

DOKUZ EYLÜL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

**PHYTOPLANKTON DYNAMICS IN THE
AEGEAN SEA: CHEMOTAXONOMY IN THE
WATER COLUMN AND DINOFLAGELLATE
CYST ASSEMBLAGE IN THE SEDIMENT**

by
Janset KAYAALP

May, 2019
İZMİR

**PHYTOPLANKTON DYNAMICS IN THE
AEGEAN SEA: CHEMOTAXONOMY IN THE
WATER COLUMN AND DINOFLAGELLATE
CYST ASSEMBLAGE IN THE SEDIMENT**

**A Thesis Submitted to the
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Resources Program**

**by
Janset KAYAALP**

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Ph.D. THESIS EXAMINATION RESULT FORM

We have read the thesis entitled “**PHYTOPLANKTON DYNAMICS IN THE AEGEAN SEA: CHEMOTAXONOMY IN THE WATER COLUMN AND DINOFLAGELLATE CYST ASSEMBLAGE IN THE SEDIMENT**” completed by **JANSET KAYAALP** under supervision of **PROF.DR. NİHAYET BİZSEL** and **PROF.DR. VERA POSPELOVA** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Doctor of Philosophy.



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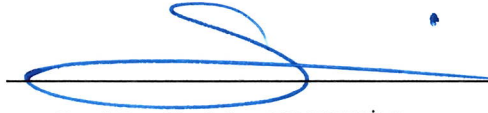
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**PHYTOPLANKTON DYNAMICS IN THE AEGEAN SEA:
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DINOFLAGELLATE ASSEMBLAGE IN THE SEDIMENT**

ABSTRACT

The Aegean Sea has a unique structure within the Mediterranean ecosystem with the complex bottom and coastal structure, various river discharges and the presence of the low salinity Black Sea water entering the Dardanelles. The aim of the research is to monitor phytoplankton pigments in the water column and modern dinoflagellate cysts in the sediment with taking environmental factors into account in the north-south gradient of the Aegean Sea. Additionally, in the Rhodes Gyre which has a special place in the Eastern Mediterranean ecosystem with its permanent “upwelling” structure, phytoplankton pigments were investigated the trophic gradient from the vicinity through the core of the gyre.

In the Aegean Sea, water column and sediment sampling was done at 10 different stations on March 2012, sediment sampling was done at 4 stations on June 2013 and in the Rhodes Gyre, water column sampling was done at 10 stations on March 2016. Collected samples were analysed by HPLC for pigment analysis. In the Aegean Sea Chl *a* ranged between BDL-2.00 µg/l. Picoplankton replaces with microplankton sized diatoms and dinoflagellates through the south. In the Rhodes Gyre, Chl *a* ranged between BDL-0.47 µg/l, maximum values have been reached in the core of the gyre and while nanoplankton has the largest proportion in the whole area microplankton prevailed over picoplankton through the core of the gyre.

Modern dinoflagellate cyst abundances ranged from 70 to 2222 cysts per gram dried sediment (max: St2, min: St1). Except for the low values observed in the Station 1C, abundances were found higher in the Black Sea influenced northern waters than the oligotrophic Eastern Mediterranean waters and while the dominancy of the autotrophic cysts in the Aegean Sea has been observed, the heterotroph cyst proportion was slightly decreasing through the south.

Keywords: Phytoplankton pigments, HPLC, modern dinoflagellate cysts, oligotrophy, Aegean Sea, Rhodes Gyre



EGE DENİZİ'NDE FİTOPLANKTON DİNAMİKLERİ: SU KOLONUNDA KEMOTAKSONOMİ VE SEDİMENTTE DİNOFLAGELLAT KİST DAĞILIMI

ÖZ

Ege Denizi karmaşık dip ve kıyı yapısı, hidrolojisi ve Çanakkale boğazı ile giriş yapan düşük tuzluluğa sahip Karadeniz suyu ile Doğu Akdeniz ekosistemi içerisinde dikkat çekmektedir. Söz konusu çalışmanın amacı, su kolonunda fitoplankton pigment maddelerinin, sedimentte modern dinoflagellat kist formları kompozisyonunun, kuzey-güney hattında ötrofi ve oligotrofi sınırlarında geçiş özelliği taşıyan Ege Denizi'nde çevresel faktörler de göz önünde bulundurularak izlenmesidir. Ayrıca Doğu Akdeniz ekosistemi içerisinde özel bir yere sahip olduğu bilinen, Rodos Siklonu'nda, “upwelling” dinamikleri sonucunda çevreden merkeze doğru görülen trofik gradient hattında, fitoplankton pigmentleri izlenmiştir.

Söz konusu amaç doğrultusunda, Ege Denizi'nde, 10 istasyondan Mart 2012'de su kolonu ve sediment örnekleme, 4 istasyondan Haziran 2013'te sediment örnekleme ve Rodos Siklonu'nda Mart 2016'da 10 istasyondan su kolonu örnekleme yapılmıştır.

HPLC ile 14 farklı pigment ölçümü gerçekleştirilmiştir. Ege Denizi'nde, Klorofil a (Chl a) değerleri; BDL-2,00 µg/l aralığında dağılım göstermiş, en yüksek konsantrasyonlar, kuzey istasyonlarından, St3'te (1,47-2,00 µg/l), en düşük değerler ise güney istasyonlarında BDL-0,01 µg/l aralığında gözlenmiştir. Kuzey-güney gradientinde, güneye doğru inildikçe pikoplanktonun, dinoflagellat ve diatom gibi mikro fitoplankton ile yer değiştirerek baskın hale geldiği gözlenmektedir. Rodos Siklonu'nda, Chl a değerleri, BDL-0,47 µg/l aralığında dağılım göstermiş, en yüksek değerler döngü merkezinde saptanmış ve nanoplankton tüm döngü alanında baskın iken döngü çevresinde ikinci sırada gelen pikoplanktonun merkeze doğru microplankton ile yer değiştirdiği gözlenmiştir.

Modern dinoflagellat kist bollukları ise ortalama 723 kist/ gram olmak üzere 70-2222 kist/gram kuru sediman aralığında deęişim göstermiştir (maksimum, İst 2C-minimum, İst 1C). 1 numaralı istasyonda görülen düşük konsantrasyon dışında, Karadeniz suyunun etkisi altında olan kuzey sularında bolluklar daha fazla iken, Doęu Akdeniz'e doęru bolluk deęerlerinin daha düşük olduęu, genel anlamda ototrof kistlerin baskın olduęu alanda, kuzeyden güneye doęru heterotrof kist oranında hafif bir düşüş olduęu gözlenmektedir.

Anahtar kelimeler: Fitoplankton pigmentleri, HPLC, modern dinoflagellate kistleri, oligotrofi, Ege Denizi, Rodos Siklonu



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CHAPTER ONE

INTRODUCTION

All marine life depends on a process which energy flows from one trophic level to another in the food chain. Autotrophs in the first trophic level which constituted mainly by planktonic primary producers, extract energy from inorganic compounds to build high energy organic molecules. Marine phytoplankton are responsible for approximately half of the total global primary production (PP) equalling about 45-59 Pg/year (Behrenfeld et al., 2001; Chavez et al., 2011; Falkowski & Raven 2007; Del Giorgio & Duarte, 2002; Keong, 2019). Phytoplankton primary production influences the carbon cycle through photosynthesis and stimulates CO₂ flux from the atmosphere to the surface ocean (Falkowski et al., 1998; Falkowski & Raven 2007; Keong, 2019). A fraction of the biologically fixed carbon will be transported to the deep ocean through the biological pump thus coupling phytoplankton production and global carbon cycle (Falkowski et al., 1998; Siegel et al., 2016). An efficient downward carbon flux has been shown to be associated with heavily ballasted phytoplankton groups such as coccolithophores (De La Rocha & Passow, 2007) and diatoms (Ragueneau et al., 2006) or species with specific life cycle strategies such as cyst formation of dinoflagellates (Dale, 2001; Fujii & Matsuoka, 2006).

Dinoflagellates are remarkable and unfamiliar organisms not only as primary producers but also having diversity of trophic strategies. Over the past few decades, dinoflagellates have aroused considerable attention primarily due to their contribution to Harmful Algal Blooms (HAB) and their potential to serve as paleoenvironmental indicators (Dale, 1996; Hallegraeff, 1993, Bizsel&Bizsel; 2015). Several studies have investigated the relationship between dinoflagellate cyst assemblages in surface sediments and environmental parameters, especially PP, Sea Surface Temperature (SST) and Sea Surface Salinity (SSS) (Elshanawany, 2010; Pospelova et al., 2008; Radi et al., 2007; Radi & de Vernal; 2004). Hence, recently deposited dinoflagellate cysts are considered as cultural eutrophication tracking signals that corresponding to the definition of “modern” sediments.

Phytoplankton size is a fundamental biological trait that influences community size structure (Petchey et al. 2008), phytoplankton community size structure, in turn, influences the carbon cycle by controlling the efficiency of the biological pump (Mousing et al., 2014). To date, the phytoplankton size classification (nano: 2-20 μm ; micro: 20-200 μm ; macro: >200 μm) introduced by Sieburth et al. (1978), has met wide acceptance as a standard procedure. Cell size estimation of phytoplankton using various new types of measurements, including flow cytometry, High Performance Liquid Chromatography (HPLC) analysis of marker pigments and DNA sequencing have started to be used widely for better evaluation (Ignatiades, 2016).

As different methods developed, it revealed that the effect of small phytoplankton on the net export might be underestimated (Richardson & Jackson, 2007). Picoplankton sized phytoplankton (<2 μm) are defined as major contributors to total photosynthetic biomass in the oceans (Booth, 1988; Li et al., 1993; Partensky et al., 1993), especially in more oligotrophic regions such as subtropical ocean gyres and the Eastern Mediterranean Sea (EastMed Sea) (Bell & Kalff, 2001; Li et al., 1993; Uysal, 2006). This smallest fraction of the phytoplankton has largely been ignored in classical light microscopy-based studies. As an average, in the whole Mediterranean Sea (Med Sea) basin, picoplankton accounts for 59% of the Total Chlorophyll a (Tot Chl a) and 65% of the PP (Siokou-Frangou et al., 2010). Within the Med Sea, a west-east gradient (Dolan et al., 1999; Ignatiades et al., 2009; Turley et al., 2000) exists because of the eutrophic character of the western part and one of the most oligotrophic area of the world in the eastern part (Azov, 1986; Krom et al., 1991; Mazzocchi et al., 1997; Souvermezoglou et al., 1992; Zohary & Robarts, 1998). In the concept of nutrients and chllorophyll a (Chl a), such gradient from eutrophy to oligotrophy can be observed both west-east and north-south. Aegean Sea has a unique structure in the Mediterranean basin with its horizontal trophic gradient due to Black Sea entrance in the north and poor EastMed Sea waters in the south.

Some remarkable regional differences have also been observed within the Mediterranean region. An important one of these systems is the “Rhodes Gyre” which is a permanent, topography controlled, cyclonic eddy and an upwelling area. It has

higher PP and richer biological diversity than those in the surrounding waters with the nutrient enrichment by the convective mixing processes. It has a distinct, rapidly changing trophic status from the periphery through the core, especially in winter.

The composition shifts of phytoplankton in the concept of changing trophic status in two special areas in the Med Sea will be discussed below but it is obvious that, a very complex system and current understanding of the processes controlling the system ranging from small to global scales is still inadequate to understand the system, as a whole. Developing a better understanding of the factors controlling processes within the marine system is essential in order to be able to sustainably manage the interaction of human activities and the earth system (Richardson et al., 2011).

1.1 Phytoplankton Pigments and Community Size Structure

Each photosynthetic phytoplankton contains different photosynthetic pigments to capture and transfer the light. Chl a which is mainly found in all marine phytoplankton and all other pigments that in composition vary from group to group, are considered as primary indicators of phytoplankton biomass and PP that form the basis of all animal production in the open sea. Phytoplankton pigments allow the chemotaxonomic composition of the phytoplankton in the ocean to be investigated with a cheaper and relatively easier method. HPLC is one of the most effective techniques currently used to discriminate and quantify pigments as it provides high sensitivity and rapid analysis.

Although spectrophotometric and fluorometric methods are routinely used today, Lorenzen & Jeffrey (1980) clearly demonstrated the necessity of incorporating a chromatographic method, especially an HPLC system, into the analytical method when the pigment structure needs to be known precisely. Thus, the methodologic studies of the microscopic organism pigments in the aquatic environment by HPLC was accelerated (Barlow et al., 1997; Furuya et al., 1998; Pinckney et al., 1996; Suzuki et al., 2015; Van Heukelem & Thomas, 2001; Vidussi et al., 1996; Wright et al., 1991). In particular, it is clear that the pigment values obtained from HPLC systems are used

in recent years (Adolf et al., 2006; Ahel et al., 1996; Aiken et al., 2009; Barlow et al., 2002; Barlow et al., 2004, Claustre et al., 2004; Ediger et al., 2006., Flander-Putrlé & Malej, 2008; Hanson et al., 2007; Higgins et al., 2006; Jin et al., 2017; Mangoni et al., 2008, Nunes et al., 2018; Pena et al., 2019; Ras et al., 2008; Schlüter et al., 2016).

Phytoplankton generally contains three types of pigment: chlorophylls, carotenoids and biliproteins. Up to date, a total of up to 50 different pigments have been identified by a variety of chromatographic methods (Jeffrey et al., 2005; Wright, 2005). Each year the number of newly identified pigments is increasing, but there is no method to resolve all the pigments that can be found in seawater (Van Heukelem & Thomas, 2001). Each method has its own advantages and limitations (Bidigare et al., 2005). The pigments and their relative sizes found in the phytoplankton groups (systematically) are summarized in Table 1.1. Although the pigment varies widely among the taxonomic groups, some are clearly considered as markers of certain groups (Wright, 2005). An important problem is that many groups contain the same pigment composition and it is impossible to distinguish them on the basis of pigment studies.

Size classification of phytoplankton based on the pigment content have been researched using mechanical separation with filters for decades but the exact fractionation of these categories is not standardized and inconsistencies can be seen across different studies. The pigment index (f) was introduced as an index of trophic status, based on seven diagnostic pigments (DP) which have been measured by HPLC related with the important taxon in phytoplankton community (Roy et al., 2011). The pigment index was reinterpreted as the proportion of microplankton by Vidussi et al. (2001), who introduced similar categories for nanoplankton and picoplankton, later modified by Uitz et al. (2006), who used multiple linear regression of a global database to determine weighting factors for each DP that were then used to calculate a weighted sum of diagnostic pigments and fractions of three size classes.

Table 1.1 The distribution of pigments related with each taxon of phytoplankton (Jeffrey et al., 2005)

(chl a: chlorophyll a, chl b: chlorophyll b, chl c1: chlorophyll c1, chl c2: chlorophyll c1, chl c3: chlorophyll c3, MgDVP: Mg 3,8-divinyl pheoporphyrin a₅ monomethyl ester, DV a: Divinyl chlorophyll a, DV b: Divinyl chlorophyll b, β,ε-car: β,ε-carotene, β,β-car: β,β-carotene, Allo: alloxanthin, 19 BF: 19'-Butanoyloxy fucoxanthin, Diadino: diadinoxanthin, Dino: dinoxanthin, Fuco: fucoxanthin, 19HF: 19'-Hexanoyloxy fucoxanthin, Lut: lutein, Neo: neoxanthin, Per: peridinin, Pras: prasinoxanthin, Viola: violaxanthin, Zea: zeaxanthin, P/cyanin: phycocyanin, P/erythrina: Phycoerythrin)

	Chlorophylls										Xanthophylls														
	chl a	chl b	chl c1	chl c2	chl c3	MgDVP	DV a	DV b	β,ε – car	β,β – car	Allo	19 BF	Diadino	Dino	Fuco	19HF	Lut	Neo	Per	Pras	Viola	Zea	P/cyanin	P/erythrin	
Cyanophyta	●									●													●	●	●
Prochlorophyta							●	●	●	●													●	●	●
Rhodophyta	●								●														●	●	●
Cryptophyta	●			●					●		●													●	●
Chlorophyceae	●	●							●															●	●
Prasinophyceae	●	●				●			●															●	●
Euglenophyta	●	●							●					●					●						●
Eustigmatophyta	●								●													●	●		●
Bacillariophyta	●		●						●					●											●
Dinophyta	●			●					●					●											●
Prymnesiophyceae	●		●		●				●							●									●
Chrysophyceae	●		●	●	●				●			●	●		●										●
Raphidophyceae	●		●	●					●				●		●										●

Picoplankton, $<2\ \mu\text{m}$, including prochlorophytes (key marker: Divinyl Chlorophyll a -DV Chl a-) and cyanobacteria (key marker: Zeaxanthin -Zea-) are defined as major contributors to the total photosynthetic biomass in the nutrient depleted, oligotrophic waters. The major pigment is DVChl a in Prochlorophyceae (Bidigare et al., 2005). The effect of picoplankton, which are important effects on aquatic ecosystems due to their small size, can be determined by the analysis of related pigments such as DV chl a and Divinyl Chlorophyll b (DV Chl b) (Jeffrey et al., 2005). From a biogeochemical point of view, size structure of phytoplankton communities is an appropriate parameter to determine the oligotrophic character of the water body. In this matter, the taxa that are difficult to identify microscopically and their effect in the food chain (Perez et al., 1996) or PP (Li, 1994) can be detected by HPLC. The use of an HPLC method in which the monovinyl and divinyl forms of Chl a cannot be separated chromatographically will cause more overestimation, especially in oligotrophic water in the presence of these pigments (Latasa et al., 1996).

Pathways of carbon transfer are influenced by phytoplankton community composition and changes in this composition, therefore, can, potentially generate cascading effects on food web dynamics. In general, phytoplankton biomass, size and diversity are controlled by various numbers of biological interactions including bottom-up processes such as competition for limiting resources (e.g. light, nitrogen, phosphorus and iron) and top-down processes such as grazing (Pace et al, 1990). Several studies showed that the microbial food web is dominant in large parts of the oligotrophic Med Sea (Christaki et al., 1996; Siokou-Frangou et al., 2002; Thingstad and Rassoulzadegan, 1995; Turley et al., 2000). The microbial system seems to be both bottom-up and top-down controlled. Siokou-Frangou et al., 2010 have reviewed the plankton studies in the Med Sea pelagic waters for last 25 years and they concluded that Med Sea is characterized by a “multivorous food web” (sensu Legendre and Rassoulzadegan, 1995), including a continuum of trophic pathways spanning from the herbivorous food web to the microbial loop and dynamically expanding or contracting along the seasons, areas and transient processes.

The most important factors that controlling the phytoplankton community size composition on the bottom up control basis, are known as nutrient availability and the temperature of the environment. The importance of small cells in the community is increasing, with temperature. This is known as temperature-size-rule (Atkinson 1994), the body size of unicellular protists are decreasing with increasing temperature. Increasing metabolic rates as a function of temperature would cause an increased resource demand. Competition for nutrients also effects the size structure, relating with the surface to volume (S/V) ratio of a cell. Small cells, have high S/V, would be more advantageous in nutrient poor waters. Despite this consistent pattern, researchers have so far failed to reach consensus on the individual effects of these factors on phytoplankton community size structure because of the negative relationship between temperature and nutrient availability in the open ocean (Mousing et al, 2014). Understanding the mechanisms controlling phytoplankton community size structure has a crucial importance for predicting the effect of climate change in the future.

1.2 Dinoflagellates, Survival Strategies and Cyst Formations

Apart from community size structure, trophic level of the taxa also has an importance to understand major trophic pathways of marine food webs. Heterotrophs have a leading role in the Med Sea food web (Bralewska & Witek, 1995; Siokou Frangou et al, 2010; Unrein et al., 2014) and one of the important groups in the microplankton size is dinoflagellates with their broad ecological roles. Beyond their trophic modes or functions as prey/predator (i.e. autotrophic, heterotrophic, mixotrophic, symbiotic, parasitic, epiphytic) (Jeong, 2010; Dagenais-Bellefeuille & Morse, 2013), several adaptation and survival strategies in stressful or unfavourable conditions, such as, encystment, carbon storage, decreased internal nitrogen pool, ornithine-urea cycle, symbiosis, transcriptional control, circadian control, mixotrophy and vertical migration (Dagenais-Bellefeuille & Morse, 2013) put them in a very special case.

Many phytoplankton species form temporary cysts or resting cysts during life cycle (Anderson et al., 1995). Cysts can be reserved in sediments for decades as potential

biodiversity reserves (Belmonte et al., 1995; Fistarol et al., 2004). Cyst formation is the adaptation of phytoplankton to reduce mortality during periods of undesirable environmental conditions. The term "cyst" is generally adopted for a non-motile cell that lacks flagella and an ability of movement. Two types of cysts are often observed in natural environment and laboratory cultures (Matsuoka & Fukuyo, 2000).

Temporary cyst: Cells that lose their flagella or lose the ability to move due to physiological events are called as temporary cysts. Loss of the flagella can occur due to mechanical effects (strong water movements, external forces such as sudden and strong impact) and increased cell density or frequency. In addition, exposure to long stress conditions can result in loss of motion.

Resting cyst: Resting cyst is a sedentary zygote (hypnosis) formed after sexual reproduction. Hypnozygotes eventually sink into the bottom of the sea or lakes. It has been reported that these cysts are based on sediments for six years under constant conditions with low oxygen and temperature (Dale, 1996). When the environmental conditions are not suitable for living organisms, the cyst can be fossilized by being compressed between layers. Temperature changes, exposure to light, transport by water masses are thought as external triggering factors for the induction of germination. However, factors such as the internal mechanism and the biological clock also control the excystment.

It is the resting cysts which are studied mainly. There are two types of permanent cysts in the sediment: living and empty cysts. Live cysts are cysts that contain protoplasm, live and can reproduce on favourable conditions. Empty cysts are described as protoplasm separated cysts (Matsuoka & Fukuyo, 2000).

Dinoflagellate cysts have become an environmental indicator that is being used today. The importance of dinoflagellate cysts can be summarized as i) adaptation to reduce mortality against abiotic stress factors and organic compounds (allelopathy) ii) ability to regenerate at the end of a sedimentation period to contribute to the recombination (genetic diversity) of the population in the water column, iii) serving as

a vector to expand geographical distribution, iv) to be used as an indicator of climate changes and evolutionary processes from geological cycles to the present time, and v) lying as a source for possible bloom events, and vi) playing as an early warning system for a possible proliferation in an area that has the presence or intensity of the certain species which can cause toxic and extreme bloom events (Fistarol et al., 2004, Matsuoka & Fukuyo, 2000; Uzar, 2010).

It is possible to evaluate the records of the cysts in the sediment at different time scales (such as looking at the past). Retrospective information can be collected on varying time scales according to the depth of the drilling cores taken from the sediment: 1) the recent few years or so, represented by near-surface sedimentation, gives us the chance to assess cyst distributions with environmental factors and potential as environmental indicators; 2) In the case of samples taken in the form of short cores, observing past for decades to centuries is suitable for observing human influence; 3) looking at millions of years in long cores from thousands of years is suitable to observe the effects of basic climatic changes; 4) Looking at hundreds of millions of years of history on the rock is essential to follow evolutionary processes (Dale, 2001).

In recent research, dinoflagellate cysts will become preferred in ecological evaluations because they occupy the basic biogeographic zones such as the benthos and are closely related to their surroundings because they contain both planktonic and benthic phases in the life cycle. The rapidly increasing human population and the environmental impact of the developing industry are the main problems faced by modern marine scientists. Micropaleontology offers retrospective data to scientists in the absence of long-term follow-up data. Dinoflagellate cyst distributions show significant changes over a period of 10 to 100 years, and these records provide ease of exploration and evaluation of climate change, marine pollution and cultural eutrophication during the last century (Dale, 2001). Hence, this recently deposited dinoflagellate cysts are the main interest of the recent researches that uses the term “modern dinoflagellate cysts”.

