the Mediterranean Sea is a biodiversity hot spot with more than 17,000 species existing (Coll *et al.*, 2010). Nutrient-enriched coastal areas and continental shelves show higher production than ultra-oligotrophic off-shore waters in the eastern Mediterranean (Figure 1.8). A northwestern to southeastern decline of biodiversity was observed (Coll *et al.*, 2010). Sea-surface chlorophyll measurements by satellites and *in situ* measurements show similar patterns with increased chlorophyll concentrations in the western basin compared to the eastern basin, and in the coastal areas compared to the offshore regions (D'Orenzio and Ribera d'Alcala, 2009; Ignatiades *et al.*, 2009; Turley *et al.*, 2000).

This increase in production, similar to eutrophic regions, is a result of the increased nutrient input by urban and industrial waste waters and agricultural activities. Anthropogenicallyinduced changes in the water composition can result in a fast and dramatic change in phytoplankton assemblages (Moncheva *et al.*, 2001; Bodeneau, 1993). Chlorophyll-a values in the eastern basin ranges between 0.1 µg/l and 0.003 µg/l (Yacobi *et al.*, 1995). Cyclonic areas and frontal zones show higher values in respect of chlorophyll-a during their high productive times (Ediger and Yılmaz, 1996). Spatial, seasonal and inter-annual variations of chlorophyll-a concentrations can be high (Siokou-Frangou *et al.*, 2010 and references within).

Picoplankton and nanoplankton contribution to the total amount of chlorophyll-a decreases if chlorophyll increases (Li, 2002). Instead, the micro-sized species of diatoms become more abundant. This effect was explained by a universal relationship of population density and organism cell-size (Li, 2002). A late winter, early spring bloom is regularly reported all over the Mediterranean (Duarte *et al.*, 1999; Siokou-Frangou *et al.*, 2010 and references within). This bloom, the DCM and the increased coastal chlorophyll-a are usually dominated by several genera, such as *Asterionellopsis*, *Chaetoceros*, *Leptocylindrus*, *Proboscia*, *Pseudonitzschia*, *Rhizosolenia*, *Thalassionema* and *Thalassiosira* (Siokou-Frangou *et al.*, 2010; Ribera d'Alcala *et al.*, 2004). This winter/spring bloom is not always visible in off-shore waters (Küçükavşar, not published data).

Since size is a very important factor under nutrient competition, bigger-celled species are mostly present in coastal areas and other nutrient-enriched waters, and might be better competitors due to their storage capacities of the cells (Finkel *et al.*, 2010). Diatoms are regularly documented as the most abundant group in the NLB (Uysal *et al.*, 2003; Eker *et al.*, 2002; Eker and Kıdeyş, 2000; Polat *et al.*, 2000). Dinoflagellates are very diverse and always present throughout the Mediterranean (Gόmez, 2006). The most common species belong to the genera of *Gymnodinium, Gyrodinium, Ceratium, Protoperidinium,* and *Oxytoxum* (Siokou-Frangou *et al.*, 2010). *Ceratium* and *Protoperidinium* are the most diverse genera in the NLB (Polat and Koray, 2007). Smaller-celled diatoms, coccolithophores (*Emiliania huxleyi*) and flagellates are more abundant during stratification in summer and they occasionally contribute to the summer blooms (Eker-Develi, 2004; Ribera d'Alcala *et al.*, 2004; Eker and Kıdeyş, 2000). Despite their high diversity in the Mediterranean, their importance regarding abundance is low. Nevertheless, the importance of flagellates regarding the food-webs is high due to their trophic strategies. Their mixotrophic and phagotrophic characteristics enable them to be simultaneously producers and consumers within the same food-web (Siokou-Frangou *et al.*, 2010).

The less diverse group of the silicoflagellates, *Dictyocha* and *Distephanus*, is generally common within off-shore phytoplankton assemblages (Estrada *et al.*, 1993). Even though bigger-celled phytoplankton species are responsible for the main amount of chlorophyll-a measured, chlorophyll and biomass are still relatively low compared to other eutrophic or blooming systems.

There is, on the other hand, a high productive bacterial community. If integrated over depth, phytoplankton and bacterial biomass are similar in the western and the eastern basins whereas the primary production and bacterial production are proportional only in the eastern basin (Turley *et al.*, 2000). Bacterial production is limited by nutrient in the east where competition for nutrients occurs (Allen *et al.*, 2002). Bacteria can be N, P, C and co-limited in the Mediterranean (Pitta *et al.*, 2005; Zohary *et al.*, 2005). High heterotrophic bacteria production, which causes a dominance of bacterial secondary production over very low primary production, results in low vertical fluxes of organic matter and an organic nutrient pool derived from heterotrophic activities instead of autotrophic activities (Allen *et al.*,2002). The dissolved organic carbon (DOC) produced from primary production serves as basic food for heterotrophic bacteria. This pathway of organic matter into bacteria is a significant part of the pelagic food web (Ducklow and Carlsson, 1992). This cycle of organic matter through DOC uptake by bacteria is referred as the "microbial loop". Heterotrophic bacteria, which are generally the most abundant species, cyanobacteria, *Synechococcus* and *Prochlorococcus*, and a variation of picoeukaryotes belong to the socalled "picoplankton" characterized by sizes between 0.2-2 µm.

This plankton group is dominant in the Mediterranean and is the reason why oligotrophic off-shore areas are productive and not ocean deserts as implied previously. Picoeukaryotes include nanoflagellates and ciliates (Siokou-Frangou *et al.*, 2010 and references within) which prey preferably on cyanobacteria and heterotrophic bacteria (Hagström *et al.*, 1988).

Synechococcus and *Prochlorococcus*, namely cyanobacteria (Cyanophyceae), are the most abundant autotrophic species in the oligotrophic off-shore waters of the eastern Mediterranean basin. With the introduction of flow cytometry these very small autotrophic organisms were detected and they gained scientific interest, especially due to their role and function in oligotrophic ecosystems, such as the Mediterranean. Their contribution to total Chl.-a reaches up to 71% in pelagic waters and 65% of the total primary production (Magazzù and Decembrini, 1995) and therefore, they play a rather crucial role of organic carbon production in the Mediterranean ecosystem (Agawin *et al.*, 1998). During autumn and winter, cyanobacteria are the most abundant at surface waters, and additionally in deeper layers under stratified summer conditions (Christiaki *et al.*, 2001).

In the coastal NLB, *Synechococcus* is the most abundant during summer and early autumn with maximum abundances of 1x10⁵ cells/ml (Uysal and Köksalan, 2006). *Prochlorococcus* exists with two ecotypes: low-light and high-light adapted. It is the smallest and, with its occupation of the water column down to 200 m and even below the euphotic zone, the most abundant photosynthetic organism in the ocean (Partensky *et al.*, 1999 and references within). are grazed on by protists and might effectively be maintained on a uniform abundance by removing the daily production (Agawin *et al.*, 1998). A positive correlation between the growth of *Synechococcus* and temperature was shown (Uysal and Köksalan, 2006; Agawin *et al.*, 1998) and would explain the increase of this species during summer months when predators are less abundant.

There is, on the other hand, a negative correlation between the growth of *Prochlorococcus* and temperature (Partensky *et al.*, 1999). Even though *Synechococcus* and *Prochlorococcus* are highly abundant in oligotrophic waters and responsible for most of the primary production in those water, they show high abundances in nutrient-rich coastal areas, as well (Uysal and Köksalan, 2006; Partensky *et al.*, 1999). Both show higher cellular C:N and N:P ratios under the replete and P-limited nutrient conditions (Bertilsson *et al.*, 2003) showing a relatively low requirement for phosphorus which, combined with slow growth, would be an advantage under constant nutrient depletion (Bertilsson *et al.*, 2003), such as in the NLB. It has been accepted for a long time that the nutritional requirements of phytoplankton species influence the stoichiometry of the sea (Falkowski *et al.*, 1998). Therefore, high amount of prochlorophyte organic matter remineralized in the deeper parts of the Mediterranean might be one explanation for the very high N:P ratios observed in the NLB.

1.4 Future challenges for the Mediterranean Sea

The main threats to the sensitive Mediterranean ecosystem are climate change, eutrophication and the introduction of alien species (Coll *et al.*, 2010). Global warming is a threat to all marine environments. The Mediterranean has only a limited connection to the global ocean through the Gibraltar Strait and is thus highly affected by warming. This makes the Mediterranean a unique miniature ocean to study the consequences of warming and other anthropogenic threats. Indeed, an increase of 0.08-0.1°C water temperature over the last 30 years was recorded (Nykjaern, 2009; Rixen, *et al.*, 2005). Warming of seawater has serious consequences. The change in temperature might, for an example, alter the thermohaline circulation within the Mediterranean, as it happened in 1989 (Lascaratos *et al.*, 1999).
A direct threat to existing ecosystems is the higher survival and acclimation of alien and invasive species, most of which were introduced through the Suez Canal from the Red Sea, beside species shifts within existing communities due to warming. In general, there will be a shift to the most tolerant species. Fu *et al.* (2007) showed that *Prochlorococcus* and *Synechococcus* abundances might be affected since *Synechococcus* growth rate increases with temperature and higher CO2 values, and under greenhouse condition simulations. This would alter the oligotrophic off-shore communities which are dominated by those two cyanobacteria. Another implication by global warming is the sea-level rise. Cazenave *et al.* (2002) reported a mean sea-level rise of 7 mm/year in the Mediterranean Sea. However, this rise as well as the fast surface water warming might be a result of inter-annual and decadal variability of the Mediterranean rather than being directly connected to global warming (Macias *et al*., 2013).

Silicate concentrations are rather stable or decrease slightly while nitrogen and phosphorus concentrations show an increasing trend over time in deep waters of the western basin (Béthoux *et al.*, 1998). The eutrophication conditions induced by nutrient inputs are a worldwide acknowledged threat by human alteration due to industrialization and agricultural developments and might turn coastal regions into silicate limited systems or systems co-limited with silicate. The result of this silicate limitation might lead to a shift of small diatoms and eventually non-siliceous species. Eutrophication might result in excessive phytoplankton blooms, harmful algal blooms (HABs), loss of oxygen due to higher bacterial productivity and fish kills.

Overexploitation threatens to planktonic species by habitat alterations through food chain top down control. Additionally many species such as the monk seal (*Monachus monachus*) and the loggerhead sea turtle (*Caretta caretta*) suffer from habitat loss in the Mediterranean due to coastal engineering, pollution or overfishing.

In the eastern Mediterranean, heterotrophic bacteria production is highly dependent on primary production products (Turley *et al.*, 2000) while primary production is highly dependent on the bacterial degraded and remineralized nutrient supplies, especially in stratified summer waters (Kress and Herut, 2001; Turley *et al.*, 2000). To get a better understanding on their complicated interactions and environmental effects on these sensitive communities which are altered with by human activities, this study concentrates on natural seasonal occurrence by monthly sampling of phytoplankton (including *Prochlorococcus* for the first time), on the impact of riverine and human (dredging) induced nutrient addition via experiments, and on the changes in nutrient concentrations in the Lamas River water.

2. Originality and Significance of this thesis

The main goal of this PhD Thesis is the comparison of phytoplankton communities of productive shelf systems with those inhabiting oligotrophic off-shore waters to biotic and abiotic changes. Edemli Time Series (ETS) has been studied on and off since 1997. None of the studies so far included micro-, nano-, and picophytoplankton species. The importance of microphytoplankton (bigger-celled phytoplankton species) for the coastal ecosystem and under eutrophic conditions has been described for several areas within the Mediterranean Sea. The Eastern Mediterranean is ultra-oligotrophic in off-shore waters and dominated by cyanobacteria (*Synechococcus* and *Prochlorococcus*), therefore they play a crucial role in the off-shore and nutrient limited ecosystem. Heterotrophic bacteria play a crucial role in the planktonic system of the eastern Mediterranean, not only as most abundant planktonic species and also as an important prey for flagellates and ciliates, creating a strongly active microbial loop, but as nutrient provider to the phototrophic primary producers as well.

Marine oligotrophic areas account for an impressive amount of global production and the transport of dissolved organic matter in the eastern Mediterranean shows how strong the plankton groups are connected. To understand the dynamics and changes of the primary producers in the NLB monthly physical, chemical and biological observations were conducted for two years (2014 and 2015). It is the first time that *Prochlorococcu*s was included into the picoplankton group. The importance of *Prochlorococcus* in the Mediterranean ecosystem reveals the necessity to understand its role and characteristics. Further it is the first time that all size-classes were counted and abundances correlated to biotic and abiotic properties per sample over time and throughout all profiles. Phytoplankton communities are connected throughout cell-sizes and trophic levels and shape the characteristics of the inhabited environment. Special attention was payed to biovolume changes of *Synechococcus* and heterotrophic bacteria as a measurable ecosystem functioning. Biovolume was correlated for each sample, each profile and over the surface transect with biotic and abiotic features of the sampling time. This way, a better understanding of limiting nutrients and ecosystem forming conditions is possible.

ETS provides a basis for nutrient influence in nature (natural and human induced ones combined) for coastal and off-shore phytoplankton communities. To gain insight into natural nutrient input nutrients carried by the Lamas River which influences the coastal ETS station over most of the year were measured be-weekly.

Additionally river water was added to coastal and off-shore phytoplankton communities and their responses in species developments and shift measured. As an example for human induced nutrient addition dumping was considered and experiments with harbor sediment added into coastal and off-shore communities were conducted to gain insight into phytoplankton responses.

The connection of physical, chemical and biological ETS data can be used in ecosystem models for climate change or eutrophication estimations.

Several studies on phytoplankton and heterotrophic bacteria communities have been conducted in NLB.

Effects of nutrients and atmospheric deposition on microphytoplankton, with special focus on the coccolithophore *E. huxleyi*, was studied by Elif Eker Develi (2004) and colleagues (2006). Spatial and temporal distribution of *Synechococcus* have been studied by Zahit Uysal, also in cooperation with Irem Köksalan and Sevim Polat and other colleagues (Uysal *et al*., 2004; Uysal, 2006; Uysal and Köksalan, 2006, 2010; Polat and Uysal, 2009). Heterotrophic bacteria were added to the investigated species using the epifluorescence microscopic cell counts in the studies of Uysal et al. (2004), Bayındırlı (2007) . Nanoflagellates were included by Ayşe Gazihan Akoğlu (2011) and Nebil Yücel (2013). The relation of these three groups with biotic (abundances) and abiotic factors have been assessed by correlation of all abundances with the factors. Nebil Yücel (2013) additionally measured heterotrophic bacteria production with ¹⁴C experiments and conducted nutrient addition experiments to examine the nutrient limitation or co-limitation.

3. Aim of this study

Following points will be examined in this PhD-Thesis:

1) Effects of physical and chemical factors on spatial and temporal abundances in coastal and off-shore phytoplankton communities, on a surface transect from on- to off-shore and with depth profiles.

-How do abiotic and biotic factors affect phytoplankton abundance, biovolume and diversity?

2) Nutrient load of the Lamas River

- How do nutrient concentrations fluctuate throughout the year?

- Is there any change compared to past measurements?

3) Natural induced nutrient input via Lamas River water

- How does river water added in different amount to coastal and off-shore phytoplankton communities effect these communities?

-Does seasonality play a role?

4) Human induced nutrient input via suspension of dredged material

- Which effect does different concentrations of dredged sediment from different influenced areas have on coastal and off-shore phytoplankton communities?

- Is there a phytoplankton community friendlier way of dumping? With reference to the dumping site in Mersin

5) Difference between natural and human induced nutrient input

- Is there a difference in changes between natural and human induced nutrient input? What are those changes if present?

4. Erdemli Time Series (ETS)

4.1 Introduction

The Northern Levantine Basin (NLB), north-eastern Mediterranean Sea, shows typical eastern Mediterranean oligotrophic features with high N:P-ratios, seasonal coastal algal blooms and low off-shore chlorophyll throughout the year (Koçak *et al*., 2010; Ludwig *et al*., 2009; Eker Develi, 2004; Moutin and Raimbault, 2002; Turley *et al*., 2000). Common nutrient profiles of high N:P-ratios in surface waters and low N:P-ratios in deep waters during summer were observed (Moutin and Raimbault, 2002; Yılmaz and Tuğrul, 1998; Ediger and Yılmaz, 1996) showing difficult and changing nutrient environments for primary producers and thus, the eastern Mediterranean is a highly fluctuating system and a real challenge for phytoplankton species.

Three major rivers discharge into and effect the Mersin Bay, which are Seyhan, Ceyhan and Göksu. Whereby the rivers Seyhan and Ceyhan contribute 85% of the dissolved organic P, N, ammonium, and Si (Koçak *et al*., 2010). Additionally there are several small rivers along the Mersin coast, such as the Lamas River. The Lamas river has a mean discharge of 3 m^3 /s, carrying low ammonium and phosphate but mentionable high N:P-ratio up to values of 279 (Koçak *et al*., 2010; Tuğrul *et al*., 2004). The discharged river water is transported by the Cilician current anti-clockwise along the coast. River discharge water combined with coastal runoff, and discharged waste water is here named as coastal influence.

Figure 4.1.1: March to June, 2015 surface chlorophyll-a average in the Levantine Basin of the eastern Mediterranean Sea, MODIS-Aqua 4 km data (https://giovanni.gsfc.nasa.gov/giovanni/).

As shown in Figure 4.1.1, coastal introduced nutrients supply the near-shore communities with constant nutrition, leading to higher production at coastal waters which can be observed via satellite chlorophyll-a observations (Raveh et al., 2015; Efrati *et al*., 2013; Volpe *et al*., 2012; D'Ortenzio and Ribera d'Alcalà, 2009). One reason for productive coastal waters and ultra-oligotrophic off-shore regions is caused by silicate influx from rivers. 90% of silicate influx into the NLB is via river discharge (Koçak *et al*., 2010). Additionally the limiting nutrients phosphorus and nitrogen are, even in low concentrations during dry seasons, replenished. Available silicate and other nutrients result in a coastal community dominated by nano- and microplankton species whose chlorophyll-a concentrations are detected by satellites (McClain, 2009 and references within). Given that pico- and nanoplankton decrease with increasing chlorophyll-a concentration (Li, 2002) diatoms and dinoflagellates are important contributors to the coastal plankton community in NLB (Polat and Aka, 2007; Eker Develi, 2004; Eker and Kıdeyș, 2000; Polat *et al*., 2000).

Off-shore phytoplankton communities on the other hand are depending on wet and dry atmospheric deposition of soluble nutrients (Herut *et al*., 1999, 2002, 2005; Markaki *et al*., 2003; Krom *et al*., 2004). Approximately 90% of dissolved inorganic nitrogen and 60% of PO4 fluxes to the NLB are airborne (Koçak *et al*., 2010). On the contrary to the first observations which claim the Mediterranean off-shore waters are low nutrient low chlorophyll (LNLC) areas and relatively life-less, it is now known that these waters are dominated by heterotrophic bacteria (Raveh *et al*., 2015; Tanaka *et al*., 2007; Thingstad and Rassoulzadegan, 1999), cyanobacteria (*Synechococcus* spp.) (Tanaka *et al*., 2007; Uysal and Köksalan, 2006), and flagellates (Siokou-Frangou *et al*., 2010 and references within). These smaller (pico-) plankton species contribute relatively low values to the amount of present chlorophyll while the contributing biomass is similar to microplankton species (Polat and Aka, 2007; Magazzù and Decembrini, 1995). The off-shore water of the Mediterranean is thus only "life-less" in respect of bigger organisms, but biologically active regarding picoplankton (Siokou-Frangou *et al*., 2010; Tanaka *et al*., 2007; Ignatiades *et al*., 2002; Yacobi *et al*., 1995).

Beside blooming events of other species, coastal and off-shore nano- and microplankton communities are dominated by nanoplankton especially *Emiliania huxleyi* (prymnesiophyceae) (Ignatiades *et al*., 2009; Eker Develi, 2004). Including picoplankton into the community, heterotrophic bacteria, *Synechococcus* and *Prochlorococcus* are the most abundant in coastal and off-shore waters throughout the year (Raveh *et al*., 2015; Yücel, 2013; Uysal and Köksalan, 2006; Uysal *et al*., 2004; Magazzù and Decembrini, 1995).

Heterotrophic bacteria in the upper layers of the Mediterranean Sea are responsible for most of the nutrient recycling (Van Wambeke *et al*., 2000). Recycled nutrients are of particular importance in LNLC areas, such as the NLB (Thingstad and Rassoulzadegan, 1999). The microbial food web is dominant in the oligotrophic Mediterranean sea (Siokou-Frangou *et al*., 2010; Turley *et al*., 2000; Christiaki *et al*., 1999; Thingstad and Rassoulzadegan, 1999). According to Hagström et al. (1988) the main carbon flux route is cyanobacteria carbon into bacterivores, such as nanoflagellates, in ecosystems with cyanobacteria dominated primary production. The abundance and biomass of autotrophic and heterotrophic nanoflagellates constitute more than 50% to the total microbial biomass in and around the Cyprus warmcore eddy in the Levantine Basin (Tanaka *et al*., 2007). *Synechococcus* is an important component of the microbial loop regulating biogeochemical cycles (Burkill *et al*., 1993; Hagström *et al*., 1988). A high primary production to total biomass ratio is typical for oligotrophic areas. Christiaki *et al* (2002) observed increasing primary production to total biomass ratios from the Balearic Sea to the East Levantine Basin. These high ratios indicate a system efficient in maintaining resources (Frontier et al., 2004 cited in Siokou-Frangou et al., 2010). Coastal communities and off-shore communities, when larger phytoplankton species bloom, might introduce a classical food-web for short terms. Wide ranges of feeding modes and food preferences of secondary producers, such as mixotrophy (Christaki *et al*., 1998) and feeding on fecal pellets (González and Smetacek, 1994), result in a multivarious food web (Legendre and Rassoulzadegan, 1995).

Throughout most of the year a DCM with increased phytoplankton biomass at 100 to 200 m depth is observed at an off-shore station with 200 m water depth (Küçükavşar, not published data). The DCM are common in the Mediterranean Sea (Ediger and Yılmaz, 1996; Estrada *et al*., 1993). DCM were found at depths with 0.5-5% surface light (Ediger and Yılmaz, 1996) and a uniform chlorophyll concentration was observed during winter-mixing events (Ediger and Yılmaz, 1996; Krom *et al*., 1992). These DCM are not represented in satellite observations and thus the oligotrophic part of the Mediterranean sea is more productive than surface chlorophyll indicates.

Erdemli Time Series (ETS) started in April, 1997. Since then, observations have been conducted regularly (monthly, weekly, sometimes even be-daily) within various projects, and similar stations and varying biological components have been measured. ETS stations are planned from the Lamas river mouth along a water column depth profile, leading offshore. ETS, which has been done for two decades on and off, shows developments and trends in environmental changes over these observation periods. Time series are necessary to compare model outputs with observational data sets.

The aim of this monthly observation is to gain a better understanding of Mersin Bay characteristics and interlinking chemical, physical and biological features. These data, especially the biological data such as bacterial biomass, are used within a biogeochemical model.

Flow-cytometric observations are new in this ETS study and thus *Prochlorococcus* is included for the first time in ETS. To understand the phytoplankton community in Mersin Bay, the pico-, nano- and microplankton abundances were included into this observation. To gain knowledge about the interaction and dependencies of phytoplankton communities in the NLB, abundances of all species were correlated with physical, chemical and biological parameters. Additionally, due to their importance in oligotrophic systems, the biomass of heterotrophic bacteria as well as cyanobacteria were correlated with chemical, physical and biological parameters.

4.2 Material and Methods

4.2.1 Sampling area

The sampling area (Fig. 4.2.1.1) lies within the Mersin Bay and is influenced by river input (Koçak et al., 2010), the Mid-Mediterranean Jet and its spontaneous occurring eddies in Mersin Bay (Malanotti-Rizzoli *et al*., 2014). The station coordinates are shown below in the Table 4.2.1. Three stations are sampled with profile, shown in red, and in total eight stations were sampled for the surface transect from on- to off-shore.

Figure 4.2.1: ETS stations in front of METU-IMS. Red stations represent deep profile stations, blue the additional transect stations.

ETS cruises were conducted with the research vessels R/V Lamas-1 (2014) and R/V Bilim-2 (2015), both belonging to ODTÜ-DBE.

4.2.2 Sample stations and coordinates

Station names are given after their total water depth. In total eight stations were sampled each month, usually within the first 2 weeks of the month. Exact station dates and which parameters were measured on each date are shown in the Table 4.2.2.

Station name	Sample depths [m]	Coordinates
ETS-20	Surface, 10, 20	36°33.570' N 34°15.628' E
$ETS-50*$	Surface	36°32.948' N 34°15.894' E
$ETS-75*$	Surface	36°32.106' N 34°16.458' E
ETS-100	Surface, 10, 25, 50, 75, 100	36°30.877' N 34°17.527' E
ETS-125*	Surface	36°29.846' N 34°18.284' E
$ETS-150*$	Surface	36°28.796' N 34°18.962' E
$ETS-175*$	Surface	36°27.704' N 34°19.509' E
ETS-200	Surface, 10, 25, 50, 75, 100, 150, 200	36°26.093' N 34°20.832' E

Table 4.2.1: Station names, coordinates and sampling depths. * represents stations which are only sampled on the surface for the transect from coast to deep off-shore waters**.**

4.2.3 General water sampling

Water samples were taken at the defined depths (Table 4.2.1) with a niskin sampling rosette, Seabird model and a total volume of eight liter per niskin bottle.

4.2.4 Physical data

Physical properties of the seawater such as salinity, temperature, density, oxygen saturation, photosyntetically active radiation (PAR) and fluorescence are measured *in situ* using a CTD (Seabird model-SBE 19) fixed to the rosette sampler. The CTD measurements were recorded during down-cast and up-cast. Down-cast average data for each meter depth were used for plotting the vertical profiles and the measurements closest to the surface for horizontal (transect) profiles. The precision of the CTD probe are \pm 0.005 °C for temperature and \pm 0.0005 S/m for conductivity. The salinity values were calculated automatically from *in situ* conductivity and temperature measurements.

Additionally the water transparency was measured *in situ* with a standard Secchi Disk, resulting in Secchi Disk Depth (SDD) for each station.

Cruise date	Station (if there was NO sampling for a parameter, the depth is written)							Samples (if a parameter was NOT taken, the parameter is written down)			
	20	50	75	100	125	150	175	200	Physical	Chemical	Biological
31.01.2014	×	×	×	×	×	×	×	×	$\pmb{\times}$	$\pmb{\times}$	×
27.02.2014	×	$\pmb{\times}$	×	$\pmb{\times}$	$\pmb{\times}$	$\pmb{\times}$	$\boldsymbol{\ast}$	$\boldsymbol{\ast}$	×	×	$\pmb{\times}$
14.03.2014	×	×	×	$\pmb{\times}$	×	×	$\boldsymbol{\ast}$	$x - A$	$\pmb{\times}$	$\pmb{\times}$	$\pmb{\times}$
04.04.2014	×	×	×	×	×	$\pmb{\times}$	×	×	$\pmb{\times}$	$\pmb{\times}$	$\pmb{\times}$
08.05.2014	×	×	×	×	×	$\pmb{\times}$	×	×	$\pmb{\times}$	$\pmb{\times}$	×
04.06.2014	$\pmb{\times}$	$\pmb{\times}$	×	$\pmb{\times}$	×	$\pmb{\times}$	$\boldsymbol{\ast}$	×	$\pmb{\times}$	$\pmb{\times}$	$\pmb{\times}$
04.07.2014	×	×	$\pmb{\times}$	$\pmb{\times}$	×	×	×	×	$\pmb{\times}$	$\pmb{\times}$	$\pmb{\times}$
05.08.2014	×	×	$x - B$	×	×	×	×	×	$\pmb{\times}$	×	$\pmb{\ast}$
15.09.2014	×	$\pmb{\times}$	×	×	×	×	×	×	$\pmb{\times}$	$\pmb{\times}$	$\pmb{\times}$
09.10.2014	×	$\pmb{\times}$	$\pmb{\times}$	$\pmb{\times}$	×	$\pmb{\times}$	$\boldsymbol{\ast}$	$\pmb{\times}$	$\pmb{\times}$	$\pmb{\times}$	$\pmb{\times}$
05.11.2014	×	$\pmb{\times}$	×	×	$\pmb{\times}$	×	$\boldsymbol{\ast}$	×	$\pmb{\times}$	$\boldsymbol{\ast}$	$\pmb{\times}$
11.12.2014	×	×	×	×					$\pmb{\times}$	$x-C$	$\pmb{\ast}$
16.01.2015	×	$\pmb{\times}$	×	×	×	×	×	×	$x - D$	$\pmb{\times}$	$\pmb{\times}$
18.02.2015	×	×	×	×	×	×	×	×	$\pmb{\times}$	$\pmb{\times}$	$\pmb{\times}$
13.03.2015	$\boldsymbol{\ast}$	$\pmb{\times}$	×	$\pmb{\times}$	$\pmb{\times}$	$\pmb{\times}$	$\boldsymbol{\ast}$	×	$\pmb{\times}$	$\pmb{\times}$	$\pmb{\times}$
07.04.2015	×	×	×	×	×	×	$\pmb{\times}$	×	×	×	$\pmb{\times}$
06.05.2015	×	×	×	×	×	×	×	×	×	$\pmb{\times}$	$\pmb{\times}$
09.06.2015	×	×	×	×	$\pmb{\times}$	×	×	×	×	$\pmb{\times}$	×
02.07.2015	×	×	×	×	×	×	×	×	$\pmb{\times}$	$\pmb{\times}$	$\pmb{\times}$
13.08.2015	$\pmb{\times}$	$\pmb{\times}$	$\pmb{\times}$	$\pmb{\times}$	×	$\pmb{\times}$	$\boldsymbol{\ast}$	×	$\pmb{\times}$	$\pmb{\times}$	$\pmb{\times}$
15.09.2015	×	×	×	×	×	×	×	×	$\pmb{\times}$	$\pmb{\times}$	$\pmb{\times}$
02.10.2015	$\pmb{\times}$	$\pmb{\times}$	×	×	×	×	×	×	$\pmb{\times}$	$\pmb{\times}$	$\pmb{\times}$
06.11.2015	×	×	×	×	×	×	×	×	×	$\pmb{\times}$	$\pmb{\times}$
07.12.2015	×	$\pmb{\times}$	$\pmb{\times}$	×	×	×	$\pmb{\times}$	×	×	$\pmb{\times}$	$\pmb{\times}$

Table 4.2.2: Sampling plan for ETS cruises in 2014 and 2015. The "X" represents sampled stations and parameters.

*A: ETS-200 at 200 m depth no chemical and biological sampling

B: ETS-75 no PAR measurement

C: ETS-100 at 100 m depth dissolved oxygen was not measured

D: ETS-175 physical parameters were not measured

4.2.5 Chemical data

4.2.5.1 Dissolved oxygen

During up-cast of the sample rosette and CTD water samples were taken at the defined depths. Initially the dissolved oxygen samples were taken into 100 ml glass bottles by using tygon plastic tubes in order to get the sample bubble-free and thus avoid contamination with air bubbles. Immediately after sampling, manganese (II) chloride and alkaline potassium iodide solutions were added and the samples were shaken until all oxygen within the sample was bound by the solutions and the sample turned orange.

Since the dissolved oxygen is not directly oxidizing the iodide ion to iodine, manganese (II) chloride was used to support a multi-step oxidation (Grasshoff *et al*., 1983). Before measuring the amount of oxygen via Winkler titration method (Strickland and Parsons, 1972) with a Metrohm 725 Oxygen Auto-Titrator Analyzer the samples were kept in the dark for at least 30 minutes. For the titration a 0.02M sodium thiosulphate solution was used (UNEP/MAP, 2005).

4.2.5.2 Inorganic nutrients

After the oxygen samples, nutrient samples were taken into, with 10% HCl pre-cleaned, high density polyethylene bottles (HDPE). The nutrient samples were deep frozen (-20°C) until they were analyzed. Nutrient concentrations (nitrate+nitrite, reactive silicate, phosphate and ammonium) were measured with the standard colorimetric methods (Strickland and Parsons, 1972) using a Bran Luebbe model four-channel auto-analyzer. Detection limits for nitrite+nitrite, silicate, phosphate and ammonium are $0.05 \mu M$, $0.3 \mu M$, 0.02 µM and 0.05 µM respectively.

4.2.5.3 Chlorophyll concentration

Sea water for chlorophyll measurements was taken into brown polyethylene bottles and filtered (between 0.5 to 1.050 L of sample volume) on white GF/F filters under dim light. After the filters were digested chlorophyll concentrations were analyzed using a conventional spectrofluorometric method (after Strickland and Pearsons, 1972) and a HITACHI fluoresence spectrophotometer F-2500. The excitation wavelength was 420 nm and the emission wavelength was 669 nm.

4.2.6 Biological data

100 ml of samples were taken into pre-cleaned borosilicate dark bottles and fixed with 2 mL 25% gluteraldehyde (final concentration of gluteraldehyde was 0.495%) and stored at room temperature in the dark. Before filtration, flow-cytrometric analyses, and light microscopic analyses the bottles were turned gently 20 times over the lit-bottom-axis to bring the settled cells into equal distribution within the sample.

4.2.6.1 Heterotrophic bacteria, *Synechococcus* and nanoflagellates counts

A volume of 10 ml of sample was filtered in dim light onto a 25 mm diameter, black, polycarbonate, nuclepore membrane filter with 0.2 µm pore size (Li and Wood, 1988; Uysal, 2000, 2001; Yucel, 2013).

During the filtration 200 µl of acridine orange (3,6-bis dimethylamino acridine from the company SIGMA) was added to the last 3 ml of the sample to stain DNA and RNA contents of the cells (Hobbie *et al*., 1977; Yucel, 2013). After the filtration the filters were immediately mounted on glass slides using non-fluorescent immersion oil and stored frozen at -20°C in the dark until counting. The acridine orange stains the DNA complex in heterotrophic bacterias and to the RNA complex in cyanobacteria leading to cell specific auto-fluorescent characteristics under different light excitations. Cells were differentiated and counted using a Nikon epifluorescence microscope (EFD3) at 1000x magnification and with a filter combination of B-2A (blue excitation – DM 505, EX 450-490, BA 520) for heterotrophic bacteria (fluorescenting green) and nanoflagellates (fluorescenting red and yellow-orange) and a filter combination of G-1A (green excitation – DM 575, EX 546/10, BA 580) for *Synechococcus* (fluorescenting orange to reddish) (Hobbie *et al*., 1977; Porter and Feig, 1980; Uysal, 2001; Yucel, 2013).

Randomly distributed 30 squares with an area of $576 \mu m^2$ each for heterotrophic bacteria and 30 visual fields with an area of $7,088.7 \mu m^2$ each for cyanobacteria and 2 diagonal stripes over the filter with an area of $37,616.5 \mu m^2$ each for nanoflagellates were counted per sample on the same filter.

The total number of cells per milliliter was calculated with the formula I given below (Edler and Elbrächter, 2010):

Formula I)
$$
\text{Cells per mL} = \left(\frac{N\left(\text{counted}\right) * \text{Area}\left(\text{total}\right)}{\text{Area}\left(\text{counted}\right) * \text{Volume}}\right)
$$

where N is the total counted number of cells

4.2.6.2 Biovolume measurements and biomass calculations

The pictures of S*ynechococcus* and heterotrophic bacteria cells were taken with a Nikon Digital Camera model DXM 1200F fixed on the epifluorecence microscope and processed on the computer to obtain size and volume of the heterotrophic bacteria and *Synechococcus* cells. The biomass was gained through conversion of biovolume to biomass using speciesdependent calculation factors of fgC per μ m³. 77 fgC/ μ m³ was suggested by Carlson *et al*. (1999) for heterotrophic bacteria and 123 fgC/µm³ by Waterburry *et al*. (1986) for cyanobacteria.

4.2.6.3 Flow cytometric analyses of Picoeukaryotes, *Synechococcus* and *Prochlorococcus* Species-cell abundance per ml for Picoeukaryotes, *Synechococcus* and *Prochlorococcus* was counted using a flowcytometer of Apogee A50-micro Flow System based on the differences in light absorption of red (633 nm) and orange (488 nm) light by species depending pigment compositions. A total volume of 150 µl with a speed of 60 µl per minute and 2 flush cycles to clean the instrument between sampling were used as the measurement set-up. Furthermore, a threshold at 39 for the red laser results was set to prevent the counting of small particles and dead cells which produce the so called background noise.

4.2.6.4 Identification of bigger phytoplankton species via light microscopy

Bigger phytoplankton cells were identified via an inverted microscope. After 24 hours of settling time in the settling chambers, with a volume of 10 or 25 ml, the cells were identified and counted. The abundance was calculated using Formula I given above. Using the abundance, the biodiversity was calculated in form of Shannon index (Shannon and Weaver, 1963) and Pielou's Evenness (Pielou, 1966).

4.2.7 Statistics

Due to non-equally distributed observations, nonparametric tests were used. Pearson correlation coefficient was used to test linear correlations between species abundances, biovolume, physical and chemical parameters (S, T, PAR, N:P). The abundances of all small-celled species were tested for their correlations with each other and the physical and chemical parameters (T, PAR, N:P). All the statistical analyses were conducted using the statistical package program SPSS.

4.3 Results

4.3.1 ETS-20 m profile station

During summer months, the salinity at the 20 m station was above 39 with a maximum at 39.4 in September, 2015. The lowest salinity was recorded in spring 2015 with a minimum salinity of 36.4 in March (Figure 4.3.1.1).

Figure 4.3.1.1: (a) Salinity [PSU], (b) temperature [$°C$] and (c) PAR [mol/m²s] profiles at all the sampling dates at the 20 m station.

As shown in Figure 4.3.1.1, the temperature at this station ranged from 14.9 in February, 2015 to 30.7 in August, 2015. In August and September, 2015, an increase of temperature in less saline surface waters was observed.

Overall, the temperature increased from winter (January, 2014 and February, 2015) to summer (September, 2014 and August, 2015), followed by a decrease to the next winter months.

The light intensity decreased with depth and penetrated the deepest in August, 2014 with still 200 mol/ $m²$ s at the 20 m water depth. The highest surface light intensity was observed from July to September, 2014 and from April to July, 2015.

The highest concentration of bioavailable nitrogen, here measured as $NO₂+NO₃$, was found during winter and early spring with a maximum value of 24 μ M at the surface and 15 μ M in 10 m depth of the 20 m station (Figure 4.3.1.2). From June to October in both years the concentration ranged from 0.08 to 0.68 µM.

Ammonium showed high concentrations during late spring and winter with a maximum concentration of 1.84 µM at the surface in January, 2014 and 2.95 µM in 20 m depth in November, 2015.

Bioavailable phosphate, in the form of PO₄, was the highest in December, 2014 (0.12 μ M) in the 20 m depth and April, 2015 (0.15 µM) at the surface. In April, 2014 and in August and October, 2015, slightly higher concentrations between 0.7 and 0.9 µM were observed at the surface of the 20 m station.

The months with the lowest concentrations of silicate throughout the water column were May, June and December, 2015 with a concentration less than $1 \mu M$. The highest concentrations of silicate were present in February, 2015 throughout the water column with values from 5.33 to 16.21 µM.

Figure 4.3.1.2: (a) $NO₂+NO₃$, (b) $NH₄$, (c) $PO₄$ and (d) Si profiles on all sample dates at the 20 m station, given in µM.

High N:P-ratios above 25 were observed during late winter/early spring in both years in the water column and low ratios below 10 were found from April to November at 20 m water depth, see Figure 4.3.1.3.

Figure 4.3.1.3: N:P-ratio on all sample dates at the 20 m station.

Oxygen measurements show highest concentrations during the first half of both years and very low concentrations from July to November, see Figure 4.3.1.4. Oxygen concentrations at the 20 m station during 2014 and 2015 ranged from a minimum of 193.5 µM in August, 2015 to a maximum of 300.2 µM in March, 2015.

Figure 4.3.1.4: (a) oxygen [µM] and (b) chlorophyll-a [µg/l] profiles on all sample dates at the 20 m station.

Chlorophyll-a concentrations were observed to be highest during early spring (February-March) with a second smaller maximum during July and August in both years and an additional one in November, 2014. Concentration values ranged from 0.01 µg/l in April, 2015 to 1.34 µg/l in February, 2015. In March, 2014 chlorophyll-a concentration values of 0.9 µg/l were measured. In November, 2014 and in July, 2015 lowest Chl.-a concentrations of 0.44 µg/l and 0.49 µg/l were measured.

Heterotrophic bacteria abundances and biomass at the 20 m station were highest during July, 2014 and August, 2015 (1.7 $x10^6$ cells/ml with a biomass of 17.5 \pm 10.6 µgC/l in July, 2014 and $1,6x10^6$ cells/ml with 20.1 ± 13.3 µgC/l in August, 2015), see Figure 4.3.1.5. There is a smaller second maximum in both years in late winter/early spring.

During November and December, 2014 cell numbers are higher in all 3 depths, but the according biomass increased only in November. Lowest abundances and biomass were measured in November, 2015 with maximum values of $3.3x10^5$ cells/ml and 2.4 ± 1.9 µgC/l.

Figure 4.3.1.5: Heterotrophic bacteria abundance in cells/ml (a) and biomass in µgC/l (b) at the 20 m station.

Cyanobacteria *(here Synechococcus* spp.) had high abundances during July, 2014, January, 2015, and a maximum number of cells per milliliter was found in August, 2015 with 2.3×10^5 cells/ml at the surface. The associated biomass maximum accounted to 11.7 ± 8.4 µgC/l. A small increase of cells numbers and biomass was observed in all depths in October 2015, see Figure 4.3.1.6. The bloom in January, 2015 did not result in an increase of biomass, as all other blooms did. The lowest abundance and biomass was found in October, 2014 with a $5x10³$ cells/ml and 0.5 ± 0.1 µgC/l at 10m water depth.

Figure 4.3.1.6: (a) fluorescence microscopical results of *Synechococcus* abundance in cells/ml and (b) biomass in µgC/l at the 20 m station.

Each sample depth (surface, 10 m, and 20 m) tested over the sampling period for Pearson Correlation revealed that heterotrophic bacteria abundance was in all sample depths significant $(p<0.05)$ negative correlated with light intensity (here PAR), and significant positive correlated with temperature, N:P-ratio, and biovolume per cell. Changes in heterotrophic bacteria biovolume showed the same significant correlations except temperature was negative, shown in Table 4.3.1.1. Correlations over all depths at each month showed a more abundant significant negative correlation with temperature and a more often significant positive correlation with N:P-ratio (both 16 times).

All other parameters for abundance and all parameters tested with biovolume did not have predominant positive or negative significant correlations.

Synechococcus abundance was significant positive correlated with temperature and biomass per cell if sample depth was analyzed over all months. The profile correlated for each month revealed a significant positive correlation with light intensity (16 out of 24 were positive). *Synechococcus* biovolume did not show a predominant correlation.

Table 4.3.1.1: Pearson Correlation analyses for heterotrophic bacteria (HB) and *Synechococcus* spp. (CB) abundances and species specific biovolumes at the 20 m station. The bracket behind the parameter gives the total number of tests, the numbers of significant (p<0.05) positive and negative (+/-) correlations out of the total number of tests are given.

Prochlorococcus abundance $(5.1x10^4 \pm 2.8x10^3 \text{ cells/ml})$ and picoeukaryotes abundance $(32.1x10³ \pm 1.2x10³$ cells/ml) were high in March, 2015. As Figure 4.3.1.7 shows, Prochlorococcus abundance was above 20x10³ cells/ml during April and May, 2014 and below at all other observation dates. Picoeukaryotes showed a fourfold smaller bloom in March, 2014 with $6.9x10^3 \pm 409$ cells/ml. *Synechococcus* (here flow-cytometric results) showed the highest abundance in October, 2015 at the surface with $4.3x10^5 \pm 54.5x10^3$ cells/ml and August, 2015 at the surface with $30.3x10^4 \pm 1.6x10^3$ cells/ml. A smaller bloom of *Synechococcus* cells was observed in July, 2014. Nanoflagellates were abundant with high numbers in July, 2014 with more than $5x10^3$ cells per milliliter in the surface and 10 m water depth. Around $3x10^3$ cells/ml were present in April, 2014 at 20 m depth, March, August and October, 2015 at the surface.

Figure 4.3.1.7: (a) *Prochlorococcus,* (b) *Synechococcus,* (c) picoeukaryotes, and (d) nanoflagellate abundances at the 20 m station, given in cells/ml. a-c are flow-cytometric results and d fluorescent microscopic results.

Statistical Pearson correlations of all species abundances with each other, depth, time, temperature, light intensity, chlorophyll-a, and N:P-ratios show a predominant significant positive correlation between *Synechococcus* and picoeukaryotes abundances. Significant positive Pearson Correlations between nanoflagellate abundances with heterotrophic bacteria, picoeukaryotes, and *Synechococcus* abundances were found. All statistical results for the 20 m station are shown in Appendix A.

Bigger-celled phytoplankton species increased from January to April, then decreased until July, had a second, smaller, maximum in August and decreased to December at the 20 m station (Figure 4.3.1.8). Until March the increase was due to a rapid increase in $\text{coccolithophores (Prymnesiophyceae)}$ abundance from January to March $(2.9x10^5$ to 1.8x10 6 cells/l), followed by a twenty-fold increase in diatom cells per liter (from 3.2x10 5 to $6.6x10⁶$ cells/l) from March to April sample date. The second maximum in August was a quarter in total cells per liter of the maximum in April. The main contributors to the second maximum were coccolithophores. Dinoflagellates had the highest abundance in April and were overall present in low numbers. The species identified and counted are given in Appendix E.

Figure 4.3.1.8: Bigger-celled phytoplankton species abundances in cells per liter at the surface of the 20 m station for 2015.

The most abundant species over all samples at the 20 m station was *Emiliania huxleyi*. Only from April to June this species is replaced by following diatom species, *Pseudo-nitzschia delicatissima*, *Chaetoceros curvisetus*, and *Leptocylindrus danicus,* ranged after the monthly order (Table 4.3.1.2).

Lowest values of Shannon Diversity Index was calculated for February which was also the month with the highest Pielou's evenness of 0.6.

Table 4.3.1.2: The most abundant species, Shannon index and Pielou's evenness of bigger-celled species abundances at the surface of the 20 m station for 2015.

Month	January	February	March	April	May	June
The most abundant species	E. huxleyi	E. huxleyi	E. huxleyi	P. delicatissima	C. curvisetus	L. danicus
Shannon index H'	0.6	2.1	1.4	1.0		1.5
H'_{max}	2.9	3.6	3.8	3.5	3.3	3.2
Shannon Diversity Index (H'_{max} - H')	2.3	1.5	2.4	2.5	2.3	1.7
Pielou's evenness J'	0.2	0.6	0.4	0.3	0.3	0.5
Month	July	August	September	October	November	December
The most abundant species	E. huxleyi	E. huxleyi	E. huxleyi	E. huxleyi	E. huxleyi	E. huxleyi
Shannon index H'	0.6	1.3	0.9	0.9	1.2	0.5
H'_{max}	2.7	3.3	3.0	2.6	3.2	2.8
Shannon Diversity Index (H'_{max} - H')	2.1	2.0	2.1	1.7	2.0	2.4
Pielou's evenness J'	0.2	0.4	0.3	0.3	0.4	0.2

4.3.2 ETS-100 m profile station

Less saline water in the surface of the 100 m station during May was observed in both years with a minimum of 37.7 in May, 2015 (Figure 4.3.2.1).

Figure 4.3.2.1: (a) Salinity [PSU], (b) temperature $[°C]$ and (c) PAR $[mol/m²s]$ profiles at all the sampling dates at the 100 m station. Black dots represent the 1 percentage light intensity depth (euphotic zone).

Higher saline water was found in the upper water column of summer months, with a maximum of 39.5 in August, 2014. Slightly less saline water was present in the deeper water directly below the less distinct halocline and the more distinct thermocline.

Temperature increased from May to September, developing a thermocline from July to October in both years. The depth of the thermocline in 2014 was around 60 m and in 2015 around 30 m. In both years a mixing of warmer water into deeper depths happened in November. The temperature of 17°C in February, 2014 represents the minimum and the temperature of 30°C in September, 2015 the maximum over the two years.

The euphotic zone (EZ), defined as the zone with more than 1% light intensity, was the shallowest in November, 2014 with 31 m water depth and the deepest in April and September, 2014 with 95 m depth.

Bioavailable nitrogen concentrations were below 0.1 µM in 2014 April surface, June, July upper and middle water column and in 2015 upper and middle water column in July, August, October and November. Maximum concentration of 14.69 µM was measured in November, 2014 in 75 m depth. Nitrogen concentration values between 0.2 and 0.4 µM were present in deeper waters in spring and summer, 2014 and spring, 2015 as well as in surface waters in May, 2014. In January/February, 2014 and February, 2015 slightly higher concentrations of nitrogen was observed along the water column (Figure 4.3.2.2).

Maximum concentration of ammonium was measured in November, 2014 with 4.94 µM at deep water and minimum concentration in April, 2014 with 0.09 µM at the surface. In April, 2015 a second smaller NH_4 maximum was observed with 2.33 μ M at 50 m water depth. All other samples had ammonium concentrations of less than 1 µM.

Bioavalable phosphate was present in concentrations at the detection limit of $0.02 \mu M$ in July and December, 2014 and November, 2015 throughout the whole water column and in surface waters in January to March and December, 2014 and July to August, 2015. Highest phosphate concentrations were found at the surface in August, 2014 with 0.09 µM. Higher $PO₄$ surface concentrations (0.07-0.08 μ M) were observed in May (both years) and September, 2015. Deep water accumulation of $PO₄$ was observed in November 2014 and April, 2015 of 0.07 µM.

High silicate concentrations were found in February, 2015 (between 1.5 and 4.09 µM). In general lower concentrations were measured during 2015, where many samples had less than 1 µM of silicate. In July, 2015 the lowest amount of silicate was measured throughout the water column. The silicate minimum concentration during this study was measured in October, 2015 in 50 m depth $(0.68 \mu M)$.

Figure 4.3.2.2: (a) $NO₂+NO₃$, (b) $NH₄$, (c) $PO₄$ and (d) Si profiles on all sample dates at the 100 m station, given in µM.

Figure 4.3.3.3: N:P-ratios on all sample dates at the 100 m station.

High N:P-ratios (>25) were measured at 75 m water depth in November, 2014, throughout the water column in February, 2015, and at 100 m depth in March and August, 2015 (Figure 4.3.3.3). Ratios below 10 were found from April to October, 2014 throughout the water column and March to December, 2015 mainly in the upper water column.

Figure 4.3.2.4: (a) oxygen [µM] and (b) chlorophyll-a [µg/l] profiles on all sample dates at the 100 m station.

Oxygen decreases in warmer months (June to November) and a minimum value of 131.5 µM was measured in August, 2015 at 10 m water depth, as shown in Figure 4.3.2.4. Higher oxygen concentrations were found in winter, spring, and deeper waters of September, October, and November in both years. The highest concentration of oxygen (256.5 µM) was measured in surface waters in May, 2014. In February, 2014 was a slight decreasedoxygen concentration measured of 180-200 µM throughout the water column.

Chlorophyll-a values ranged from 0.01 (June, 2014) to 0.70 μ g/l (May, 2014). In November, 2014 the values were above 0.45 μ g/l, except the deepest sample with 0.29 μ g/l. May and November, 2015 had chlorophyll-a concentrations above 0.1 µg/l. Low Chl.-a values were observed during summer months in upper water layers, down to 75 m in 2014 and 25 m in 2015.

Figure 4.3.2.5: Heterotrophic bacteria abundance in cells/ml (a) and biomass in µgC/l (b) at the 100 m station.

High abundances of heterotrophic bacteria were observed at the 100 m station in November, 2014 ($4x10⁵$ -8.6x10⁵ cells/ml) throughout the whole water column and a maximum of $1.1x10⁶$ cells/ml at the surface in May, 2014 (Figure 4.3.2.5). Cell numbers of heterotrophic bacteria were higher abundant in 2014 than in 2015, with a maximum of $7.5x10^5$ cells/ml at the surface and over $6.5x10^5$ cells/ml at 10 m and 25 m water depth in August and also at the surface in May.

Lowest abundance and biomass were observed during April, 2015 with $6x10^4$ to $1.2x10^5$ cells/ml and 0.4 ± 0.2 µgC/l (Figure 4.3.2.4). Highest amount of heterotrophic carbon was observed in May, 2014 with 14.7 ± 8.2 µgC/l. Biological carbon content above 5 microgramm per liter produced by living heterotrophic bacteria was observed throughout the water column in January, February, and November, 2014 and in August, 2015.

Figure 4.3.2.6: (a) fluorescence microscopical results of *Synechococcus* abundance in cells/ml and (b) biomass in µgC/l at the 100 m station.

High abundance of cyanobacteria (*Synechococcus* spp.) was found in January, 2015 throughout the whole water column of the 100 m station (Figure 4.3.2.6). The abundance decreases from the deepest sample at 100 m with $1.1x10⁵$ cells/ml to half of it at the surface with $5.2x10⁴$ cells/ml. A second maximum in October, 2015 was found at the upper water column, decreasing from the surface $(8.3 \times 10^4 \text{ cells/ml})$ to 25 m water depth sample $(4.5 \times 10^4 \text{ s})$ cells/ml). December, 2015 showed similar decrease as January, 2014 from $6.1x10⁴$ to 2.6x10⁴ cells/ml. Low abundance of *Synechococcus* (below 3x10³ cells/ml) were observed in summer months 2014 and March, 2015. In in May of both years, a higher abundance and biomass was observed at the surface water.

In May, 2014 and October, 2015 *Synechococcus* biomasses were measured with values above 4 ± 2.5 µgC/l. Biomass values above 2 µgC/l were measured at 50 to 100 m water depth in January and December, 2015.

Abundance of heterotrophic bacteria tested for correlation (Pearson correlation) per sample depth over all months reveal that out of 6 sample depths all are significantly negative correlated with light intensity (PAR) and positive correlated with temperature, see table 4.3.2.1. Four samples were significantly positive correlated with N:P-ratio. Biovolume tested for correlation showed a significant negative correlation in five cases and no predominant correlation with light intensity or N:P-ratio. Heterotrophic bacteria abundances tested by each months profile show the double number of positive significant correlations for light intensity and temperature than negative ones. Further the correlation of heterotrophic bacteria abundances and N:P-ratios were tested significantly negative for 14 months and positive for 9 months. Abundance and biovolume of heterotrophic bacteria are significantly positive correlated in eight months. Correlation of heterotrophic bacteria biovolume was tested significantly positive with light intensity in 9 months and negative in 2 months.

Synechococcus abundance and biovolume tested for each sample depth over the sampling period shows that each sample depth is significantly negative correlated with light intensity. In 5 depths biovolume and abundance are significantly positive correlated. Correlations for each month profile show predominant significant positive correlations for abundance and biomass with light intensity and temperature and negative correlations with N:P-ratios.

Table 4.3.2.1: Pearson Correlation analyses for heterotrophic bacteria (HB) and *Synechococcus* spp. (CB) abundances and species specific biovolumes at the 100 m station. The bracket behind the parameter gives the total number of tests, the numbers of significant $(p<0.05)$ positive and negative (+/-) correlations out of the total number of tests are given.

HB Sample, all months	Depth (0)	PAR (6)	T(6)	N: P(6)	Abundance $[cells/ml]$ (6)	Biovolume/cell (6)
Abundance		0/6	6/0	4/1		2/2
Biovolume/cell		0/3	0/5	3/2	2/2	
HB profile, monthly	Depth (24)	PAR (20)	T(23)	N: P(24)	Abundance $[cells/ml]$ (24)	Biovolume/cell (24)
Abundance	5/19	15/6	17/5	9/14		8/3
Biovolume/cell	3/13	9/2	3/4	4/3	8/3	
CB Sample, all months	Depth (0)	PAR (6)	T(6)	N: P(6)	Abundance $[cells/ml]$ (6)	Biovolume/cell (6)
Abundance		0/6	2/3	2/2		5/0
Biovolume/cell		0/6	2/2	2/1	5/0	
CB profile, monthly	Depth (24)	PAR (20)	T(23)	N: P(24)	Abundance $[cells/ml]$ (24)	Biovolume/cell (24)
Abundance	8/15	12/7	13/7	8/13		4/3
Biovolume/cell	1/5	7/1	5/2	2/5	4/3	

Highest abundance of *Prochlorococcus* was found in October, 2015 with $8.3 \times 10^4 \pm 4.1 \times 10^3$ cells/ml in the surface water at the 100 m station, see figure 4.3.2.7. Abundances higher than $2x10⁴$ cells/ml were observed in August, 2015 at the surface and 10 m water depth. Values below $1x10^3$ cells/ml were measured in April, 2014 and May of both years.

Flow-cytometrical measured *Synechococcus* was abundant with high cell numbers at 50 m in July, 2015 $(1.1x10^5 \pm 2.5x10^4 \text{ cells/ml})$ and 75 m in October, 2015 $(9.2x10^4 \pm 1.7x10^4$ cells/ml). In December, 2015 abundances of *Synechococcus* are higher than 3x10⁴ cells/ml throughout the water column, increasing from the surface $(3.7x10^4 \pm 1.8x10^3 \text{ cells/ml})$ to the deep $(8.1x10^4 \pm 9.8x10^3 \text{ cells/ml})$. Lowest abundances of *Synechococcus* cells were found during spring and summer months 2014 with values below $1x10^4$ cells/ml. Very low abundances of picoeukaryotes of $\leq 1x10^3$ cells/ml were found in the whole water column from April to June and September to December, 2014 and in May, July, September (except the surface) and November, 2015.

Picoeukaryotes were the most abundant in the deep water in August 2014 with $4x10^3 \pm 119$ cells/ml and in in February, 2015 with over $7x10³$ cells/ml the upper water column down to 25 m water depth and over $3.5x10^3$ cells/ml until the deep.

Nanoflagellates bloom in May, 2014 with a maximum abundance at the surface $(4x10³ \pm 67)$ cells/ml), the deeper water column ($>1x10^3$ cells/ml) of the same month, the surface waters with $>1.5x10^3$ cells/ml of July 2014, February, 2015 and October, 2015.

Figure 4.3.2.7: (a) *Prochlorococcus,* (b) *Synechococcus,* (c), picoeukaryotes, and (d) nanoflagellate abundances at the 100 m station, given in cells/ml. a-c are flow-cytometric results and d fluorescent microscopic results.
Statistical Pearson correlations of all species abundances with each other, depth, time, temperature, light intensity, chlorophyll-a, and N:P-ratios show for single sample depths over time predominant significant positive correlations between *Synechococcus*, and *Prochlorococcus* abundances and between nanoflagellate abundance and heterotrophic bacteria as well as *Synechococcus* abundances. Heterotrophic bacteria abundance is significantly positive correlated in 5 depths with chlorophyll-a concentrations. During months with strongly developed thermocline (August to October) heterotrophic bacteria and *Synechococcus* abundances are significantly positively correlated with temperature and negative with depth when tested over the whole profile for each month. Correlations overall the profile in each month reveals predominant significant positive results for the abundances of heterotrophic bacteria and nanoflagellates, as well as *Synechococcus* and *Prochlorococcus*.

All statistical results for the 100 m station are shown in Appendix B.

High abundance (6.4x10⁵ cells/l) in bigger-celled phytoplankton species during April, 2015 was mainly due to an increase in diatoms, dinoflagellates and cryptophytes. The sudden decline of the total abundance to $2.4x10^5$ cells/l in May was caused by a sharp decline of coccolithophores (fourfold), dinoflagellates (thirty-twofold), and cryptophytes, (fortyfourfold) see Figure 4.3.2.8. The second smaller autumn bloom in September was caused by an increase in coccolithophores and dinoflagellates and simultaneously showed the lowest abundance of diatoms $(1.7x10³$ cells/l). Dictyochales were only present in December with 80 cells/l.

Figure 4.3.2.8: Bigger-celled phytoplankton species abundances in cells per liter at the surface of the 100 m station for 2015.

Even though the coccolithophore *Emiliania huxleyi* was the most abundant species in most samples, except in April and May when the diatom species *Pseudo-nitzschia delicatissima* and *Chaetoceros curvisetus* had highest cell abundances (see Table 4.3.2.2). Additionally a bloom of the diatom *Lioloma pacificum* resulted in this species exceeding the abundance of *E. huxleyi* in May. This species is, compared to the other present species, big sized. SDI was the smallest in May, where Pielou's evenness was the highest, and the highest in December. Lowest Pielou's evenness was found in July and from September to December. All species identified are given in Appendix E.

Month	January	February	March	April	May	June
The most abundant species	E. huxleyi	E. huxleyi	E. huxleyi	E. huxleyi	C. curvisetus	E. huxleyi
Shannon index H'	0.9	$1.1\,$	1.7	1.3	2.3	1.6
H' max	2.6	3.1	3.2	3.2	3.3	3.2
Shannon Diversity Index (H' _{max} - H')	1.6	2.0	1.5	1.9	1.0	1.6
Pielou's evenness	0.4	0.4	0.5	0.4	0.7	0.5
Month	July	August	September	October	November	December
The most abundant species	E. huxleyi	E. huxleyi	E. huxleyi	E. huxleyi	E. huxleyi	E. huxleyi
Shannon index H'	0.9	1.4°	0.8	0.9	1.0	0.9
H' max						
	3.0	3.2	2.9	2.8	3.0	3.2
Shannon Diversity Index (H'_{max} - H')	2.1	1.9	2.1	1.9	2.0	2.3

Table 4.3.2.2: The most abundant species, Shannon index and Pielou's evenness of bigger-celled species abundances at the surface of the 100 m station for 2015.

4.3.3 ETS-200 m profile station

The salinity at the 200 m station was higher, compared to 20 and 100 m stations (Figure 4.3.3.1). The halocline started to develop from June and extended to November in both years. Lowest salinity of 37.2 was found in May, 2015 at the surface and salinity maximum of 39.5 between 10 and 50 m water depth in October, 2014. The thermocline started to develop from May on and increased till September, followed by a fast decline till November in both years.

Figure 4.3.3.1: (a) Salinity [PSU], (b) temperature $[°C]$ and (c) PAR $[mol/m²s]$ profiles at all sampling dates at the 200 m station. Light intensity is only shown until 150 m water depth and the black dots represent the 1 percentage light intensity depth (euphotic zone).

The thermocline in 2015 was half as deep as the thermocline in 2014, including the temperature maximum of 30 °C at the surface in September, 2015. Lowest temperatures of 16 °C were present in the deep waters of September and October months of both years.

From July, 2014 on 1% of surface light intensity reached around 150 m water depth and in June to July 100 m water depth in 2015. The EZ is deepest in July, 2014 with 133 m depth and shallowest in May, 2015 with 50 m water depth.

At the 200 m station high concentrations of bioavailable nitrogen were mainly found in deeper waters, below 100 m water depth (Figure 4.3.3.2). Except for March, 2015 all samples above 100 m had nitrogen concentrations below 0.8 µM. Highest concentration of nitrogen (3.11 µM) was measured in October, 2014 at 200 m depth. Minimum of nitrogen concentration measured is 0.05 µM in upper water column.

Ammonium was present in the highest concentration in May, 2015 in 100 m water depth with 0.69 µM, directly followed with the lowest concentration measured in April of 0.05 µM. In June, 2014 at 25 m and April, 2015 at 200 m concentrations above 1 µM were measured and throughout the whole water column concentrations of 0.58 to 0.8 µM in October and November, 2015. All other samples had lower concentrations.

Bioavailable phosphate was observed in higher concentrations from September, 2014 to May, 2015 in the deeper water column. During the summer months and during the winter 2014/15 surface water were at the detection limit of 0.02 μ M. The low phosphate zone deepens during summer from June to August in 2014 and June to September in 2015 and got shallower after that. Highest concentration of phosphate was found in October, 2014 at the surface $(1 \mu M)$.

Silicate concentrations ranged between 0.01 (June, 2015) to 3.20 μ M (July, 2014). The majority of samples had silicate concentrations lower than 1.5 µM.

Figure 4.3.3.2: (a) $NO₂+NO₃$, (b) $NH₄$, (c) $PO₄$ and (d) Si profiles on all sampling dates at the 200 m station, given in µM.

Figure 4.3.3.3: N:P-ratios on all sample dates at the 200 m station.

Surface water had mainly N:P-ratios below 10 in the upper water column and below 100 m water depth between 15 and 25 during both years of sampling (Figure 4.3.3.3). Subsurface samples at 10 to 25 m water depth had ratios above 15 in March, June, September, and November, 2015.

Figure 4.3.3.4: (a) oxygen [µM] and (b) chlorophyll-a [µg/l] profiles on all sample dates at the 200 m station.

Maximum and minimum values of oxygen at the 200 m station were both measured in 2015, the maximum of 261.8 µM in October at 150 m depth and the minimum of 165.5 µM in August at 100 m depth. Oxygen values below 210 µM were measured during autumn within the upper 50 m in 2014 and upper 25 m in 2015, as shown in Figure 4.3.3.4. From March to June of both years, oxygen concentrations are higher than 210 µM throughout the whole water column, with slightly lower values in deeper waters than upper ones. Very low concentrations were observed in the deep water throughout both years and in warmer months in the upper 100 m, within the thermocline.

Chlorophyll-a concentrations above 0.2 µg/l were found in May and June, 2015 in the subsurface upper water column. Elevated Chl.-a concentrations (>0.1 µg/l) were found during summer months of both years in 100-150 m water depth and in November of both years in the upper water column.

Figure 4.3.3.5: Heterotrophic bacteria abundance in cells/ml (a) and biomass in µgC/l (b) at the 200 m station.

Heterotrophic bacteria abundance and biomass at the 200 m station over the 2 years of observation are shown in Figure 4.3.3.5. During January and February in both years heterotrophic bacteria were abundant as cell numbers above $3x10⁵$ cells/ml throughout the whole water column. From June to November, 2014 and April to December, 2015 the deeper waters showed heterotrophic bacteria cell abundances below $2x10^5$ cells/ml. Heterotrophic bacteria were highly abundant in surface waters from August to October in both years, with over $7x10^5$ cells/ml in September, 2014 at 50 m depth and in August, 2015 at the surface. The maximum biomass of 8.2 \pm 3.6 µgC/l was produced by heterotrophic bacteria at the end of January, 2014 in surface waters. Less than 1 μ gC/l heterotrophic bacteria biomass was measured in April of both years and in the deeper water in May and June, 2015. In late winter in the whole water column and in summer months the upper water column carbon content of heterotrophic bacteria was above 3 µgC/l. Whereas in deeper water during summer months it was below 2.5 µgC/l.

Figure 4.3.3.6: (a) fluorescence microscopical results of *Synechococcus* abundance in cells/ml and (b) biomass in µgC/l at the 200 m station.

Abundance as well as biomass of *Synechococcus* spp. were maximal in both years in January with up to 8.2x10⁴ cells/ml and 3.4 ± 1.9 µgC/l in 2014 and up to 1.2x10⁴ cells/ml and 3.6 ± 2.3 µgC/l in 2015 (Figure 4.3.3.6). In 2014 the abundance and biomass of *Synechococcus* were lower than in 2015, where there were some small increases in abundance in the upper water column from May on with cell numbers above $10⁴$ per milliliter and biomass mainly above 5 µgC/l.

Abundances of heterotrophic bacteria tested for correlations showed that all eight sample depths tested over all months a significantly positive correlation with temperature, 7 were tested significantly negative with N:P-ratios, and 5 were significantly positively correlated with biovolume (Table 4.3.3.1). Biovolume of heterotrophic bacteria cells had in all eight sample depths a negative significant correlation with temperature.

Heterotrophic bacteria abundance correlated over the profile depths for all months showed 15 times out of 23 months tested a significant positive correlation for light intensity, 19 times a significant positive correlation with temperature and 11 times a negative correlation with N:P-ratios.

No predominant significant positive or negative correlations were found when testing biovolume in depth profiles in each month.

Table 4.3.3.1: Pearson Correlation analyses for heterotrophic bacteria (HB) and *Synechococcus* spp. (CB) abundances and species specific biovolumes at the 200 m station. The bracket behind the parameter gives the total number of tests, the numbers of significant (p<0.05) positive and negative (+/-) correlations out of the total number of tests are given.

Abundance of *Synechococcus* tested significantly positive for Pearson correlation with light intensity in all 8 sample depths and negative in 5 sample depths. *Synechococcus b*iovolume correlation with light intensity was 7 times significantly negative. When abundance was tested throughout the depth profile it was out of 23 tests 13 times significantly positive correlated with light intensity, 17 times positively with temperature and 18 times negatively with N:P-ratios. As for the single sample depth, biovolume showed no predominant significant correlation.

Highest abundances of *Prochlorococcus* were found in 100 m depth in December (3.2x10⁴ \pm 318 cells/ml) and June, $2015 (2.2x10^4 \pm 841$ cells/ml), see Figure 4.3.3.7. In 2014 the overall *Prochlorococcus* abundance was lower between 50 and 100 m water depth. During May and from August till October, 2015 higher abundances at the surface were observed as well as in summer 2015 in 150 m depth. Abundances below 500 cells/ml were present in spring and early summer in 2014.

Synechococcus (measured with flow-cytometer) was abundant with high cell numbers in October, 2015 subsurface waters (10 m depth) with $2.5x10^4 \pm 4.5x10^3$ and 150 m water depth with $1.9x10^5 \pm 1.7x10^4$ cells/ml. Low abundances of *Synechococcus*, $($ < $10x10^3$ cells/ml) were observed in the whole water column from May to August, 2014 and from March to June, 2015 at water depths below 50 m.

Picoeukaryotes were most abundant from February to April in both years with a maximum of $5.3x10^3 \pm 167$ cells/ml in February, 2015 at 100 m water depth, decreasing to the surface water with $4.4x10^3 \pm 231$ cells/ml. Values <500 cells/ml were observed in the whole water column from April to July, 2014 and in the deeper waters from May to December, 2015. High number of Picoeukaryote cells was found in January, 2014 at 25 m water depth

$(1.8x10^3 \pm 170 \text{ cells/ml}).$

Low abundances less than 100 nanoflagellate cells per milliliter were found in deep waters of June, October, and November, 2014 and in May to July, November, and December, 2015. Nanoflagellate abundances were elevated from March to May, 2014 down to 100 m water depth, February to March, 2015 in the whole water column and May (with the maximum of $1.7x10³$ cells/ml) to November, 2015 in the upper water depths.

Figure 4.3.3.7: (a) *Prochlorococcus,* (b) *Synechococcus,* (c) picoeukaryotes, and (d) nanoflagellate abundances at the 200 m station, given in cells/ml. a-c are flow-cytometric results and d fluorescent microscopic results.

Statistical Pearson correlations of all species abundances of the 200 m station with each other, depth, time, temperature, light intensity, chlorophyll-a, and N:P-ratios show a predominant significant positive correlation of heterotrophic bacteria abundance and negative of picoeukaryote abundance with temperature when tested for sampling depths over sampling period. Correlations over depth profiles for each month revealed predominant significant negative correlations of species abundances with depth and N:P-ratios and positive correlations with temperature and light intensity. Also abundances of heterotrophic bacteria, *Synechococcus*, nanoflagellates, and picoeukaryotes were predominantly significantly positively correlated with each other over sample depths and monthly profiles. All statistical results for the 200 m station are shown in Appendix C.