The increase in human population leads to a significant carbon increase both in the sea and in the atmosphere, resulting in the greenhouse effect and therefore global warming phenomena. Thus, both heat and carbon increase, results in eutrophication in the marine environment. Apart from this, as a result of the recent climate change, there are long-term heat increases or high-frequency short-term extreme events. Visible effects of these phenomena are alterations in life cycle, production, species composition and demography (Lejeusne, 2009). The most direct consequences of warming are the increase in the abundance of thermotolerant species (increase in exotic species in the Mediterranean) and the decrease in the abundance of stenothermal species, shifts in species distributions or population dynamics. The most obvious results in terms of dinoflagellates; the earlier warm-up in the annual cycle and the longer spring run, and the increase in the production of spring-bloom species and their cyst production, as well as the explosion of *Protoceratium reticulatum*, a cosmopolitan species producing Diarrhetic Shellfish Poisoning (DSP) toxin, and the increase in cyst abundance (Dale, 2001).

Studies of modern dinoflagellate cysts are important in terms of understanding the ecological role of phytoplankton and the possible threatening harmful/toxic dinoflagellate blooms. Calculation of cyst abundances is used to predict extreme outbreaks (blooms) that can occur in the region (Matsuoka & Fukuyo, 2000). Some of the reproductive events can have negative effects when carried out by species producing toxins: mass mortality of fish, the death of other creatures such as birds and whales, infection of crustacea and from there by reaching people, toxins can lead to situations that threaten human health. These extreme production events (blooms) in marine and freshwater ecosystems are now classified in the name of "Harmful Algal Blooms (HAB)" with the UNESCO/IOC_HAB decision.

1.3 Physical Framework

The Aegean Sea is a semi-enclosed basin located in the Northeastern Mediterranean (NEastMed). It is surrounded by western coast of Turkey from the east, the coasts of Greece from the north and west and the islands of Crete and Rhodes from the south. It

covers an area of 191,000 km² approximately (Göksel et al., 2015). It is connected to the Marmara and Black Seas through the Çanakkale Strait in its northern part and connected to the Levantine and Ionian Seas through several straits at the southern end (Vervatis et al., 2011). The southern boundary of the Aegean Sea is not a clear cut but the deep trough situated to the south of Crete, Karpathos and Rhodes islands can be accepted. In this manner, from this boundary through the south-east, the EastMed Sea exists. Its coastal and offshore topography is irregular and complex. The complexity of the shoreline and bottom topography is associated with the presence of a great number of islands (over 3000 islands and islets). The Aegean Sea constitutes of three major basins; the North Aegean Sea, with maximum depths up to 1500 m; the Chios Basin in the central part, with maximum depth of 1100 m; and the Cretan Sea in the south, the largest basin, with maximum depth of 2500 m. The above major basins are connected through channels shallower than 400 m (Georgiou et al., 2015).

There have been lots of studies which were carried out to understand the general physical oceanography of the Aegean Sea (Nielsen, 1912; Theocharis et al., 1993; Ünlüata, 1986). During the 90s, lots of studies concluded that significant changes of water masses in the period from 1987 to 1993 have led to the Aegean Sea waters have become densest in its known history and the outflow of dense water from the Aegean Sea, better known as the Eastern Mediterranean Transient (EMT) (Lascaratos et al., 1999; Malanotte-Rizolli et al., 1999; Roether et al., 1996). With this sudden climatic change, the source of the Eastern Mediterranean Deep Water (EMDW) have been shifted from Adriatic Sea to Aegean Sea. In the last decade, studies focused on understanding the EMT and its consequences. The exact mechanism that forced the EMT is not completely known. The possible reasons were explained in the following studies: Lascaratos et al. (1999), Roether et al. (1996), Vervatis et al. (2011) and Eronat and Sayın (2014).

The main water masses and their distribution can be summarized as explained by Gertman et al., 2006 Vervatis et al., 2011, Beşiktepe, 2015 and Georgiou et al., 2015 with totally six different water mass types. The most characteristic feature of the stratification pattern in the North Aegean Sea is the surface brackish Black Sea Water

(BSW) outflowing from the Çanakkale Strait. The BSW forms a front with the intrusion of the much saltier Levantine Surface Water (LSW) entering the Aegean through the eastern Cretan arc straits and occupy the surface waters of Central and South Aegean Sea. The intermediate layers of the North-Central Aegean are occupied by Aegean Intermediate Water (AgIW). The vertical structure of the North Aegean is comprised of a 20–70 m modified BSW surface layer, an intermediate AgIW layer down to 400 m and the locally formed North Aegean Deep Water (NAGDW). Deep and bottom layers of the Aegean Sea are filled by the locally formed three different water masses, NAGDW, Central Aegean Deep Water (CAGDW) and Cretan Deep Water (CDW) from north to south and from denser and lighter (Beşiktepe, 2015).

As the EMT event has gradually decayed, it is now evident that the 'conveyor belt' circulation in the EastMed Sea is undergoing change, as evidenced by increases in deep water temperature and nutrient concentrations mostly as a result of changes in the Aegean Sea. After EMT, all the waters of the Aegean Sea replaced with intrusion of extensive amount of water from Levantine Sea (Vervatis et al., 2011; 2013) and the above water masses are reformed. The Transient Mediterranean water (TMW) with the salinity less than 39 is located underneath the AgIW (Balopoulos et al., 1999; Theocharis et al., 1999). This water mass enters the south Aegean through the Cretan Arc Straits to balance the water left Aegean Sea during EMT.

The basin scale circulation of the Aegean Sea is cyclonic (first presented, Ovchinnikov, 1966). As in other marginal seas, the flow is also thermohaline and wind driven. The Çanakkale Strait outflow (waters of Black Sea origin, BSW) plays a very important role in surface circulation. The schematics of the Aegean Sea circulation including eddies in different scales are presented in Figure 1.1 (Bizsel et al., 2014). The outflow in the North Aegean Sea is generally cooler, fresher and with higher nutrient content and generates strong fronts with the warm, highly saline and extremely oligotrophic waters of the Aegean Sea (Gerin et al., 2014). The modified BSW entering the Aegean Sea through the Strait of Çanakkale, move towards north and circulates anticyclonically in the North Aegean Sea, then heads towards south along the Greek coast. Warm and saline Levantine waters penetrate into the Aegean through

the eastern straits of the Cretan Arc moves towards north along the coast of Turkey. LSW starts to transform extensively in the Central Aegean Sea due to cooling on the way to the north and by mixing with the saline and cold upwelling water coming from north. Existence of islands and complex topography and meteorological conditions creates meso-scale cyclonic and anti-cyclonic eddies throughout the basin. One cyclonic eddy, on the central part of the Aegean Sea forms and another one occurs in the Chios Basin (Chios Eddy) (Beşiktepe, 2015). These two eddies play a crucial role in the occurrence of the EMT as described above.

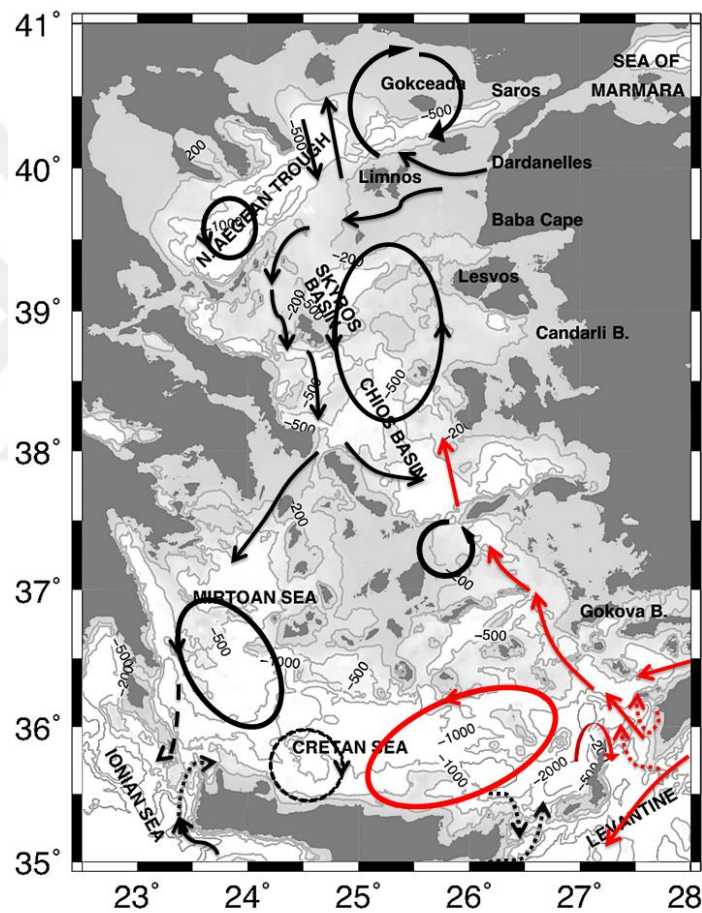


Figure 1.1 General circulation dynamics of the Aegean Sea (black Arrows: BSW. red arrows: LSW) (Bizsel et al., 2014)

The Mediterranean coast is used intensely in the general sense. Human populations are increasing, with intense anthropogenic pressure (pollution, overfishing, habitat destruction, the emergence of new species) threatening marine ecosystems. According to the modeling studies (Parry, 2000), the Mediterranean Basin is the region that will

be most affected by the ongoing global warming trend and the extreme conditions to be experienced. This makes the Mediterranean a potential model (laboratory-type environment) for monitoring physical and biological changes (Somot et al., 2008; Giorgi & Lionello, 2008; Lejeusne et al., 2009; Schroeder et al.; 2016). In the Aegean Sea, a significant temperature increase (+1 °C) has been observed in the past 20 years in a core-based study of sea surface temperature from satellites (Theocaris, 2008). In terms of all these features and the different climate change scenarios, Mediterranean region has identified as one of the most prominent climate response Hot-Spots (Giorgi, 2006).

In the southern boundary of the Aegean Sea, the EastMed Sea lays down with its characteristic of one of the most oligotrophic water masses in the world. As the Aegean Sea and the EastMed Sea thermohaline circulation is driven by net evaporation (heat loss) and influenced by wind, changes in temperature, precipitation and wind pattern are all likely to affect the physical features of the Mediterranean gyres and therefore their biogeochemical effects (Bergamasco & Malonette-Rizzoli, 2010). The upper thermocline circulation, which is embedded in the Mediterranean open thermohaline cell consist of sub-basin-scale gyres and permanent, or quasi-permanent, cyclonic and anticyclonic structures interconnected by intense jets and meandering currents (Beşiktepe et al., 2017). The existence of permanent gyres is a central biogeochemical feature of the Mediterranean Sea. An important one of these systems is the “Rhodes Gyre” which has a critical role with its permanent, topography controlled, cyclonic structure and “upwelling dynamics” in the whole Mediterranean Sea ecosystem. The Rhodes gyre occupies the area between about 34N-36N and 27.5-29.5E in the EastMed Sea. Upwelling formations in the Rhodes Gyre, provide nutrient transport from the deep to the photic zone and these results in more productive waters in the region compared to the surrounding oligotrophic waters. Concentration and differentiation are observed in lower and higher trophic levels of the ocean food chain compared to the poor Eastern Mediterranean open waters (Uysal et al., 2014). A gradient from oligotrophy to more productive trophic states from the vicinity of the Rhodes Gyre through the core would be observed like the situation in the Aegean Sea mentioned before.

1.4 Chemical and Biological Oceanography of the Area

The Mediterranean Sea is the largest European semi-enclosed sea. Recent investigations have basically confirmed the long-recognized oligotrophic nature of this sea (Krom et al., 1991; Antoine et al., 1995). According to Siokou-Frangou et al. (2010) overall results for the whole Med Sea were as follows: nutrient availability in the Med Sea is generally low, especially for phosphorous (N:P up to 60), Chl a concentrations generally displays low values (less than 0.2 $\mu\text{g Chl a}$) over large areas with a modest late winter increase, a large bloom (up to 3 $\mu\text{g/l}$) is observed throughout the late winter and spring exclusively in the northwestern area, relatively high biomass values are recorded in fronts and cyclonic gyres, nutrients and trophic state is in oligotrophic to ultra-oligotrophic scale.

Pathways of carbon transfer are influenced by phytoplankton community composition and changes in this composition, therefore, can, potentially generate cascading effects on food web dynamics. In general, phytoplankton biomass, size and diversity are controlled by a number of biological interactions including bottom-up processes such as competition for limiting resources (e.g. light, nitrogen, phosphorus and iron) and top-down processes such as grazing (Pace et al, 1990)). Several studies showed that the microbial food web is dominant in large parts of the oligotrophic Med Sea (Thingstad & Rassoulzadegan, 1995; Christaki et al., 1996; Turley et al., 2000; Siokou-Frangou et al., 2002). The microbial system seems to be both bottom-up and top-down controlled. A phosphorus addition experiment in the Eastern Mediterranean had an unexpected outcome because Chl a concentrations decreased while heterotrophic bacteria production and egg-carrying copepods numbers increased (Thingstad et al., 2005). This would be explained by the efficiency at minimizing C export to deeper waters, benefiting predators that control the plankton biomass (top-down control) (Siokou-Frangou et al., 2010). Efficient top-down control helps explain why Mediterranean fisheries are richer than anticipated based on Chl a and nutrient concentrations. In addition to efficient C export to pelagic top predators, benthic primary producers also play an important role in sustaining Eastern Mediterranean food webs by the evidence that fisheries in some Med Sea areas (i.e. North Aegean

Sea) are richer than expected on the basis of measured Chl a and nutrient concentrations (Kletou & Hall-Spencer, 2012) Siokou-Frangou et al., (2010) have reviewed the last 25 years plankton studies in the Med Sea pelagic waters and they concluded that Med Sea is characterized by a “multivorous food web” (sensu Legendre & Rassoulzadegan, 1995), including a continuum of trophic pathways spanning from the herbivorous food web to the microbial loop and these different pathways dynamically expanding or contracting along the seasons, areas and transient processes.

The existence of the most oligotrophic area of the world in the EastMed Sea (Azov, 1986; Krom et al., 1993; Mazzocchi et al., 1997; Souvermezoglou et al., 1992; Zohary & Robarts, 1998) creates a gradient in the terms of nutrients and Chl a both the west-east and the north-south directions. The western basin includes the Alboran, the Algerian-Provencal, the Tyrrhenian seas, while the eastern basin includes the Adriatic, the Ionian, the Aegean that is connected throughout the Turkish Straits System with the Black Sea and the Levant Seas. There have been several studies conducted in the Med Sea, in the concept of trophic status through west-east (Dolan et al., 1999; Marie et al., 2006; Ignatiades et al., 2009; Talarmin et al., 2015; Turley et al., 2000; van de Poll et al., 2015) and north-south (Bizsel et al., 2014; Latasa et al., 2010; Lykousis et al., 2002; Ignatiades et al., 1992; Ignatiades et al., 2009).

Ignatiades et al. (2009) conducted a large-scale research on a west-east longitudinal transect in offshore waters of the Mediterranean Sea in June-July 1999. Phosphate, nitrate and silicate values ranged between 0.01-0.18/0.01-0.26 μM , 0.03-2.61/0.07-4.59 μM and 0.71-4.82/0.56-2.95 μM in the eastern part and the western part (east/west) in the photic zone, respectively. In the study, a significant decrease of mean (over depth) temperatures (19.81–16.29 °C) and salinities (39.04-37.56) from the eastern towards the western stations was observed also. Chl a (0.08– 0.49 mg/m^3), primary production (0.05–0.22 $\text{mg C}/\text{m}^3\text{h}$) and phytoplankton abundance (1.0×10^4 - 2.2×10^4 cells/l) values also showed the same significant difference between east and west.

There are considerable amount of studies conducted in recent years on remotely sensed Chl a data to address different bio-provinces in the basin (D'ortenzio& d'alcala, 2009; Uitz et al., 2012; Basterretxea et al., 2018) and to determine the phytoplankton size classes based on different algorithms and validation of the model with in-situ pigment data measured by HPLC (Uitz et al., 2012; Navarro et al., 2014; Sammartino et al., 2015; DiCicco et al., 2017; Navarro et al., 2017) resulted generally nano-sized phytoplankton dominance in the whole basin and in the most oligotrophic regime, after nanoplankton, picoplankton playing a larger role than microphytoplankton. All of the remote sensing studies showed evident west-east and north-south trophic status gradients. Tanaka et al. (2007) reported picoplankton dominance in the Levantine Sea during May, 2001 and 2002. Larger diatoms and dinoflagellates become abundant after intermittent nutrient pulses associated with upwelling, fronts and gyres (Siokou-Frangou et al., 2010). Over 85% of Chl. a in the eastern basin is found in ultraplankton (<10µm), that comprises cyanobacteria (*Synechococcus* spp. are dominant), chlorophytes, prasinophytes and haptophytes (Denis et al., 2009). Coccolithophores are more abundant and diverse in the eastern basin (Ignatiades et al., 2009). In summer, dinoflagellates dominate the larger plankton fraction in offshore areas of the Eastern Mediterranean whereas diatoms are more prevalent during winter mixing and in inshore waters where anthropogenic eutrophication is evident (Aktan, 2011).

The bloom is less-pronounced in the Eastern Mediterranean (D'Ortenzio& d'alcala, 2009), The Chl a maxima in the eastern basin rarely exceed 0.5 µg l⁻¹ (Yacobi et al., 1995; Gotsis-Skretas et al., 1999), and the minima are as low as 0.003 µg l⁻¹ (Herut et al., 2000). Exceptions are the peak values of 1.34 µg l⁻¹ in the frontal zone of the North East Aegean Sea in April (Zervoudaki et al., 2007) and 3.07 µg l⁻¹ in a small-scale cyclonic area of the North Levantine Sea in March 1992 (Ediger and Yilmaz, 1996). In the oligotrophic areas in the Med Sea during the summer stratified period, primary production in the Eastern Mediterranean is both N and P limited, and during the winter mixing it becomes P limited (N:P up to 60) (Thingstad et al., 2005; Tanaka et al., 2011), though this limitation may be buffered by N inputs from highly populated coasts, rivers and atmospheric deposition (Krom et al., 2010; Markaki et al., 2010; Siokou-Frangou et al., 2010).

A deep chlorophyll maximum (DCM) is a permanent feature for the whole Mediterranean basin over the entire annual cycle (Siokou-Frangou et al., 2010). The DCM progressively sinks across a west to east gradient from 30m in the westernmost area (Dolan et al., 2002) to 70m in the South Adriatic Sea (Boldrin et al., 2002), down to 120m in the Levantine basin (Christaki et al., 2001; Dolan et al., 2002). This decrease in eastern part is because of the lower productivity and hence higher seawater transparency, but the level of DCM may vary considerably between cyclonic and anticyclonic areas (Ediger & Yilmaz, 1996).

Chlorophyll dynamics in the Levantine basin shows a bloom forming province around 35–36 N parallels (Uitz et al., 2012). One of the most important sub basin gyre in the Mediterranean Sea ecosystem, “Rhodes Gyre” which has a critical role with its permanent, topography controlled, cyclonic structure and “upwelling dynamics”. Upwelling formations in the Rhodes Gyre, provide nutrient transport from the deep to the photic zone and these results in more productive waters in the region compared to the surrounding oligotrophic waters. Concentration and differentiation are observed in lower and higher trophic levels of the ocean food chain compared to the poor Eastern Mediterranean open waters (Uysal et al., 2014). A gradient from oligotrophy to more productive trophic states from the vicinity of the Rhodes Gyre through the core would be observed like the situation in the Aegean Sea mentioned before. During the study period of 1996-1997, the range of Chl a was 0.01-0.07 µg/L in the surface waters and 0.04-0.35 µg/L at the DCM depth (Yilmaz, 2002).

The phytoplankton community structure in the Rhodes Gyre is depending on the nutrient related trophic status gradient which explained with the higher primary production and richer biological diversity in the centre than those in the surrounding waters (Ediger and Yilmaz, 1996; Ediger et al., 1999; Napolitano et al., 2000; Özsoy et al., 1991; 1993; Uysal et al., 2014; Yilmaz et al., 1994; Yilmaz & Tuğrul, 1998). During the study period of March 2016, the plankton abundance within photic zone has been changed in the range of about 25.000 cells/l (periphery of the gyre 200m) - 204.000 cells/l (center of the gyre DCM-75m). These relatively high biomasses were mainly due to microphytoplankton and nanophytoplankton in the center of the gyre.

The upwelling of relatively high-nutrient waters in the core of Rhodes gyre enhanced nano/microphytoplankton biomass, particularly that of diatoms. Additionally, new records for particularly small size diatom (mainly cyclotelloid species), as the dominant group in the pico-nano plankton community, have been observed for this region in the surface waters (Beşiktepe et al., 2017). Vidussi et al. (2001) investigated the phytoplankton community structure by size with phytoplankton pigments measured by HPLC in whole Mediterranean and have found that nanoplankton is the main component of phytoplankton in all area and in Rhodes Gyre, increase of the microplankton fraction is observed. In the Eastern Mediterranean phytoplankton biomass is dominated by diatom in the more productive coastal waters, while picoplankton was reported to form the most abundant group in the open sea (Eker & Kıdeys, 2000; Eker et al. 2003; Polat et al., 2000; Uysal et al., 2008; Uysal 2006; Yücel, 2013). The main components of pico-sized phytoplankton are the cyanobacterium *Synechococcus* sp. and the *Prochlorococcus* in the open water waters of East Med Sea (Uysal, 2016).

The Aegean Sea is known to have a unique structure with the complex bottom and coastal structure, various river discharges and the presence of the Black Sea water with low salinity entering the Çanakkale Strait. Effects of the Black Sea originated waters in the north and the entity of Eastern Mediterranean in the south, one of the most oligotrophic water masses in the world, cause differences in the north-south transect in terms of salinity, nutrients, and planktonic production. Ignatiades et al. (1992) have scaled different types of waters in the Aegean Sea (inshore, near shore, offshore) in a period of 1977-1984 based on nutrient concentrations. The concentrations of nitrate, nitrite, ammonium and phosphate in the offshore waters of the Rhodes island ranged between 0.02-5.06, 0.01-0.64, 0.04-12.27, 0.01-4.09 µg/l.

Küçüksezgin et al. (1995) studied open ocean waters of the Aegean Sea in 1991-94 in various months and vertical profiles showed that nitrate, nitrite, ammonium, phosphate and silicate concentrations ranged between 0.10-2.90, 0.01-0.13, 0.10-0.95, 0.01-0.16, 0.30-4.70 µM, respectively. They concluded that the highest nutrient values

were found in the northern part of the Aegean Sea and may have resulted from originating from the Black Sea and the values were increased with increasing depth.

Ignatiades et al. (2002) performed a multidisciplinary project in north and south parts of the off-shore waters of Aegean Sea in various months between 1997-1998. Mean values of phosphate, ammonium, nitrate+nitrite, silicate ranged between 0.019-0.032, 0.07-0.16, 0.29-0.62, 1.21-2.40 μM in the northern Aegean and 0.013-0.049, 0.21-0.40, 0.40-0.88, 1.18-1.63 μM in the southern part of Aegean Sea, respectively. Chl-a and primary production values were 0.26-0.38 $\mu\text{g/l}$, 0.26-1.84 $\text{mg C/m}^3\text{h}$ in the north Aegean and 0.12-0.30 $\mu\text{g/l}$, 0.17-0.51 $\text{mg C/m}^3\text{h}$ in the south Aegean, respectively. Maximum values were found in spring season during sampling periods. From the same sampling campaign Lykousis et al. (2002) summarized the nutrient, Particulate Organic Carbon (POC), chlorophyll-a, phytoplankton and mesozooplankton abundance, primary production and bacteria production levels in the Aegean Sea, all the biochemical parameters of the euphotic zone were higher in the northern part, especially during winter early spring a general biogeochemical N-S gradient was observed, implied mainly by the of Black Sea originated Water (BSW) inflow.

It is known that 38% of all marine primary production is from small phytoplankton (Agawin et al., 2000) and this value can reach 90% in oligotrophic waters (Li et al., 1983). The studies conducted on oligotrophic systems such as the Eastern Mediterranean and the Aegean Sea. Yilmaz & Beşiktepe (2010) reported that picoplankton fraction (0.7-2.7 μm) contributes more than 50% to the total chlorophyll-a concentration during the year in the open waters in the Gulf of Mersin. Polat (2006) have reported that pico+ultraplankton (<5 μm) is an important part of the phytoplankton biomass in the Iskenderun Bay and likewise in the North and South Aegean. Ignatiades et al., (2002) have shown that the picoplankton fraction (0.2–1.2 μm) predominated and accounted for the 56% to 49% of total chl a and the 51% to 41% of total primary production in the North and South Aegean Sea, respectively. Varkitzi et al. (2019) studied the dynamics of phytoplankton biomass (as Chl-a), productivity, community size structure and bloom formation along a transect of 1300

km across different pelagic environments from North Aegean to South Aegean, Levantine and Ionian seas during spring and summer 2008. An exceptional spring phytoplankton bloom which is dominated by large sized diatoms was detected in the South Aegean Sea due to cyclonic formations and a strong convection event that caused deep mixing. They have reported a clear decreasing trend of phytoplankton biomass and productivity, N:P ratios from the Aegean towards the Levantine and Ionian. Picoplankton was found as the dominant size fraction, except for the Southern Aegean in spring bloom. Nanoflagellates and small sized dinoflagellates were important phytoplankton components in the Aegean Sea, whereas in the South Aegean Sea spring bloom community structure shifted to large diatoms. Coccolithophores and small diatoms were significant components in the Levantine and Ionian seas. In the studies of microscopic analysis of the phytoplankton community in the open waters of the Aegean Sea, general taxa that have distributions depending on time and space were reported as Cyanobacteria (mainly *Synechococcus*), *Prochlorococcus*, nanoflagellata, Coccolithophorids, Diatoms and Dinoflagellates as the main component of the community changing with the method used (Brunet et al; 2007; Ignatiades, 1979; Ignatiades et al.; 2002; Karatsolis et al., 2016; Lagaria et al., 2017; Pagou & Gotsis-Skretas, 1990; Yacobi et al., 1995).

The Aegean Sea can be separated temporally into two modes of operation. First mode, there is a late winter–early spring situation when there is a maximum in phytoplankton, PP and the resultant sedimentation of POC; attributable to a classical food chain dominated by diatoms, responding to nutrient pulses. The second mode is the late summer situation, when the dominant size class of organisms is small and forms the microbial loop, resulting in DOC accumulation and minimum sedimentation rates (Fowler et al., 1991; Thingstad & Rassoulzadegan, 1995; Türkoğlu, 2015).

Studies on phytoplankton pigments detected with HPLC in the Aegean Sea open waters are also limited. Lagaria et al. (2017) investigated the phytoplankton community in different aspects (flow-cytometry, HPLC, light microscopy, C^{14} incorporation method) in the Northern Aegean Sea in three different seasons in 2013-2014. The spatio-temporal variation of Black Sea originated waters outflow was found

to greatly affect the relative contribution of pico, nano and micro sized phytoplankton to total phytoplankton biomass and production. Large cell organisms and particularly, diatoms were closely associated with the surface Black Sea masses outflowing from the Straits.

Size fractionated Chl a distribution at 2-5 m depth in offshore surface waters in the Aegean Sea in March, 2012 ranged between 0.11-1.14 mg/l while the highest values observed in northern part of the area (Bizsel, 2014). The stations that under the effect of Black Sea originated water outflow has %44, %33 and %23 of micro, nano and pico sized distribution, respectively. Central and southern part of the area showed an oligotrophic character with %1, %44 and %54 of micro, nano and pico sized distribution, respectively. During the same sampling period, phytoplankton abundances analysed with light and epifluorescence microscopy ranged between 31.148 and 2.887.824 cells/l, 4.639 and 1.081.020 cells/l and 2.57×10^7 and 8.81×10^7 cells/l for the micro, nano and picoplakton, respectively in the whole sampling area (Kısacık, 2018). The dinoflagellates cells determined with epifluorescence microscopy and the species belonging to Amphidinium, Dinophysis, Diplopsalis, Gyrodinium, Gymnodinium, Katodinium, Oblea, Polykrikos and Protoperidinium were detected as heterotrops (personal communication, Kısacık) and the heterotroph percentage (65%) was the highest in both photic and aphotic zones in north, central and south part of the Aegean Sea (Kısacık, 2018). Lagaria et al, (2017) used an all-aspect approach to calculate autotrophic biomass accurately and they have excluded the genus Amphidinium, Cochlodinium, Gyrodinium, Gymnodinium, Peridinium, Protoperidinium, Oxytoxum, Gonyaulax, Torodinium and Warnovia as heterotrophic dinoflagellates by extracting the information from literature. In the period of 2001-2003 in the four eutrophic gulfs on the Greek coastline, Ignatiades (2012) has concluded that the heterotrophic dinoflagellate population contributed the highest carbon proportion (65 %) to the stocks of phytoplankton community in contrast to the low contributions of the autotrophic (14 %) and mixotrophic (1 %) dinoflagellate species and the trophic mode information of the species extracted from literature. In recent years, similar species in the studies have assessed as strictly heterotrophs as they have no chloroplast (Ignatiades, 2012; Lagaria et al., 2017; Naustvoll, 2000) and

pigmented dinoflagellates were started to be considered as mixotrophs because no dinoflagellates are purely photoautotrophic all of the time (Barton et al., 2013; Skovgaard, 1998). The limits of these three modes are still a 'black box', however our knowledge on these flagellates is increasing with the advent of next-generation sequencing technologies, transcriptomic and even genomic tools (Dagenais-Bellefeuille & Morse, 2013; Unrein et al., 2014).

In the Mediterranean, there has been an increase in toxic dinoflagellate records, including human influences, over the past decade, and the species generally encountered are exotic species that adapt to the changing Mediterranean ecosystem (Lejeusne, 2009). It is thought that warming affects the proliferation of these harmful algae. The main concern in HAB research is a global HAB epidemic triggered by increased pollution and global warming (Smayda, 1990). The investigation of dinoflagellate cysts, which function as a reservoir at this point, is important in that it can generate ideas about future harmful blooms triggered by temperature. Dinoflagellate cysts have been used as eutrophication or pollution indicators for years depending on their trophic mode with the assumption of all transparent gonyaulacoids are autotrophic and brownish peridinoids are heterotrophs (literature-based information).

In the Western Mediterranean Sea (WMed Sea), several modern dinoflagellate cyst studies conducted mainly in the coastal part, without using acid treatment (Dhib et al., 2016; Montessoro et al., 1998; Penna et al., 2010; Rubino et al., 2000; Rubino et al., 2010; Satta et al., 2010) or using paleontological sample preparation (Fertouna-Bellakahl et al., 2014; Montessoro et al., 1998; Sangiorni et al., 2005) with assessing the relationship between environmental parameters (Ribeiro & Amorim, 2008; Zonneveld et al., 2009). A few off-shore study (Della Tommasa et al., 2004; Elshanawany et al., 2010) in the have published western part. Elshanawany et al. (2010) reported a detailed research which relates the spatial dinoflagellate cyst distribution with environmental conditions in the whole Mediterranean basin which covers the Eastern Mediterranean and Southern Aegean Sea. Seasonal sea-surface temperature sea-surface salinity, nitrate and phosphate concentration at 0 m and

annual bottom water oxygen concentration ($O_{x_{an}}$) were obtained from the World Ocean Atlas (2005) and Chl a concentrations data derived from online remote sensing observations. Linking with environmental parameters two major groups were distinguished: 1) offshore Eastern Mediterranean species (*Impagidinium* species, *Nematosphaeropsis labyrinthus*, *Pyxidinoopsis reticulata* and *Operculodinium israelianum*) related with oligotrophic, warm, saline surface water, and high oxygen bottom water concentrations 2) western Mediterranean species (*Selenopemphix nephroides*, *Echinidinium* spp., *Selenopemphix quanta*, *Quinquecuspsis concreta*, *Brigantedinium* spp. and *Lingulodinium machaerophorum*) related with higher productivity associated with lower SST, salinity, and lower bottom water oxygen concentrations.

Previous modern dinoflagellate cyst studies in the Aegean Sea are limited to Greek and Turkish coastal areas: Giannakourou et al. (2005) in Thermaikos Gulf, NW Aegean Sea; Aydin et al. (2011, 2015) in Izmir Bay, Middle East Aegean Sea; Aydin & Uzar (2013) in three different bays along the eastern coast of the Aegean Sea. Mudie et al. (2004) investigated Holocene cores of the Black-Marmara-Aegean Seas with a special emphasis on the modern dinoflagellates from the surface of the sediment cores. Two geographic groups observed according to species relative abundances. An Aegean group is characterised by the gonyaulacoid taxa and a Marmara/Black Sea group of taxa is characterised by the protoperidinioids.

1.5 Aim of the study

The aim of the research is to monitor phytoplankton pigment substances in the water column and modern dinoflagellate in the sediment with taking environmental factors into account through the trophic state gradients (eutrophy to oligotrophy) in the Aegean Sea and in the special upwelling area, Rhodes Gyre.

Monitoring the phytoplankton community changes in the Aegean Sea, which has transition characteristics at the boundaries of eutrophy and oligotrophy between the Black Sea waters originating from the north and the oligotrophic Eastern

Mediterranean in the south, will enable the creation of new and updated data belonging to the area.

The modern dinoflagellate cyst study which will be investigated with environmental parameters in the open waters of the Aegean Sea and these results will be very important in terms of innovations that the research will bring.

In this study, the depth profile of the pigments in the north-south gradient in the whole Aegean Sea will be presented and this data will provide new information for open waters of the area which has researches limited to coastal areas mainly.



CHAPTER TWO

MATERIAL AND METHODS

2.1 Study Area

In the study, samples were taken in the scope of three different projects and during different periods. Locations and details of the related projects and sampled stations have given in the Figure 2.1. The samples of this thesis research were mainly taken in March 2012 in the Aegean Sea. During the rainy season throughout a year, the Black Sea originated waters entering the Aegean Sea in the maximum discharge so the period was chosen to see the possible effect of trophic status gradient from north to south.

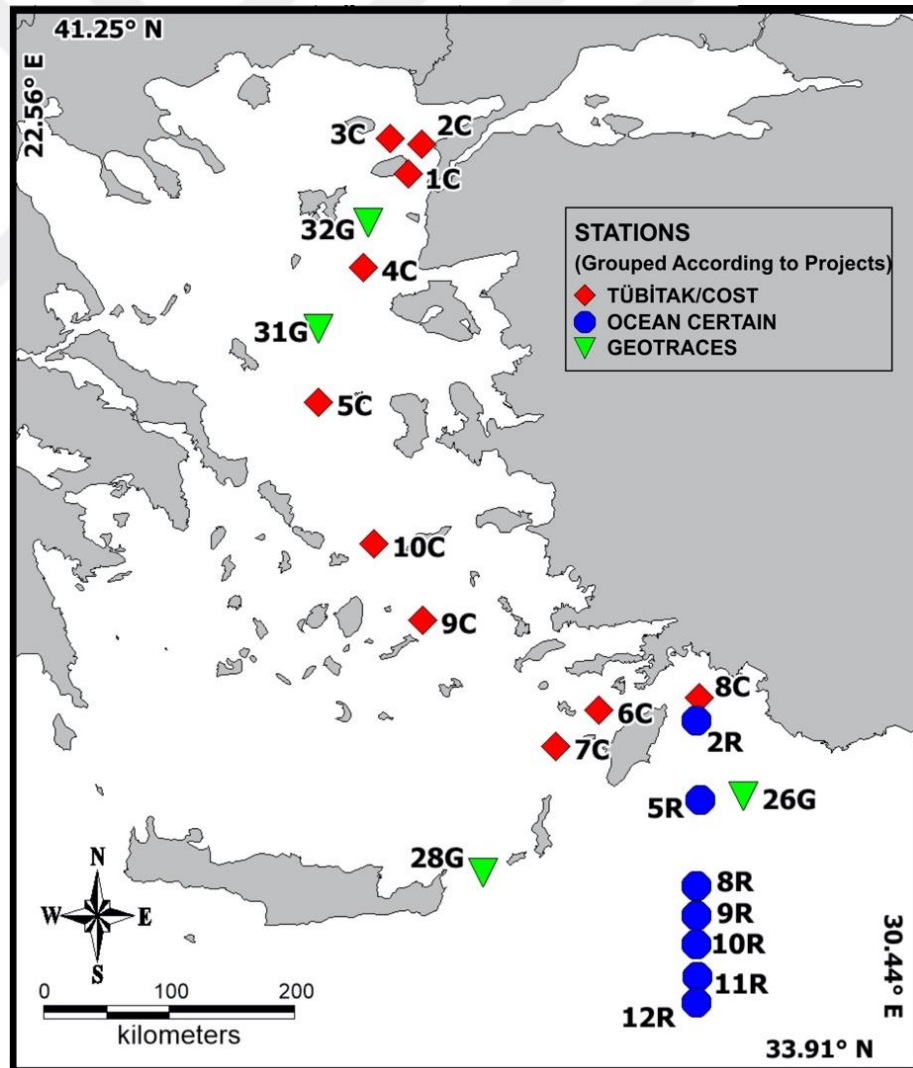


Figure 2.1 Station locations of the study area

10 different stations along the Aegean Sea were sampled in the scope of a TÜBİTAK COST action project numbered 110Y1193. In this project, water samples were taken vertically in whole column at certain depths as 0, 2, 5, 10, 25, 50, 75, 100, 200, 300, 400, 500, 750, >1000 meters depending on the depth of the station (2 m depths were only sampled in the northern stations which are under the effect of Black Sea originated waters) and physical, chemical and biological measurements were undertaken for representing the whole Aegean Sea.

On the purpose of increasing the sample number of sediment samples for statistical analysis, extra 4 stations were sampled in the frame of a global project named GEOTACES in June 2013 (Table 2.1). Two of stations located in the northern part of the Aegean Sea while the other two were in the EastMed Sea in the southern end of the Aegean Sea.

Table 2.1 Stations sampled for three different cruises. *water samples only for phytoplankton pigments

Project	Date	Station I.D.	Latitude (°N)	Longitude (°E)	Water column	Sediment sampling
TÜBİTAK COST	March 2012	1C	40.117	25.990	√	√
		2C	40.322	26.105	√	√
		3C	40.362	25.829	√	√
		4C	39.471	25.600	√	√
		5C	38.539	25.206	√	√
		6C	36.414	27.649	√	√
		7C	36.164	27.271	√	√
		8C	36.504	28.519	√	√
		9C	37.037	26.119	√	√
		10C	37.565	25.693	√	√
GEOTRACES	June 2013	26G	35.826	28.910		√
		28G	35.295	26.640		√
		31G	39.047	25.210		√
		32G	39.783	25.640		√
OCEAN CERTAIN	March 2016	2R	36.400	28.502	√*	
		5R	35.794	28.530	√*	
		8R	35.202	28.500	√*	
		9R	35.000	28.500	√*	
		10R	34.798	28.500	√*	
		11R	34.577	28.505	√*	
		12R	34.399	28.500	√*	

To discuss trophic status gradient effect on phytoplankton size distribution, another sampling was carried out in the Rhodes Gyre area as a case study. Samples for phytoplankton pigment analysis were taken from 7 stations from the vicinity of the gyre through the core in Rhodes Gyre area in the EastMed Sea in the scope of an EU 7th Framework project called OCEAN CERTAIN in March 2016 (Table 2.1). Seawater up to 200 m were sampled throughout the water column at maximum 6 different depth (10, 25, 50, 75, 100, 150, 200) and from DCM depth which is observed with fluorometer attached on CTD.

2.2 Water Column

2.2.1 Determination of Physicochemical Parameters

It should be noted that physicochemical parameters are only given for the TÜBİTAK project performed in the Aegean Sea.

The temperature, salinity and density of the seawater were measured and recorded in situ using the SBE 911 plus CTD system. The pH was measured by WTW Multi 340i (WTW GmbH & Co. KG, Weilheim). The dissolved oxygen (DO) were determined immediately on the board based on the method first proposed by Winkler (1888) and modified by Strickland & Parsons (1972).

Water samples were taken by using rosette sampler at standard depths vertically for analysis of chemically measured parameters (PO₄-3, NO₂-, NO₃-, Si(OH)₄, POC, Particulate Organic Nitrogen (PON), TSS and Chl a). Before sampling, polyethylene bottles were washed with 10% HCl and rinsed 5 or 6 times with distilled water. All samples were sealed in these pre-cleaned bottles and stored in the deep freezer until analysis.

For particulate matter (POC, PON and Chl a) analysis, the sampled water was filtered through zooplankton netting with 200 µm mesh size in order to eliminate

possible macro planktonic contamination, then they were re-filtered through the 0.7 μm pore sized glass fibre filters (Whatman GF/F).

The filters were pre-exposed to 450 °C for 2.5 h in the oven for POC and PON measurements. After filtration they were kept frozen until they were processed. Organic matter measurements were done by CHN Carlo ERBA NC2500 Elemental Analyzer (Verardo, Froelich, & McIntyre, 1990). Before the analysis, filters were dried at 50 °C and fumed with concentrated HCl acid to remove inorganic carbon and sample was placed in a tin capsule. Unfortunately, POC/PON results showed an obvious overestimation for the area and the cause couldn't be detected, hence, the results were given because of the overall bias of the whole dataset to observe the trend.

The filters for Chl a measurements were treated with 2 ml 1% MgCO_3 airtight after the filtration and measurements were carried out spectrophotometrically using T80 Plus UV/VIS Spectrophotometer by following the method of Lorenzen & Jeffrey (1980).

The determination of NO_2^- , NO_3^- , Si(OH)_4 and PO_4^{3-} were carried out using a two Channel Scalar Autoanalyzer according to Strickland & Parsons (1972) while the determination of NO_2 was carried out spectrophotometrically by use of T80 Plus UV/VIS Spectrophotometer according to Grasshoff et al. (1983).

2.2.2 Phytoplankton Pigments

Sea water samples for phytoplankton pigments analysis were filtered through 25mm diameter, 0.7 μm pore sized glass fiber filters (Whatman GF/F). Three to seven liters were filtered until the filters were clogged. After filtration, the samples were immediately preserved in liquid nitrogen (-196 °C). Extraction was carried out with 3 ml 90 % HPLC grade acetone under sonication (60 Hz for 1 minute). The samples were kept overnight (about 12 hours) in the dark at 4°C (in the refrigerator). Pigments were measured with an HPLC system (Schimadzu LC 20 AT) using a C8 column (Thermo Scientific) with a Photo-diode array (PDA) absorbance detector and a

fluorescence detector. Extract was filtered through 0.2 μm pore size filters and mixed with 1M ammonium acetate (1:1 v/v). 100 μl of buffered extracts were injected and pigments were separated with gradient elution consisted of a primary eluant (A) composed of methanol and 1M ammonium acetate (70:30 v/v), and a secondary eluant (B) consisted of 100 % methanol. Pigments were then separated at a flow rate of 1 ml min^{-1} by a linear gradient programmed as follows (minutes; % solvent A; % solvent B): (0;75;25), (1;50;50), (20;30;70), (25;0;100), (32;0;100) (Barlow et al, 1997). Pigments were detected by absorbance at 410-665 nm wavelengths. Calibration for each pigment was done with commercial standards. Calculation of pigment concentrations was based on the 'external standard' equation of Jeffrey et al. (2005). Detection limits were changed between 0.06-0.09 ng for chlorophylls and 0.02-0.06 for carotenoids.

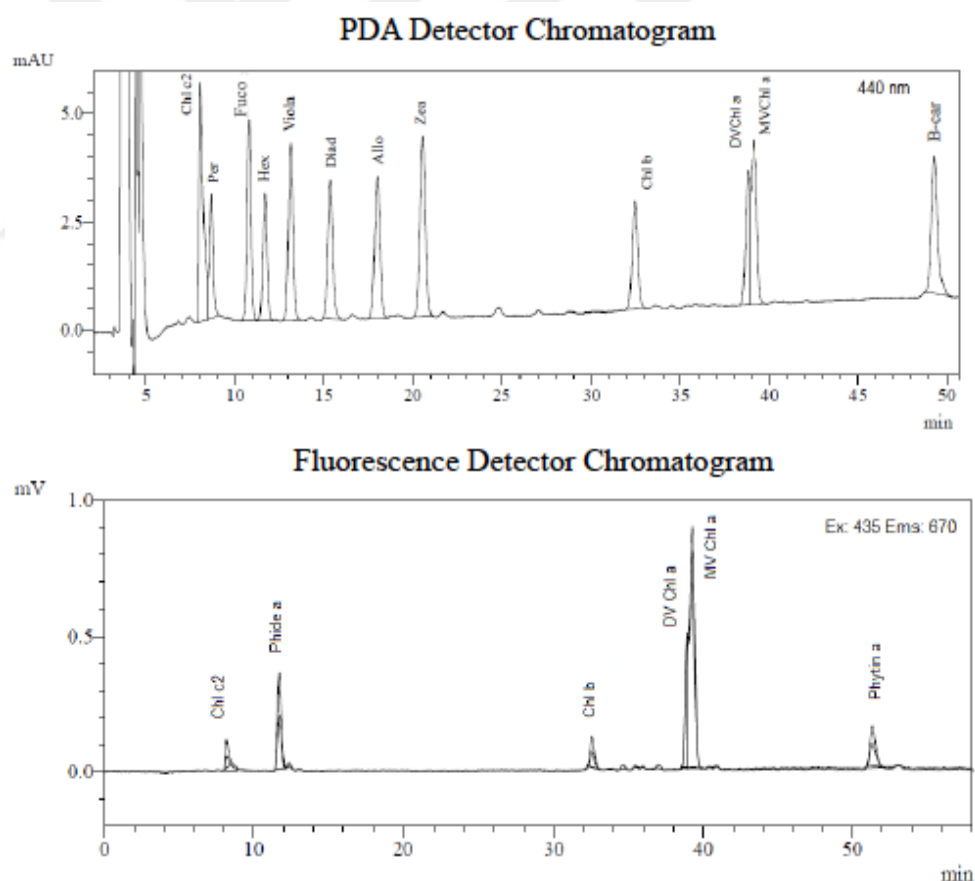


Figure 2.2 Chromatograms of the selected standard mix with Photo-diode array (PDA) absorbance detector and fluorescence detector

2.2.2.1 Classification

In this study, eighteen different pigments were measured in both Aegean Sea and Rhodes Gyre samples. Some pigments were additionally measured in the Ocean Certain project. Each pigment, abbreviations and related taxa were summarized in Table 2.2.