A strong increase in diatoms led to a bloom in May at the surface of the 200 m station, as shown in Figure 4.3.3.8. This bloom consisted of over a magnitude more diatom cells per liter and almost a magnitude higher total cell number per liter compared to cell numbers in April. Dinoflagellates are the most abundant in January and September with 17,872 and 13,104 cells/l. The maximum abundance of coccolithophores was observed in surface waters of March with $3.5x10^5$ cells/l. All species identified are given in Appendix E.

Figure 4.3.3.8: Bigger-celled phytoplankton species abundances in cells per liter at the surface of the 200 m station for 2015.

Shannon Diversity Index is lowest in June, the same month with the highest Pielou's evenness, as shown in table 4.3.3.2. The lowest Pielou's evenness was calculated for March and the highest SDI in August. Except for May, when *Chaetoceros curvisetus* is the most abundant species, *Emiliania huxleyi* dominates the 200 m station.

Month	January	February	March	April	May	June
The most abundant species	E. huxleyi	E. huxleyi	E. huxleyi	E. huxleyi	C. curvisetus	E. huxleyi
Shannon index H'	0.8	0.7	0.5	0.8	1.5	1.6
H' max	2.7	2.8	2.6	3.0	3.6	3.0
Shannon Diversity Index $(H'_{max} - H')$	1.9	2.1	2.1	2.2	2.1	1.4
Pielou's evenness	0.3	0.3	0.2	0.3	0.4	0.5
Month	July	August	September	October	November	December
The most abundant species	E. huxleyi	E. huxleyi	E. huxleyi	E. huxleyi	E. huxleyi	E. huxleyi
Shannon index H'	0.5	0.9	0.7	0.9	0.7	1.2
H' max	2.5	3.3	2.5	2.6	2.6	2.8
Shannon Diversity Index (H' _{max} - H')	2.0	2.3	1.8	1.7	1.8	1.6

Table 4.3.3.2: The most abundant species, Shannon index and Pielou's evenness of bigger-celled species abundances at the surface of the 200 m station for 2015.

4.3.4 ETS surface transect

The lowest salinity values (<37) were observed at the ETS-20 and ETS-50 stations in February and March, 2015 and at ETS-100 and ETS-200 in May, 2015, shown in Figure 4.3.4.1. The highest surface salinity value (>39.5) was observed at ETS-200 station in September, 2014.

Figure 4.3.4.1: (a) Salinity [PSU], (b) temperature [°C] and (c) PAR [mol/m2s] surface transects at all sampling dates.

The surface temperature shows a gradually increase over all stations of one month to the next, with the highest temperatures measured from July to October (maximum from ETS-20 to ETS-75 in September, 2015), followed by a steady decrease to February/March.

The light intensity at the surface was highest from July to October, 2014 and April to July, 2015. The lowest light intensities were observed along the whole transect during May, 2014, and February, March, December, 2015.

Figure 4.3.4.2: Secchi disc depths at all stations.

The SDD deepened with total water column depth and increasing temperature (Figure 4.3.4.2). The 20 m station had the lowest SDD with a minimum at 1 m in March, 2015. With 36 m SDD in 175 and 200 m stations the deepest SDD were represented in June, 2014. High SDD values for 20, 50, 75 and 100 were recorded in August, 2014 and July, 2015. Lowest values for all stations, except the 20 and 50 m stations, were measured in May, 2015.

Bioavailable nitrogen at the surface had higher concentrations at ETS stations closer to the coast, see Figure 4.3.4.3. Further off-shore than the ETS-100 station (>3.3 nautical miles distance to the coast) nitrogen values were predominantly below 0.5 µM. In summer months, from June to September, all transect stations had nitrogen concentrations $\leq 1 \mu M$. Concentrations of $NO₂+NO₃$ above 3.2 µM were found at ETS-75 (November, 2014), ETS-20 to 75 (February, 2015), and ETS-20 (March and April, 2015).

Ammonium was mostly found with elevated concentrations closer to the coast, from ETS-20 to ETS-100 stations. High concentrations of NH_4 were found at ETS-75 (1.88 μ M) and ETS-150 (1.75 µM) surface waters in November, 2014. Low ammonium concentrations were observed at the off-shore stations, from ETS-125 to ETS-200, except in October and November of both years, where elevated concentrations were observed along the whole transect.

Figure 4.3.4.3: (a) $NO₂+NO₃$, (b) $NH₄$, (c) $PO₄$ and (d) Si surface transects on all sampling dates, given in µM.

Bioavailable phosphate concentrations at the surface transect were highest from 1.8 to 4.5 nm (0.6 to 0.9 μ M) in May and at 0.2 nm (0.07 μ M) distance to the coast in September, 2014. Concentrations at the detection limit of 0.02 µM were found in February to April, 2014 and March to April, 2015. The ETS-20 station never showed values below 0.03 μ M of PO₄.

Silicate concentrations were different in the years of sampling. Very low concentrations of <1.5 µM were observed from July to September, 2015 at stations further off-shore than the ETS-75 station, in February and June, 2014 as well as in May, June and December, 2015 all surface samples had concentrations of silicate lower than 1.5 µM. Highest silicate concentrations were observed at ETS-50 in August, 2014 and ETS-20 to ETS-75 in February, 2015 with values up to 6.39 µM (ETS-50 August 2015).

Figure 4.3.4.4: N:P-ratios surface transects on all sampling dates.

Surface transects revealed that water further off-shore than the ETS-100 station with a distance of more than 3.3 nm to the coastline had predominantly N:P-ratios lower than 5. Coastal closer stations (ETS-20 to ETS-75) had N:P-ratios above 20 in winter and spring, see Figure 4.3.4.4. From June to September, 2014 all transect surface samples were with N:P<10 as well as from May to December, 2015.

Figure 4.3.4.5: (a) oxygen [µM] and (b) chlorophyll-a [µg/l] surface transects on all sampling dates.

Oxygen concentrations were high during late winter and early spring with a maximum above 250 µM at ETS20 to ETS-75 in March, 2015. Concentrations below 200 µM were found at ETS-150 in March, 2014 and ETS-100 in August, 2015. General lower values were observed in all surface stations after July, increasing with start of winter in November, December (Figure 4.3.4.5).

Surface chlorophyll-a concentrations were very low in stations further off-shore than 3.3 nautical miles. Surface chlorophyll was higher closer to the coast and the maximum was observed in February, 2015 with 1.34 µg/l at ETS-20 (0.2 nm distance to the shore) and higher than 1.0 µg/l at ETS-50 and ETS-75. The surface water 8.7 nautical miles off-shore (ETS-200 station) had concentrations between 0.07 to 0.7 µg/l. In May and November, 2014 elevated concentrations of chlorophyll-a were measured along the transect until 6.8 nm offshore (ETS-150). In both years a second increased concentration of chlorophyll was measured at the coastal stations during July/August.

Figure 4.3.4.6: Heterotrophic bacteria abundance in cells/ml (a) and biomass in µgC/l (b) surface transects at all sampling dates.

Surface transects show that high abundances of heterotrophic bacteria were mainly found from ETS-20 to ETS-100 station, see Figure 4.3.4.6. Highest abundances were found in July, 2014 and August, 2015 with cell numbers above $1.7x10^6$ cells/ml at the 20 and 50 m surface stations. Lowest abundance was found in all transect stations in April, 2015 with less than $4x10^5$ cells/ml. Slightly enhanced abundances of heterotrophic bacteria (>6x10⁵ cells/ml) at the most distant stations from the coast (ETS-150 to ETS-200) were observed in November, 2014 and May and August, 2015.

Biomass of heterotrophic bacteria was high in August, 2015 with 15.4 \pm 0.3 µgC/l. Lowest values for heterotrophic biomass values were found in April, 2014 with less than 1 µgC/l at ETS-100, ETS-125, and ETS-150.

Figure 4.3.4.7: (a) fluorescence microscopical results of *Synechococcus* abundance in cells/ml and (b) biomass in µgC/l surface transects at all sampling dates.

Cyanobacteria (here *Synechococcus* spp.) abundance and biomass are highest at coastal surface waters and in the sampling year 2015 (Figure 4.3.4.7). Only in January, 2015 are high abundances and elevated biomass values measured along the whole transect with more than 5x10⁴ cells/ml and 1 µgC/l. Maximum abundance and biomass of *Synechococcus* was found at ETS-50 with $2.6x10^5$ cells/ml and 14.3 μ gC/l. Lowest abundance and biomass values were observed from June to October, 2014 and within June, 2015 at stations further off-shore than ETS-100 with $\leq 5x10^3$ cells/ml and less than 0.1 μ gC/l.

Heterotrophic bacteria abundance at all eight surface stations were correlated significantly positive with temperature and biovolume. Biovolume was correlated significantly negative with temperature within six surface stations (Table 4.3.4.1).

Surface transects for each month reveal predominant positive significant correlations for heterotrophic bacteria abundances with temperature, N:P-ratios and biovolume and negative correlations for light intensity. Heterotrophic bacteria biovolume shows predominant significant negative correlation with temperature and positive with N:P-ratios.

Correlations of *Synechococcus* abundance of the stations are predominantly negative with light intensity, and N:P-ratios and predominantly positive with the cell-volume. In half of the surface samples biovolume was significantly positively correlated with temperature. Monthly surface transects correlations for *Synechococcus* abundances are predominantly significantly positive with temperature, N:P-ratios and biovolume and negative with distance to the shore (station) and light intensity. Biovolume of *Synechococcus* cells is predominantly significantly negatively correlated with distance to the shore (station) and positively correlated with temperature and N:P-ratios.

Table 4.3.4.1: Pearson Correlation analyses for heterotrophic bacteria (HB) and *Synechococcus* spp. (CB) abundances and species specific biovolumes for all surface transect stations. The bracket behind the parameter gives the total number of tests, the numbers of significant $(p<0.05)$ positive and negative (+/-) correlations out of the total number of tests are given.

HB Station, all months	Station (8)	PAR (8)	T(8)	N: P(8)	Abundance [cells/ml] (8)	Biovolume/cell (8)
Abundance		3/4	8/0	5/3		8/0
Biovolume/cell		1/3	1/6	3/1	8/0	
HB transect, monthly	Station (24)	PAR (24)	T(24)	N: P(24)	Abundance $[cells/ml]$ (24)	Biovolume/cell (24)
Abundance	2/18	8/12	12/9	19/4		14/3
Biovolume/cell	3/12	6/5	5/9	11/3	14/3	
CB Station, all months	Station (8)	PAR (8)	T(8)	N: P(8)	Abundance $[cells/ml]$ (8)	Biovolume/cell (8)
Abundance		1/6	3/5	0/7		7/0
Biovolume/cell		1/2	4/1	1/3	7/0	
CB transect, monthly	Station (24)	PAR (24)	T(24)	N: P(24)	Abundance $[cells/ml]$ (24)	Biovolume/cell (24)
Abundance	4/17	6/11	12/9	17/3		13/3
Biovolume/cell	4/10	2/4	9/4	7/4	13/3	

Abundance of *Prochlorococcus* was highest in spring 2015 (with cell numbers above 1x10⁴ cells/ml) from ETS-20 to ETS-75 and in May along the whole transect, except at ETS-100 where the cell number was very low with 767 ± 67 cells per milliliter. High *Prochlorococcus* in off-shore stations were observed in May, August, September, and December, 2015. Cell numbers below $1x10³$ were observed in spring 2014.

Synechococcus abundance (here measured with flow-cytometer) was higher closer to the coast than off-shore with maximum values in July, 2014, and August and October, 2015 with more than $3x10^5$ cells/ml, shown in Figure 4.3.4.8. During winter months (January to March, 2014 and October to December, 2015) cell numbers of *Synechococcus* were elevated along the whole transect. Lowest concentrations of below $1x10³$ were measured from July to October, 2014 in ETS-100 and further off-shore stations.

Pikoeukaryotes in surface waters were higher during spring in both years $(3x10^4 \pm 1.7x10^3$ cells/ml in March, 2014 and $>10^4$ cells/ml from February to April, 2015) from ETS-20 to ETS-75. Low values, even absence, were found during April to July, 2014 along the surface transect. In June and August elevated concentrations of around $1x10^3$ cells/ml were measured at coastal surface stations.

Nanoflagellates have higher abundances during spring (March both years) and summer (July, 2014 and August, 2015) with concentration of $>5x10^3$ cells/ml in July, 2014 at ETS-50 and ETS-75. In May, 2015 elevated cell numbers of up to $2x10³$ cells per milliliter were observed at the off-shore transect stations. Lowest cell abundances of nanoflagellates (<500 cells/ml) were found in off-shore transect stations in summer and autumn months of 2014.

Figure 4.3.4.8: (a) *Prochlorococcus,* (b) *Synechococcus,* (c) picoeukaryotes, and (d) and nanoflagellate abundances of the surface samples at all sampling dates, given in cells/ml. a-c are flow-cytometric results and d fluorescent microscopic results.

Statistical Pearson correlations of all species abundances of the surface transect with each other, distance to shore (station), time, temperature, light intensity, chlorophyll-a, and N:Pratios show a predominant significant negative correlation of picoeukaryote abundance with temperature when tested for sampling depths over sampling period. Correlations over depth profiles for each month revealed predominant significant negative correlations of species abundances with station and temperature for winter to early summer and autumn to winter months. Distance to shore (Station) is not significantly correlated during summer months and temperature is significantly positively correlated during summer to late autumn months. The cases of a significant correlation of species abundances with chlorophyll-a concentrations or N:P-ratios were all positive. Also abundances of heterotrophic bacteria, *Synechococcus*, nanoflagellates, and picoeukaryotes were predominantly significantly positively correlated with each other over sample depths and monthly profiles.

All statistical results for the surface transect are shown in Appendix D.

4.4 Discussion

Nutrient input into the NLB is maintained by river influx, atmospheric wet- and drydeposition, and upwelling of nutrient rich deep water (Koçak *et al*., 2010; Ludwig *et al*., 2009; Krom *et al*., 2004, 2010). In contrary to the claim the Mediterranean Sea mostly highly P-limited (Koçak *et al*., 2010; Krom *et al*., 2010, 2004, 1991; Thingstad *et al*.,2005, 1998; Béthoux *et al*., 1998) samples of ETS during 2014 and 2015 show predominantly Nlimited water, with N:P-ratios lower than Redfield's 16 (Redfield *et al*., 1963), especially in the upper water column and during summer months. Nutrient availability to autotrophic organisms shape the health and composition of the planktonic primary producer community (Weber and Deutsch, 2010; Klausmeier *et al*., 2004; Carlsson and Granéli, 1999) which in return shape the nutrient ratios in surrounding waters (Arrigo, 2005; Bertilsson *et al*., 2003; Redfield *et al*., 1963). The biomass of heterotrophs exceeds the biomass of autotrophs by an order of magnitude, showing a typical dominance of heterotrophic bacteria in unproductive environments (Uye *et al*., 1999; Gasol *et al*., 1997). This was also observed in ETS waters. Heterotrophic bacteria abundance exceeds Synechococcus abundance by an order of magnitude while biomass is double to fourfold higher at all stations and the surface profile.

Marine *Synechococcus* and *Prochlorococcus* require non-Redfield nutrient composition and thus show high cellular N:P-ratios, from 21 in nutrient replete cultures to over 100 under Plimitation (Martiny *et al*., 2013; Bertilsson *et al*., 2003). High competition characteristics in oligotrophic waters and preference for high N intake (Bertilsson *et al*., 2003) results in these species dominating the Mediterranean oligotrophic off-shore waters (Siokou-Frangou *et al*., 2010), and thus additionally supporting high N:P elemental composition of the deeper water column (Johnson *et al*., 2006; Arrigo, 2005; Bertilsson et al., 2003; Redfield *et al*., 1963).

Studies on *Synechococcus* and *Prochlorococcus* have shown that cellular N:P-ratios depend on the phase of the growth rate (Bertilsson *et al*., 2003; Heldal *et al*., 2003) and nutrient depletion of their environment (Martiny *et al*., 2013). Even though the abundance of *Synechococcus* is highly depending on available nitrogen, phosphorus is controlling its cell cycle and not nitrogen in warmer summer months (Vaulot *et al*.,1996). Although different species require nutrients in species specific ratios (Martiny et al., 2013) an N:P-ratio of 16 is still used as the threshold ratio to determine nutrient depletion.

Bioavailable N and P influx via precipitation in May, 2014 at the ETS-100 station and May, 2015 at the ETS-100 and ETS-200 station by patchy and heavy rain in Mersin area (Figure 4.4.1) resulted in an increase of *Prochlorococcus* abundances.

Figure 4.4.1: Patchy rain in off-shore waters on the 6th of May 2015. (https://giovanni.gsfc.nasa.gov/giovanni/).

Trapped river water at the surface leads to nutrient influx at those two sampling dates at ETS-100 and ETS-200 stations. The river water can be seen in turbidity measurements (see Figure 4.4.2) through the water column on this sampling date. Co-occurrence of low salinity and high turbidity indicate river water influence. Freshwater influx via precipitation does not result in higher turbidity though.

Figure 4.4.2: Turbidity and salinity profile on May 6th 2015 at ETS-200 station.

A low concentration of nitrate and "left over" phosphate in the surface water of ETS-200 station suggest that fast nitrogen uptake coupled with high requirement by *Prochlorococcus* caused this P enrichment. Atmospheric wet and dry deposition of nutrients play a crucial role for primary production in the eastern Mediterranean Sea (Koçak *et al*., 2010) and is additionally visible in September, 2015 samples, where sampling followed a 2 weeks duration of dry Sahara-dust deposition.

Elevated N:P-ratios and increased *Prochlorococcus* abundance in surface waters in September are the result of this dry-deposition.

Enhanced silicate in April, 2015 triggers the bloom at the 200 m station surface water with a strong increase of diatoms, only present within May samples and resulting in decreased silicate and increased oxygen concentrations in May in surface waters. Even though the seawater shows N-limited elemental compositions, there are arguments for a P-limited system. Low N:P-ratios are also a result of P concentrations below the detection limit of 0.02 µM and N is present in low concentrations. Observations in mesocosm experiments by Thingstad et al. (2005) showed similar difficulties with phosphorus detection limitation. Further, atmospheric deposition might lead to a removal of P by absorption on iron-rich dust particles and transportation into deeper waters, thus a higher N:P-ratio in surface waters (Krom, 1991).

The spatial distribution of abundance maxima of heterotrophic bacteria and *Prochlorococcus* within the upper water layer during stratification and *Synechococcus* at the borderline directly below stratification depths is a result of competition for scarce nutrients in stratified waters (Joint *et al*., 2002; Johnson and Howd, 2000). *Synechococcus* have the unique ability to move by propelling themselves through the water column. This motility ability is used to directly positioning themselves along low nitrogenous compound gradients (Willey and Waterbury, 1989). Deep water maxima of *Prochlorococcus* occurred especially during warmer periods at the ETS-200 station. These might be regulated by this species' preference of colder water and low-light adaptation (Zinser *et al*., 2007; Johnson *et al*., 2006; Cavender-Bares *et al*., 2001; Partensky *et al*., 1999). *Synechococcus* distribution on the other hand is less temperature controlled but depending on light penetration depth (Zinser *et al*., 2007). Yet, *Synechococcus* were found to have maxima in deeper waters (down to 150 m), and were present within 200 m water depth samples in low numbers. *Synechococcus*' ability to utilize nitrate (Moore *et al*., 2002) explains deep maxima presence at the borderline to elevated $NO₂+NO₃$ concentrations. Higher $NH₄$ concentrations and preference of ammonia as nitrogen source (Moore *et al*., 2002) explains the elevated abundances of *Synechococcus* and *Prochlorococcus* within the year 2015. Surface abundance of *Synechococcus* and *Prochlorococcus* were significantly positively correlated with N:P-ratios and throughout the profile negatively correlated with N:P-ratios. Most offshore observations record higher *Prochlorococcus* than *Synechococcus* abundances (Bertilsson *et al*., 2003; Jacquet *et al*., 1998).

In this study, however, *Synechococcus* abundances exceed *Prochlorococcus* abundances with cell numbers up to an order of magnitude difference in coastal and off-shore waters, which is common for shallow depths in the Eastern Mediterranean Sea (Zohary *et al*., 1998; Li *et al*., 1993). This might be an effect of the advantage of *Synechococcus* to use nitrate as well as ammonium as nitrogen source (Zohary *et al*., 2005; Moore *et al*., 2002) in part-time N-depleted waters albeit *Prochlorococcus* advantage of being able to grow on organic phosphorus (Partensky *et al*., 1999). If PO4 is available, *Synechococcus* is able to outcompete bacteria and eukaryotic phytoplankton species due to their superior $PO₄$ uptake kinetics (Tanaka *et al.*, 2007; Moutin *et al*., 2002). Ammonium added to phytoplankton communities from Eastern Mediterranean Sea results in an increase of chlorophyll and *Synechococcus* abundance and a decrease in *Prochlorococcus* abundance (Zohary *et al*., 2005). High abundances of heterotrophic bacteria were observed after blooms of biggercelled phytoplankton species and resulted in lower oxygen concentrations within the water column. Lack of increased remineralized elements, such as phosphorus, shows the fast uptake and thus the fast turnover time of phosphorus. Concentration of reactive phosphorus present in the water influences the turnover time. With additional P addition to a P-limited system, the turnover times become longer (Zohary *et al*., 2005).

Positive correlation of heterotrophic bacteria abundance and with chlorophyll-a concentrations and picoeukaryote abundance was found. Suppression of bigger celled phytoplankton species under favorable nutrient conditions might be caused by the ability of heterotrophic bacteria to out-compete them for available inorganic nutrients (Joint *et al*., 2002). In oligotrophic systems high biomass of heterotrophs results in a fast turn-over rate of small celled algae (Gasol *et al*., 1997). This relationship is shown in the positive correlation of picoeukaryotes and heterotrophic abundance. On the other hand organic phosphorus is rapidly mineralized by heterotrophic bacteria and provided as nutrient to the system (Parpais *et al*., 1996). There was, however, also a predominant significant correlation of heterotrophic bacteria abundance with *Synechococcus* abundance but not *Prochlorococcus* abundance. Both, heterotrophic bacteria and cyanobacteria serve nanoflagellates as food source (Christiaki *et al*., 2001) and, hence, show positive abundance correlations with their predator. Even though low-light and high-light adapted *Prochlorococcus* ecotypes are known to distribute according to their adaptation (Ting *et al*., 2002; Partensky *et al*., 1999 and references within; Moore *et al*., 1998; Urbach *et al*., 1998), it was recorded that light adaptation is not the only distribution regulating factor and both ecotypes occur with similar abundances at the surface of the water column (Johnson *et al*., 2006).

In the NLB shelf waters, heterotrophic bacteria and *Synechococcus* abundance and cell biovolumes decrease significantly with depth and the distance to the shore (station). Caused were these significant changes by a variety of factors, such as decreasing temperature and changing nutrient concentrations with depth and distance to the shore. There was no single variable found to be the exclusive cause of abundance or cell volume changes, but rather the combination of variables. *Synechococcus*, though, showed strong positive correlations for abundance and cell volume with N:P-ratios. This indicates rather N-limited conditions than P-limitation, as cyanobacteria require a higher than Redfield's amount of nitrogen.

At the coastal 20 m station, on the other hand, heterotrophic bacteria are rather N limited, as the predominant significant positive correlation of heterotrophic bacteria abundance and biovolume with N:P-ratio showed. *Synechococcus* is as often N-limited as it is P-limited, based on abundance correlation with N:P-ratio. Zohary *et al*. (2005) have shown that heterotrophic bacteria are P-limited and phytoplankton co-limited by N and P.

Co-limitation of nitrogen and phosphorus and variation of the limiting element with time also explains the sudden increase of diatoms in April but not in June, 2015. In April, 2015 measurements at the ETS-20 station showed N:P-ratios as high as 27, even though there was the highest concentration of P measured. Both elements are taken up very fast, leaving the system in an N-limited state in May followed by a Redfield-ratio state of N:P proximately 16 in June, 2015. Contrary to the expectation of another bloom due to Redfield's condition for N:P, the abundances of bigger-celled phytoplakton are decreasing, when compared to the months before. This indicates a co-limitation. The low concentrations measured for both elements in June are further indications of co-limited conditions. Both nutrients as potentially limiting nutrients have been reported for the Mediterranean Sea before (Van Wambeke *et al*., 2000; Bethoux *et al*., 1992). N and P are discussed to be co-limiting at times (Kress *et al*., 2005; Zohary *et a*l., 2005) and depending on planktonic group (Zohary *et a*l., 2005). Shifts of the limiting nutrient and between autotrophic and heterotrophic organisms show that recycling processes by heterotrophic bacteria are crucial for the nutrient supply and thus the Mediterranean planktonic ecosystem.

The sampling years 2014 and 2015 differ in precipitation amount and periods and temperature (Figure 4.4.3). This difference is reflected also in the marine environment. 2015 is colder in winter and hotter in late summer and autumn resulting in an thermocline half as deep as in 2014. Living and non-living particles are trapped within the warmer upper water mass where nutrients are depleted. Nutrients are trapped in deeper waters, circumvented to reach nutrient depleted upper water layer. Nutrient availability and light intensity play crucial roles in phytoplankton species distribution.

Trapped phytoplankton within the nutrient poor and illuminated upper water layer is highly depending on remineralization of OM as nutrient source (Abell et al., 2000). Phytoplankton directly below the thermocline has the advantage of higher nutrient availability but cope with less light intensity at ETS-100 and ETS-200 stations. This effect is called self-shading, where phytoplankton shades the deeper living species (Bindloss, 1974). ETS-200 station does not show a shallower euphotic zone depth during summer periods. This is the result of almost no phytoplankton species present during hot summer months. Precipitation leading to direct wet-deposition of nutrients in April and May in off-shore stations and indirect more river input, during winter and early spring to the ETS-20 station, resulted in changes of marine nutrient concentrations. Thus there is a difference in nutrient influx in 2014 and 2015. Especially off-shore communities depend on dry and wet-deposition as nutrient influx since river water and coastal runoff enriches predominantly the coastal area. Main period for dry-deposition of nutrients is during spring and late summer/autumn (Koçak *et al.*, 2010). The period of strong wet deposition changes, see Figure 4.4.3.

Figure 4.4.3: Monthly averages of precipitation in Erdemli and temperature in Mersin 2014 and 2015 (Mersin Meteoroloji Istasyon Müdürlüğü).

Off-shore waters are dominated by coccolithophores, considering the nano- and micro-sized groups. Hence silicate is not a limiting nutrient in off-shore waters (200 m station), this dominance is more likely controlled by P-limitation, as there is a strong increase in diatoms when phosphate in form of PO4 is available. Prymnesiophyceae, especially *Emiliania huxleyi*, are well known to be good competitors under P-limited conditions (Riegman *et al*., 2000; Egge and Heimdal, 1994). *E. huxleyi* has the highest affinity for inorganic phosphorus ever recorded and makes them strong competitors under P-limited conditions (Riegman *et al*., 2000). However, this species blooms under N-limited conditions as well (Tyrrell and Merico, 2004). This was explained by their ability to take up nitrogen from additional sources, such as amino acids, purins and urea (Palenik and Henson, 1997). Both would explain their dominance in P- and N- limited off-shore waters of the ETS during 2014 and 2015. Additionally they have the ability to accumulate P greater than 7 times the maximum steady state quota for cell phosphate due to two alkaline phosphatases bound to their cell surface which enables the cells to use phosphate esters at nanomolar concentrations levels (Smayda, 1997) and thus dominate the surface waters at ETS-100 and ETS-200. This was observed in Mersin Bay before (Eker Develi, 2004).

The most abundant diatom species *Pseud-nitzschia delicatissima*, *Chaetocero*s spp. and *Leptocylindrus danicus* are well known blooming species in the Mediterranean (Loureiro *et al*., 2009; Ribera d'Alcala *et al*., 2004; Caroppo *et al*., 2003). These species are considered rselected diatoms concerning their fast growing rate and their cell yields (Begum *et al*., 2015; Quijano-Scheggia *et al*., 2008a, 2008b; Fehling *et al*., 2005). *Pseudo-nitzschia delicatissima* blooms can be associated with domoic acid production and lead to amnesic shellfish poisoning. *P. delicatissima* was reported to contain toxic strains (Fryxell *et al*., 1997) as well as non-toxic strains (Fehling *et al*., 2005). One advantage of the genera *Pseudo-nitzschia* over other phytoplankter is the ability to use organic elements as nutient source (Howard *et al*., 2007; Hilebrand and Sommer, 1996). Loureiro et al (2009) showed that *P. delicatissima* development is favored by the presence of dissolved organic matter (DOM). Therefore *P. delicatissima* blooms are often found in-between blooms of other diatoms (Casas *et al*., 1999). Even though only a few pennate diatom species are characterized as harmful algal blooms (HABs) building species *C. curvisetus* is considered HAB building without releasing toxins into the water column instead their cell-spines break easily and accumulate in fish gills, leading to the death of the fishes (Begum *et al*., 2015). The genera *Chaetoceros* is one of the most diverse, abundant and widespread diatom genera (Li *et al*., 2017) and regularly present. *L. danicus* forms extensive blooms in coastal waters and are reported in the Mediterranean throughout the year (Eker Develi, 2004; Ribera d'Alcala *et al*., 2004).

The most abundant dinoflagellate throughout all samples belong to the genera *Heterocapsa*. The most diverse genera present was *Oxytoxum*. Both genera consist of relatively small species compared to others, such as *Tripos* spp. HABs building organisms belong with highest percentage to dinoflagellates, including *Dinophysis ovum*, *Gonyaulax spinifera*, and *Prorocentrum lima* (Moestrup *et al*., 2017), which were present within the ETS samples.

Figure 4.4.4: Shannon Diversity Index Boxplots of bigger celled phytoplankton species for all 3 ETS deep profile stations. \bigstar represents the mean.

To measure the value or state of a system on the basis of biodiversity is not meaningful. Comparing SDI of the ETS stations (Figure 4.4.4) does not show a significant difference between those even though the coastal station is biologically far more active. The Bray-Curtis-Similarities, however, show very clearly that the community at the 20 m station is different from the communities found at ETS-100 and ETS-200 stations, see Figure 4.4.5.

Figure 4.4.5: Boxplots of Bray-Curtis-Similarities [%] for all 3 ETS deep profile stations (left) where ✱ represents the mean and Bray-Curtis-Similarities separated per month (right).

As figure 4.4.5 shows, the communities of all stations are very similar from November to February. In the other months, ETS-100 and ETS-200 station communities are similar, while both differ from the community found at ETS-20 station. During the blooming time in May at the ETS-200 station the community there is less similar to the ETS-100 and higher similar to the ETS-20 station communities. Chust *et al*. (2012) reported a negative relation of similarities of three phytoplankton groups (diatoms, dinoflagellates and coccolithophores) and the distance of the sampled stations to each other. This can only be seen in the difference of the coastal and both further off-shore communities due to the small distance of the stations to each other. The stronger dissimilarity of the ETS-20 station from March to May is most probably caused by the precipitation induced river water influx and its impact on nutrient concentrations and thus an increase of diatom abundances at the ETS-20 station but not at the other two stations.

Figure 4.4.6: Dendrogram, using single linkage, of the nearest neighbor of all samples, named by month of sampling and station (20=ETS-20, 100=ETS-100, and 200=ETS-200).

Comparing phytoplankton assemblages with their nearest neighbor shows that the spring bloom sample in April of the coastal ETS-20 station is an outstanding sample with least similarity to any of the other samples, see figure 4.4.6. In total 4 cluster groups are defined, with the sample in May at the ETS-20 as a second group, similar to the third group of May sample of ETS-200 and June sample of ETS-20 stations. May was the blooming time sample of the off-shore ETS-200 station. During blooming time at the off-shore station phytoplankton assemblages resemble coastal spring and summer assemblages.

The fourth cluster group is the largest including all other samples, see figure 4.4.6. As already shown with Bray-Curtis-Similarity results, samples of ETS-100 and ETS-200 are grouped together, only autumn and winter ETS-20 samples are found to be similar to ETS-100 and ETS-200 samples. Spring and summer ETS-20 are grouped together with ETS-100 sample of April, where the highest abundance was observed, see figure 4.3.2.8.

It needs to be noted that single factors, such as abundance, can not always represent ecosystem functioning, as bacteria activity increases with P addition, with no increase in abundance visible (Zohary *et al*., 2005) and *E*. *huxleyi* biomass increases in P limited waters but not their abundance (Riegman *et al*., 2000; Paasche, 1998).

Comparing these nano- and microphytoplankton counts to the counts of previous studies is complicated due to different identification aspects. Scopes of identifications were different, names changed and the convenience of identifying the genus not however the species and giving those counts under the shortage of the genus followed by sp. This study does not differentiate species within the class Prymnesiophyceae. Since more than 90 percent belong to the species *E. huxleyi*, this species counts might include the other species of Prymnesiophyceaeas well. The method used, counting samples at 20 times magnification, is not precise enough to distinguish the different circular species accurately in the size class of *E. huxleyi* (approximately 3 µm in diameter). Thus other similar appearing cells might have been included into E. huxleyi counts and enhanced its abundances.

Furthermore, it is very important to note that all results are just a momentary state, of the sampling day and time, not representing months or overall pattern. Phytoplankton communities are patchy and fast in changing (Martin, 2003; Brentnall *et al*., 2003; Bracco *et al*., 2000; Abraham, 1998), so these monthly measurements do not represent the whole system or time-relations. Additionally, the bigger-celled phytoplankton species are often more highly concentrated in mid-deep waters (Ediger and Yılmaz, 1996) in the NLB, and thus surface samples do not represent the existing community throughout the station but only the surface.

4.5 Conclusion

The N:P-ratios measured are mainly higher than Redfield's 16 in deeper water and lower in surface water. During high precipitation times the coastal station is completely P-limited with very high N:P-ratios present in the whole water column. Increased nutrients caused by increased river influx resulted in higher cell abundances, especially of diatom cells.

Pico-sized plankton species dominated the coastal and off-shore plankton communities regarding species specific abundances. The importance of heterotrophic bacteria was also shown by the fact that the DCM depth seemed to be bound to the bacterial decomposition depth and thus appeared concurrent with oxygen reduced deeper waters. Statistical analysis revealed that heterotrophic bacteria abundance was both, N- and P-depleted, whereby a change from one to the other depletion appeared. Cyanobacteria on the other hand were mainly N-depleted regarding abundance and P-depleted regarding biovolume.

Species composition differed between coastal ETS-20 and both deeper ETS stations. ETS-100 and ETS-200 station were similar in species composition. From November to January all 3 stations were similar. The diatom bloom at the ETS-100 and ETS-200 stations in May, 2015, caused by trapped river water at the surface, led to a higher Bray-Curtis-Similarity between the ETS-200 and the ETS-20 station and grouped the off-shore station together with the coastal spring samples when nearest neighbors were compared. River water influx triggered an increase in diatom abundances, resulting in late winter and spring bloom at the coastal station. During summer months the abundance was lower but the diversity was the highest.