Table 2.2 Measured pigments: names, abbreviations and related taxa of each measured pigment. Pigments that highlighted were only measured in Rhodes Gyre case

Pigments	Abbreviations	Related taxa
Monovinyl Chlorophyll a	MV Chl a	All- except <i>Prochlorococcus</i>
Divinyl Chlorophyll a	DV Chl a	<i>Prochlorococcus</i> (Cyanobacteria)
Chlorophyll b	Chl b	Chlorophytes, Prasinophytes
Chlorophyll c2	Chl c2	Various
Chlorophyll c3	Chl c3	Prymnesiophytes, Pelagophytes (Chrysophytes)
Peridinin	Per	Dinoflagellates
19-Butanoyloxyfucoxanthin	But	Pelagophytes (major), Prymnesiophytes
Fucoxanthin	Fuco	Diatoms (major), Prymnesiophytes
19-Hexanoyloxyfucoxanthin	Hex	Prymnesiophytes (major)
Violaxanthin	Viola	Prasinophytes, Chlorophytes
Diadinoxanthin	Diadino	Various
Alloxanthin	Allo	Cryptophytes
Zeaxanthin	Zea	Cyanobacteria
Lutein	Lut	Chlorophytes (major), Prasinophytes
Beta-carotene	B-car	Various
Chlorophyllide a	Chlide a	Chl a degradation product
Phephorbide a	Phide a	Chl a degradation product
Pheophytin a	Phytin a	Chl a degradation product

Key pigments for their taxonomic correspondence are used to designate certain phytoplankton groups which are namely; fucoxanthin for Diatoms, peridinin for Dinoflagellates, 19' hexonoloyxyfucoxanthin for Prymnesiophyceae, 19' buthanoloyxfucuxanthin for Chrysophyta, alloxanthin for Cryptophyta, chlorophyll b for green flagellates (Chlorophyta), zeaxanthin for Cyanophyta and divinyl

chlorophyll a for Prochlorophyceae. The remaining are accessory pigments which are present in all phytoplankton except 3 Chl a degradation products (Chlide a, Phide a, Phytin).

Following the approach of Vidussi et al. (2001) and Uitz et al. (2006) pigments were clustered under major phytoplankton groups where Zea + DV Chl a + Chl b used to designate prokaryotic picoplankton, But-fuco + Hex-fuco + Allo to designate eukaryotic nanoflagellates, Fuco + Per to microplankton sized eukaryotes. Eight diagnostic pigments' (DP) sum is significantly correlated with Tot Chl a ($R^2=0.96$; $p<0.0000$; $n=43$) which makes DP a valid estimator of Chl a and assuming that each diagnostic pigment contributes equally to Tot Chl a. The pigment indices (f) of the chlorophyll a concentration associated with each of the three phytoplanktonic classes (f_{micro} , f_{nano} and f_{pico}) are subsequently derived according to total related pigments sum in order to express the total phytoplankton pool in terms of pigment concentration, total chlorophyll a concentration was computed as the sum of Chl a + Chlide a + DV Chl a, as the genus *Prochlorococcus* contains only the DV Chl a as its primary light harvesting pigment. As 4 extra pigments were measured in Rhodes Gyre case, it should be noted that there are a few differences in pigment sums and pigment indices. Each pigment, abbreviations, related taxon, pigment sums and pigment indices were summarized in the Table 2.3.

Table 2.3 Size classification of phytoplankton based on the pigment content method according to Uitz et al, 2006

Pigment sums	Abbreviations	Formula
Total Chlorophyll-a	Tot Chl a	MV Chl a+DV Chl a+Chlide a
Weighted sum of diagnostic pigments	DP	$0.86[\text{Zea}] + 1.01[\text{Chlb}] + [\text{DVChla}] + 0.60[\text{Allo}] + 1.27[\text{Hex}] + 0.35[\text{But}] + 1.41[\text{Fuco}] + 1.41[\text{Per}]$
Picoplankton fraction	f_{pico}	$(0.86 [\text{Zea}] + 1.01[\text{Chlb}] + [\text{DVChla}]) / \text{DP}$
Nanoplankton fraction	f_{nano}	$(0.60 [\text{Allo}] + 0.35 [\text{But}] + 1.27[\text{Hex}]) / \text{DP}$
Microplankton fraction	f_{micro}	$(1.41[\text{Fuco}] + 1.41[\text{Per}]) / \text{DP}$
Picoplankton Chl a	$\text{TChla}_{\text{pico}}$	$f_{\text{pico}} \times \text{TotChl a}$
Nanoplankton Chl a	$\text{TChla}_{\text{nano}}$	$f_{\text{nano}} \times \text{TotChl a}$
Microplankton Chl a	$\text{TChla}_{\text{micro}}$	$f_{\text{micro}} \times \text{TotChl a}$

2.2.3 Statistical Analysis

Due to lack of light measurements, the euphotic depth (Z_{eu}) couldn't be measured in the study. Thus, the euphotic depth was inferred from the Tot Chl a vertical profile, using a bio-optical model of Morel (1988) for light propagation. Due to the varying bathymetry of sampling stations and in order to obtain comparable values, integrations were performed on photic and aphotic layers which were separated based on the derivated Z_{eu} . HPLC Tot Chl a and environmental parameters within the photic and aphotic layers were integrated at each station according to the trapezoid rule. In addition, integrated values per surface area (m^2) were normalized over the respective integration depth value (m^3) for each selected layer.

To determine the spatial structure within the stations according to the physical and biochemical parameters, PCA (Principal Component Analysis) was performed. PCA aims to find combinations for certain variables to determine indices which describe the variation in the data with minimal loss of information. PCA analyses were performed by PRIMER 6.0 by normalizing the data and using the Euclidian distance matrices. Atomic ratios of nutrients were calculated STATISTICA 8.0.

Parametric statistics is based on independence. However, ecological data often violate this assumption by showing spatial dependence in terms of trends, gradients, patchiness, and random fluctuations. Spatial dependence means that the dependent variable/error term of a certain location is correlated with the same observations obtained at adjacent locations (Jansson et al., 2014). Moran's I index (Moran, 1950) was applied to characterize the Spatial Autocorrelation (SAC) of environmental parameters. Values of Moran's I range from -1 (indicating perfect dispersion) to 1 (perfect correlation). A zero value implies a random spatial pattern with no spatial autocorrelation. Positive values suggest spatially clustered patterns in adjacent sites, while negative values indicate that samples reveal very different values from the neighbouring ones (Anselin, 1995). Moran's I values and correlograms were implemented for discrete distance classes using the function "correlog" in R package "ncf" (Bjørnstad et al. 1999). The geographical distance matrix was divided into ten

distance classes to set the range of pairwise distance in each class (Legendre & Legendre, 2012). Mantel correlation coefficients were calculated at each distance class and tested for significance with a permutation test (using 999 permutations). The correlogram as a whole was considered to be significant (at a significance level of $\alpha \leq 0.05$) if at least one of its coefficients was significant at α / k , where k is the number of distance classes used (following the Bonferroni correction) (Legendre & Legendre 2012).

2.3 Sediment

2.3.1 Physicochemical parameters

Sediment samples were collected by a Box-Corer with dimensions of 50x50x50 cm. Grain size and organic matter analysis were done for the samples from the Aegean Sea in March 2012.

Sediment grain size classification were done with sieve and hydrometer and calculated as percentages according to Unified Soil Classification System (USCS). Dried and weighed sediment samples firstly separated into coarse (sand and gravel which are retained on the sieve) and fine (clay and silt) fractions by wet sieving with the 75 μm (American Society for Testing and Materials (A.S.T.M). Number 200) sieve. The fractions above (coarse) and under (fine) the sieve have dried again and serial sieving used for coarse material fractionation while hydrometer was used for fine-fraction.

Organic Carbon (OC) and Organic Nitrogen (ON) in the sediment were analysed with the same procedure for the water column. Before the analysis, sediment was dried at 50 °C and fumed with concentrated HCl acid to remove inorganic carbon and sample was placed in a tin capsule and measured by CHN Carlo ERBA NC2500 Elemental Analyzer (Verardo et al., 1990). Unfortunately, same as the situation with water column, sediment OC and TC results showed the same overestimation.

2.3.2 Modern Dinoflagellate Cysts

Sediment samples were collected with Box-Corer with dimensions of 50x50x50 cm. 0-2 cm surface sediment was sliced and the samples were stored in dark and cold conditions (+4 C°, in the refrigerator) to prevent excystment. The cleaning of sediment samples by palynological method and the training for identification were carried out in the School of Ocean and Earth Sciences University of Victoria, Canada with the co-advisor of the PhD thesis, Vera Pospelova.

In order to start the chemical treatment, varying amounts of sediment (between 0.3-11.6 grams) was subsampled according to the grain size distribution at each station. Samples were treated with distilled water to remove the salt. After washing, one Lycopodium tablet (produced in Lund University, Sweden) which contains a certain number of pollen spores, was added to the specimens to determine the proportion of spore / cyst to be used in the abundance calculations. In order to remove calcium carbonate (calcareous nanoplankton, foraminifera, etc.), 10% HCl acid was added to the samples and to remove the acid, the sample was centrifuged and washed by distilled water. HCl acid-free samples were screened through 125 and 15 µm meshes, respectively. When large material is removed with a 125 µm sieve, the cysts are collected on a 15 µm sieve and the clay is removed by passing through the lowest mesh. During the screening, the samples were kept in the ultrasonic bath for 30 seconds. Then 10 ml of 47% HF acid was added to remove the silicate containing materials (sand, diatom, silicaflagellate, etc.) for 2-3 days. Samples were washed again with HCl to remove the silicofluoride precipitated during the HF step, washed again with distilled water and transferred to storage tubes. From the cleaned samples, permanent slides were prepared with glycerine gel and the abundance values of each station were calculated by counting dinoflagellate cysts and Lycopodium tablets on a light microscope (Pospelova et al., 2005).

Species identifications were made at 40x and 60x magnifications with OLYMPUS BX53 light microscope according to the taxonomic key published by Zonneveld & Pospelova (2015).

2.3.3 Statistical analysis

To determine the diversity within the modern dinoflagellate cyst assemblages number of species, number of individuals, Shannon-Weaver diversity index, Pielou evenness index and Margalef richness index were calculated by using DIVERSE analysis in PRIMER (v.6) package program. To assess the nature of variability of the dinoflagellate cyst assemblages in the samples, PCA were performed also with PRIMER (v.6).

To relate the species community for each station with the environmental parameters modern long-term data sets were extracted from Copernicus Marine Environment Monitoring Service. This data set was most appropriate for the Aegean Sea area because of the lack of grids in remotely sensed worldwide environmental data sets due to the enormous number of islands in the area. Annual sea-surface temperature, sea-surface salinity was extracted from a data set between 1955-2014 and nitrate, phosphate concentration at surface and bottom water oxygen concentration were obtained from a data set between 1999-2016. Both data were on a 0.06x0.06 degrees resolution (Fратиanni et al, 2015).

To determine the most important parameters influencing dinocyst distributions, Canonical Correspondence Analysis (CCA) which has been using by ecologists to observe the relation between two multidimensional matrices, was performed with the normalized environmental parameters and the relative abundances of each species. Past 3 software was used for CCA analysis.

SAC analysis was done for organic matter and dinoflagellate cyst community with the same method explained for the water column parameters.

CHAPTER THREE

RESULTS

3.1 Water Column

3.1.1 Physicochemical Parameters

Physicochemical parameters were measured during March 2012 survey in the Aegean Sea and the distribution of salinity and temperature along a transect from north to south is given in Figure 3.1 and 3.2. Temperature and salinity values varied between 10.77 (St 3C) -17.31 C° (St 8C) and 34.51 psu (St 1C) - 39.16 psu (St 10C), respectively. The lowest values for both parameters were observed in the northern surface layer that Black Sea originated waters enter and the highest values were seen in the south surface layer. In general, the upper layers were more saline and warmer, while the lower layers were less cold and less salty in the southern part of the Aegean Sea. Although the surface temperature values changed around 15-17 °C, the Black Sea water inlet dropped to 14 °C in the North Aegean.

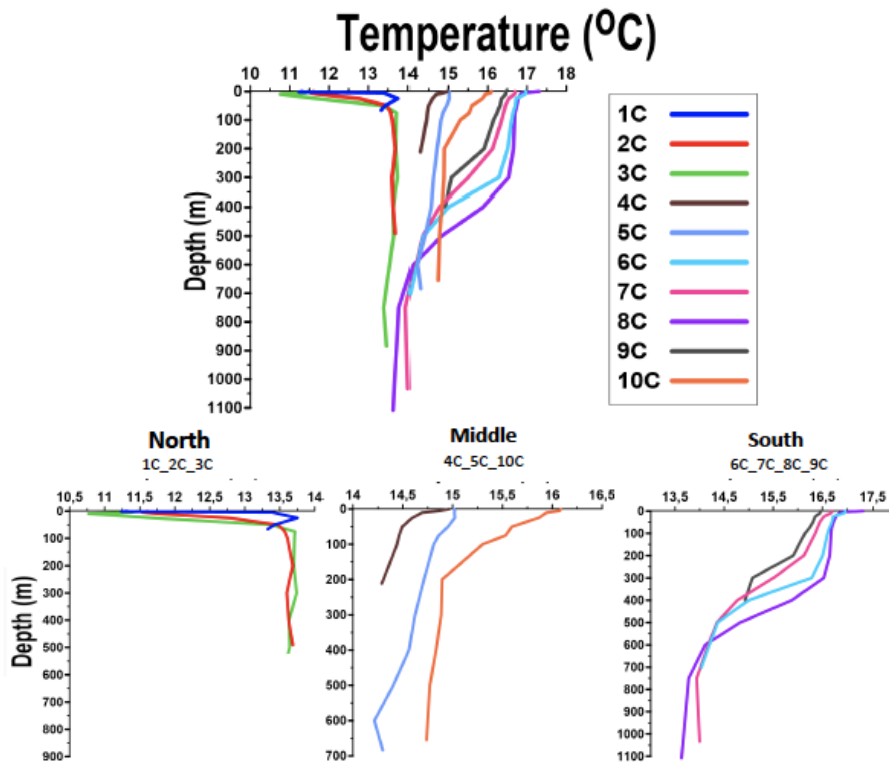


Figure 3.1 Vertical distribution of temperature in the Aegean Sea in March 2012

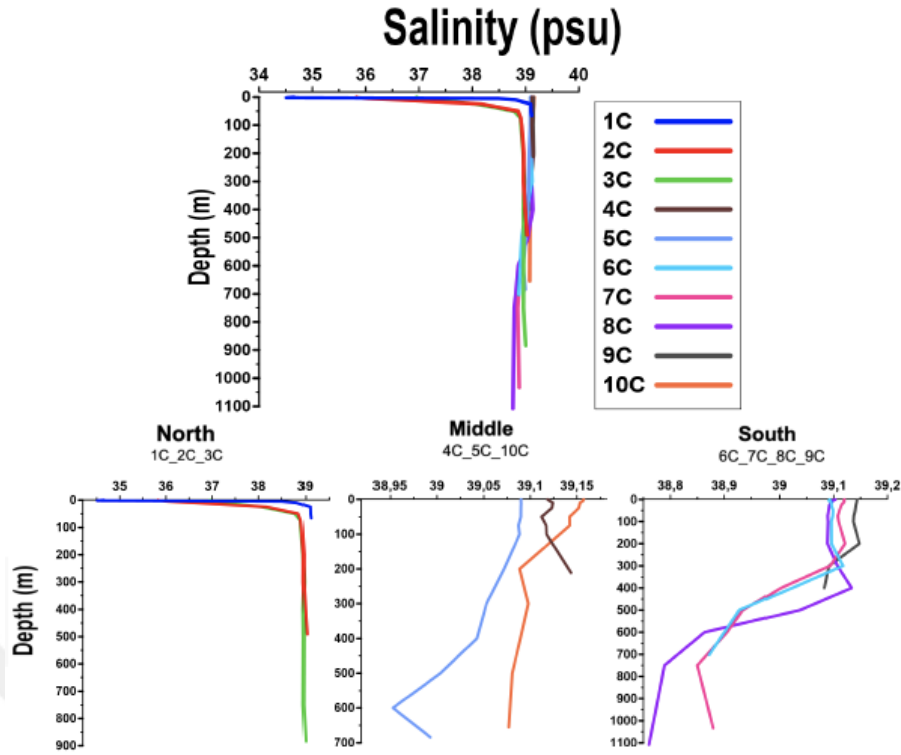


Figure 3.2 Vertical distribution of salinity in the Aegean Sea in March 2012

With a general definition, it can be said that March 2012 cruise was the end of a typical winter season for the Aegean Sea in the scope of salinity and temperature values (Figures 3.1 and 3.2). In order to understand the differences between regions within the Aegean Sea, temperature, salinity and density profiles and T-S diagram were assessed and in terms of physical properties, the period of March 2012 can be divided into 3 different regions; North Aegean where the stations 1C, 2C, and 3C located, Middle Aegean where the stations 4C, 5C and 10C located and South Aegean where the stations 6C, 7C, 8C and 9C located (Figure 3.3). In the North Aegean, surface waters were cooler and less salty with the Black Sea originated water effect. South Aegean profiles showed an Eastern Mediterranean water type with its mixed surface layers till around 300 meters and salinity/temperature values that decreasing with depth. Among these regions, the most striking area is Middle Aegean. T-S diagram showed that water masses in the Middle Aegean are denser than other regions due to the lower temperature and higher salinity and vertical profiles showed deep water mixing in this middle region.

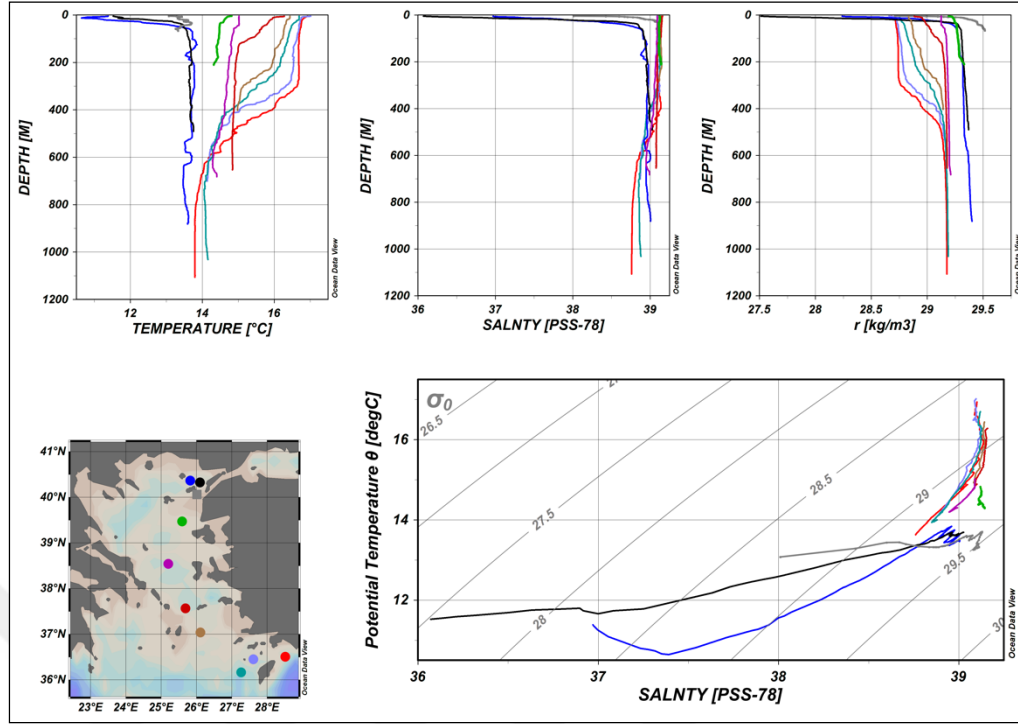


Figure 3.3 Salinity, temperature and density profiles of each station and T-S diagram of the Aegean Sea (Bizsel et al, 2014)

DO values in the study ranged from 4.17 (St 8C bottom depth-1108m-) to 6.75 ml/l (St 1C surface-0m-). In this range of change, a significant decrease from surface to bottom was observed at St 6C, 7C and 8C which were deepest and southernmost stations. The pH values were varied between 8.26 (St 7C bottom -1033m-) and 8.37 (St 1C 0m, St 3C 0m, 10m). The pH change overlaps with high DO values (Figure 3.4).

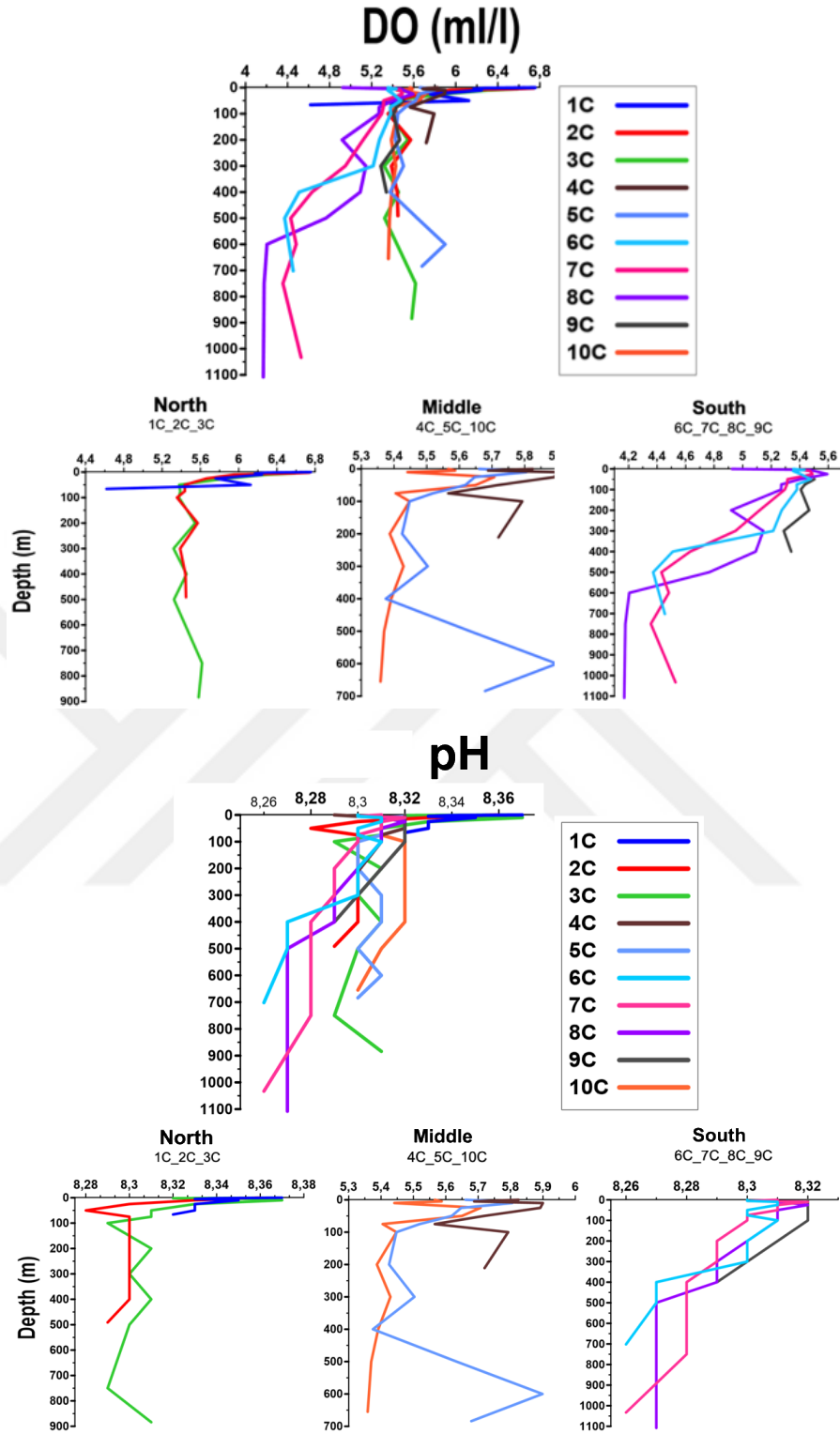


Figure 3.4 Vertical distribution of Dissolved Oxygen (DO) (top) and pH (bottom) in the Aegean Sea in March 2012

TSS values ranged between 0.3-7.0 mg/l. Minimum value was observed in the south at St 9C surface and maximum values was observed in the north at 2 and 5 meters of

depth of St 1C which showed highest TSS values in the whole water column (min 6.33 mg/l at surface) (Figure 3.5). St 1C is the closest station to Çanakkale Strait opening so it would be the most effected station by the particulate matter load of Black Sea originated waters. The reason for the increase in the bottom depths in some stations (St 3C, 7C, 9C and 10C) may be caused by the resuspension of the sediment. The increase that observed after 200 m in the water column in St 5C and 9C can be explained by the vertical mixing observed at middle region of the Aegean Sea.