Coccolithophore species (*Emiliania huxleyi*) seemed to be higher abundant compared to the observations of Eker Develi from 1999 to 2001 (2004). This could be a result of either a change in the environmental conditions. The start of the Erdemli sewage water discharge or changes in elemental river water nutrient compositions due to human activities, such as new dams build in the rivers Seyhan and Ceyhan resulting in less silicate availability along Mersin coast, might show effects in species shifts and new dominating species within the bigger-celled phytoplankton species. Additionally to higher abundance of coccolithophores HABs building species of the genus *Oxytoxum* (dinoflagellate) seem to have become more diverse than in the previous study. The diatom genus *Chaetoceros* on the other hand seems to be less diverse in species.
5. Nutrient concentrations and effects of the Lamas River water

5.1 Introduction

Rivers regulate the marine biodiversity and productivity, especially in the oligotrophic Mediterranean by introducing nutrients into the marine system (Koçak *et al*., 2010; Ludwig *et al*., 2009). They play a crucial role in nutrient cycles, as in phosphorus, nitrogen and silicate cycles. Before the Aswan Dam was build the Eastern Mediterranean was mainly supplied with freshwater by the Nile river. Now the influx of freshwater from Nile is less than the river discharge into the Cilician Basin by much smaller rivers (Pinardi et al., 2005; Drinkwater and Frank, 1994). The NLB is river-fed by four major rivers: Orontes, Seyhan, Ceyhan, and Göksu River (from east to northwest). Additionally, several small rivers and only seasonally active streams discharge into the Northern Levantine Basin. The Cilician current flows anti-clockwise along the coast of NLB and transports discharged river water along with it. Close to the Middle East Technical University-Marine Science Institute (METU-IMS) is the river mouth of the Lamas River. This river is discharging throughout the whole year into the Mediterranean sea with a mean discharge of 3 m³/s (Koçak *et al.*, 2010). This river and its discharge characteristics and changes of nutrient loads play an important role for the coastal plankton community (Cloern *et al*., 1983). The Lamas River is small in relation to the other rivers flowing into the NLB.

The main source of freshwater into NLB are Seyhan and Ceyhan. Over 85% of $PO₄$, the dissolved silicate and NO₃ originate from these freshwater sources (Koçak *et al.*, 2010). Even though its total influence to the river-originated nutrition at the off-shore waters in NLB might be small, the influence at the closer coastal area can be observed visually as the color of the sea differs within the plum region. The marine area supplied by the rivers change depending river water flow and marine currents. Occasionally river water reaches further off-shore and thus might effect the off-shore phytoplankton communities as well. Thus, regular scientific cruises were conducted by METU-IMS since 1997 starting in front of the Lamas River mouth and leading off-shore to observe the influence of the Lamas river to the local marine ecosystem.

Nutrients carried by rivers into the sea drive the coastal communities. This nutrient load can be both, positive when it increases production in an oligotrophic area and negative when it causes eutrophication due to anthropogenic sources. The semi-enclosed Mediterranean Sea is one of the oligotrophic water bodies in the world and the Levantine Basin is known to be the most oligotrophic (Lakkis *et al*., 2002).

This oligotrophic state of the Mediterranean Sea is due to the anti-estuarine circulation carrying nutrient-rich deep water into the Atlantic Ocean and causing hereby a loss of those nutrients from the system (Béthoux *et al*., 1998). Koçak et al. (2010) showed that although in average 90% of DIN (Dissolved Inorganic Nitrogen) and 60% of $PO₄$ transported into the NLB was due to atmospheric input, 90% of silicate derived from the river discharges. Despite nutrient load from the atmosphere to the whole surface area, relatively high levels of chlorophyll-a is mostly observed via satellite only within coastal regions and occasionally further off-shore between Turkey and Cyprus (Lakkis *et al*., 2002) and thus shows a further sign why coastal communities are depending on riverine inputs. And thus in Mersin Bay region, rivers are the main source of nutrients for the marine coastal primary producers (Koçak *et al*., 2010; Ludwig *et al*., 2009; Krom et al., 2004, 2010).

N:P-ratios higher than Redfield Ratio are a common characteristic of the Mediterranean Sea and, except for a short time between 1975 and 1992, the Levantine Basin was throughout phosphate limited (Ludwig *et al.,* 2009). N:P-ratios are also much higher than Redfield Ratio in both, atmospheric and riverine inputs (Koçak *et al*., 2010; Tuğrul *et al*., 2004; Herut, 2002; Kouvarakis et al., 2002; Ludwig *et al*., 2002).

Riverine nutrient inputs of 5 rivers showed a deficiency in phosphate in all rivers with N:Pratios ranging from 18 to 279 (Koçak *et al*., 2010). Additionally between 1992 and 2001 the dissolved inorganic nitrogen fluxes via atmosphere increased in concentration while the dissolved inorganic phosphorus concentrations decreased (Herut, 2002), which explains a high N:P-ratio in the entire water body and not only the coastal river influenced area. The increase of N and P fertilizer led to an anthropogenic increase of these nutrients transported to the marine environment by river water (Yunev *et al*., 2007; Moncheva *et al*., 2001; Sur *et al*., 1994). Simultaneously there is a decrease in silicate export of rivers due to hydrological alterations, such as damming and diversion and hence longer water residence time in reservoirs behind dams (Humborg *et al*., 2008; Ittekkot *et al*., 2000; Milliman, 1997). Even though all nutrients get trapped in those reservoirs, downstream human activities compensate for nitrogen and phosphorous loss but not for silicate (Ittekkot *et al*., 2000). After the Aswan High Dam started to perform dissolved silicate was reduced by almost 200 µmol/L in the Nile estuary (Whaby and Bishara, 1980). Both, increase in N and P and decrease in Si may result in regional silicate limitation in coastal waters (Da Cunha *et al*., 2007; Humborg et al., 2000). One consequence of less silicate supply to the marine environment is a shift from diatoms to non-silicious plankton species, such as flagellates and cyanobacteria (Humborg *et al*., 2000; Egge and Aksnes, 1992).

Coastal phytoplankton communities are mostly dominated by the bigger-celled phytoplankton species such as diatoms and flagellates (Yücel, 2013; Eker Develi, 2004; Eker Develi *et al*., 2002). Diatom species require silicate to reproduce since their valves consists of hydrated silicon dioxide.

Since the main sources of silicate are land-based and not due to atmospheric deposition (Koçak *et al*., 2010) the coastal and off-shore phytoplankton communities differ. Off-shore phytoplankton communities of the eastern Mediterranean consist mainly of cyanobacteria (*Synechococcus* and *Prochlorococcus*), coccolithophores, and small celled dinoflagellates and diatoms. Coastal communities exist mainly of bigger celled plankton species, whereby they only exceed cyanobacteria in biomass, not in abundance (chapter 4, Aktan, 2011; Ignatiades, 2009; Uysal and Köksalan, 2006; Eker Develi *et al*., 2002; Uysal *et al*., 2002; Eker and Kıdeyş, 2000; Polat *et al*., 2000; Fogg, 1995). New primary production in estuaries is mainly due to diatom growth (Dugdale and Wilkerson, 1998) and even coastal zones represent less than 10% of the total oceanic area their total contribution of oceanic new primary production is between 30-50% (Pearl, 1995). Nutrient input results in biomass changes within phytoplankton communities in the Mediterranean, particularly in microphytoplankton (Duarte et al., 2000).

Sudden addition of nutrients to a depleted system can be defined as a stress factor. Systems with unpredictable stress factors tend to consist of r-selected life strategists (Greenslade, 1983). Fisher (1977) stated that open-ocean diatoms and estuarine diatoms differ in their response to stress and that estuarine algae are adapted to rapid fluctuating conditions. Estuarine communities are highly resistant to a variety of physical and chemical disturbances (Fischer, 1977). Depending on phytoplankton community, origin of the community and stress factor shifts of species and changing production (enhanced or inhibited) might lead to changes of abundances and biomass of phytoplankton groups when river water is introduced into the communities.However, in total less than 2% of the overall primary production in the Mediterranean Sea is sustained by the river transported nutrients (Ludwig *et al*., 2009).Enhanced or reduced nutrient load have a direct effect on the marine ecosystem, starting by primary producers, such as changes in biodiversity, blooms, harmful algal blooms (HABs), and eutrophication, which then effect higher trophic levels with hypoxia and fish kills (Howarth *et al*., 2011; Bodeanu, 1993). Eutrophication and HABs are common features in estuaries and coastal ecosystems (Anderson *et al*., 2002; Nixon, 1995; Caperon *et al*., 1971).

River water does not only alter the nutrient state of the marine system but also temperature, salinity, turbidity while it introduces freshwater and coastal plankton species. All these changes by the river water might affect coastal ecosystems different from off-shore ecosystems.

Nutrients carried by river water and phytoplankton assemblages are not stable and hence the effect might change depending on season and phytoplankton assemblage origin.

To investigate these differences, observations of nutrient concentrations carried by the Lamas River were measured biweekly and effect of Lamas River water, added in logarithmic increase of concentrations to coastal and off-shore phytoplankton communities in 3 different seasons, investigated. The summer experiment had to be stopped due to a technical failure in the dark-light and temperature regulation of the culture room.

5.2 Material and Methods

Biweekly samples (3 parallels) from the Lamas River were taken for nutrient analyzes close to the river mouth from February, 2015 to February, 2016. The samples were filtered through sterile syringe filters with a pore size of 0.2µm (Minisart NY 25 from Satorius Stedium Biotech) into pre-cleaned (with 10% HCl) 15 ml polypropylene centrifuge tubes (ISOLAB) and deep frozen until analyzed. Nutrient concentrations (nitrate+nitrite, reactive silicate, phosphate and ammonium) were measured after standard colorimetric methods (Strickland and Parsons, 1972) using a Bran Luebbe model four-channel auto-analyzer. Detection limits for nitrate+nitrite, silicate, phosphate and ammonium are as follows: 0.05 μ M, 0.3 μ M, 0.02 μ M and 0.05 μ M respectively.

For the experimental part, on- and off-shore water was taken during ETS at the ETS-20 and the ETS-200 station (see chapter 4 for detailed information) from 3 to 5 m water depth and filtered through a 200 µm pore size net to exclude bigger zooplankton predators. On the same day, Lamas River water was taken at the same location as the biweekly nutrient samples. Both, the river water and the communities were acclimated for 3-4 days under experimental conditions before the experiments were set up.

This experiment was repeated in 3 seasons (fall, winter, spring). The river water concentration was added in a log scale and is shown in Table 6.2.1. Controls in triplets are river water free communities from on- and off-shore. The communities were hold in room temperature 21 \pm 1 °C and a controlled dark-light cycle of 12:12 hours. Each treatment had a total volume of 5 liter and was set up in triplets (3 parallels). The position of every culture was randomly changed every 24 hours to prevent effects of the set-up-location. Every 24 hours the communities were gently turned to distribute settled down cells in the water column again.

Salinity in communities was measured after the river water addition and all salinity values are shown in table 6.2.2.

Table 6.2.2: Salinity [PSU] of all set-ups after river water was added.

Treatment		Coastal control Off-shore control 6% river water		12% river water	24% river water	48% river water
Salinity [PSU]	$39.3 + 0.3$	$39.2 + 0.1$	$37.0 + 0.5$	$34.5 + 0.3$	$30.0 + 0.1$	$20.5 + 0.4$

Every 24 to 48 hours samples for chlorophyll-a and small celled phytoplankton species (flow-cytrometric measurements) were taken with a plastic syringe. Chlorophyll signal was measured using a Fluorometer, Turner Design Model blue. Calibration of the flourometer was done before the first experiment.

Differences in light absorption of red (633 nm) and orange (488 nm) light by species depending pigment compositions using a flowcytometer of Apogee A50-micro Flow System resulted in species-cell abundance per ml for picoeukaryotes, *Synechococcus* and *Prochlorococcus*. A total volume of 150 µl with a speed of 60 µl per minute and 2 flush cycles to clean the instrument between sampling was used as measurement set-up. Further a threshold at 39 for the red laser results was set to prevent the counting of small particles and dead cells, producing the so called background noise.

At the beginning and the end of the experiment nutrient samples were taken. Those samples were taken into with 10% HCl pre-cleaned high density polyethylene bottles (HDPE) and processed the same way as the biweekly river water nutrient samples, described above.

Samples for bigger-celled species identification were taken into 50 ml pre-cleaned borosilicate dark bottles and fixed with 1 ml 25% gluteraldehyde (final concentration of gluteraldehyde was 0.495%) and stored under room temperature in the dark until identification. After 24 h settling time in settling chambers, the bigger cells were identified with help of an inverse light microscope and the abundance calculated. Abundance numbers are used for the biodiversity (Shannon Index and Pielou's evenness) calculations, representing the shift in the communities.

Non-parametric statistical Kruskal-Wallis H Tests, or so called one way ANOVA, was used to test for differences in nutrient and chlorophyll-a concentrations between different set-ups in each experiment, nutrients at the beginning of the experiment and chlorophyll-a at the end of the experiment. Additionally Wilcoxon Signed Rank Tests were done to compare the related samples of nutrient concentrations at the beginning and the end of each experiment. All statistical tests were conducted with SPSS.

5.3 Results

Figure 5.3.1 shows the nutrient concentration changes within the sampling period, where nitrogen decreased. An overall increase of ammonium over the year was observed. The average silicate transported by the Lamas River declined slightly during summer time to ca. 60 μ M and was higher during wet seasons (winter 2015 around 115 μ M and around 85 μ M in winter 2016) whereby nitrogen in form of $NO₂+NO₃$ showed a sharp decline in December, 2015 from 115.07 to 85.61 µM. There was a strong decline in early May, 2015 from over 100 μ M to 90 μ M.

Ammonium increased from June on during summer and dropped in September to 1.08 µM followed by a peak in October at 5.52 µM. Phosphorus was present with the lowest values $(0.07 - 0.35 \mu)$ of all the measured inorganic nutrients and with higher concentrations (around 30 µM) during winter and spring 2015.

Figure 5.3.1: Biweekly nitrogen (dark blue), silicate (green), phosphate (red) and ammonium (light blue) concentrations in μ M (N and Si concentrations according to the left Y-axis and PO₄ and ammonium concentrations according to the right Y-axis). The black bar distinguishes between the beginning of the measurements in February 2015 and the end in February 2016. Error bars show the standard deviation of 3 parallel measurements.

Si:N-ratios are lower than Redfield's ratio, except in December samles, where it was above with a ratio of 1.35. The average Si:N-ratio is 0.66. The lowest N:P with 285 in November 2015 and the highest N:P-ratio of 1,487 was found in mid August 2015.

Figure 5.3.2: Biweekly N:P- and SI:N-ratios of the Lamas River nutrients. The black bar distinguishes between the beginning of the measurements in February 2015 and the end in February 2016.

Chlorophyll-a increased over time and stronger with the concentration of river water added within all manipulated communities, see Figure 5.3.3. Starting concentrations of chlorophyll-a were 0.13 ± 0.02 µg/l within coastal communities and 0.08 ± 0.01 µg/l within off-shore communities. All, coastal and off-shore, treatments consisting of 48% river water had a lag-phase of 48 hrs followed by the strongest increase to 1.51 ± 0.13 µg/l (coastal) and 1.59 ± 0.18 µg/l (off-shore).

The same amounts of river water present led to higher chlorophyll-a increase in coastal than in respective off-shore communities. An increase in chlorophyll-a with a river water proportion of 6% to 0.68 ± 0.17 µg/l (coastal) and 0.20 ± 0.01 µg/l (off-shore), 12% to 1.09 \pm 0.2 µg/l (coastal) and 0.36 \pm 0.04 µg/l (off-shore), and 24% to 1.32 \pm 0.36 µg/l (coastal) and 0.69 ± 0.03 µg/l (off-shore) was observed.

Albeit abundances of picoeukaryotes for 48% river water treatments showed similar trends over time, including a lag-phase of 48 hrs followed by a steep growth to $8.2x10^3 \pm 737$ (coastal) and $10x10^3 \pm 1.9x10^3$ (off-shore) cells/ml, the other treatments did not. However, the initial cell numbers (coastal: $1.1x10^3 \pm 175$ and off-shore: 997 \pm 172 cells/ml) as well as the abundance after 312 hrs were increased and higher in coastal communities (coastal: control with $2.7x10^3 \pm 643$, 6% with $3.2x10^3 \pm 1.6x10^3$, 12% with $5.1x10^3 \pm 929$ cells/ml and off-shore: control with $1.5x10^3 \pm 58$, 6% with $1.8x10^3 \pm 400$, 12% with $3.3x10^3 \pm 529$ cells/ml), except for those with 24% river water where the abundance in off-shore $(7.6x10³$ \pm 1.1x10³ cells/ml) was higher than in coastal $(4.3x10^3 \pm 1.7x10^3$ cells/ml) communities.

Figure 5.3.3: Chlorophyll-a concentrations [µg/l] and picophytoplankton species abundances in October for coastal (C and filled circles) and off-shore (O and non-filled circles) communities with different amount of Lamas River water addition, the error-bars represent standard deviations.

Contrariwise initial *Synechococcus* abundance was higher within off-shore $(3.1x10⁴ +$ 6.1x10³ cells/ml) than coastal (2.5x10⁴ \pm 586 cells/ml) communities. In general there was an increase for all treatments after 312 hrs, except within the off-shore 48% river water treatment, which decreased to $1x10^4 \pm 1.9x10^3$ cells/ml. While the abundance doubled itself with each increasing step of river water in coastal communities after 312 hrs (6%: 6.6x10⁴ ± 1.3x10⁴, 12%: 1.6x10⁵ \pm 5.4x10⁴, 24%: 3.4x10⁴ \pm 4.4x10⁴ and 48%: 7.2x10⁵ \pm 8.3x10⁴ cells/ml) there was a small increase from the initial abundance in off-shore communities in 6% (2.7x10⁴ \pm 4.2x10³ cells/ml) and 12% (3.2x10⁴ \pm 6.2x10³ cells/ml), followed by a double increase with 24% (6.2x10⁴ \pm 11x10³ cells/ml) river water concentration.

Prochlorococcus showed no general response in abundance changes to the amount of river water mixed with seawater. Both control (coastal: from $4.4x10^3 \pm 751$ to $3x10^3 \pm 1.8x10^3$ cells/ml and off-shore: from $4.4x10^3 \pm 608$ to $2.3x10^3 \pm 520$ cells/ml) and 6% river water groups (coastal: from $5.1x10^3 \pm 651$ to $3.5x10^3 \pm 814$ cells/ml and off-shore: from $7x10^3 \pm 165$ $1.6x10^3$ to $4.2x10^3 \pm 833$ cells/ml) as well as off-shore 48% (from $7.9x10^3 \pm 1.2x10^3$ to $2.5x10³ \pm 462$ cells/ml) communities declined from initial cell numbers to abundance after 312 hrs.

Coastal 48% and off-shore 24% communities showed an increase in *Prochlorococcus* abundance (coastal 48% from $8.1x10^3 \pm 854$ to $1.9x10^4 \pm 1.6x10^3$ and off-shore 24% from $6x10^3 \pm 436$ to $8x10^3 \pm 1.4x10^3$ cells/ml) after 312 hrs. Coastal 12% and 24% and off-shore 12% communities showed no change in abundance after 312 hrs.

Variations between the three parallels were generally higher with higher dilution with river water and get stronger over time.

Nitrogen and silica showed a higher and ammonium a lower initial concentration with higher river water percentage for all treatments, as shown in Figure 5.3.4. However there was no trend for phosphate. Within coastal 48% communities nitrogen decreased from 0.36 \pm 0.25 to 0.22 \pm 0.23 µM, off-shore control groups from 0.84 \pm 0.97 to 0.13 \pm 0.09 µM and increased within coastal 48% communities from 28.68 ± 17.62 to 39.82 ± 8.0 μ M.

Phosphate increased in most treatments whereby coastal 12% communities showed the strongest increase from 0.03 ± 0.01 to 0.07 ± 0.01 µM. Silica however decreased in concentrations over time in coastal control (2.35 \pm 0.21 to 1.74 \pm 0.36 µM), 48% (46.69 \pm 1.99 to 30.04 \pm 1.26 µM), off-shore control (1.09 \pm 0.44 to 0.79 \pm 0.08 µM) and increased within off-shore 48% (27.47 \pm 18.71 to 38.81 \pm 7.14 μ M) communities.

Even though differences of nutrient concentrations could be seen when plotted as boxplots, Wilcoxon Signed Rank Tests for related samples (beginning – end) were non-significant for all October experiment set-ups.

Significant Kruskal-Wallis H Test (one way ANOVA on ranks) results for independent samples of nutrient concentrations after Lamas River water was added to October communities are shown in table 5.3.1. Adjusted Test statistics and adjusted P-values were non-significant for all tests. The total statistic output on the other hand was significant, so normal test statistics and normal P-values are given in Table 5.3.1. All Kruskal-Wallis H Test results are given in Appendix F.

Figure 5.3.4: Boxplots of nutrient concentrations [µM] at the start and the end for October RWE. □ represents the mean and \times the minimum and maximum values.

Table 5.3.1: Significant non-parametric statistical results (p<0.05) of Kruskal-Wallis H Test between the different October experiment set-ups for all initial nutrient concentrations and chlorophyll-a concentrations at the end of the experiment.

Nutrient	Sample 1 - Sample 2	Test Statistic	Std. Error	P-value	
$NO2+NO3$	O -control $- O$ -24	-18.667	7.188	.009	
	O -control $ O$ -48	-20.333		.005	
	C -control $-C-24$	-20.333		.005	
	C -control $-C$ -48	-24.667		.001	
	$C-6 - C-24$	-14.333		.046	
	$C-6 - C-48$	-18.667		.009	
NH ₄	O -control $ O$ -48	21.333	7.187	.003	
	$C-24 - C-48$	17.667		.014	
PO ₄	Non-significant for all samples				
Si	O -control $- O$ -24	-21.000	7.188	.003	
	O -control $ O$ -48	-20.000		.005	
	C -control $-C-24$	-19.000		.008	
	C -control $-C$ -48	-23.000		.001	
	$C-6 - C-24$	-14.667		.041	
	$C-6 - C-48$	-18.667		.009	
Chlorophyll-a	O -control $ O$ -48	-24.000	7.188	.001	
	$O-6 - O-48$	-23.000		.001	
	$O-12 - O-48$	-18.333		.011	
	C -control – C -48	$-16,000$.026	

Bigger-celled phytoplankton communities were generally dominated by diatoms (Table 5.3.2). River water addition resulted in an increase of total abundance of species, except in coastal 6%, where it resulted in a decrease when compared to the belonging control groups. Off-shore communities showed a higher total abundance with 6%, 24% and 48% river water dilution than related coastal communities. Species belonging to chlorophyta were only present in both communities with highest amount of river water dilution and there was a general increase for diatom, coccolithophore, cryptophyta and dinoflagellate abundance with higher river water dilution for coastal and off-shore communities.

Biodiversity measurements showed a decrease in Shannon index and increased in Pielou's evenness for coastal communities with increased river water dilution. Shannon index decreased with river water dilution in off-shore communities but increased with 48% dilution and Pielou's evenness decreased with higher river water dilution. Strongest changes for Shannon index and Pielou's evenness were within the 48% treatments when compared to control groups. In off-shore control groups *Emiliania huxleyi* was the most abundant species and in all off-shore river water dilution the diatom *Nitzschia tenuirostris*.

For the coastal communities chain building diatoms of the genus *Chaetoceros* (*C. curvisetus* and *C. tortissimus*) were most abundant, except with 6% river water dilution, where it is the coccolithophore *Emiliania huxleyi*.

Table 5.3.2: Shifts and biodiversity changes of bigger-celled phytoplankton groups for Lamas River water addition in October. Percentage represent the concentration of Lamas river water within the communities.

Dilution of winter coastal and off-shore communities with Lamas river water resulted in an increase of chlorophyll-a concentrations after a lag-phase of 96 hrs (Figure 5.3.5). Whereby initial concentrations within communities from the same origin were similar (coastal: $0.20 \pm$ 0.01 μ g/l, off-shore: 0.14 \pm 0.01 μ g/l), only with high dilution (48%) both groups had lower concentrations (coastal: 0.13 ± 0.01 µg/l, off-shore: 0.11 ± 0.01 µg/l).

An increase over time until 408 hrs was observed in all communities and higher increase was observed with higher dilution factors (coastal: 6% to 0.41 ± 0.14 , 12% to 0.38 ± 0.12 , 24% to 0.34 \pm 0.15, 48% to 2.02 \pm 1.48 µg/l and off-shore: 6% to 0.42 \pm 0.05, 12% to 0.39 \pm 0.08, 24% to 0.52 \pm 0.12, 48% 1.42 \pm 0.75 µg/l).

Figure 5.3.5: Chlorophyll-a concentrations [µg/l] and picophytoplankton species abundances in January for coastal (C and filled circles) and off-shore (O and non-filled circles) communities with different amount of Lamas River water addition, the error-bars represent standard deviations.

There was no general trend for picoeukaryote abundances over time with amount of dilution or community origin. Picoeukayote abundance decreased in coastal control (from $4.6x10^3 \pm$ 306 to 3x10³ \pm 1.2x10³ cells/ml), coastal 6% (from 4.6x10³ \pm 902 to 3.3x10³ \pm 321 cell/ml), coastal 12% (from $4.3x10^3 \pm 723$ to $2.9x10^3 \pm 1.5x10^3$ cells/ml), and off-shore 24% (from $3.3x10^3 \pm 503$ to $2.5x10^3 \pm 800$ cells/ml). An increased amount of cells were present in both 48% groups (coastal: from $2.5x10^3 \pm 611$ to $4.7x10^3 \pm 1.5x10^3$ cells/ml, off-shore: from $3x10^3 \pm 306$ to $9.6x10^3 \pm 5.4x10^3$ cells/ml).

Average initial abundances of *Synechococcus* were similar within coastal $(5.3 \times 10^4 \pm 3.5 \times 10^3)$ cells/ml) and off-shore $(4.6x10^4 \pm 1x10^4 \text{ cells/ml})$ communities and decreased over time to approximately half the initial abundances, with an exception for both high dilution groups, where average initial abundances were lower (coastal 48% with $3.1x10^4 \pm 3.9x10^3$ and offshore with $3.3x10^4 \pm 1.6x10^3$ cells/ml) and increased to a double for coastal 48% (6.6x10⁴ \pm $4.9x10⁴$ cells/ml) and to $4.3x10⁴ \pm 4.2x10⁴$ cells/ml. Both high dilution groups showed very high variations for *Synechococcus* abundances within the parallels after 408 hrs.

Average Prochlorococcus initial abundances increased in coastal (control: $9.6 \times 10^3 \pm$ 2.3x10³, 6%: $1.1x10^4 \pm 2.5x10^3$, 12%: $1.2x10^4 \pm 3.7x10^3$, 24%: $1.4x10^4 \pm 4.8x10^3$ and 48%: $1.9x10^4 \pm 5.5x10^3$ cells/ml) and off-shore (control: $7.4x10^3 \pm 451$, 6%: $9.4x10^3 \pm 757$, 12%: $7.4x10^3 \pm 265$, 24%: $8.9x10^3 \pm 361$ and 48%: $1.5x10^4 \pm 493$ cells/ml) communities with higher levels of dilution and decreased over time within all communities with the strongest decrease in off-shore 48% communities to $7.8 \times 10^3 \pm 153$ cells/ml. Variations between the three parallels of the treatments were higher with higher dilution and get stronger with time within coastal communities and are generally lower within off-shore communities.

Ammonium concentrations increased over time within all communities and decreased with higher river water dilution while phosphate initial concentrations were also increasing with higher dilution but decreasing with time in all communities and were respectively higher within coastal communities, see Figure 5.3.6. Nitrogen concentrations showed no change with time but river water dilution. Initial concentrations were higher with higher dilution in off-shore and coastal communities and higher in off-shore communities than coastal ones with same dilution factor, except 48% dilution communities, where the coastal communities (43.59 \pm 5.75 µM) resulted in a higher concentration than off-shore ones (28.30 \pm 18.96 μ M). Silica initial concentrations were similar within all communities (2.85 \pm 0.32 μ M) and increased overtime, except in both control groups where initial concentration were lower and decreased with time (coastal from 2.49 \pm 1.18 to 1.13 \pm 0.17 µM and off-shore from 2.30 \pm 1.12 to 0.82 \pm 0.15 µM). The increase in silica strengthened with higher dilution factors in all other treatments.

Variations of nutrient concentrations increased between the 3 parallels with higher dilution factor in coastal and off-shore communities.

Even though differences of nutrient concentrations can be seen when plotted as boxplots (Figure 5.3.6), Wilcoxon Signed Rank Tests for related samples (beginning – end) are nonsignificant for all January experiment set-ups.

Figure 5.3.6: Boxplots of nutrient concentrations [µM] at the start and the end for January RWE. □ represents the mean and \times the minimum and maximum values.

Significant Kruskal-Wallis H Test (one way ANOVA on ranks) results for independent samples of nutrient concentrations after Lamas River water was added to January communities are shown in Table 5.3.3. Adjusted Test statistics and adjusted P-values were non-significant for all tests. The total statistic output on the other hand was significant, so normal test statistics and normal P-values are given in Table 5.3.3. All Kruskal-Wallis H Test results are given in Appendix F.

Table 5.3.3: Significant non-parametric statistical results (p<0.05) of Kruskal-Wallis H Test between the different January experiment set-ups for all initial nutrient concentrations and chlorophyll-a concentrations at the end of the experiment.

Bigger celled phytoplankton communities were dominated by coccolithophores within all communities except the coastal community with 48% river water which was dominated by diatoms and thus the most abundant species within this communities was *Chaetoceros curvisetus* and not, as in all others, *Emiliania huxleyi*. There was a strong increase in species number and total abundance of diatom species within the coastal 48% groups, shown in an almost fivefold increase of total number of cells per liter (Table 5.3.4).

Species belonging to Chlorophyta and Dyctiochales were only present in coastal communities and the latter with high river water dilution. Dinoflagellate abundances increased within coastal communities and were highest within the 24% groups $(4.2 \times 10^4$ cells/l). Coccolithophores decreased in coastal communities with higher river water dilution and had the highest abundance of 9.8×10^5 cells/l within off-shore 48% communities. Cryptophyta decreased in off-shore and increased in coastal communities with increasing dilution. SDI increased and Pielou's evenness decreased with higher river water dilution within off-shore communities. Increased dilution level within coastal communities on the other hand resulted in a decrease in Shannon diversity index and an increase in Pielou's evenness.

Table 5.3.4: Shifts and biodiversity changes of bigger-celled phytoplankton groups for Lamas river water addition in January. The percentage represent the concentration of Lamas river water within the communities.

	Off-shore control	Off-shore 6%	Off-shore 12%	Off-shore 24%	Off-shore 48%
Total number of species	30	27	31	34	25
Total number of cells/l	793,216	1,011,136	944,416	925,248	1,108,680
Most abundant species	E. huxleyi	E. huxleyi	E. huxleyi	E. huxleyi	E. huxleyi
Bacillariophyceae cells/l	141,680	62,160	320,800	438,496	124,800
Prymnesiophyceae cells/l	615,264	931,712	605,056	446,368	976,720
Cryptophyceae cells/l	30,624	9,280	13,920	12,064	2,320
Pyrrophyceae cells/l	5,648	7,984	4,640	28,320	4,840
Dictyochophyceae cells/l	$\overline{0}$	$\mathbf{0}$	$\mathbf{0}$	Ω	Ω
Chlorophyceae cells/l	$\overline{0}$	Ω	Ω	Ω	Ω
Shannon Diversity Index (H'max-H')	2.4	2.9	2.0	1.4	2.6
Pielou's evenness J'	0.3	0.1	0.4	0.6	0.2
	Coastal control	Coastal 6%	Coastal 12%	Coastal 24%	Coastal 48%
Total number of species	26	27	28	33	37
Total number of cells/l	793,216	1,042,112	919,872	526,360	4,380,160
Most abundant species	E. huxleyi	E. huxleyi	E. huxleyi	E. huxleyi	C. curvisetus
Bacillariophyceae cells/l	21,440	122,928	354,480	52,600	3,848,880
Prymnesiophyceae cells/l	848,192	912,224	555,872	403,680	491,840
Cryptophyceae cells/l	6,496	5,568	8,352	27,840	18,560
Pyrrophyceae cells/l	2,736	928	1,088	42,240	18,560
Dictyochophyceae cells/l	$\mathbf{0}$	464	80	$\mathbf{0}$	$\mathbf{0}$
Chlorophyceae cells/l	$\mathbf{0}$	Ω	$\mathbf{0}$	Ω	2,320
Shannon Diversity Index (H'max-H')	3.0	2.6	1.9	2.0	1.8

Diluting spring-bloom (April) phytoplankton communities with river water (April experiment) resulted in an instant growth, with high dilution after a 48 hrs lag-phase, of chlorophyll-a, see Figure 5.3.7. While most coastal communities, including control, showed a maximum in chlorophyll-a concentrations between 144 and 192 hrs (control from 0.43 \pm 0.03 µg/l to 0.62 \pm 0.11µg/l, 6% from 0.44 \pm 0.03 µg/l to 0.88 \pm 0.13 µg/l, 12% from 0.43 \pm 0.05 μ g/l to 0.80 ± 0.33 μ g/l, 24% from 0.31 ± 0.05 μ g/l to 0.74 ± 0.24 μ g/l), followed by a decrease to 288 hrs, high river dilution resulted in a slow, but steady increase with time in chlorophyll-a from 0.31 ± 0.05 µg/l to 0.62 ± 0.19 µg/l after 240 hrs and then a decrease to half the concentration (0.23 \pm 0.05 µg/l) at 288 hrs. Off-shore communities showed however no change over time (control and 6% river water) or a steady increase throughout the experiment in chlorophyll-a (12% from 0.25 \pm 0.02 µg/l to 0.39 \pm 0.16 µg/l, 24% from 0.22 \pm 0.02 µg/l to 0.42 \pm 0.26 µg/l and 48% from 0.20 \pm 0.02 µg/l to 1.47 \pm 0.17 µg/l).

Figure 5.3.7: Chlorophyll-a concentrations [µg/l] and picophytoplankton species abundances in April for coastal (C and filled circles) and off-shore (O and non-filled circles) communities with different amount of Lamas River water addition, the error-bars represent standard deviations.

Due to a technical defect in temperature control of the culture room, this experiment was stopped after 288 hrs, so the off-shore communities do not reach their stationary growth phase.

Picoeukaryote abundances resulted in an increase over time in coastal communities (control from 2.1x10 3 ± 252 to 4.6x10 3 ± 839 cells/ml, 6% from 2.9x10 3 ± 451 to 4.9x10 3 ± 346 cells/ml, 12% from 2.6x10³ \pm 624 to 5.1x10³ \pm 702 cells/ml, and 24% from 2.1x10³ \pm 300 to $3.2x10^3 \pm 839$ cells/ml) and a decrease in off-shore communities (control from 2.4x10³ \pm 473 to $1.5x10^3 \pm 306$ cells/ml, 6% from $3.8x10^3 \pm 265$ to $1.8x10^3 \pm 265$ cells/ml, 12% from $2.7x10^3 \pm 321$ to $2.2x10^3 \pm 208$ cells/ml, and 24% from $3.4x10^3 \pm 265$ to $1.9x10^3 \pm 907$ cells/ml), except with 48% river water dilution for both community origins. While off-shore communities decreased steadily, coastal ones peaked after 96 hrs (48%), 144 hrs (12%) and 192 hrs (control, 6%, and 24%).

Synechococcus abundances increased first with higher river water dilution but decreased with higher dilution (24 and 48% coastal and 48% off-shore). Off-shore communities resulted in higher abundance of *Synechococcus* cells per milliliter at the end of the experiment. Throughout all communities *Synechococcus* increased till the end of the experiment and showed the highest abundance with intermediate river water dilution within off-shore communities (6% from $2.2 \times 10^4 \pm 2.3 \times 10^3$ to $5.3 \times 10^4 \pm 1.1 \times 10^4$ cells/ml, 12% from $1.9x10^4 \pm 1.3x10^3$ to $6.1x10^4 \pm 1.8x10^4$ cells/ml, and 24% from $1.4x10^4 \pm 115$ to $5x10^4 \pm 1.5x10^4$ 1.8x10⁴cells/ml). Further, there was an increase in the standard deviation with time in all treatments.

Prochlorococcus abundance showed no general trend to dilution with river water in neither communities. *Prochlorococcus* peaked but did not change to the end within all coastal communities, except with 12% where the dilution led to an overall increase from $5.1x10^3 \pm$ $1.1x10³$ to $8x10³ \pm 1.3x10³$ cell/ml. Off-shore communities with no (from $2.3x10³ \pm 265$ to $4.1x10^3 \pm 58$ cells/ml) or low dilution (6% from $4.1x10^3 \pm 862$ to $7.9x10^3 \pm 2.3x10^3$ cells/ml and 12% from 4.5x10³ \pm 208 to 6.1x10³ \pm 1.1x10³ cells/ml) also resulted in an increase over time whereby high dilution with 48% river water resulted in a peak after 96 hrs (from $8.1x10^3 \pm 1.1x10^3$ to $1x10^4 \pm 1x10^3$ cells/ml) followed by a decrease to $5.7x10^3 \pm 2.6x10^3$ cells/ml after 288 hrs.

Figure 5.3.8: Boxplots of nutrient concentrations [µM] at the start and the end for April RWE. □ represents the mean and \times the minimum and maximum values.

Initial nitrogen and silicate concentrations increased with percentage of river water dilution while initial concentrations of phosphate and ammonium decreased within communities, as shown in Table 5.3.8. Nitrogen concentration increased till the end of the experiment in all diluted communities, but decreased within coastal control (from 0.18 ± 0.11 to 0.06 ± 0.0 μ M) and off-shore control (from 0.44 \pm 0.14 to 0.14 \pm 0.09 μ M).

The ammonium concentration decreased with time within control and intermediate (12%) dilution but increased with 24 to 48% dilution in both communities. All coastal communities showed a decrease in phosphate concentration with time. Off-shore control (from 0.12 \pm 0.04 to 0.20 \pm 0.01 µM) and 6% (from 0.09 \pm 0.06 to 0.16 \pm 0.02 µM) dilution communities showed an increase and all higher diluted communities showed a decrease of phosphate over time. Control and 6% river water dilution showed a slight decrease or no change of silicate over time whereas all other dilution factors resulted in an increase in coastal and off-shore communities.

Table 5.3.5: Significant non-parametric statistical results (p<0.05) of Kruskal-Wallis H Test between the different April experiment set-ups for all initial nutrient concentrations and chlorophyll-a concentrations at the end of the experiment.

Nutrient	Sample 1 - Sample 2	Test Statistic	Std. Error	P-value	
$NO2+NO3$	O -control $- O$ -24	-22.333	7.188	.002	
	O -control $- O$ -48	-24.667		.001	
	$O - 6 - O - 24$	-15.333		.033	
	$O-6 - O-48$	-17.667		.014	
	C -control $-C-24$	-17.000		.018	
	C -control $-C$ -48	-20.000		.005	
	$C-6 - C-48$	-16.333		.023	
NH ₄	Non-significant for all samples				
PO ₄	C -control $-C-12$	19.500	7.188	.007	
	C -control $-C-24$	18.000		.012	
	$C-6 - C-12$	20.167		.005	
	$C-6 - C-24$	18.667		.009	
Si	O -control $- O$ -24	-19.333	7.188	.007	
	O -control $ O$ -48	-22.000		.002	
	$O - 6 - O - 24$	-15.000		.037	
	$O - 6 - O - 48$	-17.667		.014	
	C -control $- C$ -12	-14.333		.046	
	C -control $-C-24$	-20.000		.005	
	C -control $-C$ -48	-22.667		.002	
	$C-6 - C-24$	-15.000		.037	
	$C-6 - C-48$	-15.000		.037	
Chlorophyll-a	Non-significant for all samples				

Even though differences of nutrient concentrations could be seen when plotted as boxplots, Wilcoxon Signed Rank Tests for related samples (beginning – end) were non-significant for all April experiment set-ups.

Significant Kruskal-Wallis H Test (one way ANOVA on ranks) results for independent samples of nutrient concentrations after Lamas River water was added to April communities are shown in table 6.3.5. Adjusted Test statistics and adjusted P-values were non-significant for all tests. The total statistic output on the other hand was significant, so normal test statistics and normal P-values are given in Table 6.3.5. All Kruskal-Wallis H Test results are given in Appendix F.

Table 5.3.6: Shifts and biodiversity changes of bigger-celled phytoplankton groups for Lamas river water addition in April. The percentage represent the concentration of Lamas river water within the communities.

A very strong increase in diatom cells per liter in the communities was the result of river water dilution, see table 5.3.6. While coccolithophores showed only slight changes within off-shore communities, an increase with added amount of river water (from 6.8×10^5 cells/l within the control to 1.2x10⁶ cells/l within 24% dilution) but a drop to a quarter $(3.7x10⁵$ cells/l) with 48% dilution was observed in coastal communities.

Further an increase of dilution factor resulted in an increase of Pyrrophyceae in off-shore communities. Cryptophyceae showed the highest abundance within 48% diluted communities and lowest within 12 and 24% communities.

The coccolithophore *Emiliania huxleyi* was the most abundant species in all communities, except the off-shore 48%, where the chain building diatom *Chaetoceros curvisetus* was most abundant, as shown in Table 6.3.6. SDI was smallest in off-shore 24% and coastal 48% communities. Whereby Pielou's evenness J' was greatest in off-shore 6% and 24% communities.

5.3.1 Statistical problems

Non-parametrical Wilcoxon signed Rank test for all nutrient concentration differences at the start and the end of the experiment in all experiments show non-significant results, even though differences of nutrient concentrations can be seen when plotted as boxplots. These non-significant results are due to very low sample sizes and less sensitivity in nonparametrical testing.

5.4 Discussion

There was a decrease of 23% in the average silicate concentration from the year 2003 (Tuğrul *et al*., 2004) to 2015 (this study) from 99.1µM to 75.88 µM. Meanwhile the concentration of nitrate increased 25% from 89.34 μ M to 114.61 μ M, which led to a decrease of Si:N-ratio. The average ratio of 0.66 in recent measurements is below the Redfield ratio of 0.93 and might be an indication of silicate depletion in the future at the coastal environment. During a long term seasonal sampling from January 1999 to December 2007 an overall average N:P-ratio of 279 and a Si:N-ratio of 1.1 was measured (Koçak *et al*., 2010). The N:P-ratios measured in this study are > 284.3 for all measurements. Increase of N:P- and decrease of Si:N-ratios suggest that phosphate is still the main controlling factor, yet for a diatom dominated coastal phytoplankton community silicate depletion might lead to a non-diatom dominated community (Humborg *et al*., 2000; Egge and Aksnes, 1992).

The spring bloom observed from April to June in the 2015 ETS coastal station was mainly driven by diatom species (4.3.1.8) and might have been controlled by silicate decrease in river water. However, the late summer bloom in August, 2015 was dominated by coccolithophores, not by diatoms, even though silicate concentrations were as high as during spring discharged by the Lamas River water.

A decrease in riverine silicate is often caused by damming and human-made water alterations (Aigars et al., 2014; Humborg et al., 2006). There are no dams built on the Lamas River. On the other hand two hydroelectric power plants were built between 2007 and 2009 on the Lamas River, which take up some of the river water via big pipes (http://www.camcocleanenergy.com/case-studies/lamas-hydroelectric-power-plant-case-

study) and thus influence the total volume transported by the Lamas River.

The volume plays a crucial role since it influences the speed of the water transport and thus the flushing time and solubility of chemicals within it (Sigleo and Frick, 2003). Silicate is less soluble compared to nitrate and the latter increases with water flow and speed, showing no dilution effect (Hill *et al*., 1999) while silicate is reported to decrease during high rain and flood times (Sigleo and Frick, 2003). However, nitrogen shows similar behavior as described in the literature, and silicate shows a higher concentration in rainy and high water carrying months than in the relatively slow flowing summer months, which is similar to the results of Aigars *et al*. (2014) for the Daugava River in Lativa. Slower water flow causes longer water residence time and might have the same silicate reduction effect as reservoirs behind dams. Nevertheless, changes in Si:N-ratios seen in this study are more likely caused by human induced nitrogen input.

The reduced freshwater discharge from rivers for the Mediterranean Sea results in a reduction of elements particularly originating from natural sources like erosion and this was also shown for the NLB where Lamas River is situated (Ludwig et al., 2009).

Additionally the agricultural needs of water and increase of fertilization around the river have changed in recent years due to a shift from citrus tree farms and small vegetable fields to greenhouse banana farms (Emekli et al., 2010). Banana plants need a higher amount of water and the surrounding fields in Limonlu area are watered via small channels supplied by Lamas River water. Molle *et al*. (2008) showed that in Amman, Jordan, the total water cost for banana farms are approximately 3 times higher than for citrus orchards and 5 times higher than for vegetable farms. Furthermore, there is a non-neglectable amount of pesticides and fertilizers used in the surrounding greenhouses. This might explain the decrease in PO_4 during summer time in addition to natural causes. Changes in water quality due to agricultural developments are well known (Hinsby *et al*., 2012; Ludwig *et al*., 2009) and an explanation for the recent increase of nitrate. Even though eutrophication might not be a danger for the small coastal area fed with nutrients by the Lamas River, silicate depletion and high N:P-ratios might lead to a more oligotrophic system in the coming years. River water discharge to marine systems results in multiple changes in the medium, such as temperature, salinity, and nutrient concentrations and has thus a strong influence on phytoplankton communities, even on the existence of summer blooms (Cloern *et al*., 1983). Already small environmental changes may lead to changes in phytoplankton assemblages (Lichtman *et al*., 2012; Finkel *et al*., 2010 and references within) and might affect the whole local food web (Frederiksen *et al*., 2006). Characteristics of river water as well as plankton communities change with the season and hence effects of the same river to the same area differ throughout the year (Aigars *et al*., 2014; Ludwig *et al*., 2009; Spatharis *et al*., 2007; Doğan-Sağlamtimur and Tuğrul, 2004; Sieglo and Frick, 2003; Cloern *et al*., 1983). As shown in chapter 4 of this thesis, precipitation and consequently the river discharge vary and on the other hand the phytoplankton communities change seasonally. Small seasonal changes in river water nutrient concentrations were measured in addition. All these variations resulted in different responses to river water addition by the present phytoplankton communities, especially in October. The strongest increase in chlorophyll-a was triggered by river water addition into the off-shore community in October and April. In January, addition of river water resulted in an increase of chlorophyll-a in the off-shore and the coastal treatments consisting of 48% river water (Table 6.4.1).

It was obvious that in October the increased river water addition led to a steeper positive slope in Chl.-a, picoeukaryotes, *Synechococcus* and *Prochlorococcus*, except the latter had a steeper negative slope in off-shore waters. In the April experiment, intermediate river water addition caused the steepest slopes. The experiment in January showed that both, *Synechococcus* and *Prochlorococcus* decrease rather than increase and only high river water addition led to a positive slope in *Synechococcus*.

Late winter/spring and autumn blooms are very common in the Mediterranean (Raveh *et al*., 2015; Spatharis et al., 2007). During these natural occurring blooming times, the phytoplankton community is more diverse (see chapter 4; Spatharis *et al*., 2007) and thus react very fast and strong to favorable changes, such as nutrient additions via added river water. Chlorophyll measurements of the April experiment indicate that the coastal communities were at the end of a bloom when the experiment started. The initial chlorophyll-a concentrations are higher than in the other two experiments and a short but fast increase in chlorophyll-a was observed in coastal communities, followed by an equally short and fast decrease. The off-shore 48% treatment showed a steady increase of chlorophyll-a until the end of the experiment, even exceeding chlorophyll-a concentrations of coastal treatments. In October on the other hand, the chlorophyll-a measurements indicate an initial phytoplankton community which was directly at or shortly before their blooming time.

An increase of chlorophyll-a with increased river water concentration was observed, showing that the initial species were limited in nutrients and react with an increase in biomass and thus chlorophyll-a when those limiting nutrients are added. Chlorophyll-a concentrations as a measure of biomass is questionable, since chlorophyll-concentrations are depending on available nutrient concentrations and other factors (Eker-Develi *et al*., 2006). Diatoms exposed to Si:N-ratio of 1 show lower chlorophyll increase but higher cell-number than those exposed to a N:P-ratio of 4 (Gilpin *et al*., 2004). Despite this, chlorophyll-a concentrations are often used as control for the state of a phytoplankton community.

Low concentrations of silicate and nitrogen were measured in the initial sea water in January as well as in the added river water. Additionally the communities in January consist mainly of coccolithophores, except the coastal 48% river water treatment and fast growing diatom species in the other two experiments led to an increase in diatom abundances (Figure 5.4.1). Since coastal and off-shore communities were very similar during winter months, and thus in January, both community origins are more alike, also with river water added than in the other experiments (Figure 5.4.3).

Initial communities in October and April were limited in nitrogen with N:P-ratio below 5 and the April coastal communities were additionally silicate-limited with Si:N-ratio of 0.7. After addition of river water, all phytoplankton communities were exposed to P-limited waters with N:P-ratios > 30 and silicate saturation with Si:N-ratios of \sim 1. Highest N:P-ratios of > 1,000 were found in October communities with 48% river water.

While phosphorous in the April 48% treatments decrease, nitrogen and silicate increase, indicating that remineralization took place. Communities within the January experiment were initially exposed to highly N-limited environment, after river water addition as well and turn to the end of the experiment to P-limitation, whereby communities with more than 6% river water had phosphorus concentrations at the detection limit of 0.02 μ M. The change in nutrient limitation might be caused by bacterial activity and the remineralization of organic matter after a certain time. The fast turn-over rate of phosphate (Benitez-Nelson and Buesseler, 1999), which is most probably taken up very efficient by the dominant species *E. huxleyi*, resulted in a P-limited system. The high N-limitation at the beginning in January might be one reason for no growth of diatoms in most of the communities, the high abundance of *E. huxleyi* and the decrease of *Prochlorococcus* and *Synechococcus*. Cyanobacteria, here *Synechococcus* and *Prochlorococcus*, are known to be nitrogen limited (Martiny *et al*., 2013; Bertilsson *et al*., 2003) and heterotrophic bacteria as well as biggercelled species are P-limited or P- and N-co-limited (Moutin and Raimbault, 2002; Zohary and Robarts, 1998) in the Mediterranean and also the NLB (see chapter 4).

Spatharis *et al*. (2007) recorded that environmental condition dependent phytoplankton assemblages exist, leading to different kind of blooms. They state that after a nutrient peak in surface waters a bloom occurs, dominated by a single small diatom species in a low diverse community, as it was seen in the October and the April experiments. The species *Nitzschia tenuirostris* and species from the genera *Chaetoceros* were the only dominant species in all experiments not belonging to coccolithophores. *N. tenuirostris* is known as a blooming small diatom species especially in the Black Sea region (Balycheva, 2014; Đakovac *et al*., 2004) and has been recently reported to be one of the major contributing species in therms of abundance to the bulk phytoplankton in Mersin Bay area (Tuğrul *et al*., 2015; Uysal *et al*., 2014). *Chaetoceros* species are well known bloomer in the Mediterranean (Eker Develi, 2004; Polat et al., 2000; Mura et al., 1996; Ignatiades et al., 1995). The only freshwater species found was *Closterium parvulum* in both October 48% treatments and the coastal 48% treatment in January, see figure 6.4.1.

Closterium parvulum is exclusive a freshwater species (Brook, 1981) and thus very sensitive to salinity, explaining why it was only found in treatments consisting of 48% river water and a salinity of approximately 20.5.

Figure 5.4.1: Bigger-celled phytoplankton group abundances in cells per liliter for all experiments. The letter O represents off-shore, the letter C coastal communities, and the percentages the amount of river water added at the beginning of the experiments.

Prochlorococcus species are highly sensitive to temperature reaching their maximum when surface waters are stratified during hot summer months (Zubkov *et al*., 2000).

The maximum growth rates for two strains of *Prochlorococcus* were shown to be between 25 and 27 °C (Johnson *et al*., 2006). All three seasonal experiments were conducted with the same water temperature of 21 \pm 1 °C, which is below the optimum temperature of *Prochlorococcus* and might have been the reason for its lack of growth or decrease in most of the experiments (Table 5.4.1). Tolerance differences to salinity might also play a crucial role for species sorting after river water was added (Kirst, 1989) and hence loss of rare species from the system.

The amount of nutrients carried by rivers into the sea are altered by humans, with intense consequences for phytoplankton assemblages, such as eutrophication when too many nutrients reach the sea water (Ludwig *et al*., 2009; Yunev *et al*., 2007; Moncheva *et al*., 2001; Uysal and Sur, 1995; Sur *et al*., 1994) or limitation when damming causes loss of nutrients as it is the case for silicate (Aigars et al., 2014; Ludwig *et al*., 2009; Humborg et al., 2006).

Water discharge volume and speed influences the concentration of nutrients, the salinity, and the temperature in the affected seawater, as well as the size of the area it affects. During rainy seasons the river water can occasionally be carried on top of the seawater out to offshore regions, as it was the case in May, 2015 (Chapter 4).

Further, during rainy seasons, the carried water is very turbid (brown-yellowish color) and affects the phytoplankton community. Cloern showed in 1987 that a negative relationship of turbidity and phytoplankton biomass exists, which is mainly caused by light attenuation of particles carried by the river (Cole *et al*., 1992).

Month	Treatment	Chlorophyll-a $[\mu g^* \hat{l}^{-1} d^{-1}]$	Picoeukaryotes [cells*ml ⁻¹ d ⁻¹]	Synechococcus $[cells*ml^{-1}d^{-1}]$	Prochlorococcus [cells*ml ⁻¹ d ⁻¹]
October	$C - C$				-113
	$C - 6$	0.04		2,846	-123
	$C - 12$	0.09		10,179	105
	$C - 24$	0.11		23,990	128
	$C - 48$	0.11	2,467	122,842	1,333
	$O - C$		144		-209
	$O - 6$				-442
	$O - 12$		-11		606
	$O - 24$	0.04	333	6,954	-724
	$O - 48$	0.12	800	69,142	$-1,892$
January	$C - C$		-98	$-2,127$	-418
	$C - 6$			$-1,788$	-382
	$C - 12$		-76	$-1,398$	-398
	$C - 24$		-78	$-1,737$	-567
	$C - 48$	0.19	127	2,022	-308
	$O - C$		47	$-2,151$	-276
	$O - 6$			$-1,529$	-290
	$O - 12$		49	$-1,649$	-182
	$O - 24$		-49	-610	-210
	$O - 48$	0.09	384	584	-418
April	$C - C$		883	325	
	$C - 6$	0.07	800	883	292
	$C - 12$	0.06	1,100	1,222	567
	$C - 24$	0.07	694	444	-111
	$C - 48$	0.05	153	1,964	272
	$O - C$		-81	1,000	153
	$O - 6$		-167	2,633	319
	$O - 12$		-42	3,497	131
	$O - 24$		-122	3,033	44
	$O - 48$	0.13	917	2,142	-200

Table 5.4.1: Comparison of Chlorophyll-a, picoeukaryotes, *Synechococcus* and *Prochlorococcus* slopes in all three RWE. Positive slopes are given in black and negative slopes in red.

River water addition leads to less similar phytoplankton assemblages as in the control groups (Figure 5.4.2). The smaller the difference of river water contribution to the communities, the more similar they are.

The most similar are the control and the low river water communities, whereby the coastal intermediate river water communities C12 and C24 show also high similarities.

Figure 5.4.2: Bray-Curtis-Similarities [%] for all experiments between the same origin communities of one experiment (left) and detailed between all coastal communities (right). The first letter "O" represents Off-shore and the first letter "C" represents coastal communities. The second letters "C" represents control treatments and the numbers represent the percentage of river water within the community.

October communities are the least and January communities are the most similar. January off-shore communities are all very similar to each other, compared to the other two seasons. Additionally are January off-shore and coastal communities when existing of the same amount of river water the most similar to each other (Figure 6.4.3). In October the communities between coast and off-shore differ the most, whereas they are more than 75% similar in January.

Figure 5.4.3: Bray-Curtis-Similarities [%] for all experiments between off-shore and coastal communities of one experiment. The first letter "O" represents Off-shore and the first letter "C" represents coastal communities. The second letters "C" represents control treatments and the numbers represent the percentage of river water within the community.

While October and April communities are less than 50 percent similar to each other, January communities are only below the 50% mark if the community consisted of 48% river water, see figure 5.4.3. This shows that in January the water column was well mixed (chapter 4) and thus the initial nutrient conditions similar for coastal and off-shore communities resulting in very similar phytoplankton assemblages.

5.5 Conclusion

Changes in water flux due to two hydroelectric power plants and agricultural habits of the rural area around the Lamas river mouth led to an increase in nitrogen concentration and a slight decrease in silicate concentration within the Lamas River water. Consequently the Si:N-ratio decreased compared to previous ones from 1.1 to 0.66. Simultaneously the N:Pratio increases from 279 to 284. These changes in nutrient composition might lead to a shift in coastal phytoplankton assemblages to a less silicious species based community since the diatom dominated coastal community is depending on silicate influx from the river. Most important, the Lamas River should not be addressed as a "natural" river anymore. Human induced nutrient changes are measurable and influence the local coastal ecosystem.

The experiments showed that the amount of river water influencing phytoplankton communities plays a role as well as the community origin it affects. Off-shore communities tend to change into coastal-like communities after river water was added, shown in high Bray-Curtis-Similarities with increased river water concentration. Seasonality of the phytoplankton assemblages additionally affect the amount of influence of river water. The coastal and off-shore communities in January had high Bray-Curtis-Similarities.

Winter communities have the highest Bray-Curtis-Similarities in natural occurring assemblages, see chapter 4, and thus coastal and off-shore nano- and micro-sized phytoplankton was similar when the experiment was set-up. Only high river water addition led to a growth within the coastal community and caused hereby the difference in assemblages seen in Figure 5.4.3.

Increase of Chl.-a measured as growth of the phytoplankton communities was mainly caused by an increase in diatom species. This also resembles the measurements in natural habitat, as shown in chapter 4. River water can thus still be used as a "natural" nutrient source.

River water has severe effects on several environmental conditions. It does not only enhance nutrient concentrations but alters salinity, temperature, and turbidity. All these changes are stress and community shaping environmental factors. All these factors were studied extensive in cultured phytoplankton and under natural conditions. Species dependent salinity, temperature and light intensity preferences and optima affect the existing community. In this study only the effect of nutrients was investigated and the other implications by river water addition neglected.

Further, limitation of nutrients to Phytoplankton is defined after Redfield Ratios (RR) with 16 for N:P and 0.94 for Si:N (Redfield *et al*., 1963). Even though species of different phytoplankton groups vary in their nutrient ratio preference for growth (Arrigo, 2005; Klausmeier *et al*., 2004; Geider and LaRoche, 2002), the average of all species nutrient composition is 16:1 and 0.94 for N:P and Si:N after RR (Klausmeier *et al*., 2004).
6. Sediment addition experiment

6.1 Introduction

Dumping waste into the Sea is a common way of waste management. Since the 1950's the thereby accruing threats to the ecosystem were noticed and first steps for protection taken. In 1970 the National Environmental Policy Act (NEPA) established the President's Council on Environmental Quality (CEQ) which has played a pioneer role in scientific based regulating of ocean dumping. Other countries followed this example (e.g. The London Convention, 1972; Oslo Dumping Convention, 1972) controlling and preventing marine pollution on an international basis. Still, the dumping of dredged material is regulated country and area specific but not prohibited as many other wastes are, such as radioactive waste in 1975 (London Convention Protocol, 1975). Permits for ocean disposal of dredged material, regulations and the control of such is country dependent. There had been a lot of scientific studies and work done on the subject of suitable disposal sites in European and American waters (Kapsimalis *et al.*, 2013, 2010; Fredette and French, 2004; Essink,1999) and lead to international treaties and protocols (PIANC, 1998). Despite the scientific interest for decades with this topic in many other countries, this project, and thus this study, is a case project for Turkish waters.

Natural transport of ocean or riverine sediment occasionally results in deposition in strategically important places, such as ports. Origin of these sediments and exposure to substances during and after transportation to the dredged area determines not only grain sizes but also other biological and chemical characteristics of the sediment, such as concentrations of metals or toxins and organic matter (Moll and Mansfield, 1991; Gorsline, 1984). Sediment around Samandağ harbor is transported and influenced by the Orontes, or so called Asi or Al asi, River. Formed in the Baq'a (Bekaa) Valley in Lebanon, this 404 km long river flows through Lebanon, Syria and Turkey and discharges into the Mediterranean south of the port of Samandağ. Agricultural, industrial and urban activities along the river basin result in deterioration of water quality and eutrophication in the middle and lower reaches (UN-ESCWA and BGR, 2013). Sediment from Mersin Bay on the other hand is highly influenced by industrial and urban activities. Mersin is a large city with a population of approximately one million inhabitants and one of the major harbors of Turkey. Pollutions and toxins are often occurring in ports and harbors with high densities of ships and distributed by those into surrounding waters (Körbahti and Artut, 2010; Gabrielides *et al*., 1990).

Smaller marinas in rural areas without a direct influence by major rivers, industrial and agricultural activities or cities, as the Tirtar harbor, can be considered to exhibit predominantly natural (non-toxic) sediment.

To deepen and maintain harbor entrances and navigation channels dredging is required. Dredged material disposal into the Sea, however, often results in harmful impacts on the natural marine environment (Choi *et al*., 2005; Moll and Mansfiels, 1991). Accumulated nutrients, metals, organic matter and other toxins within the sediment represent a threat to most marine organisms (Nayar *et al*., 2004; Salomons *et al*., 1987; Salomons and Förstner, 1980). Contaminated sediment disposal into the marine ecosystem affects the ecosystem in various ways, depending on the nature and amount of contaminants (Reisch, 1980) and often leads to shifts in species composition or vanishing of marine species (Nayar *et al*., 2004; Monteiro *et al*., 1995; Moll and Mansfield, 1991).

While impacts on effects of dumped dredged material on benthic organisms are well studied (van der Wal et al., 2011; Nichols *et al*., 1990), studies on pelagic phytoplankton communities are scarce.

Transportation, resuspension and survival of benthic or settled pelagic phytoplankton species within the dredged material show high capability of out-competing the naturally occurring species in disposal site waters (Nalewajko and Murphy, 1998). Additional factors affecting the communities at the dumping site are manifold, such as turbidity, transported organic matter, toxins, nutrients and metals (Miller *et al*., 2010; Cao *et al*., 2007; Nayar *et al*., 2004; Salomons *et al*., 1987).

Increased water turbidity caused by resuspension of dumped sediments is time and area limited and can be reduced to a minimum by seasonal limited and duration controlled dredging (Essink, 1999). Dredged sediments release up to high quantities of carried substances into the marine environment, and hence influences the chemical properties of the marine environment. These affects might be of short duration if dumping is not regularly repeated (Boran *et al*., in prep.) but turn from temporary to permanent change of the dumping site environment if dumping is happening for long periods.

Variation in chemical compounds, quantity and toxicity within dredged sediments and species specific responses to introduced substances and environmental changes results in various responses of the pelagic phytoplankton community, complicating general conclusions (Miller *et al*., 2010; Duarte et al., 2000; Lewis, 1995).

Opposed results for bioassays testing pelagic bacteria and phytoplankton production correlation with added sediment (Choi *et al*., 2005; Moll and Mansfield, 1991; Severn *et al*., 1989) are the results of those unpredictable variations within the sediment and species specific responses within the communities.

Ecological status and site specific hydrographic characteristics challenge the generalization of environmental and ecological impacts of dredged material dumping (Bolam *et al*., 2006). Especially transported metals within the sediment result in a shift to metal resistant species (Monteiro *et al*., 1995), whereby *Synechococcus* sp. shows a high sensitivity to metals (Miao *et al*., 2005). Heterotrophic bacteria increase, autotrophic bacteria and phytoplankton decrease in abundance and production, when exposed to heavy metals from dredged and resuspended sediments (Nayar *et al*., 2004). Inhibitory substances introduced by sediment disposal might cause lower bacterial and phytoplankton production (Choi *et al*., 2005). However, every change in the nutritional environment might be followed by a competition for resources and result in shifts and out-competition of key species, leading to a change at the bottom level of the Mediterranean food web.

Comparing phytoplankton communities within dumping sites with reference areas is scientifically not conclusive due to patchiness and natural variability (Choi *et al*., 2005). Mesocosm experiments show a compelling potential to gain a better understanding how pelagic phytoplankton is influenced by dumped dredged material even though natural variability in physical and chemical water characteristics like mixing and resuspension of matter in the water column can not be reproduced.

The aim of this study within the framework of TÜBITAK DIPTAR project (Tuğrul *et al*., 2015) was to gain an insight into effects of dredged material dumping on local pelagic phytoplankton communities resulting in advices for least harmful dumping site locations. Further, to understand different effects of sediments from disparate influenced areas, sediment originated from three areas influenced by river discharge (Samandağ), human and industrial activities (Mersin) and natural (Tirtar) were chosen and compared.

6.2 Material and Methods

Surface sediments were taken from Samandağ, Mersin and Tirtar harbors with a Van Veen grab. Each sediment was mixed and put in plastic bags (for the experiment and metal analyzes) and glass jars (for POC and PON analyzes). The plastic bags were deep frozen and the glass jar was stored in a cold fridge until the experimental set-up and further analyzes.

On- and off-shore water was taken during ETS from the ETS-20 and the ETS-200 station from 3 to 5 m water depth and filtered through a 200 µm pore size net to exclude zooplankton predators. Then the communities were acclimated for 3-4 days under experimental conditions before the experiments were set up. Starting day (set-up day after acclimation) was $17th$ of May 2005 for the Samandağ experiment, $9th$ of June 2015 for the Mersin experiment and $15th$ of May 2016 for the Tirtar experiment.

The communities were hold in room temperature 21 ± 1 °C and a controlled dark-light cycle of 12:12 hours. Each treatment had a total volume of 5 liter and was set up in triplets (3 parallels). The position was randomly changed every 24 hours to prevent effects of the setup-location. Every 24 hours the communities were gently turned to distribute settled down cells in the water column again.

The amount of sediment added in wet-weight (WW), depending on the total amount of sediment available, to 20 l of on- and off-shore community. After 4 hours of settling time for the sediment, the upper sediment free water was taken out and distributed into the different treatments to gain final concentrations of low and high amount of sediment added (Table 6.2.1). Controls were left sediment water free.

Sediment origin		Conc. / L (low) [gWW] $ $ Conc. / L (high) [gWW]
Mersin harbor (urban influenced)	1.5	
Samandağ harbor (river + human influenced)	3.75	22.5
Tirtar harbor (natural)		

Table 6.2.1: Amount of sediment added into all three sediment addition experiments

Every 24 to 48 hours samples for nutrients, chlorophyll-a and small celled phytoplankton species (flow-cytometric measurements) were taken with a plastic syringe. Nutrient samples were taken first into with 10% HCl pre-cleaned high density polyethylene bottles (HDPE). The samples were deep frozen (-20°C) until they were analyzed.

Nutrient concentrations (nitrate+nitrite, reactive silicate, phosphate and ammonium) were measured after standard colorimetric methods (Strickland and Parsons, 1972) using a Bran Luebbe model four-channel auto-analyzer. Detection limits for nitrate+nitrite, silicate, phosphate and ammonium are as follows: 0.05 μ M, 0.3 μ M, 0.02 μ M and 0.05 μ M respectively.

Chlorophyll-a and small celled phytoplankton samples were taken into 25 ml scintvials after gently mixing of communities via 10 times turning over the lit-bottom-axis. Chlorophyll signal was measured using a Turner Design Model blue Fluorometer. Calibration of the flourometer was done before the first experiment. For this water of all experiments was filtered (between 1 to 2 liter of sample volume) on white GF/F filters under dim light and, after filters are digested (after Strickland and Parsons, 1972), analyzed using a conventional spectrofluorometric method with a HITACHI fluoresence spectrophotometer F-2500. The excitation wavelength was 420 nm and the emission wavelength was 669 nm. Samples of the same waters were measured simultaneously with the fluorometer resulting in a calibration curve.

Picophytoplankton species (picoeukaryotes, *Synechococcus* and *Prochlorococcus*) were counted every 24-48 hours via flow-cytrometry. Differences in light absorption of red (633 nm) and orange (488 nm) light by species depending pigment compositions using a flowcytometer of Apogee A50-micro Flow System resulted in cell-concentrations per ml for picoeukaryotes, *Synechococcus* and *Prochlorococcus*. A total volume of 150 µl with a speed of 60 µl per minute and 2 flush cycles to clean between sampling was used as set-up. Further a threshold in red was set at 39 to prevent the counting of small particles and dead cells, the so called background noise. Samples for bigger-celled species identification were taken into 50 ml pre-cleaned borosilicate dark bottles, fixed with 1 ml 25% gluteraldehyde (final concentration of gluteraldehyde was 0.495%) and stored until identification. After 24 h settling time in settling chambers, the bigger cells were identified with help of an inverse light microscope and the abundance calculated. Abundance was used for the biodiversity (Shannon Index and Pielou's evenness) calculations, representing the shift in the communities. A small amount of sediment was weighted wet and after drying in the oven at 50°C until all water evaporated to gain wet-weight/dry-weight ratios (WW/DW).

Additionally metals and organic nutrients within the sediment were measured for a better comparison of differences on sediment origin. Metal analysis of sediment samples were performed according to the method described in EPA 3051 A. 0.1 g of dried samples were transferred to Teflon covered bottles and 5.0 mL concentrated nitric acid was added to each sample.

After 5 minutes, Teflon covered bottles were closed and put in the microwave at 120 ˚C. Samples were digested for 15 minutes and cooled down to room temperature. Then, 2.0 mL of concentrated hydrofluoric acid was added to each sample and a second digestion applied at 120 ˚C for 15 minutes. After the samples reached room temperature, 0.6 g of boric acid was added to each sample and the final digestion step was applied at 120 ˚C for 15 minutes in a microwave, followed by cooling to room temperature.

To gain a final volume of 50 mL distilled water was added and the samples were analyzed by Perkin Elmer Model (NexION® 350X) Inductively Coupled Plasma Mass Spectrometer (ICP-MS).

The freeze dried samples for TC, TOC and TN measurements were analyzed by the Vario El Cube Elementar Model CHN analyzer via dry oxidation method (Grasshoff *et al*., 1983, UNEP/MAP, 2006). For homogenization of the samples, dried and powdered sediment was sieved through 63µm pore size and defined amounts put into pre-combusted (6 hours at 400°C) silver (TOC) or tin (TC and TN) cups. 10 µl of distilled water was added to each sample and to remove inorganic carbon in form of $CO₂$ from the samples for TOC measurements 10 µl of 20% HCl (vol/vol). HCl was added until all inorganic carbon was removed. TC and TN measurements did not include this acid-adding step. After a drying period of one day in 60-70°C all silver cups were compacted and analyzed with the autosampler of CHN analyzer (Nieuwnhuize *et al*., 1994). Prepared standards with acetanilide (71.09% C, 10.36% N) were used for quantitative determination of TC, TN and TOC concentrations within the sediments.

Non-parametrical statistical Kruskal-Wallis H Tests, or so called one way ANOVA, was used to test for differences in nutrient and chlorophyll-a concentrations between different set-ups in each experiment, nutrients, and chlorophyll-a at the beginning of the experiment and at the end of the experiment. Additionally Wilcoxon Signed Rank Tests were done to compare the related samples of nutrient concentrations at the beginning and the end of each experiment. All statistical tests were conducted with SPSS.

6.3 Results

WW/DW-ratios show that Tirtar sediment had the most water stored within it, with a ratio of 1.714 and Mersin sediment the least (WW/DW = 1.488), see table 6.3.1.