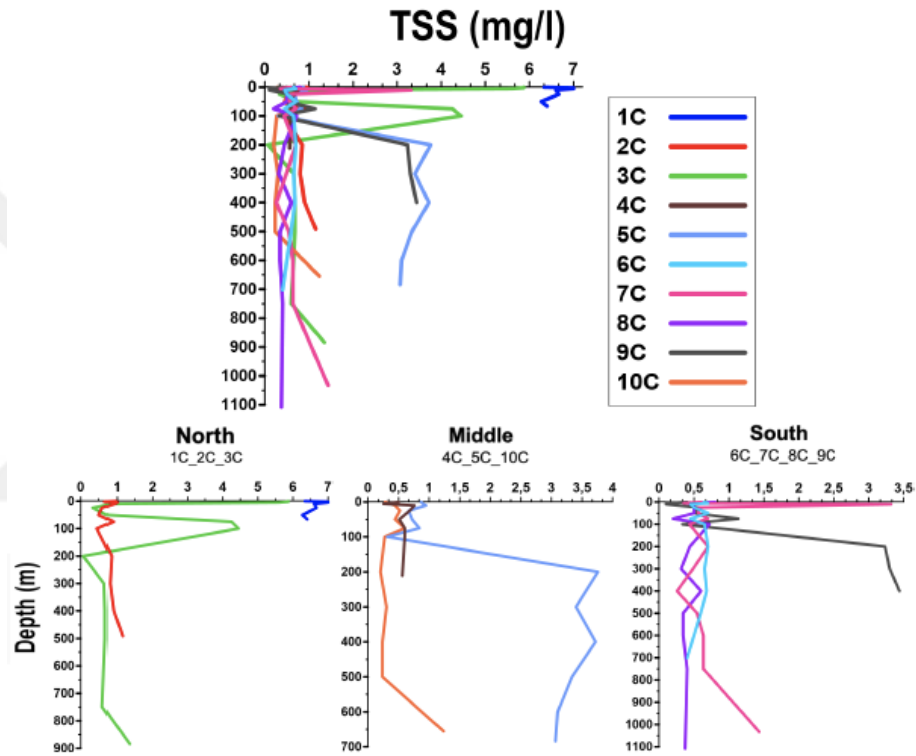


Figure 3.5 Vertical distribution of TSS in the Aegean Sea in March 2012

The spatial distributions of nitrate (NO_3^-) and nitrite (NO_2^-), phosphate (PO_4^{3-}) and silicate (Si(OH)_4) have given in Figure 3.6 and 3.7. NO_3^- values generally vary from BDL-12 μM at all stations from the surface to the bottom layers. However, at some stations, maximum values over this range was observed (5C- 200m: 23 μM and 7C- 300m: 29 μM). It is noteworthy emphasize that these increases were observed in deep layers in the southern and central stations rather than northern region.

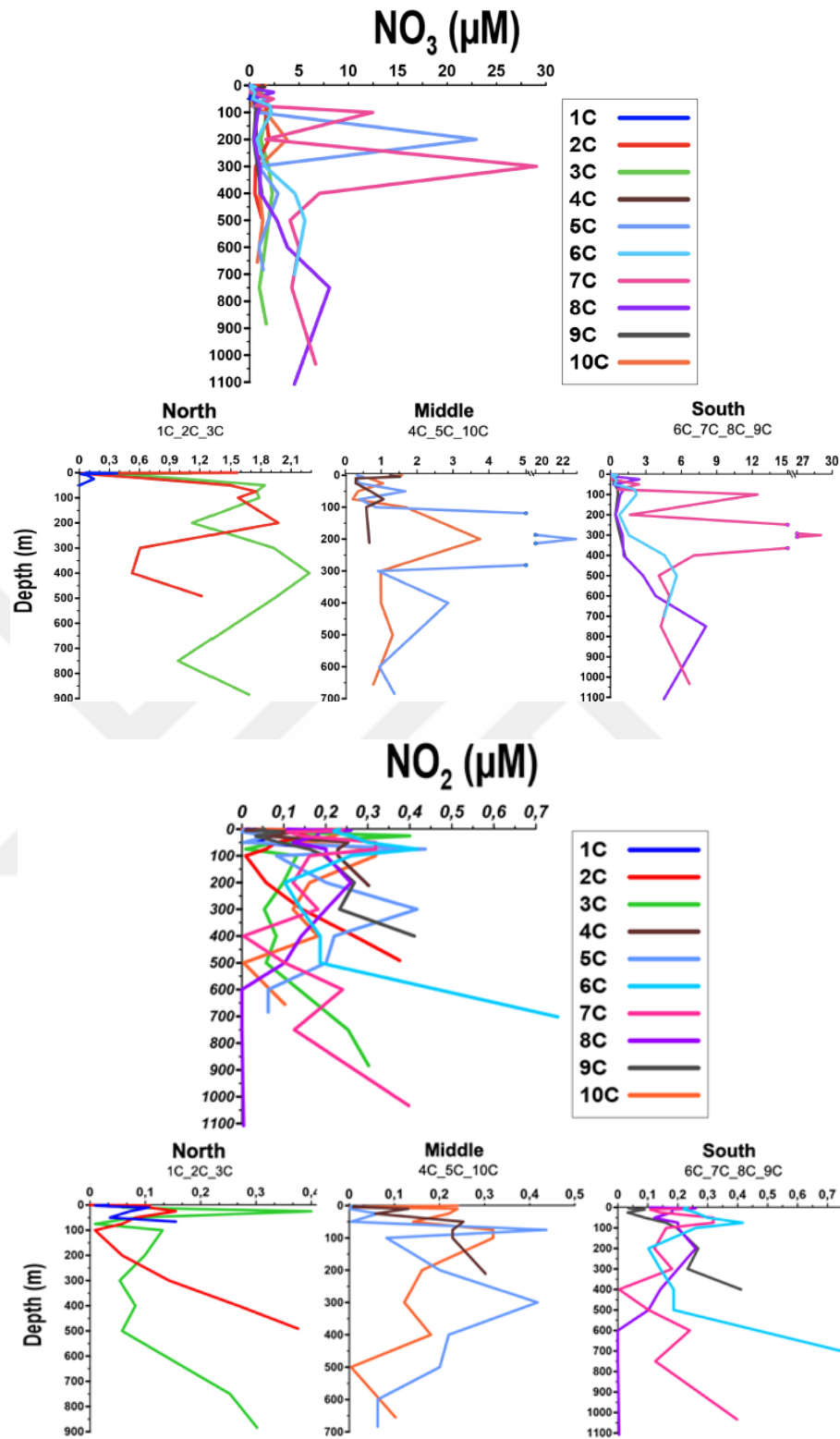


Figure 3.6 Vertical distribution of nitrate (NO_3^-) (top) and nitrite (NO_2) (bottom) in the Aegean Sea in March 2012

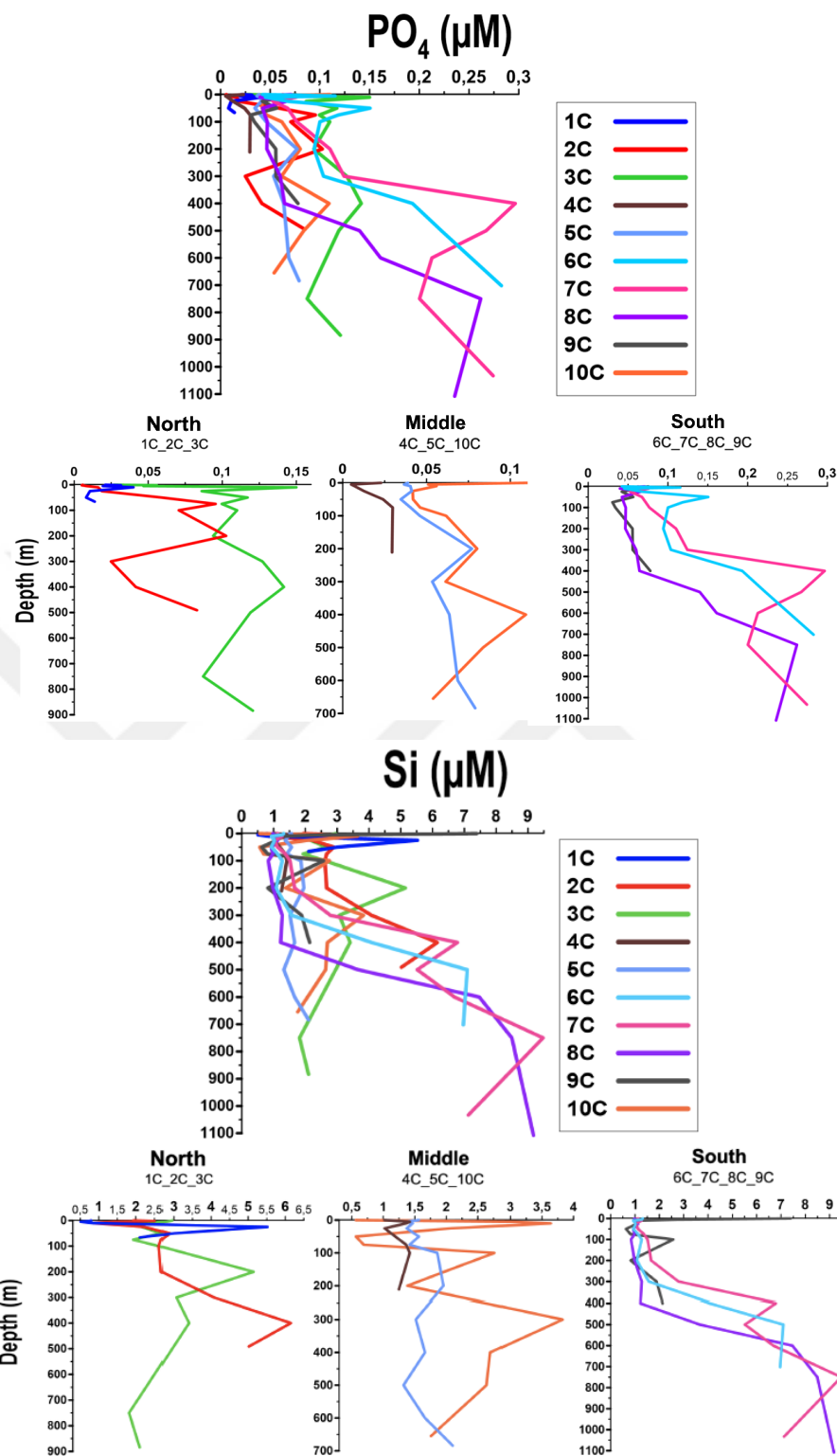


Figure 3.7 Vertical distribution of phosphate (PO_4^{3-}) (top) and silicate ($\text{Si}(\text{OH})_4$) (bottom) in the Aegean Sea in March 2012

The NO_2 values range from BDL-0.75 μM in all stations from the surface to the bottom layers. The maximum values were observed in northern surface waters and in

station 6C bottom water where the dissolved oxygen is relatively low. Although the overall range of PO_4^{3-} values of 0,01-0,30 μM , it is seen that the values generally do not exceed 0.1 μM . As the case observed with nutrients that containing inorganic nitrogen, PO_4^{3-} values were also increased in deep layers at stations 6C, 7C, and 8C. The overall range of $\text{Si}(\text{OH})_4$ values was determined as 0.51-9.48 μM . The maximum values were measured as > 9 μM at St 7C, 750 m and in bottom water at St 8C (Fig. 3.5). Similar to nitrate and phosphate, a slight increase in silicate values can be distinguished in the vertical distributions of the southern stations.

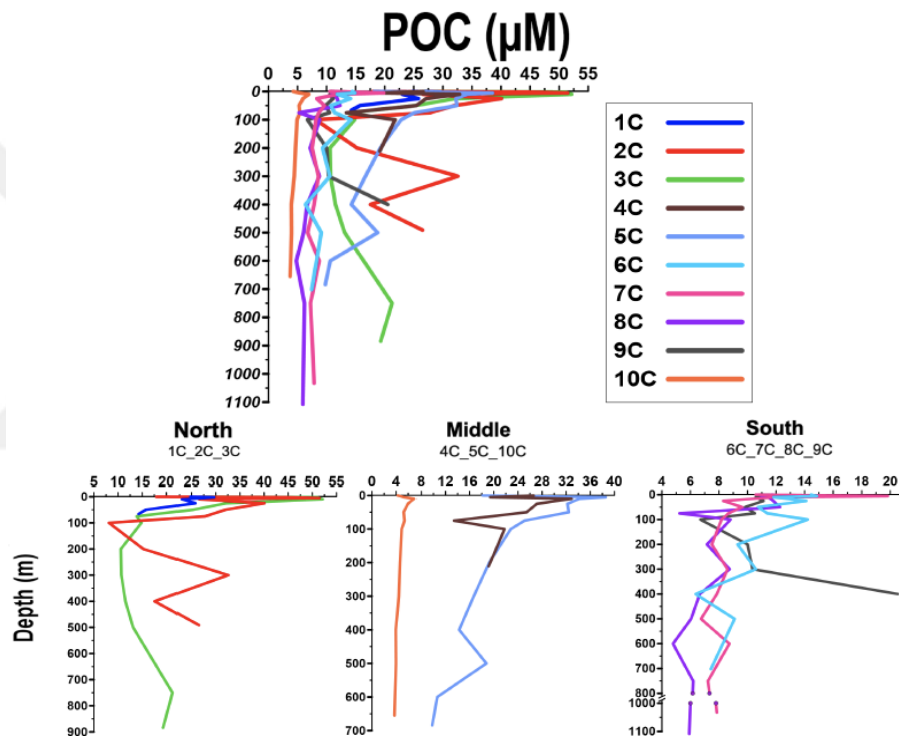


Figure 3.8 Vertical distribution of POC in the Aegean Sea in March 2012

Particulate Organic Carbon (POC) values ranged from 3.73 to 52 μM and (Figure 3.8). Maximum values were observed generally in northern region and the minimum values were observed in entire water column of St 10C. It should be noted that POC/PON results showed an obvious overestimation close to coastal water ranges because of an undetected measurement problem.

3.1.2 Phytoplankton Pigments

3.1.2.1 Aegean Sea

Chl a measurements done by spectrophotometry and HPLC were compared and these two variables are significantly correlated ($Y = 0.404X + 0.167$; $r = 0.653$; $p < 0.05$ and $n = 38$) (Figure 3.9). Spectrophotometric measurements generally have higher values than HPLC measurements (Figure 3.10).

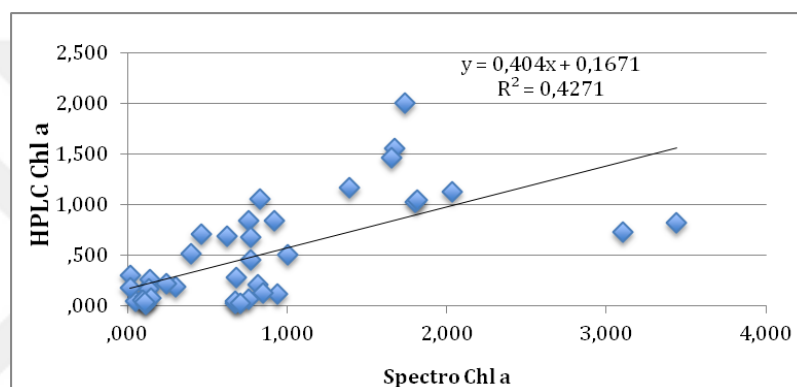


Figure 3.9 Scatter plot of Chl a values measured by HPLC against measured by spectrophotometer in March 2012 in the Aegean Sea

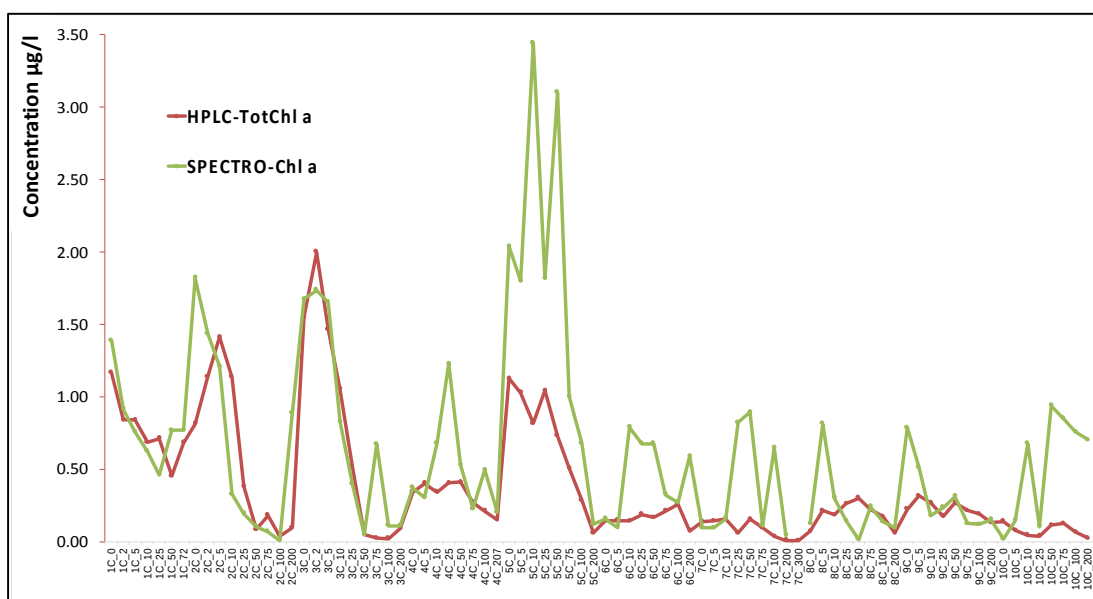


Figure 3.10 Comparison of Chl a values measured by spectrophotometer and HPLC

Distribution pattern in the first 5 station in the northern area and at stations 6C, 7C, 8C, 9C and 10C in the south differs from each other. Overestimation of spectrophotometric measurement is much higher in the southern area than the northern, which can be explained by the fact that the spectrophotometric method is less sensitive at low concentration.

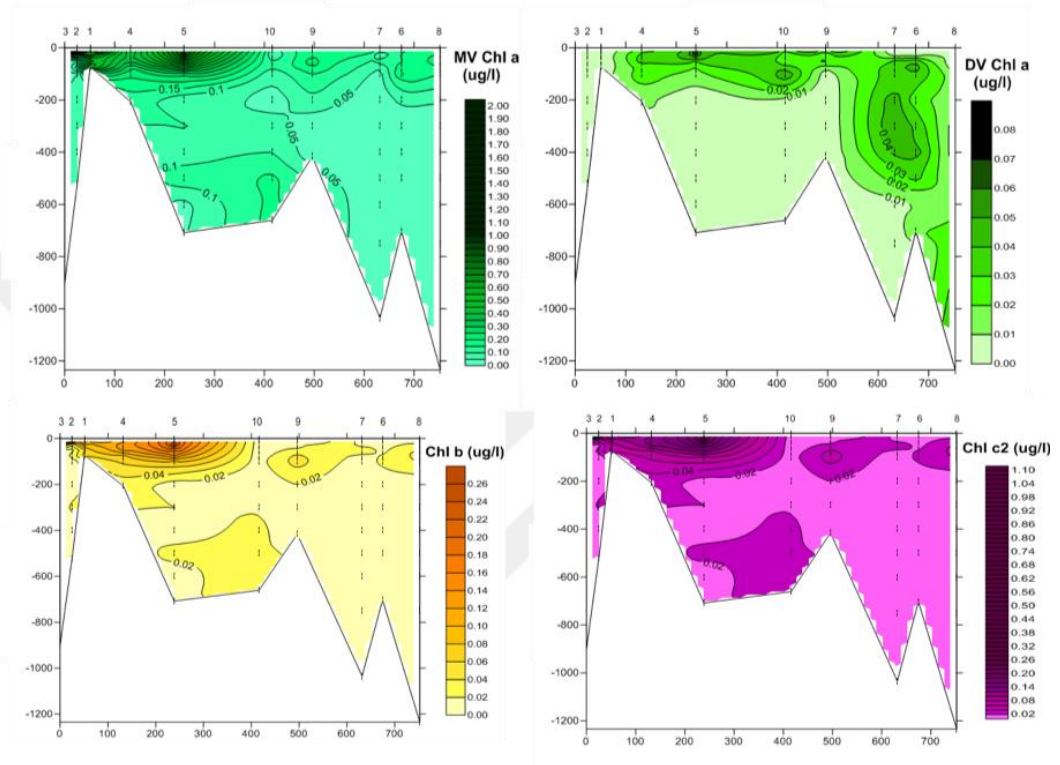


Figure 3.11 Concentrations of chlorophylls ($\mu\text{g/l}$) in the Aegean Sea in March 2012. (Station numbers aligned from North to South (y axis gives the distance in km))

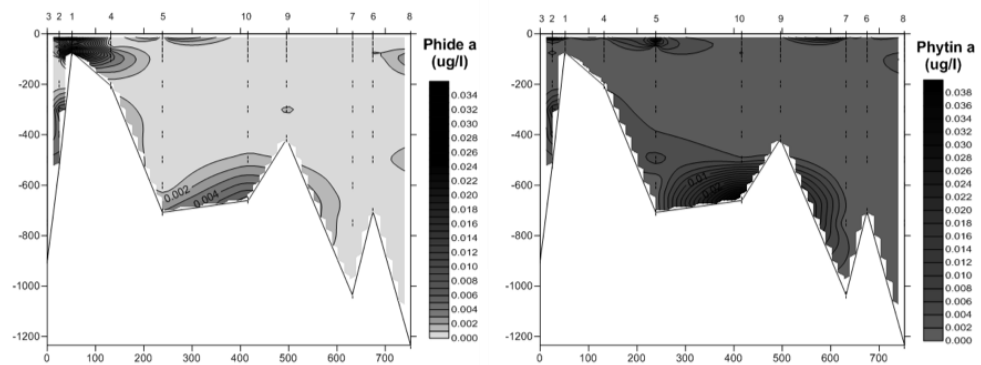


Figure 3.12 Concentrations of Chl a degradation products ($\mu\text{g/l}$) in the Aegean Sea in March 2012. (Station numbers aligned from North to South (y axis gives the distance in km))

The distribution of all pigments measured by HPLC is shown as transect from north to south in Figures 3.11 and 3.12 and 3.13. Chlorophylls ranged between, Chl a; 0.001-1.999 µg/l, DV Chl a; BDL-0.067 µg/l, Chl b; BDL-0.265 µg/l, Chl c2; BDL-1.091 µg/l, respectively. Chl a degradation products ranged between; Phide a; BDL-0.034 µg/l, Phytin a; BDL-0.043 µg/l respectively (Figure 3.11 and 3.12). Surface MV Chl a values decreased from north through south. On the other hand, DV chl a values showed an inverse trend through south with relatively high values in deeper zones. There has been an increase after 300 meters at 5C, 10C and northern station 3C which is similar to vertical trends of PO₄, NO₃, POC and TSS which is related to the vertically homogenous characteristic of the central part of the Aegean Sea.

Carotenoid vertical distributions as transects in the Aegean Sea in March 2012 were given in the Figure 3.13. Photoprotective carotenoids ranged between: β-car; BDL-0.057 µg/l, Diad; BDL-0, Allo; BDL-0.081 µg/l, BDL-0.028 µg/l and Zea; BDL-0.150 µg/l while photosynthetic carotenoids ranged between: Fuco; BDL-0.453 µg/l, Per; BDL-0.142 µg/l, Hex; BDL-0.316 µg/l, ,066 µg/l. All surface carotenoid values decreased from north through south except Zea. Zea showed a similar trend with DV Chl a.

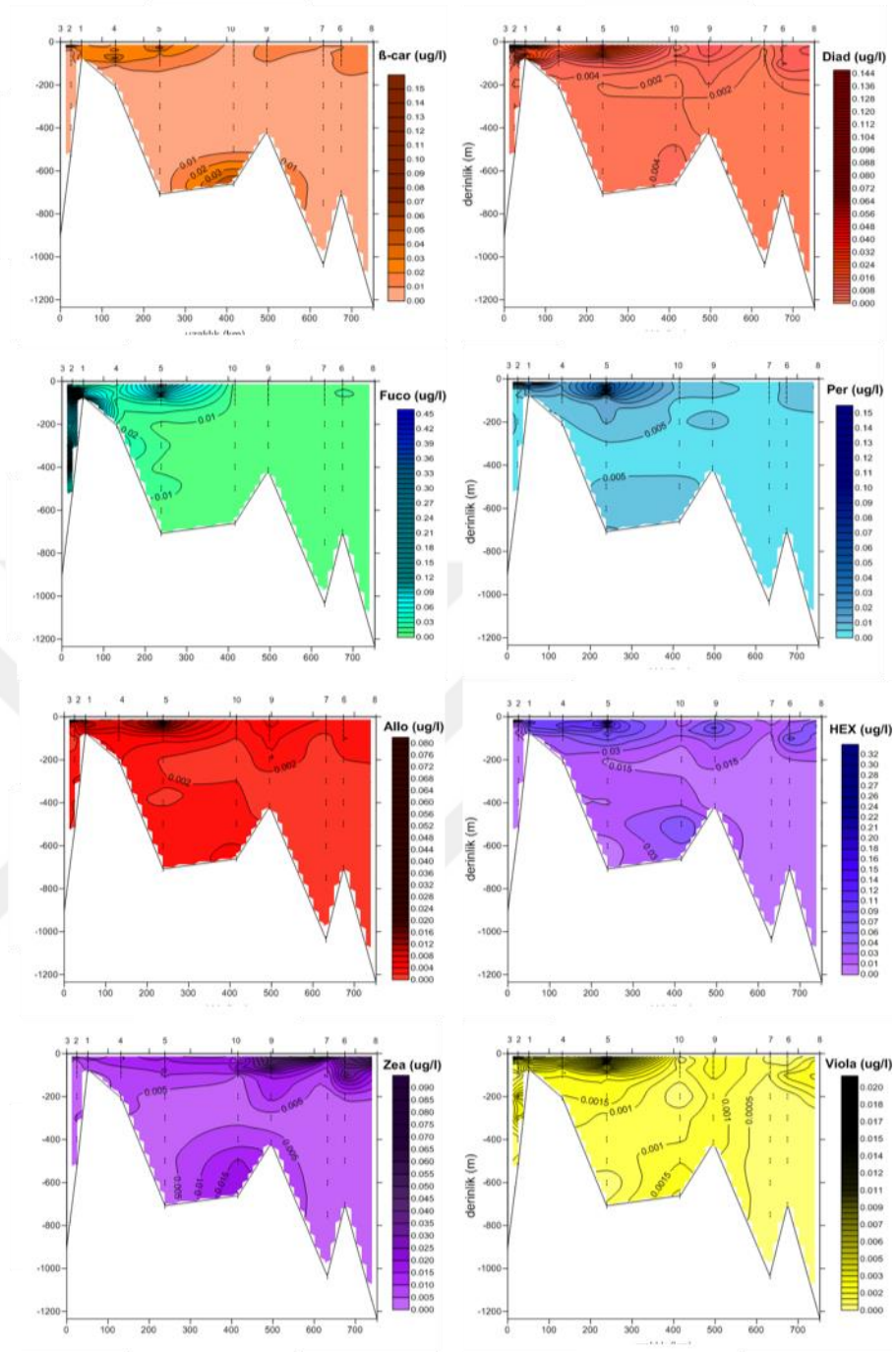


Figure 3.13 Concentrations of Carotenoids ($\mu\text{g/l}$) in the Aegean Sea in March 2012. (Station numbers aligned from North to South (y axis gives the distance in km))

Tot Chl a vertical distribution in the photic zone in the Aegean Sea is given in Figure 3.14. Vertical profiles of each diagnostic pigment (DP) (Peridinin, Fucoxanthin, Fuco-hex, Alloxanthin, Zeaxanthin, Chlorophyll b and DV Chlorophyll a) were given in the Figure 3.15 as vertical distributions in the photic zone. As the concentrations lowers after 100 meters and it is the maximum Z_{eu} depth for all stations (derived by Morel, 1988) the vertical profiles have been cut to see the distribution clearly. All diagnostic pigments and Tot Chl a have the similar vertical trends as DP (diagnostic pigments summation) is a valid estimator of Chl a. In norther stations (1C, 2C, 3C), highest values observed in the first 25 meters which is affected by Black Sea, the values decreasing fast with increasing depth. In the middle stations (4C, 5C, 10C), values are lower than north but a vertically homogenous structure observed.

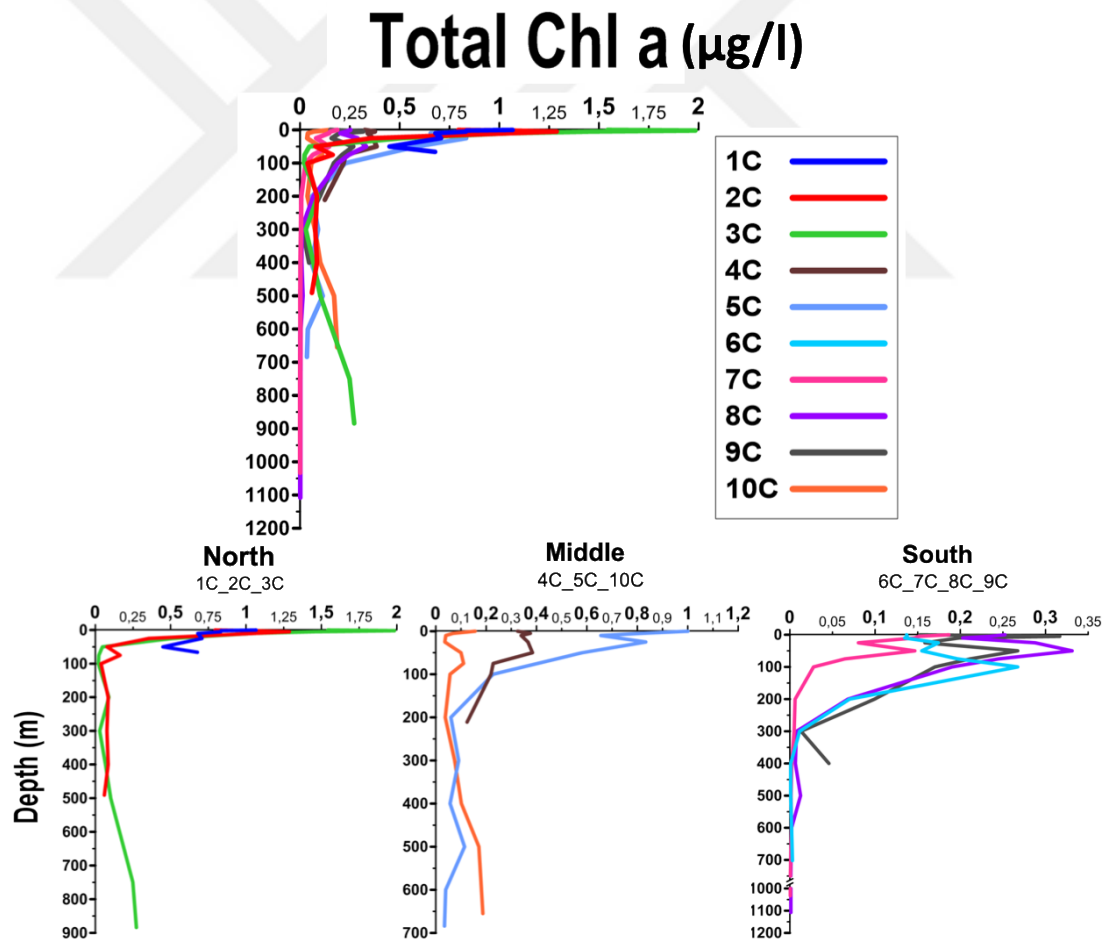


Figure 3.14 Vertical distribution of Tot Chl a ($\mu\text{g/l}$) in the Aegean Sea in March 2012

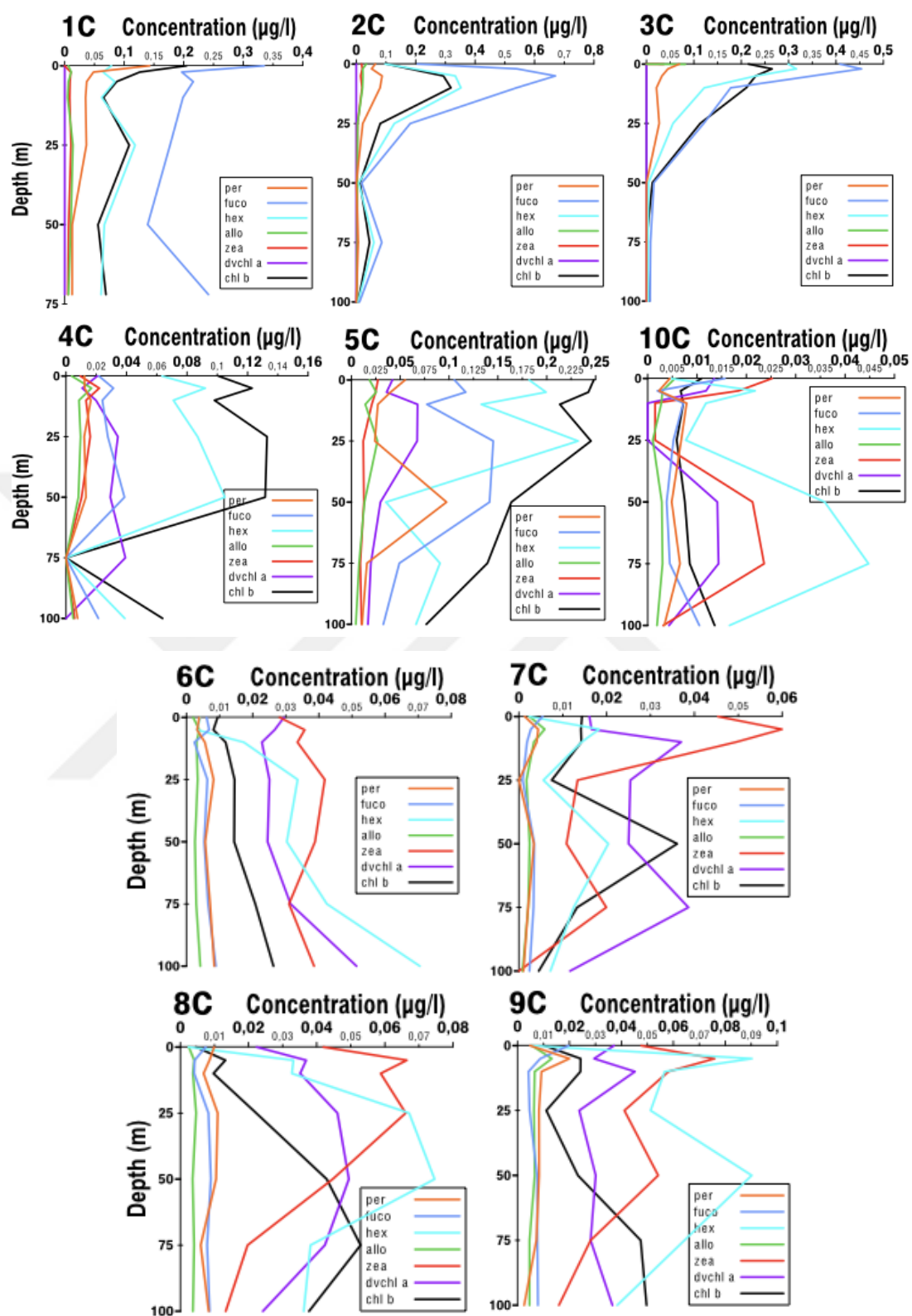


Figure 3.15 Vertical profiles of diagnostic pigments (µg/l) in the photic zone of Aegean Sea in March 2012

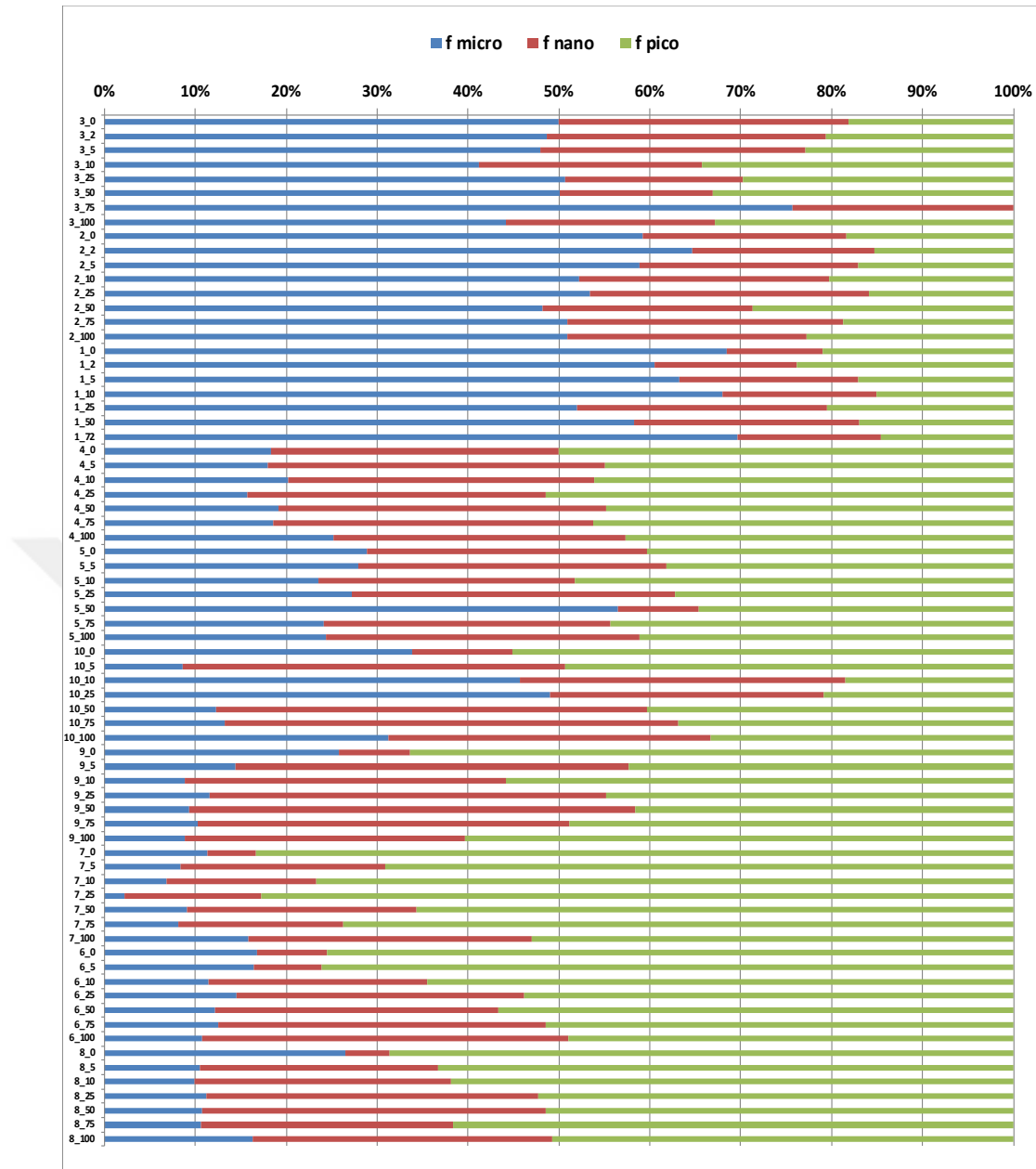


Figure 3.16 Biomass proportions (%) of each size class in percentage at each depth of stations in the Aegean Sea in March 2012

All chlorophylls except DV Chl a and all carotenoids except Zea have higher percentages at the stations 1C, 3C and 5C in the north. DV Chl a, key pigment of the prochlorophytes, and Zea, key pigment of the cyanobacteria, have higher percentages at 10C and 8C at the southern stations. At the southern stations, Per, key pigment of dinoflagellates, and Fuco, the key pigment of the diatoms, are lower than the north. The biomass proportions of each size classes which are derived from DP (Uitz et al., 2006) also confirm that in the southern waters of the Aegean Sea, picoplankton have

been prevailed over microplankton, confirming that this region has a more pronounced oligotrophic water feature (Figure 3.16).

3.1.2.2 Rhodes Gyre

Tot Chl a is ranged between BDL-0.47 $\mu\text{g/l}$ in the upper 200m in the water column of the Rhodes Gyre in March 2016 and concentrations for each depth is represented in Figure 3.17. In the core of the gyre at stations 9R, 10R, 11R and 12R maximum values have been reached at DCM and the minimum DCM values were observed at stations 2R and 5R. Fluorescence results shows significant correlations with Chl a concentrations ($R^2=0.77$; $p<0.0000$; $n=43$). Both Chl a and fluorescence results show that relatively high values (0.2-0.4 $\mu\text{g/l}$) have been observed down to 150m in northernmost stations (1, 2 and 3) and getting shallower towards south (60-70 meters at stations 11, 12). Only at station 5R, a second increase (at 150 m) after 50 m has been observed. In Figure 3.18, vertical profiles of the Chl a concentrations of each station given.

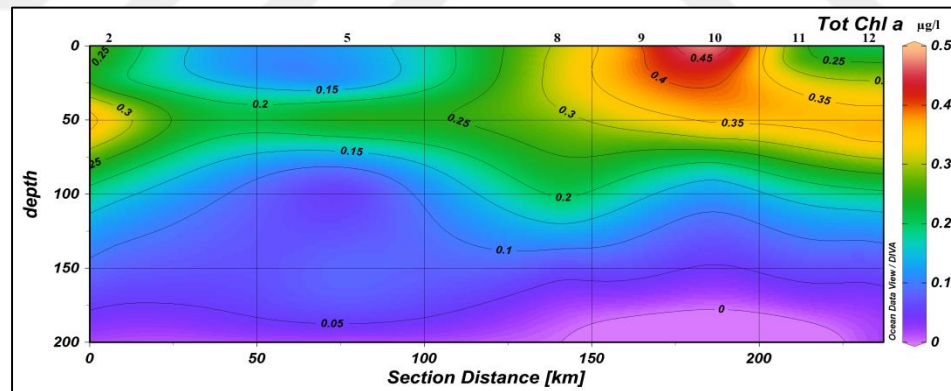


Figure 3.17 Tot Chl a ($\mu\text{g/l}$) distribution along the transect in Rhodes Gyre in March 2016

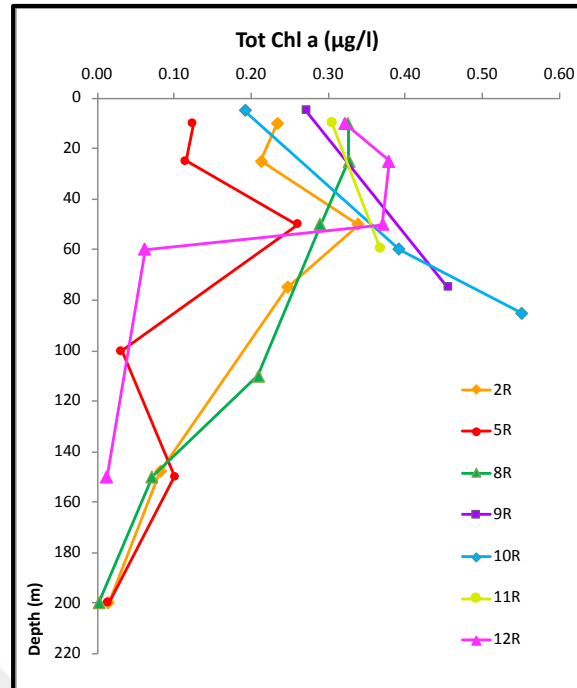


Figure 3.18 Vertical profiles of Tot Chl a ($\mu\text{g/l}$) in each station in the Rhodes Gyre in March 2016

In Figure 3.19, as bars represent the Tot Chl a concentration on related depth of the station, areas were stacked into pieces by related portion of the size classes. In addition, independent from the concentration, the details of the biomass proportion (f %) of each size classes in Tot Chl a is given in Figure 3.20. Nanoplankton proportion in Chl a is the largest in the whole area while picoplankton and nanoplankton dominance over microplankton in the border of the eddy changes through the core as nanoplankton and microplankton are the prevailing groups. In the study area for all depths, the average pigment (n=43) composition within each class as follows: (1) picophytoplankton were composed of 39% of Zea, 31% of DV Chl a and 22% of Chl b; (2) nanophytoplankton were composed of 31% of But-fuco 63% of Hex-fuco and 6% of Allo; (3) microphytoplankton were composed of 83% Fuco and 17% Per.