Table 6.3.1: Wet-weight to dry-weight ratios of all sediments**.**

Sediment origin	Wet-weight / dry weight
Mersin	1.49
Samandağ	1.52
Tirtar	1.71

Except for chrome, all metal concentrations were highest within Tirtar sediment and higher in Mersin than Samandağ originated sediment as shown in table 6.3.2. Cadmium and aluminum are slightly higher within Tirtar originated sediment (0.49 and 42.4 g/kg) than the concentration within Mersin originated sediment (0.38 and 38.8 g/kg). Samandağ sediment carried less than half the concentration of nickel, copper, zinc, cadmium and aluminum than Tirtar sediment. Chrome concentrations were highest within Mersin (372.0 mg/kg) and lowest within Tirtar (278.1 mg/kg) originated sediment.

Table 6.3.2: Metals concentrations, total carbon, organic carbon and total nitrogen concentrations within sediments originated from Mersin, Samandağ and Tirtar ports.

Parameter per unit sediment	Cr [mg/kg]	Mn [mg/kg]	Fe $[g/kg]$	Co [mg/kg]	Ni [mg/kg]
Mersin	372.0	644	45.7	35.2	683
Samandağ	297.0	577	30.7	32.6	384
Tirtar	278.1	901	56.4	55.2	946.5
Parameter per unit sediment	Cu [mg/kg]	Zn [mg/kg]	Cd [g/kg]	Pb [mg/kg]	Al $[g/kg]$
Mersin	24.7	76.7	0.38	22.8	38.8
Samandağ	17.4	40.8	0.194	4.98	17.7
Tirtar	45.2	109.7	0.49		42.4
Parameter per unit sediment	$TC \text{ [mmol/g]}$	TOC [mmol/g]	TN [mmol/g]		
Mersin	4.52	0.37	0.03		
Samandağ	3.48	1.01	0.09		
Tirtar	4.99	1.10	0.07		

TOC and TN were lowest concentrated within Mersin originated sediment (0.37 and 0.03 mmol/g) and TC within Samandağ originated sediment with 3.48 mmol/g, as shown in Table 6.3.2. Total and organic carbon were highest within Tirtar originated sediment (4.99 and 1.10 mmol/g) and total nitrogen within Samandağ originated sediment with 0.09 mmol/g.

Mersin originated sediment addition led to an increase in chlorophyll-a in all communities. High and low addition to the coastal communities was immediately followed by an increase in chlorophyll-a whereas off-shore communities showed the typical lag-phase, 120 hrs for high addition and 192 hrs for low addition, see figure 6.3.1. Overall Chl.-a concentrations were higher throughout the experiment in coastal communities with same amount of sediment added.

Figure 6.3.1: Chlorophyll-a concentrations and picoplankton abundances for Mersin sediment addition over time. Filled circles and the letter C represent the coastal and non-filled circles and the letter O the off-shore communities, the error-bars represent standard deviations.

Higher added sediment results in a faster and higher increase of Chl.-a. Coastal communities had maximum chlorophyll-a concentrations after 96 hrs with 5.97 ± 0.12 µg/l for high and 120 hrs with 1.96 \pm 0.22 µg/l for low addition while off-shore communities showed maximal Chl.-a values after 216 hrs with 4.12 \pm 0.85 µg/l for high and 312 hrs with 1.26 \pm 0.28 µg/l for low addition.

Chlorophyll concentrations differed significantly $(p<0.01)$ at the end of the experiment between both controls and both high sediment addition treatments (Table 7.3.3).

High background noise due to small sediment particle was the reason to exclude the flowcytrometric measurements of picoeukaryotes, *Synechococcus* and *Prochlorococcus* for the first 48 hrs. Development in picoeukaryote abundances was higher with higher added sediment amount and in coastal communities. There was a constant increase in abundance for picoeukaryotes. The stationary phase for high sediment addition into the coastal communities was not reached at the end of the experiment. Sediment added in low concentration to coastal and high amount added to offshore waters resulted in a peak of picoeukaryote abundance at 144 hrs / 216 hrs with $9.2x10^3 \pm 3.1x10^3$ / $2x10^4 \pm 6.7x10^3$ cells per ml. Low amount added to off-shore communities was followed by a 120 hrs lag phase and a slight but steady increase until a peak at 264 hrs with an abundance of 6.4x10³ \pm 2.1x10³ cells per ml, a slight decrease at 312 hrs and the maximum abundance with $6.8x10³$ \pm 2.3x10³ cells per ml at 360 hrs and thus the end of the experiment.

Synechococcus abundance was higher within coastal communities and higher added sediment whereby the difference in coastal and off-shore communities for high additions were small. Changes in *Synechococcus* abundances throughout the experiment were minimal except an increase for coastal low addition and coastal control communities. Coastal communities with high amount of Mersin sediment added showed a slight drop in *Synechococcus* abundance at the end of the experiment. Even though the lowest *Prochlorococcus* abundance was in the control groups in respect to community origin, the off-shore low treatment showed the same number of cells as the control group at several times (48, 192 and 216 hrs). Off-shore treatments with both, high and low, sediment addition resulted in two peaks for *Prochlorococcus* abundance after 96 hrs with $1.6x10^4 \pm$ 5.4x10³ (high) and $4.8x10^3 \pm 1.6x10^3$ (low) and 216 (high) and 264 (low) hrs with $1.1x10^4 \pm 1.6x10^4$ $3.5x10³$ (high) and $4.3x10³ \pm 1.4x10³$ (low) cells per milliliter.

Overall, the variation for Chl.-a and pico-phytoplankton between the three parallels increased with amount of sediment added.

Figure 6.3.2: Nutrient changes for Mersin sediment addition over time. Filled circles and the letter C represent the coastal and non-filled circles and the letter O the off-shore communities, the error-bars represent standard deviations.

Nutrient changes with sediment addition and uptake during the experiment are shown in Figure 6.3.2. A general decrease for nitrogen (coastal high, low and control and off-shore high and control), phosphate (high additions for both community origins) and silica (all treatments except off-shore low and control) was observed over the duration of the experiment. Silica and ammonium concentrations increased with sediment addition, significant differences are shown in table 6.3.3, whereby silica was higher in addition to coastal (5.68 \pm 3.66 µM) than off-shore treatments (3.77 \pm 0.33). Phosphate was higher in high sediment addition to off-shore (with 0.25 ± 0.03 µM) than coastal treatments (0.30 \pm 0.15 μ M), as well is ammonium (C-high: 1.09 \pm 0.25 and O-high 0.39 \pm 0.44 μ M). The high concentration of phosphate in coastal and off-shore high sediment added treatments decreased fast within the first 144 hrs, in C-high to 0.04 ± 0.04 µM and for O-high even down to the detection limit of 0.02 \pm 0.01 µM. A peak of ammonium at 48 hrs for high addition treatments was followed by a sharp decline over the following 96 hrs, within Chigh from 3.02 ± 0.84 to 0.33 ± 0.28 µM and O-high from 3.16 ± 0.93 to 0.11 ± 0.04 µM. Variations for high sediment addition and off-shore communities between the three parallels were high, especially for ammonium measurements.

Figure 6.3.3: Boxplots of nutrient concentrations $[\mu M]$ at the start and the end for Mersin sediment addition. \Box represents the mean and \times the minimum and maximum values.

Even though differences of nutrient concentrations can be seen when plotted as boxplots, shown in Figure 7.3.3, Wilcoxon Signed Rank Tests for related samples (beginning – end) are non-significant for all Mersin sediment set-ups.

Kruskal-Wallis H Test (one way ANOVA on ranks) for independent samples of nutrient concentrations after Mersin port sediment was added are shown in table 6.3.3. Adjusted Test statistics and adjusted P-values were non-significant for all tests. The total statistic output on the other hand was significant, so normal test statistics and normal P-values are given in Table 6.3.3.

Nutrient	Sample 1 - Sample 2	Test Statistic	Std. Error	P-value		
$NO2+NO3$	Non-significant for all Samples					
NH ₄	O -control $ O$ -low	4.354	.566			
	O-control - O-high	-13.167	4.354	.002		
	O -low $ O$ -high	-10.667	4.354	.014		
	C-control - C-low	4.833	4.354	.267		
	C -control – C -high	-3.667	4.354	.400		
	C -low $ C$ -high	-8.500	4.354	.051		
PO ₄	O -control $ O$ -low	-1.167	4.298	.786		
	O-control - O-high	-10.167	4.298	.018		
	O -low $ O$ -high	-9.000	4.298	.036		
	C-control - C-low	3.167	4.298	.461		
	C -control – C -high	-6.833	4.298	.112		
	C -low $- C$ -high	-10.000	4.298	.020		
Si	O -control $ O$ -low	-4.333	4.350	.319		
	O-control - O-high	-11.667	4.350	.007		
	O -low $ O$ -high	-7.333	4.350	.092		
	C -control $- C$ -low	-3.333	4.350	.443		
	C-control – C-high	-8.667	4.350	.046		
	C -low $ C$ -high	-5.333	4.350	.220		
Chlorophyll-a	O -control $ O$ -low	-6.333	4.359	.146		
	O-control - O-high	-12.000	4.359	.006		
	O -low $ O$ -high	-5.667	4.359	.194		
	C -control $- C$ -low	-5.000	4.359	.251		
	C-control - C-high	-11.667	4.359	.007		
	C -low $ C$ -high	-6.667	4.359	.126		

Table 6.3.3: Non-parametrical statistical results of Kruskal-Wallis H Test between the different Mersin experiment set-ups for all initial nutrient concentrations and chlorophyll-a concentrations at the end of the experiment. Statistical significant differences ($p < 0.05$) are given in bold.

Coastal and off-shore treatments showed an increase in abundance of diatoms (Bacillariophyceae), coccolithophores (Prymnesiophyceae), dinoflagellates (Pyrrophyceae) and cryptomonads (Cryptophyceae) with sediment addition, whereby diatoms increased the strongest (Table 6.3.4). Coastal treatments showed higher abundance of cells compared to same treated off-shore treatments. Silicoflagellates (Dictyochophyceae) were only present within the coastal control group and disappeared with sediment addition.

	Off-shore control	Off-shore low	Off-shore high
Total number of species	22	25	18
Total number of cells/l	104,192	2,364,080	4,352,320
Most abundant species	E. huxleyi	N. tenuirostris	P. delicatissima
Bacillariophyceae cells/l	16,640	1,793,360	3,751,440
Prymnesiophyceae cells/l	71,456	348,000	373,520
Cryptophyceae cells/l	12,992	180,960	192,560
Pyrrophyceae cells/l	3,104	41,760	34,800
Dictyochophyceae cells/l	Ω	$\mathbf{0}$	Ω
Shannon Diversity Index (H'max-H')	1.8	0.9	1.2
Pielou's evenness J'	0.4	0.7	0.6
	Coastal control	Coastal low	Coastal high
Total number of species	29	26	26
Total number of cells/l	1,246,304	3,953,280	6,897,360
Most abundant species	C. curvisetus	P. delicatissima	P. delicatissima
Bacillariophyceae cells/l	1,081,120	3,382,560	5,941,520
Prymnesiophyceae cells/l	123,424	412,960	714,560
Cryptophyceae cells/l	25,984	129,920	218,080
Pyrrophyceae cells/l	12,992	27,840	23,200
Dictyochophyceae cells/l	2,784	$\mathbf{0}$	$\mathbf{0}$
Shannon Diversity Index (H'max-H')	1.3	1.6	1.9

Table 6.3.4: Shifts and biodiversity changes of bigger-celled phytoplankton genera after Mersin sediment addition.

In off-shore communities the Shannon diversity Index (SDI) increased with low sediment addition but decreased again when addition amount is higher. Pielou's evenness J' showed the same trend. In coastal communities SDI and J' increases in coastal and decreases in offshore communities. Additionally SDI increased and J' decreased with increasing amount of sediment added in off-shore communities. The most abundant species in off-shore treatments changed from the control (*Emiliania huxleyi*) to low (*Nitzschia tenuirostris*) to high (*Pseudo-nitzschia delicatissima*) and in coastal treatments from *Chaetoceros curvisetus* in the control to *Pseudo-nitzschia delicatissima* in both sediment addition treatments.

Coastal communities spiked with Samandağ harbor sediment had higher concentrations of Chl.-a and higher Picoeukaryotes and *Prochlorococcu*s abundances. Further they showed an increase in Chl.-a for high addition with one peak after 120 hrs at 4.62 ± 0.44 µg/l and two peaks for low addition after 48 hrs with 4.63 ± 0.13 and 120 hrs with 4.46 ± 0.92 µg/l (see figure 6.3.4).

An overall decrease of Chl.-a was observed for the coastal control group with first an increase from 3.27 ± 0.24 to 4.07 ± 0.3 µg/l after 24 hrs of the experiment.

Off-shore communities showed a lag-phase of 48-72 hrs, followed by an Chl.-a increase for high and low addition. High sediment added off-shore communities reached the plateau phase after 384 hrs with a Chl.-a concentration of 2.76 \pm 0.78 µg/l. Chlorophyll-a differences between the treatments at the end of the experiment are all non-significant. The difference of the off-shore control and off-shore high sediment addition treatments show a p-value of 0.056, see table 7.3.5.

Picoeukaryote abundances for all coastal communities started with lower numbers when sediment is added (control: $2.8x10^4 \pm 9,4x10^3$, low: $2.5x10^4 \pm 8.5x10^3$, high: $1.6x10^4 \pm 1.6x10^4$ $5.4x10³$) and decreased over time while addition to off-shore communities resulted in an enhancement with sediment added, strongest for high addition which increased from $3.2x10³$ \pm 1.1x10³ to a peak after 384 hrs with 2.1x10⁴ \pm 7x10³ cells/ml.

Figure 6.3.4: Chlorophyll-a concentrations and picoplankton abundances for Samandağ sediment addition over time. Filled circles and the letter C represent the coastal and non-filled circles and the letter O the off-shore communities, the error-bars represent standard deviations.

Synechococcus cell counts showed an enhancement with amount of sediment added for coastal and off-shore communities.

Whereby there was no difference in cell numbers due to the community origin. Whilst all off-shore communities and low addition and control coastal communities fluctuated but showed no further increase or decrease during the experiment, high sediment addition to coastal communities resulted in an increase from $1.1x10^5 \pm 3.4x10^4$ to $5.5x10^5 \pm 1.8x10^5$ cells/ml. *Prochlorococcus* on the other hand showed an elevated number of cells per milliliter with amount of added sediment in all treatments (C-control: $1.8x10^4 \pm 6x10^3$, Clow: $3.1x10^4 \pm 1x10^4$, C-high: $5.6x10^4 \pm 1.9x10^4$, O-control: $2.6x10^3 \pm 856$, O-low: $3.1x10^3$ \pm 1x10³, O-high: 2.4x10⁴ \pm 8x10³) and decreased over time in all coastal (C-control: 6.3x10³ \pm 2.1x10³, C-low: 8.9x10³ \pm 3x10³, C-high: 1.1x10⁴ \pm 3.6x10³) and high addition off-shore $(3.4x10³ \pm 1.2x10³)$ communities while low addition and control off-shore communities fluctuated but do neither decrease nor increase. The higher the amount of sediment added, the higher the variations between the three parallels.

Figure. 6.3.5: Nutrient changes for Samandağ sediment addition over time. Filled circles and the letter C represent the coastal and non-filled circles and the letter O the off-shore communities, the error-bars represent standard deviations.

Sediment addition resulted in higher starting concentrations of nitrogen and silicate whereby offshore communities had lower nitrogen but higher silicate concentrations (Figure 6.3.5). Even though off-shore communities resulted in higher nitrogen concentrations if sediment is added in higher amounts, coastal control group showed higher nitrogen than communities with low amount of sediment added throughout the duration of the experiment. Within the first half of the experiment nitrogen decreased for all off-shore communities (control: 0.26 \pm 0.02 to 0.05 \pm 0 µM after 240 hrs, low: 0.43 \pm 0.2 to 0.08 \pm 0.04 µM after 240 hrs, high: 0.53 ± 0.15 to 0.22 ± 0.03 µM after 192 hrs).

This decrease was followed by an increase till the end of the experiment (control: 0.66 ± 0.4) µM, low: 1.54 \pm 0.78 µM, high: 0.51 \pm 0.49 µM). Coastal control and low addition communities decreased from 1.58 \pm 0.47 to 0.68 \pm 0.14 µM (control) and 1.45 \pm 0.08 to 0.24 \pm 0.06 µM (low) while high sediment addition decreased from 1.73 \pm 0.11 to 1.03 \pm 0.15 µM after 192 hrs, followed by an increase to 1.83 ± 1.01 µM.

Ammonium concentrations decreased over time in coastal communities with sediment addition from 1.30 \pm 0.25 to 0.51 \pm 0.26 µM (low) and 1.81 \pm 0.01 to 0.81 \pm 0.12 µM (high). The coastal control groups showed a decrease from 1.10 ± 0.21 to 0.32 ± 0.16 μ M after 144 hrs and then an increase to 2.60 \pm 0.87 µM. Ammonium concentrations in offshore communities increased over the duration of this experiment from 0.58 ± 0.1 to 2.16 ± 1.1 0.78 µM (control), 1.75 ± 0.14 to 4.63 ± 0.9 µM (low) and 1.51 ± 0.06 to 4.44 ± 4.65 µM (high).

Phosphate increased slightly in all treatments except in off-shore high communities, where it decreased from 0.11 ± 0.01 to 0.02 ± 0.01 μ M and increased respectively stronger in coastal communities with low addition from 0.03 ± 0.01 to 0.14 ± 0.03 µM.

Silica concentrations were higher with amount of sediment added in coastal and off-shore high communities. Within all coastal communities silica concentrations decreased first (from 0.14 ± 0.01 to 0.09 ± 0 µM after 96 hrs in control, 0.28 ± 0.02 to 0.06 ± 0.02 µM after 120 hrs in low and from 1.09 \pm 0.07 to 0.12 \pm 0.07 µM after 144 hrs in high) and increased to the end of the experiment (to 0.55 ± 0.11 µM for control, 0.45 ± 0.04 µM for low and 1.4 \pm 0.41 µM for high). Off-shore communities showed a reverse trend for silica concentrations, first an increase until 240 hrs (from 1.01 ± 0.02 to 1.8 ± 0.08 µM in control, 1.01 ± 0.02 to 1.83 ± 0.53 µM in low and from 1.98 ± 0.06 to 2.96 ± 0.31 µM in high) followed by a decrease (to 0.92 \pm 0.38 µM for control, 0.95 \pm 0.44 µM for low and 1.27 \pm 0.54 µM for high) till the end of the experiment.

Variations between the three parallels increased, shown in higher standard error bars, with the duration of the experiment and were generally higher in off-shore communities.

Figure 6.3.6: Boxplots of nutrient concentrations μ M at the start and the end for Samandağ sediment addition. \Box represents the mean and \times the minimum and maximum values.

Even though differences of nutrient concentrations can be seen when plotted as boxplots, shown in figure 6.3.6, Wilcoxon Signed Rank Tests for related samples (beginning – end) are non-significant for all Samandağ sediment set-ups.

Kruskal-Wallis H Test (one way ANOVA on ranks) for independent samples of nutrient concentrations after Samandağ port sediment was added are shown in table 6.3.5. Adjusted Test statistics and adjusted P-values were non-significant for all tests. The total statistic output on the other hand was significant, so normal test statistics and normal P-values are given in Table 6.3.5.

Nutrient	Sample 1 - Sample 2	Test Statistic	Std. Error	P-value
$NO2+NO3$	O -control $ O$ -low	-3.667	4.359	.400
	O -control – O -high	-5.333	4.359	.221
	O -low $ O$ -high	-1.667	4.359	.702
	C-control – C-low	1.333	4.359	.760
	C -control – C -high	-2.333	4.359	.592
	C -low $ C$ -high	-3.667	4.359	.400
NH ₄	O-control - O-low	-13.000	4.357	.003
	O -control – O -high	-8.333	4.357	.056
	O -low $ O$ -high	5.667	4.357	.193
	C -control $ C$ -low	-2.333	4.357	.592
	C-control - C-high	-10.333	4.357	.018
	C -low $ C$ -high	-8.000	4.357	.066
PO ₄	O -control $-O$ -low	-7.000	4.097	.088
	O-control – O-high	-12.000	4.097	.003
	O -low $ O$ -high	-5.000	4.097	.222
	C -control $ C$ -low	6.000	4.097	1.43
	C -control – C -high	.000	4.097	1.000
	C -low $ C$ -high	-6.000	4.097	.143
Si	O -control $ O$ -low	.000	4.352	1.000
	O -control – O -high	-7.500	4.352	.085
	O -low $ O$ -high	-7.500	4.352	.085
	C -control – C -low	-3.000	4.352	.491
	C-control – C-high	-12.000	4.352	.006
	C -low $- C$ -high	-9.000	4.352	.039
Chlorophyll-a	O -control $ O$ -low	-3.000	4.359	.491
	O -control – O -high	-8.333	4.359	.056
	O -low $ O$ -high	-5.333	4.359	.221
	C-control - C-low	-5.667	4.359	.194
	C -control – C -high	-4.000	4.359	.359
	C -low $ C$ -high	1.667	4.359	.702

Table 6.3.5: Non-parametrical statistical results of Kruskal-Wallis H Test between the different Samandağ experiment set-ups for all initial nutrient concentrations and chlorophyll-a concentrations at the end of the experiment. Statistical significant differences ($p < 0.05$) are given in bold.

Throughout all communities diatoms were dominant for the bigger phytoplankton species whereby the abundance of diatom cells increased with amount of sediment added to offshore communities, it decreased in coastal communities, as did the abundance of coccolithophores, dinoflagellates and cryptophyta (except for low addition into coastal communities where these groups increased slightly). Thus the total number of cells increased (off-shore) and decreased (coastal) accordingly (Table 6.3.6).

Shannon diversity index and Pielou's evenness decreased with low and increased slightly with higher addition of sediment in off-shore communities. In coastal communities however both biodiversity factors increased with addition of sediment. The most abundant species for all treatments was *Pseudo-nitzschia delicatissima*, except in high sediment addition to offshore communities where there was a shift to *Nitzschia tenuirostris*.

Table 6.3.6: Shifts and biodiversity changes of bigger-celled phytoplankton genera after Samandağ sediment addition.

Communities spiked with Tirtar harbor sediment showed higher Chl.-a concentration, picoeukaryotes, *Synechococcus* and *Prochlorococcus* abundances relating to the amount of sediment added with generally higher concentrations/abundances in coastal communities, see figure 6.3.7. Chlorophyll-a concentrations decreased in coastal control (from 1.87 ± 0.02) to 0.52 \pm 0.06 µg/l) and low sediment addition communities (from 2.12 \pm 0.02 to 1.02 \pm 0.08 µg/l) but increased for coastal high sediment addition (from 2.67 ± 0.04 to 4.29 ± 0.72 µg/l) and slightly in all off-shore communities (control: 0.14 ± 0.01 to 0.37 ± 0.08 µg/l, low: 0.16 ± 0.01 to 0.91 ± 0.13 µg/l, high: 0.14 ± 0 to 1.67 ± 0.12 µg/l).

Chlorophyll of both high sediment addition treatments were significantly different from both control treatments, shown in table 7.3.7. Abundance of picoeukaryotes decreased from $1.9x10^4 \pm 6.2x10^3$ to $6x10^3 \pm 2x10^3$ cells/ml in coastal control and to $1.4x10^4 \pm 4.6x10^3$ cells/ml in coastal low sediment addition communities, while high sediment addition in coastal communities resulted in an increase to $2.9x10^4 \pm 9.6x10^3$ cells/ml.

Figure 6.3.7: Chlorophyll-a concentrations and picoplankton abundances for Tirtar sediment addition over time. Filled circles and the letter C represent the coastal and non-filled circles and the letter O the off-shore communities, the error-bars represent standard deviations.

Picoeukaryotes doubled in off-shore low addition communities from $3.1x10^3 \pm 1x10^3$ to $6.4x10³ \pm 2.1x10³$ cells per ml and resulted in a slightly higher abundance with higher sediment addition $(7.6x10^3 \pm 2.6x10^3 \text{ cells/ml})$ at the end of the experiment.

With reference to a starting abundance of $6.7x10^4 \pm 2.2x10^4$ *Synechococcus* cells per milliliter in coastal communities the abundance enhanced threefold $(2.2x10^5 \pm 7.2x10^4$ cells/ml) with low, fivefold $(3.5x10^5 \pm 1.2x10^5$ cells/ml) with high sediment addition and decreased to $9.7x10^3 \pm 3.2x10^3$ cells/ml in the control groups. The strongest increase regarding the starting abundance of $1.9x10^4 \pm 6.4x10^3$ cells/ml showed high sediment addition to off-shore communities with a sevenfold increase to $1.4x10^5 \pm 4.7x10^4$ cells/ml.

Low addition off-shore communities resulted in a slight increase of *Synechococcus* abundance to 2.5x10⁴ \pm 8,422 cells/ml and a decrease for off-shore control groups to 3.1x10³ \pm 1x10³ cells/ml over time was observed.

Prochlorococcus abundance halved within coastal control communities from $1.5x10^4 \pm$ 4.9x10³ to 6.9x10³ ± 2.3x10³ cells/ml while it doubled to 3.2x10⁴ ± 1.1x10⁴ cells/ml in low and sixfolded to $8.3x10^4 \pm 2.8x10^4$ cells/ml in high sediment addition coastal communities. Sediment addition to off-shore communities resulted in an increase from $3.1x10^3 \pm 1x10^3$ cells/ml to $7.8x10^3 \pm 2.6x10^3$ cells/ml (low) and $4.1x10^4 \pm 1.4x10^4$ cells/ml (high). Variations within the three parallels increased with amount of sediment added and are set-up depending higher in coastal communities.

Nutrient measurements showed an overall decrease in nitrogen and silicate while phosphate decreased within the first week and increased afterwards (Figure 6.3.8).

Figure 6.3.8: Nutrient changes for Tirtar sediment addition over time. Filled circles and the letter C represent the coastal and non-filled circles and the letter O the off-shore communities, the error-bars represent standard deviations.

Nitrogen concentration decreased for the coastal control from 0.89 ± 0.74 to 0.54 ± 0.2 μ M, coastal low from 0.46 \pm 0.23 to 0.27 \pm 0.03 µM, coastal high from 0.55 \pm 0.12 to 0.49 \pm 0.04 µM, off-shore control from 0.5 ± 0.31 to 0.33 ± 0.03 µM, off-shore low from 0.46 ± 0.03 0.44 to 023 \pm 0.03 µM and off-shore high communities from 0.43 \pm 0.16 to 0 29 \pm 0.02 µM but increased within the latter communities to 0.76 ± 0.54 µM within the last 24 hrs.

Ammonium concentrations increased in all communities (coastal control: 3.4 ± 2.38 to 4.92 \pm 1.46 µM, coastal low: 1.99 \pm 0.54 to 3.88 \pm 1.49 µM, off-shore control: 2.01 \pm 1.93 to 3.07 \pm 0.78 µM, off-shore low: 2.42 \pm 0.37 to 3.88 \pm 0.84 µM and off-shore high: 5.92 \pm 0.54 to 8.48 \pm 4.03 µM), except with high sediment addition to coastal communities where no change between start and end of the experiment was observed.

Phosphate decreased throughout all communities the first 144-216 hrs and increased again till the end of the experiment whereby the end-concentration was very similar to the beginning one in each community. Only a small decrease between starting and ending concentrations of 0.05 μ M in coastal high and a slight increase in off-shore control (0.2 μ M) and off-shore high $(0.3 \mu M)$ communities was noted.

Silica concentrations increased over time within the coastal low communities from 0.91 \pm 0.11 to 1.03 \pm 0.19 µM and decreased in all other communities (coastal control: 1.04 \pm 0.2 to 0.8 ± 0.03 µM, coastal high: 1.53 ± 0.32 to 0.73 ± 0.23 µM, off-shore control: 1.41 ± 1.11 to 1.11 ± 0.02 µM and coastal low: 1.53 ± 0.2 to 1.38 ± 0.31 µM). The strongest decrease to half the beginning concentration however was found in high sediment addition off-shore communities $(2.67 \pm 0.31 \text{ to } 1.35 \pm 0.33 \text{ µM}).$

The standard errors show higher variations between the three parallels of the off-shore communities.

Figure 6.3.9: Boxplots of nutrient concentrations [µM] at the start and the end for Tirtar sediment addition. \Box represents the mean and \times the minimum and maximum values.

Even though differences of nutrient concentrations can be seen when plotted as boxplots, shown in Figure 6.3.9, Wilcoxon Signed Rank Tests for related samples (beginning – end) are non-significant for all Tirtar sediment set-ups.

Kruskal-Wallis H Test (one way ANOVA on ranks) results for independent samples of nutrient concentrations after Tirtar port sediment was added are shown in table 6.3.7. Adjusted Test statistics and adjusted P-values were non-significant for all tests. The total statistic output on the other hand was significant, so normal test statistics and normal Pvalues are given in Table 6.3.7.

Table 6.3.7: Non-parametrical statistical results of Kruskal-Wallis H Test between the different Tirtar experiment set-ups for all initial nutrient concentrations and chlorophyll-a concentrations at the end of the experiment. Statistical significant differences ($p < 0.05$) are given in bold.

Nutrient	Sample 1 – Sample 2	Test Statistic	Std. Error	P-value		
$NO2+NO3$	Non-significant for all Samples					
NH ₄	Non-significant for all Samples					
PO ₄	Non-significant for all Samples					
Si	O -control – O -low	-2.000	4.359	.646		
	O -control – O -high	-7.333	4.359	.092		
	O -low $ O$ -high	-5.333	4.359	.221		
	C -control $- C$ -low	1.333	4.359	.760		
	C -control – C -high	-7.000	4.359	.108		
	C -low $ C$ -high -8.333 4.359		.056			
Chlorophyll-a	O -control $ O$ -low	-6.333	4.359	1.46		
	O-control - O-high	-10.333	4.359	.018		
	O -low $ O$ -high	-4.000	4.359	.359		
	C -control $- C$ -low	-5.667	4.359	.194		
	C-control – C-high	-13.667	4.359	.002		
	C -low $ C$ -high	-8.000	4.359	.066		

Bigger celled phytoplankton species abundance increased with amount of sediment added and was respectively higher in coastal communities, see Table 6.3.8. Coccolithophores dominated off-shore control, off-shore low and coastal control communities whereas offshore high, coastal low and coastal high were dominated by diatoms. With higher amount of sediment added the abundance of diatoms, coccolithophores, cryptophyta and dinoflagellates increased in off-shore communities while coastal communities showed a decrease in coccolithophores and dictyochales and an increase in diatoms and cryptophyta. Dinoflagellates showed the highest abundance in low sediment addition to coastal waters.

Table 6.3.8: Shifts and biodiversity changes of bigger-celled phytoplankton genera after Tirtar sediment addition.

While Pielou's evenness showed higher numbers in sediment added communities, except in off-shore low, Shannon diversity index decreased with sediment amount added in coastal communities. In off-shore communities low sediment addition resulted in a higher SDI and a lower SDI with high sediment added. *Emiliania huxleyi*, a coccolithophores species, was the most abundant species within all off-shore and coastal control communities and the diatom *Leptocylindrus danicus* within coastal low and coastal high communities.

6.3.1 Statistical problems

Non-parametrical Wilcoxon signed Rank test for all nutrient concentrations differences at the start and the end of the experiment for all experiments were done. Even though differences of nutrient concentrations can be seen when plotted as boxplots, an example is shown in Figure 6.3.1.1., Wilcoxon signed Rank test are non-significant for all 3 experiments due to very low sample sizes.

6.4 Discussion

Sediment from a more natural place, not influenced by human activity or bigger rivers, show no impact on pelagic phytoplankton when added to the off-shore community even though the metal concentrations were the highest in the sediment originated from Tirtar and claimed "natural sediment". However, an increase in chlorophyll-a was observed in coastal communities after sediment was added. Since the growth in Chl.-a is due to growth in bigger-celled species, especially diatom species, see Figure 7.4.1, and off-shore waters contain mainly small celled species (see chapter 4 of this thesis; Yücel, 2013; Sioukou-Frangou,2010; Agawin *et al*., 2000), this result is a very strong argument to move dumping areas further into off-shore waters. The general growth of resuspended transported species was observed in bigger-celled species (Nalewajko and Murphy, 1998). The risk that those species take over the disposal site communities in Mediterranean Sea is relatively small since the off-shore waters are most of the time highly N and P-limited (see chapter 4 of this thesis; Koçak *et al*., 2010; Kress *et al*., 2005; Thingstad *et al*., 2005). Further, cyanobacteria do not recover, if they are transported within sediment from nutrient rich coastal waters to P-limited off-shore waters (Nalewajko and Murphy, 1998) and thus cause no harm to the existing off-shore plankton community.

The slopes of chlorophyll-a and species development after sediment was added show clearly that with high amount of sediment added the impact was more severe (Table 6.4.1). Steepest slopes were found in coastal communities, with the exception of the experiment with sediment from Mersin harbor where the off-shore communities showed steeper slopes for picoeukaryotes and *Synechococcus*. *Synechococcus* and *Prochlorococcus* seem to profit from sediment addition and grow faster in off-shore waters, supporting the hypothesis of their advantage over bigger plankton species in nutrient uptake and thus resulting in faster turnover rates of these species. The intermediate amount of Samandağ harbor sediment added led to a positive growth in *Synechococcus* and *Prochlorococcus* in off-shore communities, while high amount added led to a strong decrease. Picoeukaryotes, on the other hand, increase in growth by a factor of 100. The high negative slope of *Prochlorococcus* after sediment originated from Samandağ harbor was added is most probably a result of sediment particles measured at the first measurement since it is the first measurement leading to these high negative results.

Lag phases are typical for phytoplankton species growth curves and the duration is species dependent (Spies, 1987). A lag-phase in community growth of 192 hrs in off-shore treatments suggests that due to less bigger-celled species reaction of the communities is either missing or not shown in chlorophyll development.

Growth and blooming of *Pseudo-nitzschia* species does not always show in a chlorophyll-a increase and are often short lasting of about one week (Quiroga, 2006). Additionally does chlorophyll not always resemble the growth of the plankton community (Eker-Develi *et al*., 2006)

Sediment origin	Treatment	Chlorophyll-a $[\mu g*l^{-1}d^{-1}]$	Picoeukaryotes $[cells*ml^{-1}d^{-1}]$	Synechococcus [cells*ml ⁻¹ d ⁻¹]	Prochlorococcus $[cells*ml^{-1}d^{-1}]$
Mersin	$C - C$	0.09	322	3,806	356
	$C - L$	0.36	494	7,700	1,933
	$C - H$	1.44	3,322	944	5,644
	$O - C$		181	-143	33
	$O - L$	0.16	675	376	-69
	$O - H$	0.19	5,567	2,042	454
Samandağ	$C - C$	-0.11	$-1,646$	5,533	$-1,475$
	$C - L$	0.03	$-1,175$	7,350	$-2,713$
	$C - H$	0.57	-579	56,000	$-5,608$
	$O - C$	0.03	-9	-232	74
	$O - L$	0.05	14	754	119
	$O - H$	0.24	1,478	$-5,608$	$-1,065$
Tirtar	$C - C$	-0.11	$-1,067$	$-4,797$	-647
	$C - L$	-0.09	-397	12,308	1,461
	$C - H$	0.31	847	23,364	5,661
	$O - C$		-61	-939	51
	$O - L$	0.11	196	363	273
	$O - H$	0.23	263	25,417	7,393

Table 6.4.1: Comparison of Chl.-a, picoeukaryotes, *Synechococcus* and *Prochlorococcus* slopes in all three SAE. Positive slopes are given in black, negative slopes in red.

Suppression of species growth by metal input could be an other explanation (Najar *et al*., 2004). Metal input might explain the lacking growth and/or decline of cyanobacteria. Cyanobacteria are influenced faster and stronger by metal addition due to cell-wall specific characteristics (Miao *et al*., 2005). High metal concentrations were measured within sediment from Tirtar port and might be the reason why growth was inhibited for a long time, shown in the longest lag-phases. Total organic matter content of the Tirtar sediment was higher than of the Samandağ sediment, showing that less organic matter for bacterial activities and thus nutrient source were not the reason for the late reply of the phytoplankton community.