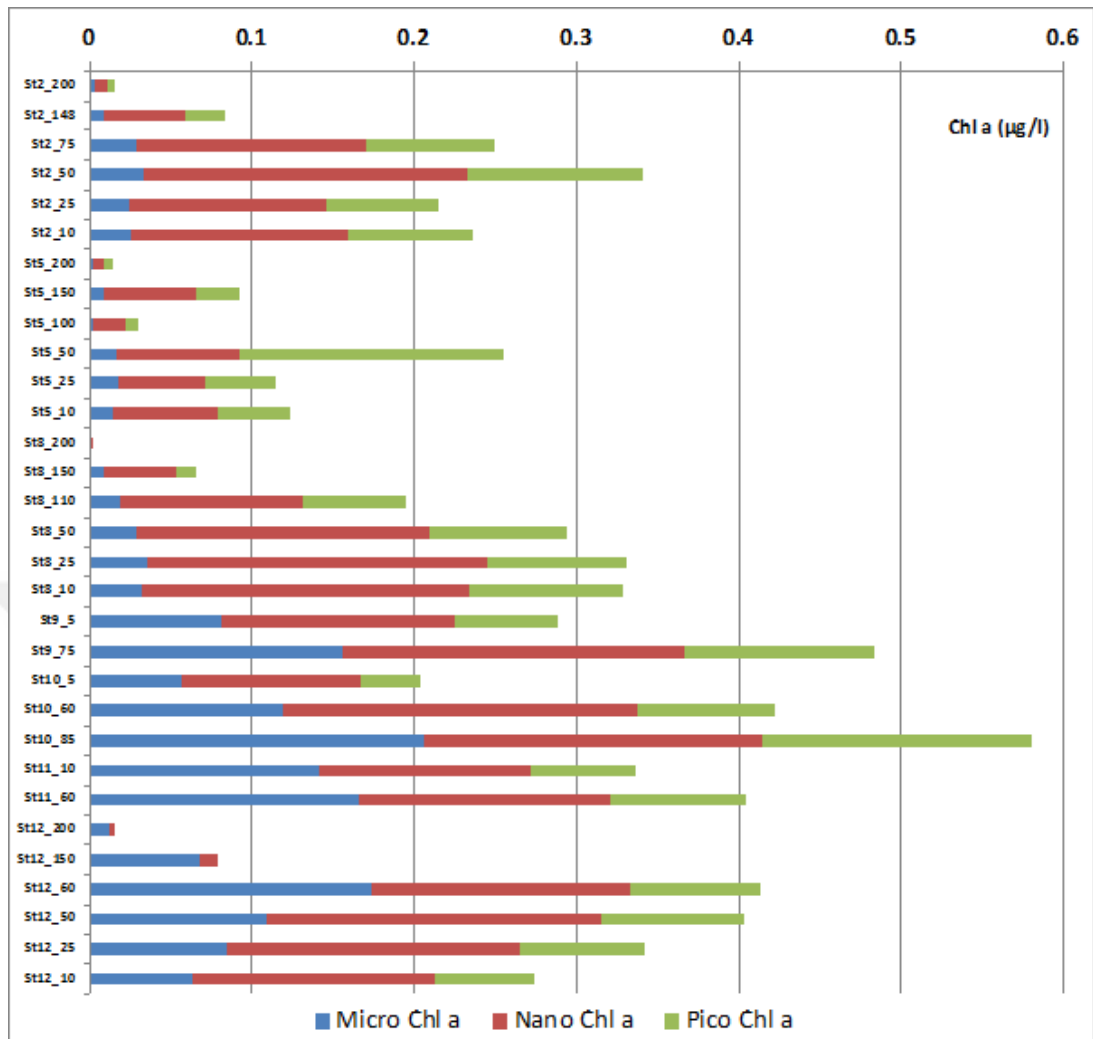


Figure 3.19 Pico, Nano and Microplankton Chl a (µg/l) at each depth of the station in Rhodes Gyre in March 2016

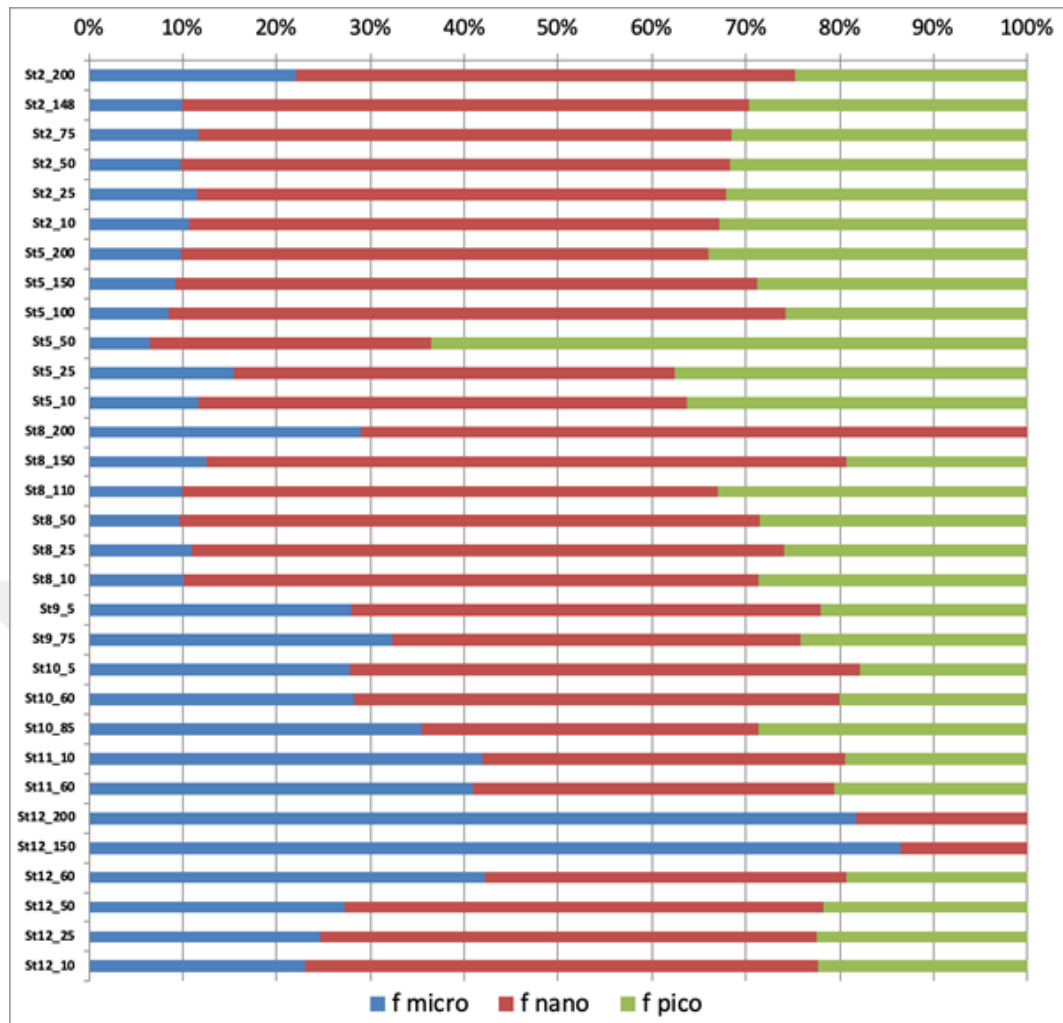


Figure 3.20 Biomass proportions (%) of each size class at each depth of stations in the Rhodes Gyre in March 2016

Seven diagnostic pigments (Zea, DV Chl a, Chl b, But-fuco, Hex-fuco, Allo, Fuco, Per) of phytoplankton groups ranged between 0,0006-0,0543, BDL-0,0827, BDL-0,0540, 0,011-0,0580, 0,0007-0,1195, BDL-0,0145, 0,0001-0,0580, BDL-0,0537 $\mu\text{g/l}$, respectively. Prymnesiophyceae that represented by Hex-fuco is the dominant group at all stations. Maximum values of DV Chl a at St5 50 m and Per at St12 60 m are striking. Fuco concentrations are low in the northern stations but increasing towards the core of the gyre at the stations 9, 10, 11 and 12 and maximum values are at the DCM depths of this stations (Figure 3.20). The Chl a degradation products were generally at low concentrations with the ranges BDL-0,0688; BDL-0,0053 and BDL-0,0907 $\mu\text{g/l}$ for Chlide a, Phide a and Phytin a, respectively.

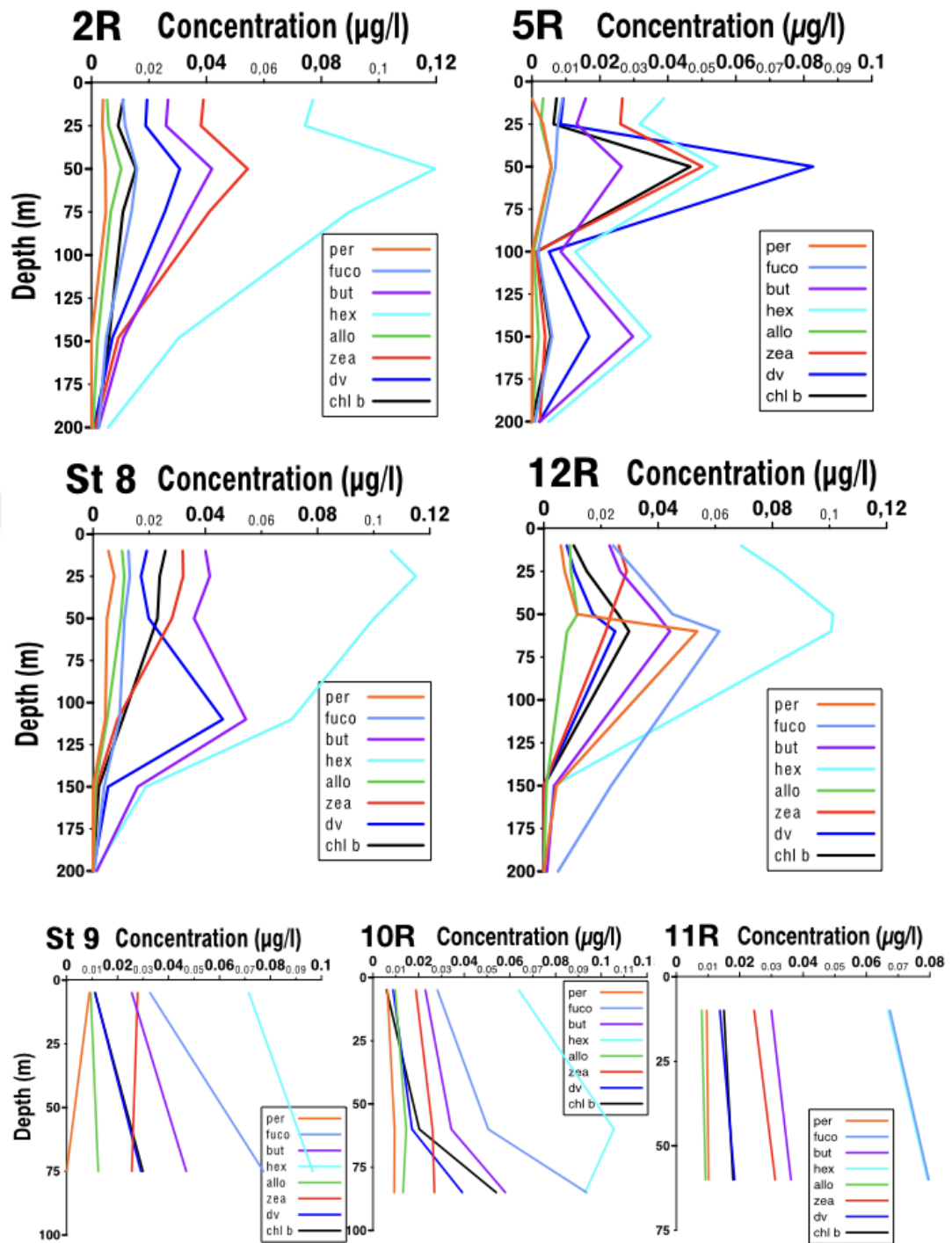


Figure 3.21 Vertical profiles of eight diagnostic pigments (µg/l) in the photic zone of Rhodes Gyre in March 2016

3.1.3 Statistical Analysis in the Water Column

The euphotic depth was inferred from the Tot Chl a vertical profile, using a bio-optical model of Morel (1988) for light propagation. Due to the varying bathymetry of sampling stations and in order to obtain comparable values, integrations were performed on photic and aphotic layers. HPLC Tot Chl a and environmental parameters within the photic and aphotic layers were integrated at each station according to the trapezoid rule. In addition, integrated values per surface area (m²) were normalized over the respective integration depth value (m³) for each selected layer. For PCA and SPA analysis in the water column, integrated data were used.

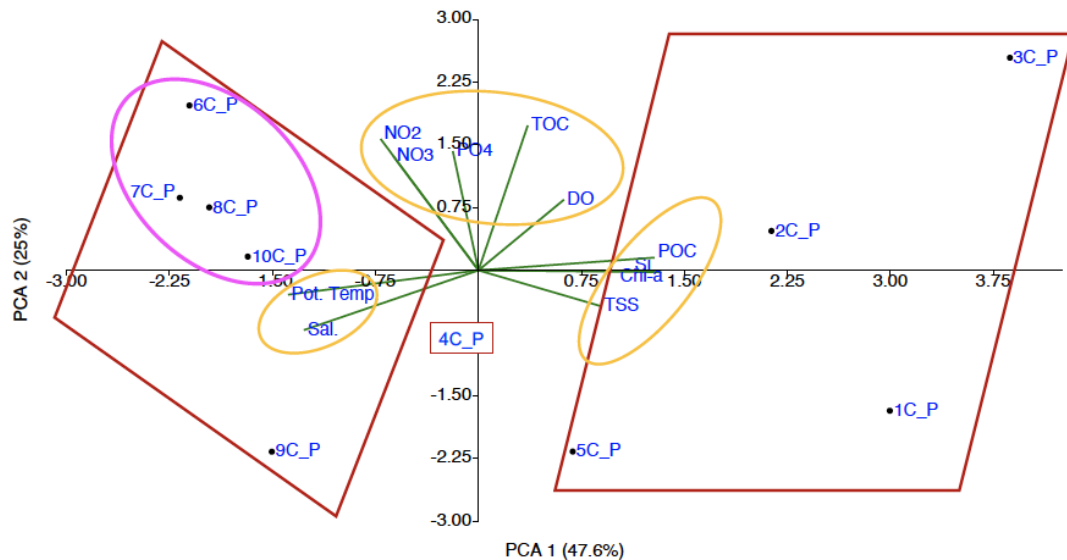


Figure 3.22 PCA diagram showing variance in the photic zone in the Aegean Sea in March 2012

To determine the parameters that have the largest variation in photic and aphotic layers PCA (Principal Component Analysis) was performed (Figure 3.22 for photic zone, Figure 3.23 for aphotic zone). In the photic zone, the first three principal component axis explains 82.2% of the total variance; first principal component (PC1), second principal component (PC2) and third principal component (PC3) explains 47.6%, 25% and 9.6% of the variance, respectively. 48% of the variance is related to temperature, salinity, Chl a, POC and silicate, 25% of the variance is related to nitrite and TOC. According to PC1, stations in the Aegean Sea photic zone separated into

two groups as north (1, 2, 3 and 5) and south (6, 7, 8, 9, 10). Photic zone of St 4C not affected from variables related with PC1. PC1 correlated positively with Chl a, POC, TSS, silicate and negatively with temperature and salinity so these variables affect the separation of the stations as north and south. PC2 correlated positively with nutrients, TOC and DO, negatively with salinity (Table 3.1). In the diagram, a group that ordinated in the more negative side of the PC1 and positive side of the PC2 is including the photic layer of the southern stations (6 ,7, 8, 9) and related with relatively higher salinity, temperature values and lower nutrient, oxygen concentrations.

Table 3.1 The eigenvectors of the variables for the first three principle components in the photic zone of the Aegean Sea in March 2012.

	PC 1	PC 2	PC 3
Chl a	0.40411	-0.0067719	0.0080027
Temperature (°C)	-0.42076	-0.088243	-0.050796
Salinity (psu)	-0.38583	-0.21655	0.082213
DO (ml/l)	0.18898	0.25617	0.64935
TSS (mg/l)	0.27067	-0.13	-0.12506
NO ₃ (μM)	-0.18816	0.41374	0.4181
NO ₂ (μM)	-0.21619	0.47639	-0.02051
PO ₄ (μM)	-0.056655	0.43196	-0.5267
Si (μM)	0.39138	-0.0025066	-0.18257
POC (μM)	0.38915	0.046069	0.14738
TOC (μM)	0.10889	0.52526	-0.21357

In the aphotic zone of the Aegean Sea, the first three principal component axis explains 84.8% of the total variance; first principal component (PC1), second principal component (PC2) and third principal component (PC3) explains 49.9%, 20.9% and 13.8% of the variance, respectively. 50% of the variance is related to Chl a, nitrate, phosphate and DO, 20% of the variance is related to temperature, salinity, silicate and nitrite. PC1 separated stations 6C, 7C and 8C from the other stations distinctively. These stations ordinated at the more negative side of the PC1 axis and related with relatively higher nutrient concentrations. PC1 correlated positively with DO, Chl a, POC, TSS and negatively with nutrients. Aphotic zone of St 10C not affected from variables related with PC1. PC2 correlated positively with nitrite, temperature and salinity and negatively with nutrients (Table 3.2). In the diagram station 1C ordinated in the very positive side other PC1 and related with relatively higher particulate matter (Chl a, POC, TSS) concentrations.

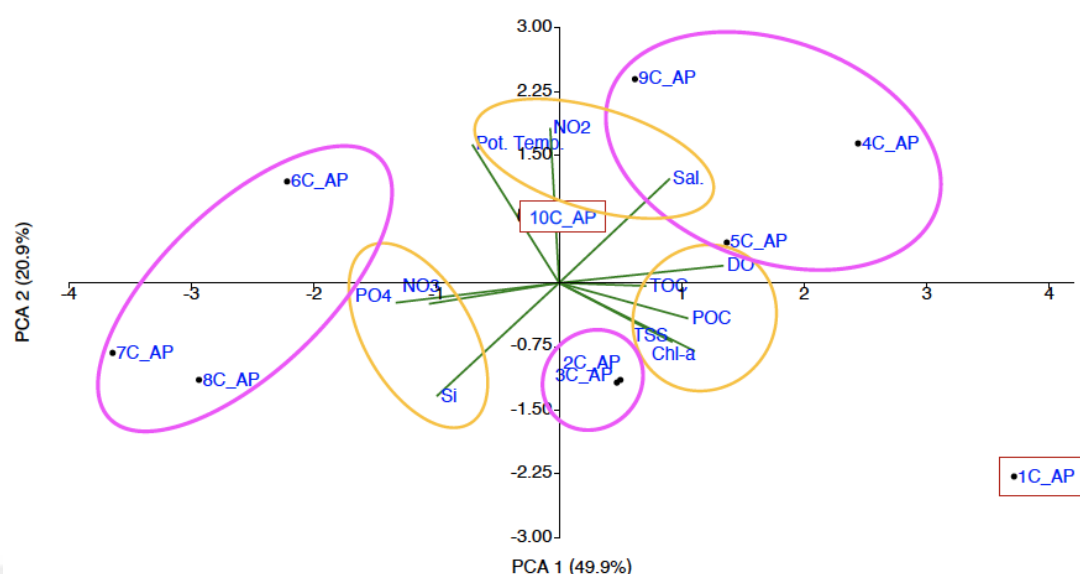


Figure 3.23 PCA diagram showing variance in the aphotic zone in the Aegean Sea in March 2012

Table 3.2 The eigenvectors of the variables for the first three principle components in the photic zone of the Aegean Sea in March 2012.

	PC 1	PC 2	PC 3
Chl a	0.33815	-0.24563	-0.2729
Temperature (°C)	-0.21633	0.49702	-0.25247
Salinity (psu)	0.27594	0.37478	-0.3287
DO (ml/l)	0.40978	0.061945	0.089732
TSS (mg/l)	0.28236	-0.21261	-0.30829
NO ₃ (μM)	-0.32431	-0.075314	0.21992
NO ₂ (μM)	-0.022179	0.55688	0.26089
PO ₄ (μM)	-0.40716	-0.071277	0.085901
Si (μM)	-0.30494	-0.40724	-0.11032
POC (μM)	0.32166	-0.12774	0.39487
TOC (μM)	0.21717	-0.010929	0.59895

To determine the spatial structure of the Aegean Sea, Moran's I values and correlograms were used for Spatial Autocorrelation Analysis. Resulted correlograms for the environmental variables in Aegean Sea in March 2012 are given in Figure 3.24. Temperature, DO, salinity and silicate typically showed gradient-like structure, where correlation values were positive at short distances, but negative at longer distances. Density and phosphate showed nearly gradient-like structure with negative autocorrelation in the first distance class and positive autocorrelation in the last distance class. Nitrite exhibited patchlike structures while nitrate was showing no oscillations, Moran's I values were generally around zero. Particulate matter (TSS, Chl

a and POC) showed significant spatial autocorrelation. TSS was showing no oscillations while Chl a and POC indicated a patchy structure (large oscillations), indicating decreasing positive spatial autocorrelation at small spatial scales (i.e., till 150 km), whereas at intermediate scales (i.e., at around 200-250 km), autocorrelation significantly increasing within a sharp decrease at around 320 km and oscillated at negative side at largest spatial scales.

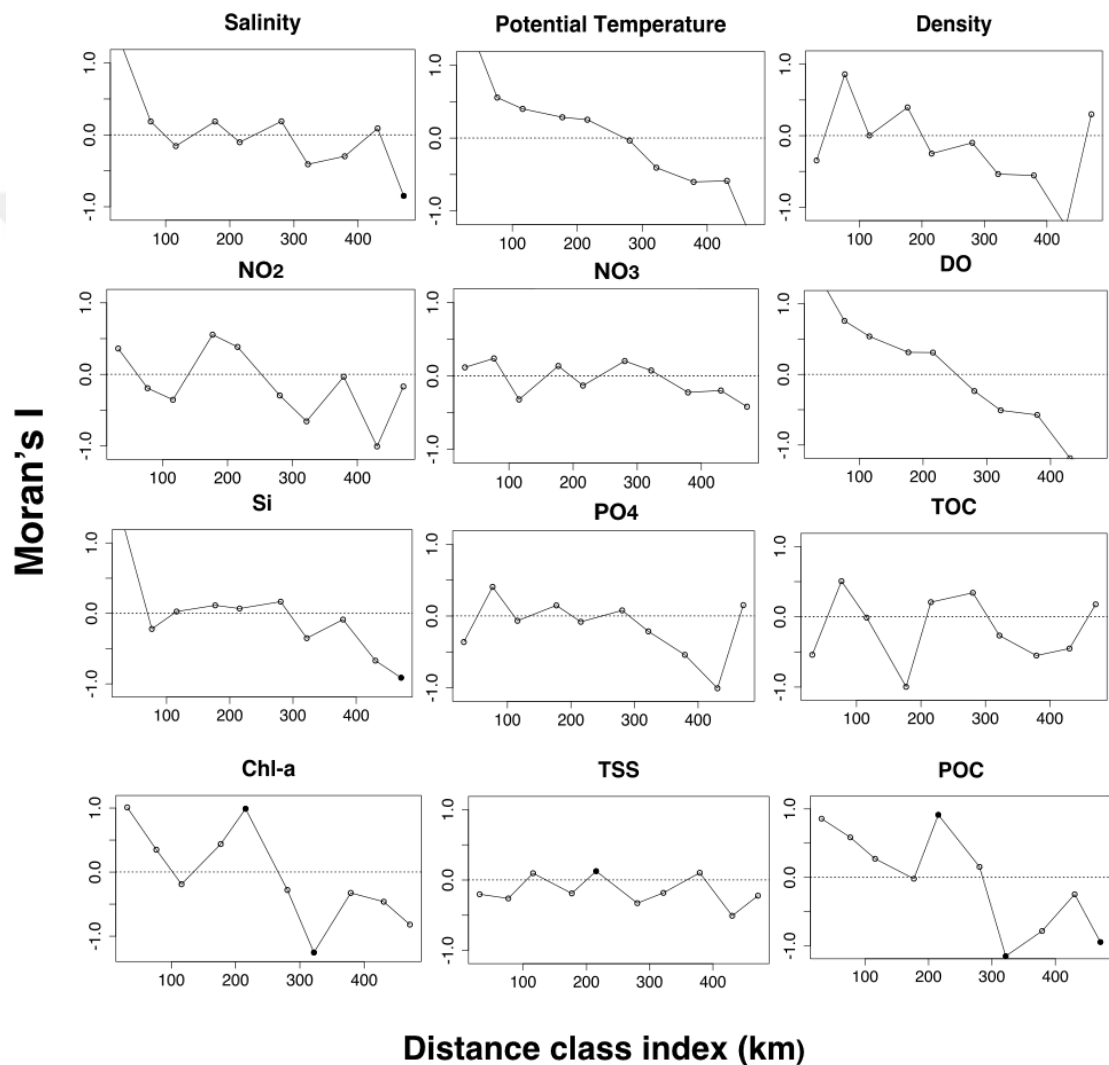


Figure 3.24 Spatial autocorrelation of environmental variables in the Aegean Sea based on Moran's I with 999 permutations. The solid circles indicate the significantly autocorrelated ($p < 0.05$)

3.2 Sediment

3.2.1 Physicochemical Parameters

Organic carbon and nitrogen percentages in total in the sediment results were given in the figure 3.25. The highest OC% values are measured as 12%, 8% and 9% in St 3C, 4C and 6C, respectively. Except the maxima, values were usually less than 5%. Values were quite high for open sea as the overall CHN results in the water column. The maximum OC values were higher than the values observed in a fish farming area in Gulf of Ildiri (9%) in the Aegean Sea.