If inhibition is lethal or long lasting, it will result in a damage to the existing plankton community (Monteiro *et al*., 1995) and newly formed niches will hardly be taken over by other species due to nutrient limitation. Especially in the oligotrophic Mediterranean Sea heterotrophic bacteria and cyanobacteria production is very important (Yücel, 2013; Siokou-Frangou *et al*., 2010; Agawin et al., 2000; Li et al., 1993). Off-shore communities consist of small-celled picoplankton species, such as heterotrophic bacteria, *Synechococcus* and *Prochlorococcus* (Yücel, 2013; Siokou-Frangou *et al*., 2010). Thus the impact on the natural communities in off-shore waters will be less severe.

Sinking rates of sediment and dilution factors in off-shore waters are additional arguments for this recommendation. Several types of models show the sinking rate for sediment size and environmental dependent conditions (Morris, 2000). Using STFATE model with typical Mersin port sediment and Mersin Bay environmental conditions in two possible dumping sites at 50 and 150 m water column depth results in faster sinking rates and less remaining clay particles in the water column at 150 m column depth dumping site (Tuğrul *et al*., 2015; Boran *et al*., in prep.).

Sediment originated close to a river mouth is less toxic regarding metals and organic matter and result in a less strong impact on pelagic phytoplankton than originated close to human influenced areas such as industrial cities or heavily used ports. Mersin port sediment had not only high concentration of metals and organic matter, it resulted in chlorophyll-a concentrations one µg/l higher within all mesocosms. Tirtar harbor sediment does not lead to a decrease in *Prochlorococcus* but an increase. Nevertheless, dumped dredged material has the capability to change functional communities at the bottom of marine food webs.

Pelagic plankton communities are very variable in time and space (Martin, 2003; Brentnall *et al*., 2003; Bracco *et al*., 2000; Abraham, 1998) and thus communities within the dumping zone compared to a nearby reference zone are not reliable. The increase in measured Chl.-a can be related to an increase with higher concentration of sediment added, mainly by diatoms (Figure 6.4.1). The high diatom abundance in the off-shore control treatments and the reduction of cells in the coastal treatments of the Samandağ experiment might be due to the phase of the initial community, e.g. being at the end of a blooming-phase when this experiment was conducted. *Pseudo-nitzschia delicatissima* was the most abundant species in all Samandağ communities, even the initial off-shore communities. This small diatom species is often present in the Mediterranean and known to bloom easily (chapter 4, Quiroga, 2006).

Sediment added to off-shore communities led to the biggest changes in phytoplankton assemblages and consequently to communities the least similar to the initial ones, as shown in Figure 6.4.2. Coastal communities were less similar to each other, whereby both communities with added, high and low, are the most similar ones.

This shows that communities, when triggered by sediment addition, do not only grow regarding abundances but also shift in composition. Samandağ experiment communities were very similar, despite sediment addition. This supports the idea that the initial community of the Samandağ sediment experiment was at the end of a blooming phase.

Fig. 6.4.1: Bigger-celled phytoplankton group abundances in cells per liter for all experiments. The letter O represents off-shore, the letter C coastal communities, -C represents control, -L represents low, and -H represents high addition treatments.

Additionally there is low similarity between high addition treatments when Samandağ sediment was added. Mersin or Tirtar sediment addition leads to more alike communities. Bray-Curtis-Similarities between control groups are very low for all experiments, but increase with added sediment in Mersin and Tirtar experiments (Figure 6.4.2).

Inorganic phosphate and silicate concentrations are higher with amount of sediment added and thus are supporting phytoplankton growth on an elemental level. The Mediterranean Sea is highly P limited (Koçak *et al*., 2010; Krom *et al*., 2010; 2004; 1991; Thingstad et al.,2005/1998; Béthoux *et al*., 1998) and hence any kind of P addition leads to an increase in phytoplankton species (Caron et al., 2000).

Figure 6.4.2: Bray-Curtis-Similarities [%] for all experiments between the same origin communities of one experiment (a) and between coastal and off-shore communities with the same treatment of each experiment (b). The first letter "O" represents Off-shore and the first letter "C" represents coastal communities. The second letters "C" (control), "L" (low), and "H" (high) represent the amount of sediment added into the community.

Heterotrophic bacteria increase with phosphate addition and can induce a decrease of phytoplankton species due to their better competition ability (Joint *et al*., 2002). Silicate is getting more into scientific focus since its concentration is decreasing and it might turn into the limiting nutrient (Egge and Aksnes, 1992), also in the Mediterranean Sea (Koçak *et al*., 2010). Silicate influx reduction caused by damming of the bigger rivers supplying the NLB with nutrients (Seyhan and Ceyhan) might lead to similar problems as occurred after the Aswan High Dam was build in the Nile river. Silicate at the coastal region decreased drastically and a shift to smaller species, especially in the group of diatoms, was observed (Whaby and Bishara, 1980).

The results of these sediment addition experiments implement that a dumping area in offshore waters would result in less impact to the pelagic communities in the NLB.

It is very important to note here, that reactive metal concentrations were not measured within the water of the different treatments, just in the sediment before addition resulting in a lack of data and the knowledge of how much metal was actually released into the water and thus was available to the communities.

Further, bacterial production was only measured as abundance of cyanobacteria. Heterotrophic bacteria were not included within these experiments but contribute together with cyanobacteria significantly to the total production in Mersin Bay off-shore waters.

These results are effective for the used local phytoplankton communities. Results of similar experiments will differ with community origin and assemblages at the experimental set-up as well as with different sediment added. Thus these results and conclusions regard the results of the experiments conducted.

6.5 Conclusion

Higher amount of sediment added resulted in a stronger effect regarding total growth in Chl.-a and slope steepness of the measured species abundances. Growth of chlorophyll-a measured seems to resemble diatom growth. The shift to diatom dominated phytoplankton assemblages, excluding pico-sized species, and was observed in all treatments after sediment was added. Long lag-phases in off-shore communities and a simultaneous increase in nutrients with Chl.-a increase (end of lag-phase) showed that the production in off-shore communities was rather regenerated production where species use remineralized nutrients as source of nutrition. Bacterial activity could be seen in the in the increase of nutrients after a certain time span passed. The communities had higher Bray-Curtis-Similarities after sediment was added and control groups were the least similar. This is a further evidence that coastal communities with high amount of big-sized phytoplankton species are stronger effected by sediment disposal.

Seasonal timing of dumping plays a crucial role as well. Species depending blooming times in the NLB should be considered to maintain the natural fluctuation. *Synechococcus* is most abundant in winter months, as well as bigger-celled species while *Prochlorococcus* prefers warmer months (Zubkov *et al*., 2000). Therefore dumping in summer months, if it can not be prevented, should happen closer to the coast and during winter times in off-shore waters. Increased turbidity caused by dumping can be neglected as an effect on phytoplankton, since it will be local and short lasting (Essink, 1999; Boran *et al*., in prep.). Bigger-celled phytoplankton species are, on the other hand, most diverse during summer months and the chance that an opportunistic species is present and might use the nutrient input via sediment to bloom is higher during summer months (chapter 4). During winter months, the difference between the coastal and off-shore communities is minimal and thus the dumping location plays a smaller role if mixing does not occur. If mixing is strong, dumping in deeper waters where re-suspension might not occur throughout the whole water column and sunken sediment stays at the bottom, would be recommended.

7. General Conclusion

The oligotrophic Mediterranean Sea has been a frequently studied sea due to its fast reaction to regional and global changes. It is, contrary to most of the other marine oligotrophic environments, highly P-limited (Krom et al., 1991) and regarded as oligotrophic but still biologically active, shown by the regularly occurring DCM, which is triggered by regenerated production in summer and autumn period (Puji-Pay *et al.*, 2011; Ediger and Yılmaz, 1996). Nutrient, water layer and planktonic turn-over rates are strongly linked and still not fully understood in the Mediterranean. Human activities drive coastal nutrient input and the local eutrophication-like conditions. Since its unique condition marks the Mediterranean as a miniature ocean, it is frequently used to predict future changes for the world's ocean. Therefore, the effect of abiotic and biotic factors on plankton communities needs to be understood as plankton does represent the first trophic layer and the direct link to elemental nutrient conditions in the marine environment.

The two year ETS observations show that the off-shore zone of NLB is highly dominated by bacteria and small-celled phytoplankton species. They fuel the off-shore ecosystem, not only by their dominance and thus as an important food source but in case of heterotrophic bacteria additionally as a nutrient providing source. A strong heterotrophic bacterial activity was observed especially after blooms of bigger-celled species but also with increase in cyanobacteria abundance and resulted in a decrease of oxygen, also directly below the DCM. In general, primary and bacterial production are proportional (Turley *et al*., 2000) but under conditions of lower primary production than bacterial production low vertical OM fluxes occur (Allen *et al*., 2002). And hence, without the remineralized nutrients in off-shore waters nutrient depletion would be more severe. *Synechococcus* and *Prochlorococcus* are always present within the two year observations in the coastal and off-shore stations. Both are key organisms for the offshore water ecosystems. Their dominance in abundance over photoautotrophic plankton in coastal water looses importance due to their relatively smaller biomass, in comparison to bigger-celled species. Nevertheless heterotrophic bacteria and cyanobacteria are of high importance to the food web, the microbial loop and the nutrient ratios of the Mediterranean deep waters. Positive correlations of cyanobacteria and heterotrophic bacteria with their predators, i.e. nanoflagellates, show the importance of them as food source for the next trophic layer.

As phytoplankton is dependent on nutrient input into the Mediterranean, especially the oligotrophic NLB, the observed nutrient ratios show expected fluctuations depending on river flux, distance to the coastal influence and atmospheric wet-and dry-deposition.

Nutrient uptake by phytoplankton was observed in the upper water column and nutrient regeneration by heterotrophic bacteria in the deeper water. High N:P-ratios have been found in the deeper water at the 200 m station. This strongly suggests a P-limited system, in which cyanobacteria influence high environmental N:P-ratios with their high N:P-cell content. Surface samples, where light and temperature are not changing, reveal a positive correlation of *Synechococcus* and heterotrophic bacteria with high N:P-ratios showing that they might be rather N-limited than P-limited. Correlated depth profile samples resulted in preliminarily negative correlations with N:P-ratios.

This negative correlation is more likely due to changing physical factors with depth, such as light and temperature and an increase of N:P in deeper waters, where abundances of both species are naturally lower. Furthermore, negative and positive correlations for both species suggest a co-limitation or changing of the limiting nutrient with time and condition.

Environmental factors influence not only the abundance of species but also their biomass. The biomass as a measure of ecosystem functioning measure is common since all size classes of one trophic level can be observed in the same unit and thus compared. While the biovolume of heterotrophic bacteria is negatively correlated with temperature its abundance is positively correlated with temperature. Meanwhile, cyanobacteria cells increase in volume with increasing temperature and N:P-ratios. In both cases cell abundance and volume are mostly positively correlated, which shows that the intra-specific competition has a small effect on abundance and the inter-specific competition and grazing might be dominant biotic factors controlling the abundance of these two plankton groups.

Bigger-celled species are mainly dominant in coastal waters in which higher nutrient influx appears due to river input and coastal influence. These species are controlled primarily by nutrient availability and secondarily by grazing. Blooming events follow the increased nutrient input caused by the increased coastal influence, winter mixing or atmospheric wetand dry-deposition, especially in early spring and late summer. Diatom species with fast turn-over rates, such as *P. delicatissima*, *Chaetoceros* spp. and *L. danicus* were the most abundant species. The relatively small size of *Achnanthes* spp., *Bacteriastrum delicatulum*, *Thalassionema nitzschioides*, *Sceletonema costatum*, *Nitzschia tenuirostris* and *Thalassiosira* spp. results in the presence of these species throughout the year at all the stations. The importance of *Emiliania huxleyi* for the local ecosystem is visible since this species is not only always present but also most of the year dominating the off-shore community. Small flagellates dominate the flagellate group. This group is relatively small regarding abundances but can make up a big part of the off-shore community since the diatoms are less abundant in these waters.

Nutrients seem to be the main factor controlling the phytoplankton assemblages in the NLB. The ETS stations are located from coastal to off-shore waters, starting in front of the Lamas River estuary, which contributes nutrients to the coastal marine environment. Consequently this river contributes nutrients and defines the development of the phytoplankton community at the coastal ETS station. Nutrient concentrations carried by Lamas River were measured to observe trends in nutrient concentrations and fluctuation over a one-year span. When compared to previous measurements, N:P-ratios increased while there is a decrease in Si:N-ratios. The cause of this change is an overall increase of nitrogen within the river water. Agricultural activities along the Lamas River have shifted from vegetable gardens and citrus tree farms to banana plantations. Banana fields require fertilization and a lot of watering. Thus the river water flow decreases while nutrients washed into the river increase. Both have a severe impact on the amount of nutrients carried to the marine coastal ecosystem. A shift from big-celled species to smaller-celled diatoms and eventually nonsiliceous phytoplankton species, such as flagellates or coccolithophores might be the future of the coastal phytoplankton community if Si:N increases further.

River water added to coastal and off-shore phytoplankton communities has a nonpredictable but visible influence on coastal and off-shore phytoplankton communities. The effect on phytoplankton communities is depending mainly on the initial existing phytoplankton assemblage and the nutrients transported by the river into the coastal marine environment. The influx of river water is essential for phytoplankton species, especially in the oligotrophic Mediterranean and leads to higher production at the coastal zone. If river water reaches the off-shore waters, it leads to a similar effect as at the coastal environment: growth in phytoplankton, especially increase in abundance of small diatoms, and thus increase in chlorophyll, but less strong than in coastal waters. River water intrusion has additional to nutrient influx several severe effects on the local environmental conditions, such as temperature, salinity and turbidity changes. Species specific preferences for ranges in these conditions might lead to shifts in the community due to competitive advantages and disadvantages of species after river water intrusion.

To understand the effect of the human-induced nutrient addition, sediments from the disparately influenced areas (Samandağ, influenced by river discharge, Mersin, influenced by human and industrial activities, and non-influenced Tirtar) were chosen and compared. The strength of the effect on pelagic phytoplankton groups is depending on the dredged material origin. The human-influenced sediments (from a city or a river with agriculture alongside) have a stronger impact on the environment regarding chlorophyll-a changes and blooming of bigger-celled phytoplankton species, and thus need to be dumped with more care.

These results suggest that the dumping site in Mersin would be less harmful to the pelagic phytoplankton communities if moved further off-shore and conducted during winter months when species abundance is lowest. Lack of nano- and micro-sized phytoplankton species present in the off-shore water plankton community results in longer lag-phases and thus slower and smaller growth when dredged material is dumped. Additionally the seasonality of plankton groups should be considered and blooms are smaller in off-shore waters than coastal ones in the Mersin Bay area.

Fast turnover rates of cyanobacteria and heterotrophic bacterioplankton and their fast recovery after disturbance are a further reason to dump in the off-shore waters where these are the most common primary producers and plankton species.

Comparing natural and human induced nutrient addition reveals that human induced addition led to a stronger increase in chlorophyll and altered the communities more severely. The lag-phases of chlorophyll growth in river water added communities are shorter than in sediment added communities. The strongest increase in chlorophyll-a measured after river water addition was $0.13 \mu g^{*1}d^{-1}$ and after sediment addition was $0.31 \mu g^{*1}d^{-1}$.

Nevertheless, the off-shore communities seem to utilize nutrients originating from sediment more likely, even though the long lag-phase indicates regenerated production rather than new production. For both nutrient addition experiments, the composition in phytoplankton communities is important and since those are very variable, experiments might differ according to the set-up conditions.

8.1 Suggestions for future research

Future research at ETS should include profiles of big-celled phytoplankton, not only surface observations. It was obvious in monthly samples of ETS-200 station that in summer months the main phytoplankton bulk was present in subsurface waters, shown by the DCM present throughout the year at the ETS-200 station, except during winter mixing months and in the samples of Selin Küçükavşar, not published data.

In addition, all species should be measured in volume, so the biomass of the whole community can be used in models or to describe the ecosystem. To do so a camera system attached to the inverse microscope would be preferable to lessen the work load. Pictures of phytoplankton species can then be further analyzed on the computer and volumes can be measured.
To gain a better insight into the effect of biotic and abiotic factors on heterotrophic bacteria and cyanobacteria cell volumes additional experiments with controlled environmental conditions should be done in the lab with only one or two factors changing. Further, experiments on nutrient addition and phytoplankton shifts should include bacteria as well. In this study, heterotrophic bacteria were not included due to time restriction and work load. Hence, to understand their essential role within the Mediterranean plankton community and their interaction with the other species this group should be included in the observations.

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Appendix A

Table A1: Pearson correlation results of heterotrophic bacteria abundance, biovolume, depth, PAR, temperature and N/P ratios of single depths over time of the ETS-20 station. Significant correlations are bold.

Depth			PAR	T	N to P	Abundance	Biovolume
$\mathbf{0}$	Abundance	Pearson correlation	$-.051**$	$.241**$	$.149**$	$\mathbf{1}$	$.114***$
		Sig. (2-tailed)	.002	.000.	.000		.000
		N	3928	4371	4371	4371	4371
	Biovolume	Pearson correlation	$-.057**$	$-.120**$	$.040**$	$.114**$	$\mathbf{1}$
		Sig. (2-tailed)	.000	.000	.008	.000	
		N	3928	4371	4371	4371	4371
10	Abundance	Pearson correlation	$-.202**$	$.095***$	$.208**$	$\mathbf{1}$	$.085***$
		Sig. (2-tailed)	.000	.000.	.000		.000
		N	3636	4000	4000	4000	4000
	Biovolume	Pearson correlation	$-.060**$	$-.177**$	$.088**$	$.085***$	$\mathbf{1}$
		Sig. (2-tailed)	.000	.000	.000	.000	
		N	3636	4000	4000	4000	4000
20	Abundance	Pearson correlation	$-.155***$	$-.114**$	$.117**$	$\mathbf{1}$	$.140**$
		Sig. (2-tailed)	.000	.000.	.000		.000
		N	3250	3753	3753	3753	3753
	Biovolume	Pearson correlation	$-.009$	$-.181**$	$.115***$	$.140**$	$\mathbf{1}$
		Sig. (2-tailed)	.592	.000.	.000	.000	
		N	3250	3753	3753	3753	3753

Table A2: Pearson correlation results of heterotrophic bacteria abundance, biovolume, depth, PAR, temperature and N/P ratios of each ETS-20 station profile. Significant correlations are bold.

Date			Depth	PAR	T	N to P	Abundance	Biovolume
		N	394	394	394	394	394	394
	Biovolume	Pearson correlation	$.109*$	$-.131**$	$-.108*$	$-.124*$	$-.133**$	1
		Sig. (2-tailed)	.030	.009	.032	.014	.008	
		N	394	394	394	394	394	394
Dec 15	Abundance	Pearson correlation	$-.853**$.973**	$-.999**$.996**	$\mathbf{1}$	$.101*$
		Sig. (2-tailed)	.000	.000	.000	.000		.013
		N	592	592	592	592	592	592
	Biovolume	Pearson correlation	$-.109**$	$.109**$	$-.099*$	$.097*$	$.101*$	$\mathbf{1}$
		Sig. (2-tailed)	.008	.008	.016	.018	.013	
		N	592	592	592	592	592	592

Table A3: Pearson correlation results of *Synechococcus* spp. abundance, biovolume, depth, PAR, temperature and N/P ratios of single depths over time of the ETS-20 station. Significant correlations are bold.

Depth			PAR	T	N to P	Abundance	Biovolume
$\mathbf{0}$	Abundance	Pearson correlation	$.424**$	$.476***$	$-.279**$	$\mathbf{1}$.307**
		Sig. (2-tailed)	.000	.000	.000.		.000
		$\mathbb N$	571	874	874	874	874
	Biovolume	Pearson correlation	.108**	.179**	$-.222**$	$.307**$	$\mathbf{1}$
		Sig. (2-tailed)	.010	.000	.000.	.000.	
		N	571	874	874	874	874
10	Abundance	Pearson correlation	$-.345**$	$.471***$.042	$\mathbf{1}$.148**
		Sig. (2-tailed)	.000	.000	.096		.000
		N	1277	1558	1558	1558	1558
	Biovolume	Pearson correlation	$-.067*$	$.135***$	$-.015$	$.148**$	$1\,$
		Sig. (2-tailed)	.016	.000	.547	.000.	
		N	1277	1558	1558	1558	1558
20	Abundance	Pearson correlation	$-.418**$	$.265***$	$.213**$	$\mathbf{1}$	$.181***$
		Sig. (2-tailed)	.000	.000	.000		.000
		N	1255	1557	1557	1557	1557
	Biovolume	Pearson correlation	$-.108**$	$.209**$	$-.037$	$.181***$	$\mathbf{1}$
		Sig. (2-tailed)	.000	.000	.147	.000	
		$\mathbb N$	1255	1557	1557	1557	1557

Table A4: Pearson correlation results of *Synechococcus* spp. abundance, biovolume, depth, PAR, temperature and N/P ratios of each ETS-20 profile. Significant correlations are bold.

Date			Depth	PAR	T	N to \boldsymbol{P}	Abundance	Biovolume
		Sig. (2-tailed)	.622	.420	.396	.888	.427	
		$\mathbf N$	129	129	129	129	129	129
Oct 15	Abundance	Pearson correlation	$.336**$	$-.679**$	$-.676**$	$-.981**$	$\mathbf{1}$.018
		Sig. (2-tailed)	.000	.000	.000	.000		.793
		$\mathbf N$	209	209	209	209	209	209
	Biovolume	Pearson correlation	$-.138*$.100	.100	$-.047$.018	$\mathbf{1}$
		Sig. (2-tailed)	.047	.151	.149	.495	.793	
		$\mathbf N$	209	209	209	209	209	209
Nov 15	Abundance	Pearson correlation	$1.000**$	$-0.953**$	$-.999**$	$-.405**$	$\mathbf{1}$	-0.068
		Sig. (2-tailed)	.000	.000	.000.	.000.		.488
		$\mathbf N$	107	107	107	107	107	107
	Biovolume	Pearson correlation	$-.068$.066	.068	.031	-0.068	$\mathbf{1}$
		Sig. (2-tailed)	.489	.502	.489	.754	.488	
		$\mathbf N$	107	107	107	107	107	107
Dec 15	Abundance	Pearson correlation	$-.373**$	$.646**$	$-.850**$.878**	$\mathbf{1}$	$-.084$
		Sig. (2-tailed)	.000	.000	.000	.000		.277
		$\mathbf N$	170	170	170	170	170	170
	Biovolume	Pearson correlation	$-.258**$	$.184*$	$-.093$.075	$-.084$	$\mathbf{1}$
		Sig. (2-tailed)	.001	.017	.228	.328	.277	
		$\mathbf N$	170	170	170	170	170	170

Table A5: Pearson correlations of abundances of heterotrophic bacteria (HB), cyanobacteria (CB), nanoflagellates (NF), picoeukaryotes (Picoeuk), *Synechococcus* (Synecho), and *Prochlorococcus* (Prochloro) with each other, depth, light intensity (PAR), temperature (Temp) and N/P-ratios (NtoP) of each ETS-20 sample depth. Significant correlations are bold.

Table A6: Pearson correlations of abundances of heterotrophic bacteria (HB), cyanobacteria (CB), nanoflagellates (NF), picoeukaryotes (Picoeuk), *Synechococcus* (Synecho), and *Prochlorococcus* (Prochloro) with each other, depth, light intensity (PAR), temperature (Temp) and N/P-ratios (NtoP) of of each ETS-20 station profile. Significant correlations are bold.

Appendix B

Table B1: Pearson correlation results of heterotrophic bacteria abundance, biovolume, PAR, temperature and N/P ratios of single depths over time of the ETS-100 station. Significant correlations are bold.

Depth			PAR	T	N to P	Abundance	Biovolume
$\bf{0}$	Abundance	Pearson correlation	$-.177**$.188**	$.091**$	$\,1$.191**
		Sig. (2-tailed)	.000	.000	.000		.000
		N	3129	3250	3319	3319	3319
	Biovolume	Pearson correlation	$-.169**$	$-.052**$	$.108**$.191**	$\mathbf{1}$
		Sig. (2-tailed)	.000	.003	.000	.000	
		N	3129	3250	3319	3319	3319
10	Abundance	Pearson correlation	$-.301**$	$.319**$	$-.038*$	$\mathbf{1}$.030
		Sig. (2-tailed)	.000	.000	.037		.095
		N	2848	3012	3102	3102	3102
	Biovolume	Pearson correlation	$-.069**$	$-.090**$	$.047**$.030	$\mathbf{1}$
		Sig. (2-tailed)	.000	.000	.009	.095	
		$\mathbf N$	2848	3012	3102	3102	3102
25	Abundance	Pearson correlation	$-143**$.382**	.279**	$\mathbf{1}$	$-.047**$
		Sig. (2-tailed)	.000	.000	.000		.008
		$\mathbf N$	3010	3160	3203	3203	3203
	Biovolume	Pearson correlation	$-.010$	$-.031$	$-.070**$	$-.047**$	$\mathbf{1}$
		Sig. (2-tailed)	.582	.077	.000	.008	
		N	3010	3160	3203	3203	3203
50	Abundance	Pearson correlation	$-0.145**$.485**	$.183**$	$\mathbf{1}$	$-.046*$
		Sig. (2-tailed)	.000	.000	.000		.012
		N	2789	2924	2996	2996	2996
	Biovolume	Pearson correlation	$-.005$	$-.130**$	$.038*$	$-.046*$	$\mathbf{1}$
		Sig. (2-tailed)	.784	.000	.039	.012	
		$\mathbf N$	2789	2924	2996	2996	2996
75	Abundance	Pearson correlation	$-.166**$	$.543**$	$.671**$	$\,1\,$	$-.038$
		Sig. (2-tailed)	.000	.000	.000		.054
		N	2322	2440	2528	2528	2528
	Biovolume	Pearson correlation	$-.044*$	$-.123**$	$-.065**$	$-.038$	$\mathbf{1}$
		Sig. (2-tailed)	.033	.000	.001	.054	
		N	2322	2440	2528	2528	2528
100	Abundance	Pearson correlation	$-.267**$	$.252**$	$-.028$	$\mathbf{1}$.089**
		Sig. (2-tailed)	.000	.000	.154		.000
		$\mathbf N$	2259	2388	2514	2514	2514
	Biovolume	Pearson correlation	$-.015$	$-.178**$.029	$.089**$	$\mathbf{1}$
		Sig. (2-tailed)	.463	.000	.148	.000	
		N	2259	2388	2514	2514	2514

Table B2: Pearson correlation results of heterotrophic bacteria abundance, biovolume, depth, PAR, temperature and N/P ratios of each ETS-100 station profile. Significant correlations are bold.

Date			Depth	PAR	T	N to P	Abundance	Biovolume
		$\mathbf N$	884	884	884	884	884	884
	Biovolume	Pearson correlation	$-.007$.059	.010	.014	.011	$1\,$
		Sig. (2-tailed)	.825	.081	.770	.668	.740	
		N	884	884	884	884	884	884
Aug 15	Abundance	Pearson correlation	$-.982**$.706**	.980**	$-.696**$	$\mathbf{1}$	$.160**$
		Sig. (2-tailed)	.000	.000	.000	.000		.000
		N	1228	1228	1228	1228	1228	1228
	Biovolume	Pearson correlation	$-.163**$	$.184**$	$.154**$	$-.088**$	$.160**$	$\mathbf{1}$
		Sig. (2-tailed)	.000	.000	.000	.002	.000	
		N	1228	1228	1228	1228	1228	1228
Sep 15	Abundance	Pearson correlation	$-.867**$.739**	.936**	$-.714**$	$\mathbf{1}$	$-.057$
		Sig. (2-tailed)	.000	.000	.000	.000		.088
		N	891	891	891	891	891	891
	Biovolume	Pearson correlation	.051	-0.09	$-.069*$.041	$-.057$	$1\,$
		Sig. (2-tailed)	.130	.778	.039	.224	.088	
		N	891	891	891	891	891	891
Oct 15	Abundance	Pearson correlation	$-.961**$.529**	.978**	$-.729**$	$\mathbf{1}$.027
		Sig. (2-tailed)	.000	.000	.000	.000		.386
		N	1011	1011	1011	1011	1011	1011
	Biovolume	Pearson correlation	$-.029$.011	.028	$-.023$.027	$1\,$
		Sig. (2-tailed)	.352	.737	.376	.462	.386	
		N	1011	1011	1011	1011	1011	1011
Nov 15	Abundance	Pearson correlation	$-.642**$	$.224**$.837**	$-.754**$	$\,1$	$-.067$
		Sig. (2-tailed)	.000	.000	.000	.000		.074
		N	710	710	710	710	710	710
	Biovolume	Pearson correlation	$-.026$.061	$-.023$.029	$-.067$	$\mathbf{1}$
		Sig. (2-tailed)	.485	.105	.532	.448	.074	
		N	710	710	710	710	710	710
Dec 15	Abundance	Pearson correlation	.733**	$-.150**$	$-.766**$	$-.317**$	$\mathbf{1}$	$-.038$
		Sig. (2-tailed)	.000	.000	.000	.000		.237
		N	992	992	992	992	992	992
	Biovolume	Pearson correlation	$-.073*$	$.086**$	$.081*$	$-.024$	$-.038$	$\mathbf{1}$
		Sig. (2-tailed)	.021	.007	.010	.457	.237	
		N	992	992	992	992	992	992

Table B3: Pearson correlation results of *Synechococcus* spp. abundance, biovolume, PAR, temperature and N/P ratios of single depths over time of the ETS-100 station. Significant correlations are bold.

Depth			PAR	T	N to P	Abundance	Biovolume
		Sig. (2-tailed)	.000	.000	.000		.000
		N	1085	1172	1211	1211	1211
	Biovolume	Pearson correlation	$-.168**$.001	$-.025$	$.123**$	$\,1\,$
		Sig. (2-tailed)	.000	.970	.380	.000	
		N	1085	1172	1211	1211	1211
50	Abundance	Pearson correlation	$-.382**$	$-.220**$	$-.016$	$\mathbf{1}$	$.163**$
		Sig. (2-tailed)	.000	.000	.581		.000
		$\mathbf N$	995	1097	1150	1150	1150
	Biovolume	Pearson correlation	$-.234**$	$-.091**$	$.134**$	$.163**$	$\mathbf{1}$
		Sig. (2-tailed)	.000	.003	.000	.000	
		N	995	1097	1150	1150	1150
75	Abundance	Pearson correlation	$-.400**$	$.069*$	$.116**$	$1\,$	$.075*$
		Sig. (2-tailed)	.000	.024	.000		.012
		$\mathbf N$	969	1075	1110	1110	1110
	Biovolume	Pearson correlation	$-.121**$	$-.106**$	$-.072*$	$.075*$	$\mathbf{1}$
		Sig. (2-tailed)	.000	.000	.017	.012	
		$\mathbf N$	969	1075	1110	1110	1110
100	Abundance	Pearson correlation	$-.280**$.385**	$-.226**$	$1\,$.019
		Sig. (2-tailed)	.000	.000	.000		.546
		N	963	1018	1062	1062	1062
	Biovolume	Pearson correlation	$-.117**$	$-.033$	$.133***$.019	$1\,$
		Sig. (2-tailed)	.000	.292	.000	.546	
		$\mathbf N$	963	1018	1062	1062	1062

Table B4: Pearson correlation results of *Synechococcus* spp. abundance, biovolume, depth, PAR, temperature and N/P ratios of each ETS-100 profile. Significant correlations are bold.

Date			Depth	PAR	T	N to P	Abundance	Biovolume
	Biovolume	Pearson correlation	$-.309**$	$.227**$	$.325***$	$-.203**$	$.330**$	$\mathbf{1}$
		Sig. (2-tailed)	.000	.000	.000	.000	.000	
		N	305	305	305	305	305	305
Nov 15	Abundance	Pearson correlation	$-.831**$.298**	.941**	$-.837**$	$\mathbf{1}$.026
		Sig. (2-tailed)	.000	.000	.000	.000		.684
		N	240	240	240	240	240	240
	Biovolume	Pearson correlation	$-.075$.089	.081	$-.084$.026	$\mathbf{1}$
		Sig. (2-tailed)	.249	.169	.214	.197	.684	
		N	240	240	240	240	240	240
Dec 15	Abundance	Pearson correlation	.985**	$-.590**$	$-.983**$	$-.499**$	$\mathbf{1}$	$.124*$
		Sig. (2-tailed)	.000	.000	.000	.000		.016
		N	374	374	374	374	374	374
	Biovolume	Pearson correlation	$.118*$	$-.117*$	$-136**$	$-.071$	$.124*$	$\mathbf{1}$
		Sig. (2-tailed)	.022	.023	.009	.171	.016	
		N	374	374	374	374	374	374

Table B5: Pearson correlations of abundances of heterotrophic bacteria (HB), cyanobacteria (CB), nanoflagellates (NF), picoeukaryotes (Picoeuk), *Synechococcus* (Synecho), and *Prochlorococcus* (Prochloro) with each other, light intensity (PAR), temperature (Temp) and N/P-ratios (NtoP) of each ETS-100 sample depth. Significant correlations are bold.

Table B6: Pearson correlations of abundances of heterotrophic bacteria (HB), cyanobacteria (CB), nanoflagellates (NF), picoeukaryotes (Picoeuk), *Synechococcus* (Synecho), and *Prochlorococcus* (Prochloro) with each other, depth, light intensity (PAR), temperature (Temp) and N/P-ratios (NtoP) of of each ETS-100 station profile. Significant correlations are bold.

Date	Variable		HB	СB	NF	Picoeuk	Synecho	Prochloro
Jan 14								
		Depth Pearson Correlation	-257	$-.654$	$-.041$	$-0.975**$	-0.610	-559
		Sig. (2-tailed)	,623	,159	,939	,001	,199	,249
		N	6	6	6	6	6	6
	Temp	Pearson Correlation						
		Sig. (2-tailed)						
		N						
		PAR Pearson Correlation						
		Sig. (2-tailed)						
		N						
		Chl Pearson Correlation	-287	,923**	-275	,497	,910*	,761
		Sig. (2-tailed)	,581	,009	,597	,316	,012	,079
		N	6	6	6	6	6	6
		NtoP Pearson Correlation	,212	,724	$-.011$,970**	,669	,581
		Sig. (2-tailed)	,687	,104	,983	,001	,146	,227
		N	6	6	6	6	6	6
		HB Pearson Correlation	$\,1\,$	$-.036$,891*	,343	-0.072	-360
		Sig. (2-tailed)		,946	,017	,506	,892	,483
		N	6	6	6	6	6	6
		CB Pearson Correlation	$-.036$	$\,1\,$	-162	,672	,995**	,854*
		Sig. (2-tailed)	,946		,759	,143	,000	,030
		N	6	6	6	6	6	6
		NF Pearson Correlation	,891*	-162	$\,1$,095	$-.206$	-592
		Sig. (2-tailed)	,017	,759		,858	,696	,216
		N	6	6	6	6	6	6
		Picoeuk Pearson Correlation	,343	,672	,095	$1\,$,622	,537
		Sig. (2-tailed)	,506	,143	,858		,187	,272
		$\mathbf N$	6	6	6	6	6	6
	Synecho	Pearson Correlation	$-.072$,995**	$-.206$,622	$\mathbf{1}$,882*
		Sig. (2-tailed)	,892	,000	,696	,187		,020
		$\mathbf N$	6	6	6	6	6	6
		Prochloro Pearson Correlation	$-.360$	$.854*$	-592	,537	,882*	$\mathbf{1}$
		Sig. (2-tailed)	,483	,030	,216	,272	,020	
		N	6	6	6	6	6	6
Feb 14								
		Depth Pearson Correlation	,793	-0.054	$-0.845*$,973**	-,117	,805
		Sig. (2-tailed)	,060	,920	,034	,001	,825	,053
	Temp	N Pearson Correlation	6	6	6	6 -337	6	6
		Sig. (2-tailed)	-760 ,080	$-.876*$,335 ,516	,514	$-.817*$,047	-180 ,733
		$\mathbf N$,022				
			6	6	6	6	6	6
		PAR Pearson Correlation						
		Sig. (2-tailed)						
		$\mathbf N$						
		Chl Pearson Correlation	,698	$-.030$	$-.835*$,943**	$-.054$,856*
		Sig. (2-tailed)	,123	,955	,038	,005	,919	,030
		N	6	6	6	6	6	6
		NtoP Pearson Correlation	-552	-0.307	,605	$-.818*$	-352	-772

Date Variable		HB	CB	NF	Picoeuk	Synecho	Prochloro
	Sig. (2-tailed)	,256	,554	,203	,047	,494	,072
	N	6	6	6	6	6	6
	HB Pearson Correlation	$\mathbf{1}$,427	-765	,718	,306	,428
	Sig. (2-tailed)		,398	,076	,108	,555	,397
	N	6	6	6	6	6	6
	CB Pearson Correlation	,427	$1\,$,127	$-.003$,983**	$-.047$
	Sig. (2-tailed)	,398		,810	,995	,000	,929
	N	6	6	6	6	6	6
	NF Pearson Correlation	-765	,127	$1\,$	-,793	,208	$-.470$
	Sig. (2-tailed)	,076	,810		,060	,693	,347
	N	6	6	6	6	6	6
	Picoeuk Pearson Correlation	,718	$-.003$	-793	$1\,$	$-.028$,880*
	Sig. (2-tailed)	,108	,995	,060		,958	,021
	N	6	6	6	6	6	6
	Synecho Pearson Correlation	,306	,983**	,208	$-.028$	$\,1$	$-.016$
	Sig. (2-tailed)	,555	,000	,693	,958		,976
	N	6	6	6	6	6	6
	Prochloro Pearson Correlation	,428	$-.047$	$-.470$,880*	$-.016$	$\mathbf{1}$
	Sig. (2-tailed)	,397	,929	,347	,021	,976	
	N	6	6	6	6	6	6
Mar 14							
	Depth Pearson Correlation	$-.409$	$-.880*$	$-.916*$	$-.810$	$-.878*$,879*
				,010		,021	
	Sig. (2-tailed)	,421 6	,021		,051		,021
	N		6	6	6	6	6
Temp	Pearson Correlation	,209	,799	,561	,356	,857*	$-.930**$
	Sig. (2-tailed)	,691	,056	,247	,489	,029	,007
	N	6	6	6	6	6	6
	PAR Pearson Correlation						
	Sig. (2-tailed)						
	N						
	Chl Pearson Correlation	,333	,158	,106	,220	,110	,106
	Sig. (2-tailed)	,519	,764	,842	,676	,836	,842
	N	6	6	6	6	6	6
	NtoP Pearson Correlation	,012	-590	$-.420$	-317	-0.668	,742
	Sig. (2-tailed)	,982	,218	,407	,541	,147	,091
	N	6	6	6	6	6	6
	HB Pearson Correlation	$\,1$,632	,567	,641	,485	-169
	Sig. (2-tailed)		,178	,240	,170	,330	,749
	N	6	6	6	6	6	6
	CB Pearson Correlation	,632	$1\,$,835*	,696	.983**	$-.865*$
	Sig. (2-tailed)	,178		,039	,125	,000	,026
	$_{\rm N}$	6	6	6	6	6	6
	NF Pearson Correlation	,567	,835*	$1\,$,952**	,784	-,697
	Sig. (2-tailed)	,240	,039		,003	,065	,124
	N	6	6	6	6	6	6
	Picoeuk Pearson Correlation	,641	,696	,952**	$\mathbf{1}$,604	-0.473
	Sig. (2-tailed)	,170	,125	,003		,205	,343
	N	6	6	6	6	6	6
	Synecho Pearson Correlation	,485	,983**	,784*	,604	$1\,$	$-0.935**$
	Sig. (2-tailed)	,330	,000	,065	,205		,006
	N	6	6	6	6	6	6

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Appendix C

Table C1: Pearson correlation results of heterotrophic bacteria abundance, biovolume, PAR, temperature and N/P ratios of single depths over time of the ETS-200 station. Significant correlations are bold.

Depth			PAR	T	N to P	Abundance	Biovolume
0	Abundance	Pearson correlation	.239**	.452**	$-.072**$	$\mathbf{1}$	$.116**$
		Sig. (2-tailed)	.000	.000	.000		.000
		N	2551	2818	2818	2818	2818
	Biovolume	Pearson correlation	$-.002$	$-.058**$	$-.034$	$.116**$	$\mathbf{1}$
		Sig. (2-tailed)	.929	.002	.074	.000	
		N	2551	2818	2818	2818	2818
10	Abundance	Pearson correlation	$.101**$.445**	$-.257**$	$\mathbf{1}$	$-.005$
		Sig. (2-tailed)	.000	.000	.000		.804
		N	2467	2783	2783	2783	2783
	Biovolume	Pearson correlation	$-.070**$	$-.040*$	$-.004$	$-.005$	$\mathbf{1}$
		Sig. (2-tailed)	.001	.034	.833	.804	
		N	2467	2783	2783	2783	2783
25	Abundance	Pearson correlation	$.167**$.530**	$-.129**$	$\,1$	$-.012$
		Sig. (2-tailed)	.000	.000	.000		.496
		N	2748	3034	3034	3034	3034
	Biovolume	Pearson correlation	$-.079**$	$-.080**$.001	$-.012$	$\mathbf{1}$
		Sig. (2-tailed)	.000	.000	.950	.496	
		N	2748	3034	3034	3034	3034
50	Abundance	Pearson correlation	$-.004$.559**	$-.038$	$\,1$	$-.037$
		Sig. (2-tailed)	.842	.000	.050		.056
		N	2354	2668	2668	2668	2668
	Biovolume	Pearson correlation	$-.014$	$-.102**$.023	$-.037$	$\mathbf{1}$
		Sig. (2-tailed)	.504	.000	.227	.056	
		N	2354	2668	2668	2668	2668
75	Abundance	Pearson correlation	$-.303**$.471**	$-.216**$	$\mathbf{1}$	$.111***$
		Sig. (2-tailed)	.000	.000	.000		.000
		N	2050	2283	2283	2283	2283
	Biovolume	Pearson correlation	$.044*$	$-.054**$	$-.021$	$.111***$	$\mathbf{1}$
		Sig. (2-tailed)	.048	.009	.306	.000	
		N	2050	2283	2283	2283	2283
100	Abundance	Pearson correlation	$-.138**$.338**	$-.063**$	$\mathbf{1}$	$.044*$
		Sig. (2-tailed)	.000	.000	.004		.046
		N	1778	2062	2062	2062	2062
	Biovolume	Pearson correlation	.025	$-.118**$	$-.068**$	$.044*$	$\mathbf{1}$
		Sig. (2-tailed)	.297	.000	.002	.046	
		N	1778	2062	2062	2062	2062
200-150	Abundance	Pearson correlation	$-.518**$.580**	$-.189**$	$\,1$	$.077**$
		Sig. (2-tailed)	.000	.000	.000		.003
		N	1267	1462	1462	1462	1462
	Biovolume	Pearson correlation	$-.024$	$-.109**$.042	.077**	$\,1$
		Sig. (2-tailed)	.394	.000	.106	.003	
		N	1267	1462	1462	1462	1462
200	Abundance	Pearson correlation	$-.355**$	$.612**$	$-.483**$	$\,1\,$.098**
		Sig. (2-tailed)	.000	.000	.000		.000
		N	1134	1375	1375	1375	1375
	Biovolume	Pearson correlation	.027	$-.133**$	$.057*$.098**	$1\,$
		Sig. (2-tailed)	.355	.000	.033	.000	

$n =$ \sim 1077 13/J +134 10 / U 137 J $- - -$ ___ ___	1375
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Table C2: Pearson correlation results of heterotrophic bacteria abundance, biovolume, depth, PAR, temperature and N/P ratios of each ETS-200 station profile. Significant correlations are bold.

Date			Depth	PAR	T	N to P	Abundance	Biovolume
		Sig. (2-tailed)	.059	.807	.303	.760	.044	
		N	521	521	521	521	521	521
Jul 15	Abundance	Pearson correlation	$-.689**$	$.063*$.381**	$-.502**$	$\,1$.046
		Sig. (2-tailed)	.000	.029	.000	.000		.113
		$\mathbf N$	1212	1212	1212	1212	1212	1212
	Biovolume	Pearson correlation	$-.026$.016	.012	$-.013$.046	$\mathbf{1}$
		Sig. (2-tailed)	.363	.571	.666	.663	.113	
		$\mathbb N$	1212	1212	1212	1212	1212	1212
Aug 15	Abundance	Pearson correlation	$-.919**$.927**	.955**	$-.426**$	$\,1\,$	$.127**$
		Sig. (2-tailed)	.000	.000	.000	.000		.000
		N	1089	1089	1089	1089	1089	1089
	Biovolume	Pearson correlation	$-.078*$	$.156**$	$.142**$.036	$.127**$	$\mathbf{1}$
		Sig. (2-tailed)	.010	.000	.000	.239	.000	
		N	1089	1089	1089	1089	1089	1089
Sep 15	Abundance	Pearson correlation	$-0.954**$.775**	.936**	$-.389**$	$\mathbf{1}$	$-.095**$
		Sig. (2-tailed)	.000	.000	.000	.000		.001
		N	1141	1141	1141	1141	1141	1141
	Biovolume	Pearson correlation	$.112**$	$-.047$	$-.093**$.037	$-.095**$	$\mathbf{1}$
		Sig. (2-tailed)	.000	.109	.002	.217	.001	
		N	1141	1141	1141	1141	1141	1141
Oct 15	Abundance	Pearson correlation	$-0.932**$.803**	.958**	$-.625**$	$1\,$.057
		Sig. (2-tailed)	.000	.000	.000	.000		.065
		N	1049	1049	1049	1049	1049	1049
	Biovolume	Pearson correlation	$-.031$	$.116**$	$.068*$	$-.006$.057	$\mathbf{1}$
		Sig. (2-tailed)	.320	.000	.027	.858	.065	
		$\mathbb N$	1049	1049	1049	1049	1049	1049
Nov 15	Abundance	Pearson correlation	$-0.913**$	$.814**$.921**	$-.389**$	$\,1$.039
		Sig. (2-tailed)	.000	.000	.000	.000		.303
		N	712	712	712	712	712	712
	Biovolume	Pearson correlation	$-.041$	$-.031$	$.087*$	$-.059$.039	$\mathbf{1}$
		Sig. (2-tailed)	.275	.413	.020	.117	.303	
		N	712	712	712	712	712	712
Dec 15	Abundance	Pearson correlation	$-.732**$.296**	.922**	$-.671**$	$\,1$.006
		Sig. (2-tailed)	.000	.000	.000	.000		.859
		$\mathbb N$	830	830	830	830	830	830
	Biovolume	Pearson correlation	$-.016$	$-.010$.006	$-.025$.006	$1\,$
		Sig. (2-tailed)	.645	.780	.859	.469	.859	
		N	830	830	830	830	830	830

Table C3: Pearson correlation results of *Synechococcus* spp. abundance, biovolume, PAR, temperature and N/P ratios of single depths over time of the ETS-200 station. Significant correlations are bold.

	Biovolume	Pearson correlation	$-.107**$.018	.050	.032	$\,1\,$
		Sig. (2-tailed)	.002	.568	.119	.319	
		$\mathbf N$	809	956	956	956	956
25	Abundance	Pearson correlation	$-.298**$	$-.218**$.031	$\mathbf{1}$.050
		Sig. (2-tailed)	.000	.000	.333		.124
		N	810	961	961	961	961
	Biovolume	Pearson correlation	$-.232**$	$-.062$	$.100**$.050	$\mathbf{1}$
		Sig. (2-tailed)	.000	.053	.002	.124	
		$\mathbf N$	810	961	961	961	961
50	Abundance	Pearson correlation	$-.421**$	$-.212**$.062	$\mathbf{1}$.135**
		Sig. (2-tailed)	.000	.000	.063		.000
		N	781	896	896	896	896
	Biovolume	Pearson correlation	$-.242**$	$-.047$	$.088**$.135**	$\mathbf{1}$
		Sig. (2-tailed)	.000	.159	.008	.000	
		N	781	896	896	896	896
75	Abundance	Pearson correlation	$-.471**$	$.140**$	$-.075*$	$\mathbf{1}$	$.103**$
		Sig. (2-tailed)	.000	.000	.022		.001
		N	818	943	943	943	943
	Biovolume	Pearson correlation	$-.157**$.014	$-.012$	$.103**$	$\mathbf{1}$
		Sig. (2-tailed)	.000	.661	.720	.001	
		N	818	943	943	943	943
100	Abundance	Pearson correlation	$-.306**$	$.186**$	$-.172**$	$\mathbf{1}$	$-.003$
		Sig. (2-tailed)	.000	.000	.000		.936
		N	698	826	826	826	826
	Biovolume	Pearson correlation	$-.104**$	$-.035$	$-.043$	$-.003$	$\mathbf{1}$
		Sig. (2-tailed)	.006	.315	.214	.936	
		N	698	826	826	826	826
150	Abundance	Pearson correlation	$-.359**$.839**	$-.215**$	$\mathbf{1}$.046
		Sig. (2-tailed)	.000	.000	.000		.224
		N	568	711	$711\,$	711	711
	Biovolume	Pearson correlation	$-.191**$	$.079*$	$-.092*$.046	$\mathbf{1}$
		Sig. (2-tailed)	.000	.035	.015	.224	
		N	568	711	711	711	711
200	Abundance	Pearson correlation	$-0.272**$	$.843**$	$-.322**$	$\mathbf{1}$	$.085*$
		Sig. (2-tailed)	.000	.000	.000		.033
		N	535	633	633	633	633
	Biovolume	Pearson correlation	$-.174**$	$.086*$.054	$.085*$	$1\,$
		Sig. (2-tailed)	.000	.031	.177	.033	
		$\mathbf N$	535	633	633	633	633

Table C4: Pearson correlation results of *Synechococcus* spp. abundance, biovolume, depth, PAR, temperature and N/P ratios of each ETS-200 profile. Significant correlations are bold.

Date			Depth	PAR	T	N to P	Abundance	Biovolume
Sep 15	Abundance	Pearson correlation	$-.844**$.881**	.950**	$-.447**$	$\mathbf{1}$.037
		Sig. (2-tailed)	.000	.000	.000	.000		.539
		$\mathbb N$	275	275	275	275	275	275
	Biovolume	Pearson correlation	-0.068	$-.006$.066	.011	.037	$\mathbf{1}$
		Sig. (2-tailed)	.262	.924	.273	.860	.539	
		N	275	275	275	275	275	275
Oct 15	Abundance	Pearson correlation	$-.751**$.916**	.907**	$-.450**$	$\mathbf{1}$	$.262**$
		Sig. (2-tailed)	.000	.000	.000	.000		.000
		$\mathbb N$	348	348	348	348	348	348
	Biovolume	Pearson correlation	$-.195**$.232**	.253**	$-.081$	$.262**$	$\mathbf{1}$
		Sig. (2-tailed)	.000	.000	.000	.133	.000	
		$\mathbf N$	348	348	348	348	348	348
Nov 15	Abundance	Pearson correlation	$-0.926**$.556**	.989**	$-.660**$	$\mathbf{1}$.062
		Sig. (2-tailed)	.000	.000	.000	.000		.285
		$\mathbb N$	296	296	296	296	296	296
	Biovolume	Pearson correlation	$-.109$.087	.078	$-.074$.062	$\mathbf{1}$
		Sig. (2-tailed)	.062	.134	.179	.203	.285	
		$\mathbf N$	296	296	296	296	296	296
Dec 15	Abundance	Pearson correlation	$-.602**$.192**	.798**	$-.515**$	$\mathbf{1}$.091
		Sig. (2-tailed)	.000	.001	.000	.000		.121
		$\mathbf N$	294	294	294	294	294	294
	Biovolume	Pearson correlation	$-.105$.051	$.138*$	$-.068$.091	$\mathbf{1}$
		Sig. (2-tailed)	.072	.386	.018	.244	.121	
		$\mathbf N$	294	294	294	294	294	294

Table B5: Pearson correlations of abundances of heterotrophic bacteria (HB), cyanobacteria (CB), nanoflagellates (NF), picoeukaryotes (Picoeuk), *Synechococcus* (Synecho), and *Prochlorococcus* (Prochloro) with each other, light intensity (PAR), temperature (Temp) and N/P-ratios (NtoP) of each ETS-100 sample depth. Significant correlations are bold.

Table C6: Pearson correlations of abundances of heterotrophic bacteria (HB), cyanobacteria (CB), nanoflagellates (NF), picoeukaryotes (Picoeuk), *Synechococcus* (Synecho), and *Prochlorococcus* (Prochloro) with each other, depth, light intensity (PAR), temperature (Temp) and N/P-ratios (NtoP) of of each ETS-200 station profile. Significant correlations are bold.

Date	Variable		HB	CB	NF	Picoeuk	Synecho	Prochloro
		Sig. (2-tailed)	.035	.916	.073	.851	.762	.001
		$\mathbf N$	8	8	8	8	8	8
	HB	Pearson Correlation	$\mathbf{1}$	$-.111$.422	$-.110$	$-.054$	$-.524$
		Sig. (2-tailed)		.793	.298	.795	.899	.183
		N	8	8	8	8	8	8
		CB Pearson Correlation	$-.111$	$\mathbf{1}$.417	.438	.994**	$-.040$
		Sig. (2-tailed)	.793		.304	.277	.000	.926
		N	8	8	8	8	8	8
		NF Pearson Correlation	.422	.417	$\mathbf{1}$.500	.462	$-.443$
		Sig. (2-tailed)	.298	.304		.207	.250	.272
		N	8	8	8	8	8	8
		Picoeuk Pearson Correlation	$-.110$.438	.500	$\,1\,$.398	$-.037$
		Sig. (2-tailed)	.795	.277	.207		.328	.931
		N	8	8	8	8	8	8
		Synecho Pearson Correlation	$-.054$.994**	.462	.398	$\mathbf{1}$	$-.113$
		Sig. (2-tailed)	.899	.000	.250	.328		.790
		N	8	8	8	8	8	8
	Prochloro	Pearson Correlation	$-.524$	$-.040$	$-.443$	$-.037$	$-.113$	$\mathbf{1}$
		Sig. (2-tailed)	.183	.926	.272	.931	.790	
		N	8	8	8	8	8	8
Jul 14								
		Depth Pearson Correlation	$-.358$	$-.132$	$-.890**$	$-.622$	$-.289$.433
		Sig. (2-tailed)	.384	.755	.003	.100	.487	.284
		$\mathbf N$	8	8	8	8	8	8
	Temp	Pearson Correlation	$-.139$	$-.033$.669	.537	.068	-263
		Sig. (2-tailed)	.742	.939	.070	.170	.874	.530
		N	8	8	8	8	8	8
		PAR Pearson Correlation	$-.047$	$-.279$.725*	.519	$-.146$	$-.297$
			.912	.503	.042	.187	.730	.475
		Sig. (2-tailed) N	8	8	8	8	8	8
		Chl Pearson Correlation	$-.025$.001	$-.606$.100	$-.039$.602
		Sig. (2-tailed)	.952	.998	.112	.814	.927	.115
		N	8	8	8	8	8	8
	NtoP	Pearson Correlation	$-.578$	$-.281$	$-.725*$	$-.712$	$-.438$.227
		Sig. (2-tailed)	.133	.500	.042	.048	.278	.589
		N	8	8	8	8	8	8
		HB Pearson Correlation	$1\,$.267	.572	.141	.397	$-.540$
		Sig. (2-tailed)		.523	.139	.740	.330	.168
		$\mathbf N$	8	8	8	8	8	8
		CB Pearson Correlation	.267	$\mathbf{1}$	$-.062$.280	.979**	$-.425$
		Sig. (2-tailed)	.523		.885	.501	.000	.294
		$\mathbf N$	8	8	8	8	8	8
		NF Pearson Correlation	.572	$-.062$	$\mathbf{1}$.337	.102	$-.546$
		Sig. (2-tailed)	.139	.885		.414	.810	.161
		$\mathbf N$	8	8	8	8	8	8
		Picoeuk Pearson Correlation	.141	.280	.337	$\mathbf{1}$.412	$-.094$
		Sig. (2-tailed)	.740	.501	.414		.311	.826
		N	8	8	8	8	8	8
		Synecho Pearson Correlation	.397	.979*	.102	.412	$\mathbf{1}$	$-.508$
		Sig. (2-tailed)	.330	.000	.810	.311		.198
		N	8	8	8	8	8	8
		Prochloro Pearson Correlation	$-.540$	$-.425$	$-.546$	$-.094$	$-.508$	$\mathbf{1}$

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Appendix D

Station			PAR	T	N to P	Abundance	Biovolume
50	Abundance	Pearson correlation	$-.340**$	$.307**$	$.052**$	$\mathbf{1}$	$.154***$
		Sig. (2-tailed)	.000	.000	.001		.000
		N	3660	3976	3976	3976	3976
	Biovolume	Pearson correlation	$-.078**$	$-.063**$	$.108**$	$.154**$	$\mathbf{1}$
		Sig. (2-tailed)	.000	.000	.000	.000	
		N	3660	3976	3976	3976	3976
75	Abundance	Pearson correlation	$-.190**$	$.220**$	$-.045**$	$\mathbf{1}$.125**
		Sig. (2-tailed)	.000	.000	.004		.000
		N	3608	4199	4199	4199	4199
	Biovolume	Pearson correlation	$-.068**$	$-.055**$	$-.028$.125**	$\mathbf{1}$
		Sig. (2-tailed)	.000	.000	.067	.000	
		N	3608	4199	4199	4199	4199
125	Abundance	Pearson correlation	$-.212**$	$.077**$	$.247**$	$\mathbf{1}$.125**
		Sig. (2-tailed)	.000	.000	.000		.000
		N	3096	3481	3481	3481	3481
	Biovolume	Pearson correlation	.009	$-.116**$	$.160**$	$.125***$	$\mathbf{1}$
		Sig. (2-tailed)	.622	.000	.000	.000	
		N	3096	3481	3481	3481	3481
150	Abundance	Pearson correlation	$.079**$.178**	.215**	$\mathbf{1}$	$.052**$
		Sig. (2-tailed)	.000	.000	.000		.004
		N	2808	3003	3003	3003	3003
	Biovolume	Pearson correlation	.031	$-.020$	$-.013$	$.052**$	$\mathbf{1}$
		Sig. (2-tailed)	.100	.279	.471	.004	
		N	2808	3003	3003	3003	3003
175	Abundance	Pearson correlation	$.056**$	$.235***$	$-.235**$	$\mathbf{1}$	$.056**$
		Sig. (2-tailed)	.005	.000	.000		.003
		N	2495	2784	2912	2912	2912
	Biovolume	Pearson correlation	$.076**$	$-.179**$	$-.028$	$.056**$	$\mathbf{1}$
		Sig. (2-tailed)	.000	.000	.132	.003	
		N	2495	2784	2912	2912	2912

Table D1: Pearson correlation results of heterotrophic bacteria abundance, biovolume, PAR, temperature and N/P ratios over time of the surface station samples. Significant correlations are bold.

Table D2: Pearson correlation results of heterotrophic bacteria abundance, biovolume, PAR, temperature and N/P ratios of transect over time. Significant correlations are bold.

Date			PAR	T	N to P	Abundance	Biovolume
Jan 14	Abundance	Pearson correlation		$-.828**$.535**	$\mathbf{1}$.005
		Sig. (2-tailed)		.000	.000		.879
		N		736	805	805	805
	Biovolume	Pearson correlation		$.116***$	$-.110**$.005	$1\,$
		Sig. (2-tailed)		.002	.002	.879	
		N		736	805	805	805
Feb 14	Abundance	Pearson correlation		$-.881**$.914**	$\mathbf{1}$.157**
		Sig. (2-tailed)		.000	.000		.000
		N		820	820	820	820
	Biovolume	Pearson correlation		$-.217**$	$.151***$.157**	$1\,$
		Sig. (2-tailed)		.000	.000	.000	
		N		820	820	820	820
Mar 14	Abundance	Pearson correlation		$-.899**$.698**	$\mathbf{1}$.167**

Date			PAR	T	N to P	Abundance	Biovolume
	Biovolume	Pearson correlation	$-.110**$.089**	$.051*$.087**	$\mathbf{1}$
		Sig. (2-tailed)	.000	.000	.010	.000	
		N	2523	2523	2523	2523	2523
Sep 15	Abundance	Pearson correlation	.849**	$.307**$	$.211**$	$\mathbf{1}$	$-.122**$
		Sig. (2-tailed)	.000	.000	.000		.000
		N	1623	1623	1623	1623	1623
	Biovolume	Pearson correlation	$-.136**$	$-.024$.087**	$-.122**$	$\mathbf{1}$
		Sig. (2-tailed)	.000	.328	.000	.000	
		N	1623	1623	1623	1623	1623
Oct 15	Abundance	Pearson correlation	$-.672**$	$.514**$	$.117**$	$\mathbf{1}$.029
		Sig. (2-tailed)	.000	.000	.000		.240
		N	1631	1631	1631	1631	1631
	Biovolume	Pearson correlation	$-.030$	$-.014$	$-.074**$.029	$\mathbf{1}$
		Sig. (2-tailed)	.230	.564	.003	.240	
		N	1631	1631	1631	1631	1631
Nov 15	Abundance	Pearson correlation	$-.216**$.596**	$.079*$	$\mathbf{1}$	$-.018$
		Sig. (2-tailed)	.000	.000	.010		.563
		N	1061	1061	1061	1061	1061
	Biovolume	Pearson correlation	.139**	$-.043$.016	$-.018$	$\mathbf{1}$
		Sig. (2-tailed)	.000	.163	.602	.563	
		N	1061	1061	1061	1061	1061
Dec 15	Abundance	Pearson correlation	$-.708**$	$-.806**$	$.522**$	$\mathbf{1}$.192**
		Sig. (2-tailed)	.000	.000	.000		.000
		N	1201	1201	1201	1201	1201
	Biovolume	Pearson correlation	$-.109**$	$-.186**$	$.180**$.192**	$\mathbf{1}$
		Sig. (2-tailed)	.000	.000	.000	.000	
		N	1201	1201	1201	1201	1201

Table D3: Pearson correlation results of *Synechococcus* spp. abundance, biovolume, PAR, temperature and N/P ratios of surface stations over time. Significant correlations are bold.

Station			PAR	T	N to P	Abundance	Biovolume
	Biovolume	Pearson correlation	.009	$.069*$	$-.050$	$.146**$	1
		Sig. (2-tailed)	.785	.020	.093	.000	
		N	1009	1145	1145	1145	1145
175	Abundance	Pearson correlation	$-.183**$	$-.294**$	$-.166**$	$\mathbf{1}$	$.091**$
		Sig. (2-tailed)	.000	.000	.000		.004
		N	786	925	1013	1013	1013
	Biovolume	Pearson correlation	$-.079*$	$-.033$	$-.053$	$.091**$	$\mathbf{1}$
		Sig. (2-tailed)	.028	.310	.091	.004	
		N	786	925	1013	1013	1013

Table C4: Pearson correlation results of *Synechococcus* spp. abundance, biovolume, depth, PAR, temperature and N/P ratios of each transect. Significant correlations are bold.

Date		Distance	PAR	m	N to P	Abundance	Biovolume
	.,	415	415	415	415	415	-415

Table C5: Pearson correlations of abundances of heterotrophic bacteria (HB), cyanobacteria (CB), nanoflagellates (NF), picoeukaryotes (Picoeuk), *Synechococcus* (Synecho), and *Prochlorococcus* (Prochloro) with each other, depth, light intensity (PAR), temperature (Temp) and N/P-ratios (NtoP) of each surface station. Significant correlations are bold.

Table B6: Pearson correlations of abundances of heterotrophic bacteria (HB), cyanobacteria (CB), nanoflagellates (NF), picoeukaryotes (Picoeuk), *Synechococcus* (Synecho), and *Prochlorococcus* (Prochloro) with each other, distance, light intensity (PAR), temperature (Temp) and N/P-ratios (NtoP) of of each ETS transect. Significant correlations are bold.

Date Variable		HB	CB	NF	Picoeuk	Synecho	Prochloro
	Sig. (2-tailed)	.275	.120	.359	.260	.098	.289
	N	8	8	8	8	8	8
	HB Pearson Correlation	$\mathbf{1}$.895**	.991**	.279	.887**	$-.590$
	Sig. (2-tailed)		.003	.000	.504	.003	.124
	N	8	8	8	8	8	8
	CB Pearson Correlation	.895**	$\mathbf{1}$	$.841**$.630	.999**	$-.603$
	Sig. (2-tailed)	.003		.009	.094	.000	.114
	N	8	8	8	8	8	8
	NF Pearson Correlation	.991**	$.841**$	$\mathbf{1}$.186	$.830*$	$-.600$
	Sig. (2-tailed)	.000	.009		.659	.011	.116
	N	8	8	8	8	8	8
	Picoeuk Pearson Correlation	.279	.630	.186	$\mathbf{1}$.635	$-.156$
	Sig. (2-tailed)	.504	.094	.659		.091	.712
	N	8	8	8	8	8	8
	Synecho Pearson Correlation	.887**	.999**	$.830*$.635	$\mathbf{1}$	$-.606$
	Sig. (2-tailed)	.003	.000	.011	.091		.111
	N	8	8	8	8	8	8
	Prochloro Pearson Correlation	$-.590$	$-.603$	$-.600$	$-.156$	-606	$\mathbf{1}$
	Sig. (2-tailed)	.124	.114	.116	.712	.111	
	N	8	8	8	8	8	8
Aug 14							
	Station Pearson Correlation	$-.118$	$-.633$	$-.746*$	$-.638$	$-.654$	$-.601$
	Sig. (2-tailed)	.780	.092	.034	.089	.079	.115
	N	8	8	8	8	8	8
	Chl Pearson Correlation	.227	.887**	$.861**$	$.724*$.914**	.685
	Sig. (2-tailed)	.589	.003	.006	.042	.001	.061
	N	8	8	8	8	8	8
	Temp Pearson Correlation	.007	$-.500$	$-.721*$	$-.437$	$-.488$	$-.508$
	Sig. (2-tailed)	.988	.207	.044	.280	.220	.199
	N	8	8	8	8	8	8
	NtoP Pearson Correlation	.130	.958**	.852**	.673	.935**	.754*
	Sig. (2-tailed)	.759	.000	.007	.068	.001	.031
	N	8	8	8	8	8	8
	HB Pearson Correlation	$\mathbf{1}$.364	.466	.745	.328	.512
	Sig. (2-tailed)		.375	.245	.034	.428	.195
	N	8	8	8	8	8	8
	CB Pearson Correlation	.364	$\,1$.883**	.797*	.980**	$.822*$
	Sig. (2-tailed)	.375		.004	.018	.000	.012
	N	8	8	8	8	8	8
	NF Pearson Correlation	.466	.883**	$\,1$	$.833*$	$.832*$.936**
	Sig. (2-tailed)	.245	.004		.010	.010	.001
	N	8	8	8	8	8	8
	Picoeuk Pearson Correlation	$.745*$.797*	$.833*$	$1\,$	$.751*$	$.814*$
	Sig. (2-tailed)	.034	.018	.010		.032	.014
	$\mathbf N$	8	8	8	8	8	8
	Synecho Pearson Correlation	.328	.980**	$.832*$	$.751*$	$\mathbf{1}$	$.722*$
	Sig. (2-tailed)	.428	.000	.010	.032		.043
	$\mathbf N$	8	8	8	8	8	8
	Prochloro Pearson Correlation	.512	$.822*$.936**	$.814*$	$.722*$	$\mathbf{1}$
			.012	.001	.014	.043	
	Sig. (2-tailed)	.195					

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Appendix E

Appendix F

Table F1: Kruskal-Wallis H Test (one way ANOVA on ranks) results for independent samples of nutrient concentrations after Lamas River water was added for all 3 River water experiments.

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Curriculum Vitae

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Date of birth: 02.08.1984 Place of Birth: Dharmsala Kangra, India Gender: Female Citizenship: German Marital status: Married Maiden name: Schulze

EDUCATION

 Dissertation, Middle East Technical University - Institute for Marine Sciences, 2017 Concentrations: Phytoplankton, Nutrient effects on phytoplankton communities, Nutrient cycles

Thesis: Natural and human induced nutrient impacts on phytoplankton communities in Mersin Bay, NE Mediterranean

- M.Sc., Oceanography, GEOMAR, University of Kiel, 2013 Concentrations: Biology, Ecology, Planktology Thesis: Experimental tests of dispersal frequency on phytoplankton diversity in source- sink metacommunities
- B.Sc., Biology, Alfred Wegener Institute (AWI), University of Bremen, 2010 Concentrations: Marine Biology, Ecology, Planktology Thesis: Copepod grazing rates on several diatoms considering biomechanical aspects

EXPERIENCES

- Research assistant 2011-2013 GEOMAR, Lab work and preparation of mesocosm experiments
- Research assistant 2008-2010 Alfred-Wegener-Institute, preparing and attending international bionic meetings, lab work in Bremerhaven, Helgoland and Kiel, and preparing and attending the exhibition at Hannover Messe 2010
- Research assistant 2006-2008 University of Bremen, Assisting the bachelor course "Struktur und funktion von wirbellosen Tieren"

PROFESSIONAL QUALIFICATIONS

- Knowledge of reverse light microscopical identification of phytoplankton species
- Fluorescence microscopy of *Synechococcus* spp. and heterotrophic bacteria
- Knowledge of Flow-cytometry (Apogee A50 Micro)
- Knowledge of Statistica and SPSS
- **•** Efficient helper on board research vessels

PUBLICATIONS

 Friedrichs L, Hörnig M, **Schulze L**, Bertram A, Jansen S, Hamm C (2013) Size and biomechanic properties of diatom frustules influence food uptake by copepods. Mar Ecol Prog Ser 481:41-51. https://doi.org/10.3354/meps10227

PRESENTATIONS AND POSTERS

 L.J. Schulze, S. Tugrul, Z Uysal (2016). Effect of dredged material dumping into local (pico-) phytoplankton groups of NE Mediterranean. CIESM Congress 2016, Kiel, article 0285

FELLOWSHIPS

- February 2013-September 2017 TUBITAK 2215 scholarship for international students in Turkey
- 2013-2015 Lecturer and assistant in the TUBITAK project "Denizimi Taniyorum ve Koruyorum" at METU-IMS (Erdemli) and Dokuz Eylül Üniversitesi (Izmir)
- 18-26 July 2014 field experiment in flow-cytometry within the TUBITAK project MENAPHY at Stazione Zoologica Anton Dohrn, Naples, Italy
- February 2013 Internship at the Institute of Zoology, University of Innsbruck, Austria, under the supervision of Prof. Dr. Bert Hobmayer
- September 2010-August 2011 DAAD fellowship "Sino-German Master Programme in Biological Oceanography", Ocean University of China, Qingdao, China, and GEOMAR, Kiel, Germany

SKILLS AND QUALIFICATIONS

- Fluent in English, moderate in Chinese and Italian, beginner in Spanish and Turkish
- Good organization skills

INTERESTS

- Diatoms and their ecological role
- Diving and snorkeling
- Hiking and camping
- Sports (soccer, swimming, running)
- **•** Traveling