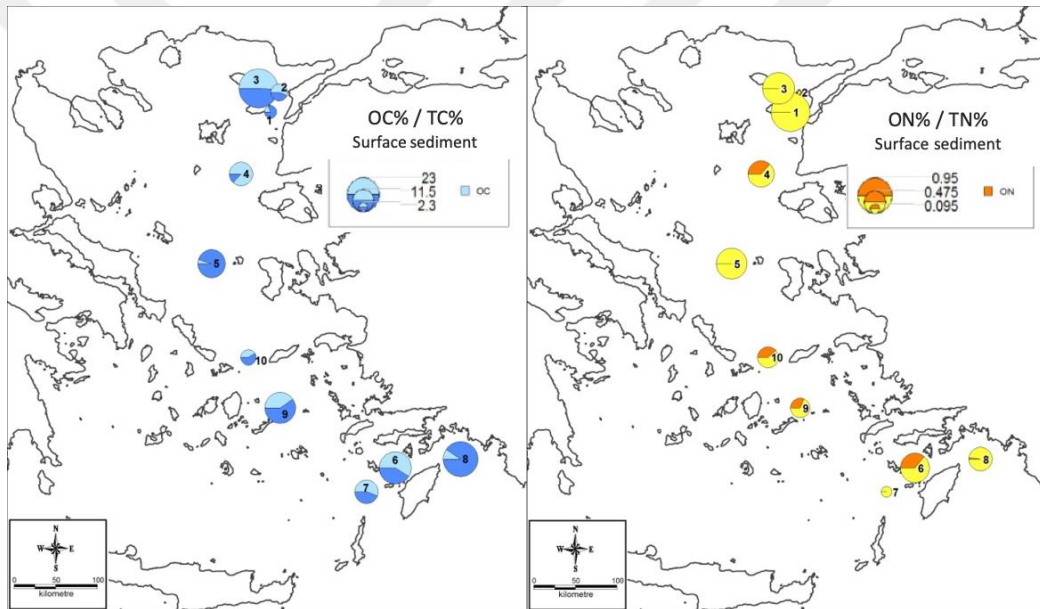


Figure 3.25 Sediment OC (a) and ON (b) percent distribution in the Aegean Sea in March 2012

Sediment grain size classification were given in the Figure 3.26. Only in the station 1C, there is no information of silt/clay proportions in the fine fraction according to the procedure that the hydrometer analysis is redundant for the samples of dominated by coarse material. Station 2C and 4C have higher percentage of sand than the other stations. Except the stations 1C, 2C and 4C, the other stations have a higher silt/clay content. In deeper stations in the south high numbers of foraminiferal linings have been observed.

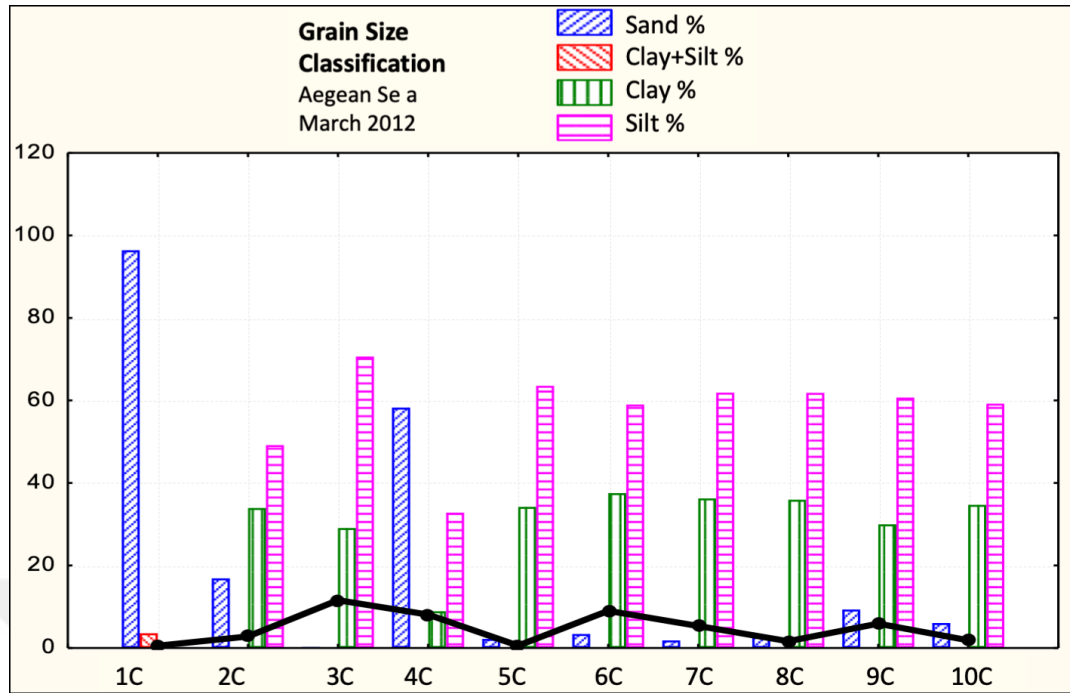


Figure 3.26 Sediment grain size distribution in the Aegean Sea in March 2012

3.2.2 Dinoflagellate Cysts

A dinoflagellate cyst community with high diversity has been observed in Aegean Sea which comprising different kind of water masses. Totally 14 sediment samples from two different sampling periods were collected. 12 of the stations were located in Aegean Sea while only two of them from Eastern Mediterranean Sea. Modern dinoflagellate cyst abundances ranged from 70 to 2222 cysts per gram dried sediment (max: St2C, min: St1C) with an average value of 723 cysts/g (Table 3.2). Station 1C sediment showed different characteristics of the grain size that 93% of the sediment was bigger than 120 μm and content of the coarse material was mainly sand and shells. This may be associated with the position of the northernmost station in the entrance of the Çanakkale Strait. Except for the low values observed in the station 1C, abundances were found higher in the Black Sea influenced northern waters than the oligotrophic Eastern Mediterranean waters.

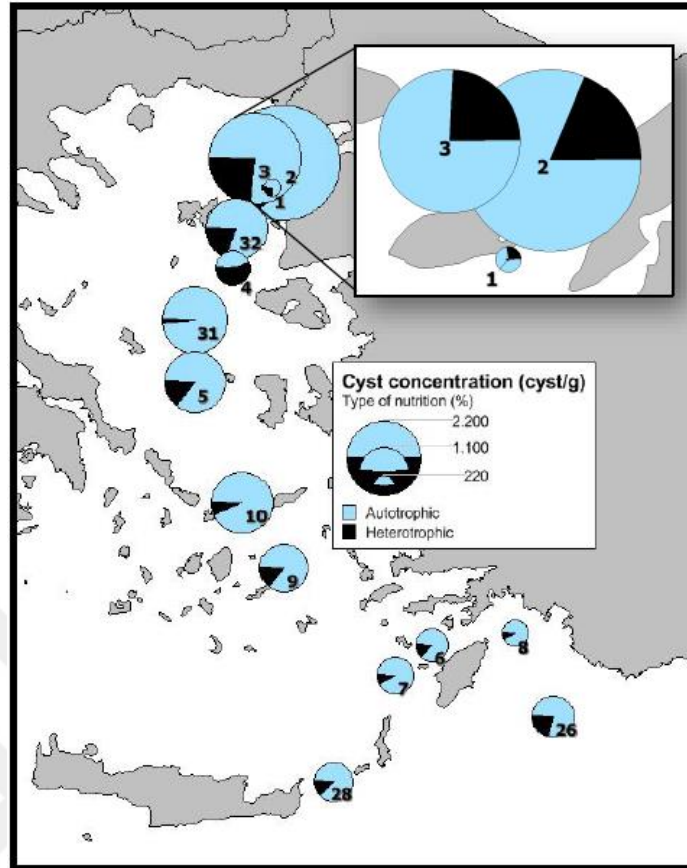


Figure 3.27 Heterotrophic and autotrophic proportions of cyst concentrations in the Aegean Sea

Figure 3.27 illustrates the pattern of the heterotrophic/autotrophic-related taxon in relation to geographical location of sites. The relative abundance of cysts produced by autotrophic dinoflagellates were dominant at all stations and their abundance ranged from 73 to 98%, except St 4C (45%). While the dominance of the autotrophic cysts is clear, the heterotrophic cyst proportion in the dinoflagellate cyst assemblages was slightly decreasing through the south.

Table 3.3 Taxonomic citation of dinoflagellate cysts used in this study

Cyst species (paleontological name)	References	Dinoflagellate thecate affinity (biological name)	HAB sp
AUTOTROPHIC TAXA			
Gonyaulacaceae			
cyst of <i>Alexandrium</i> spp.	Halim 1960 emend. Balech 1990	<i>Alexandrium</i> spp.	yes
cf. <i>Ataxiodinium choane</i>	Reid 1974	<i>Gonyaulax spinifera</i> complex	no
<i>Achomosphaera andalousiense</i>	Jan du Chêne 1977, emend. Jan du Chêne & Londeix 1988	? <i>Gonyaulax</i> sp. indet.	no
<i>Bitectatodinium tepikiense</i>	Wilson 1973	<i>Gonyaulax spinifera</i>	no
<i>Impagidinium aculeatum</i>	(Wall 1967) Lentin et Williams 1981	<i>Gonyaulax</i> sp. indet.	no
<i>Impagidinium</i> cf. <i>paradoxum</i>	(Wall 1967) Stover et Evitt 1978	<i>Gonyaulax</i> sp. indet.	no
<i>Impagidinium</i> cf. <i>patulum</i>	(Wall 1967) Stover et Evitt 1978	<i>Gonyaulax</i> sp. indet.	no
<i>Impagidinium</i> cf. <i>plicatum</i>	Versteegh et Zevenboom 1995	<i>Gonyaulax</i> sp. indet.	no
<i>Impagidinium striatum</i>	(Wall 1967) Stover et Evitt 1978	<i>Gonyaulax</i> sp. indet.	no
<i>Impagidinium velorum</i>	(Wall 1967) Stover et Evitt 1978	<i>Gonyaulax</i> sp. indet.	no
<i>Impagidinium</i> sp. T1	Indeterminant	<i>Gonyaulax</i> sp. indet.	no
<i>Impagidinium</i> sp. T2	Indeterminant	<i>Gonyaulax</i> sp. indet.	no
<i>Impagidinium</i> spp.	Stover et Evitt 1978	<i>Gonyaulax</i> sp. indet.	no
<i>Lingulodinium machaerophorum</i>	(Deflandre and Cookson 1955) Wall 1967	<i>Lingulodinium polyedrum</i>	yes
<i>Nematosphaeropsis labyrinthus</i>	(Ostenfeld 1903) Reid 1974	<i>Gonyaulax spinifera</i> complex	no
<i>Operculodinium centrocarpum</i>	sensu Wall et Dale 1966	<i>Protoceratium reticulatum</i>	yes
<i>Operculodinium israelianum</i>	(Rossignol 1962) Wall 1967	? <i>Protoceratium reticulatum</i>	no
<i>Operculodinium janduchenei</i>	Head et al. 1989	Unknown	?
<i>Operculodinium</i> spp.	Wall 1967	<i>Protoceratium</i> spp.	no
<i>Polysphaeridium zoharyi</i>	(Rossignol 1962) Bujak et al. 1980	<i>Pyrodinium bahamense</i>	yes
<i>Pyxidinosia reticulata</i>	(McMinn et Sun 1994) Marret et de Vernal 1997	Unknown	?
<i>Spiniferites cruciformis</i>	Wall and Dale in Wall et al. 1973	Gonyaulacaceae	no
<i>Spiniferites</i> cf. <i>hyperachantus</i>	(Deflandre and Cookson 1955) Cookson and Eisenack 1974	<i>Gonyaulax</i> sp. indet.	no
<i>Spiniferites</i> cf. <i>lazus</i>	Reid 1974	<i>Gonyaulax</i> sp. indet.	no
<i>Spiniferites</i> cf. <i>multisphaerus</i>	Price and Pospelova 2014	Unknown	no
<i>Spiniferites mirabilis</i>	(Rossignol 1964) Sarjeant 1970	<i>Gonyaulax spinifera</i> complex	no
<i>Spiniferites ramosus</i>	(Ehrenberg 1838) Mantell 1854	<i>Gonyaulax spinifera</i> complex	no
<i>Spiniferites</i> cf. <i>ristingensis</i>	Head 2007	Unknown	no
<i>Spiniferites</i> sp. T1	Indeterminant	<i>Gonyaulax</i> sp. indet.	no
<i>Spiniferites</i> spp.	Mantell, 1850 emend. Sarjeant, 1970	<i>Gonyaulax</i> spp.	no
Suessiaceae			
cyst of <i>Biecheleria</i> spp.	Moestrup, Lindberg & Daugbjerg, 2009	<i>Biecheleria</i> spp.	
Peridiniaceae			
cyst of <i>Pentapharsodinium dalei</i>	Indelicato et Loeblich III 1986	<i>Pentapharsodinium dalei</i>	no

Table 3.3 continues

Cyst species (paleontological name)	References	Dinoflagellate thecate affinity (biological name)	HAB sp
HETEROTROPHIC TAXA			
Diplopsalidaceae			
<i>Dubridinium</i> spp.	Reid, 1977	Diplopsalid group	no
Protopteridiniaceae			
<i>Brigantedinium carioense</i>	(Wall, 1967) Lentin and Williams, 1993	<i>Protopteridinium avellanum</i>	no
<i>Brigantedinium irregulare</i>	Matsuoka 1987b	<i>Protopteridinium denticulatum</i>	no
<i>Brigantedinium simplex</i>	Wall 1965 ex Lentin & Williams 1993	<i>Protopteridinium conicoides</i>	no
<i>Brigantedinium</i> spp.	Reid, 1977 ex Lentin and Williams, 1993	? <i>Protopteridinium</i> spp.	no
<i>Echinidinium</i> spp.	Zonneveld, 1997	<i>Protopteridinium</i> spp. indet.	no
<i>Echinidinium</i> cf. <i>delicatum</i>	Zonneveld, 1997	<i>Protopteridinium</i> sp. indet.	no
cyst of <i>Gymnodinium nolleri</i>	Ellegaard and Moestrup, 1999	<i>Gymnodinium nolleri</i>	no
cyst of <i>Gymnodinium</i> spp.	Stein, 1878	<i>Gymnodinium</i> spp.	no
<i>Lejeunecysta marieae</i>	(Harland in Harland et al., 1991) Lentin et Williams, 1993	<i>Protopteridinium</i> sp. indet.	no
<i>Peridinium ponticum</i>	Wall and Dale in Wall et al., 1973	<i>Protopteridinium</i> sp. or ? <i>P. divergens</i>	no
cyst of <i>Protopteridinium oblongum</i>	Wall and Dale, 1968	<i>Protopteridinium oblongum</i>	no
<i>Protopteridinium</i> sp. type F	Indeterminant	<i>Protopteridinium</i> sp. indet.	no
<i>Protopteridinium</i> spp.	Bergh, 1881	<i>Protopteridinium</i> sp. indet.	no
<i>Quinquecuspidata concreta</i>	(Reid, 1977) Harland, 1977	<i>Protopteridinium leonis</i>	no
<i>Selenopemphix nephroides</i>	(Benedek 1972) Benedek et Sarjeant, 1981	<i>Protopteridinium subinermis</i>	no
<i>Selenopemphix undulata</i>	Verleye et al., 2011	<i>Protopteridinium</i> sp. indet.	no
<i>Selenopemphix quanta</i>	(Bradford, 1975) Matsuoka, 1985	<i>Protopteridinium conicum</i>	no
<i>Stelladinium stellatum</i>	(Wall) Reid 1977	<i>Protopteridinium compressum</i>	no
<i>Stelladinium</i> cf. <i>robustum</i>	Zonneveld 1997	? <i>Protopteridinium compressum</i>	no
<i>Stelladinium</i> spp.	Bradford 1975	<i>Protopteridinium</i> spp.	no
<i>Trinovantedinium appplanatum</i>	(Bradford 1977) Bujak et Davies 1983	<i>Protopteridinium pentagonum</i>	no
<i>Votadinium</i> cf. <i>calvum</i>	Reid 1977	<i>Protopteridinium oblongum</i>	no

In total, 56 different taxa belonging to 25 genera and 5 families, identified and quantified. Nomenclature for modern dinoflagellate cysts and thecate names refers to Zonneveld & Pospelova (2015). The list of dinoflagellate cysts identified with their paleontological names, abbreviations and biological affinities with the taxonomic references and toxicity information is given in the Table 3.3. Images of all dinoflagellate cyst taxa recorded in this study are provided in Figure 3.28-32.

Table. 3.4 Abundances of species, total cyst counts and concentrations in each station and station depths in the Aegean Sea

Station ID	1C	2C	3C	4C	5C	31G	32G
Depth (m)	71	429	880	256	688	220	95
Total Cyst Counted	299	303	310	309	316	200	338
Total Cyst Concentration (cysts/g)	70	2077	1531	252	749	202	272
Autotrophic species							
<i>Alexandrium</i> spp.	6.7	0.8	0.3	0.6	0.0	0.0	0.3
cf. <i>Ataxiodinium choane</i>	0.3	0.0	0.0	0.0	0.0	0.3	0.0
<i>Achomosphaera andalousiense</i>	0.0	1.5	0.0	0.0	0.6	0.0	0.3
<i>Biecheleria</i> spp.	25.8	8.6	3.2	0.3	3.5	0.9	2.4
<i>Bitectatodinium tepikiense</i>	0.7	2.3	2.3	0.0	0.9	1.2	0.6
<i>Filisphaera filifera</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Impagidinium aculeatum</i>	0.0	0.8	1.0	2.3	20.6	3.0	0.3
<i>Impagidinium</i> cf. <i>paradoxum</i>	0.0	0.2	0.6	0.3	2.5	1.5	0.3
<i>Impagidinium</i> cf. <i>patulum</i>	0.0	0.0	0.0	1.0	0.3	0.3	0.3
<i>Impagidinium</i> cf. <i>plicatum</i>	0.0	0.0	0.0	0.0	0.0	0.3	0.0
<i>Impagidinium striatum</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Impagidinium velorum</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Impagidinium</i> spp.	0.0	0.2	0.0	0.3	4.7	10.4	0.3
<i>Lingulodinium machaerophorum</i>	3.3	4.2	9.0	1.3	3.5	2.7	5.8
<i>Nematosphaeropsis labyrinthus</i>	0.0	0.8	1.6	2.6	4.4	7.7	0.3
<i>Operculodinium centrocarpum</i>	20.7	22.8	29.7	17.2	16.8	22.3	32.8
<i>Operculodinium israelianum</i>	0.0	1.2	0.6	2.3	2.5	7.4	1.2
<i>Operculodinium janduchenei</i>	0.0	0.0	0.0	0.0	0.0	0.3	0.0
<i>Operculodinium</i> spp.	0.3	0.0	0.0	0.0	0.0	0.0	0.0
<i>Pentapharsodinium dalei</i>	0.3	0.3	0.0	0.0	0.3	0.6	0.0
<i>Polysphaeridium zoharyi</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Pyxidinosia reticulata</i>	0.0	0.0	0.0	0.0	0.0	0.6	0.0
<i>Spiniferites cruciformis</i>	0.0	0.5	0.0	0.0	0.0	0.0	0.0
<i>Spiniferites hyperachantus</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.6
<i>Spiniferites</i> cf. <i>latus</i>	0.0	0.3	1.3	0.0	0.6	1.8	0.9
<i>Spiniferites</i> cf. <i>multisphaerus</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Spiniferites mirabilis</i>	1.7	5.3	2.9	2.6	5.7	6.0	6.4
<i>Spiniferites ramosus</i>	4.7	14.6	6.8	1.3	2.8	5.7	6.1
<i>Spiniferites</i> cf. <i>ristingensis</i>	0.0	0.3	0.0	0.0	1.6	0.9	0.9
<i>Spiniferites</i> spp.	7.4	16.4	14.2	13.3	13.3	24.1	19.8
Heterotrophic species							
<i>Brigantedinium cariacense</i>	0.0	0.0	0.3	0.0	0.0	0.0	0.0
<i>Brigantedinium irregulare</i>	0.0	0.2	0.0	0.0	0.0	0.0	0.0
<i>Brigantedinium simplex</i>	0.3	0.2	0.0	0.0	0.0	0.0	0.0
<i>Brigantedinium</i> spp.	13.7	7.3	12.3	12.3	9.2	0.9	7.0
<i>Dubridinium</i> spp.	0.0	0.0	0.3	0.0	0.0	0.0	0.0
<i>Echinidinium</i> spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.3
<i>Echinidinium</i> cf. <i>delicatum</i>	0.3	0.2	0.0	0.0	0.0	0.0	0.0
<i>Gymnodinium nolleri</i>	0.7	1.5	0.6	39.2	0.0	0.3	8.2
<i>Gymnodinium</i> spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Lejeunecysta marieae</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Peridinium ponticum</i>	0.0	0.2	0.3	0.0	0.0	0.0	0.0
<i>Protoperidinium oblongum</i>	0.0	0.2	0.0	0.0	0.0	0.0	0.0
<i>Protoperidinium</i> type F	0.0	0.0	0.0	0.0	0.0	0.0	0.3
<i>Protoperidinium</i> spp.	1.0	0.7	0.0	0.0	0.0	0.0	0.0
<i>Quinquecupis concreta</i>	0.0	0.3	0.0	0.0	0.0	0.0	0.3
<i>Selenopemphix nephroides</i>	1.0	0.5	0.6	1.3	2.5	0.0	1.2
<i>Selenopemphix undulata</i>	0.3	0.2	0.0	0.0	0.9	0.3	0.0
<i>Selenopemphix quanta</i>	7.4	3.6	6.8	0.3	0.0	0.0	1.5
<i>Stelladinium stellatum</i>	1.0	0.8	0.0	0.3	0.0	0.0	0.0
<i>Stelladinium</i> cf. <i>robustum</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Stelladinium</i> spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Trinovantedinium applanatum</i>	0.3	1.6	1.3	0.0	2.5	0.6	0.9
<i>Votadinium</i> cf. <i>calvum</i>	0.3	0.3	0.6	0.0	0.0	0.0	0.0
Spiny brown	0.7	1.0	2.6	0.0	0.0	0.0	0.0
Unknown cyst	1.0	0.2	0.6	1.3	0.0	0.0	0.6

Table. 3.4 continues

Station ID	6C	7C	8C	9C	10C	26G	28G
Depth	704	1079	1384	416	600	>4000	1100
Total Cyst Counted	283	307	312	296	349	336	329
Total Cyst Concentration (cysts/g)	142	441	724	364	334	874	746
Autotrophic species							
<i>Alexandrium</i> spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
cf. <i>Ataxiodinium choane</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Achomosphaera andalousiense</i>	0.0	0.0	0.4	0.0	0.0	0.0	0.0
<i>Biecheleria</i> spp.	0.0	0.0	0.4	0.3	0.0	11.5	0.0
<i>Bitectatodinium tepikiense</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Filisphaera filifera</i>	0.0	0.0	0.0	0.0	0.6	0.3	0.0
<i>Impagidinium aculeatum</i>	15.0	26.9	21.6	17.3	14.4	27.4	20.6
<i>Impagidinium</i> cf. <i>paradoxum</i>	1.5	3.3	1.4	1.3	0.6	2.7	2.9
<i>Impagidinium</i> cf. <i>patulum</i>	1.5	4.4	3.2	0.3	0.0	9.5	2.0
<i>Impagidinium</i> cf. <i>plicatum</i>	0.0	0.3	0.0	0.7	0.3	0.0	0.6
<i>Impagidinium striolatum</i>	0.5	0.0	0.0	0.0	0.0	0.0	0.0
<i>Impagidinium velorum</i>	0.0	0.0	0.0	0.0	0.0	0.3	0.3
<i>Impagidinium</i> spp.	4.5	9.8	4.6	6.2	3.8	8.1	7.4
<i>Lingulodinium machaerophorum</i>	6.0	3.3	11.0	9.1	23.4	4.1	2.9
<i>Nematosphaeropsis labyrinthus</i>	5.5	3.6	3.9	5.9	3.5	3.0	3.7
<i>Operculodinium centrocarpum</i>	2.5	1.5	0.7	7.8	14.7	1.0	12.3
<i>Operculodinium israelianum</i>	8.5	12.1	9.5	6.8	6.1	0.3	7.7
<i>Operculodinium janduchenei</i>	0.5	0.6	0.4	0.0	0.3	0.0	0.6
<i>Operculodinium</i> sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.3
<i>Pentapharsodinium dalei</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Polysphaeridium zoharyi</i>	0.0	0.0	0.0	0.0	0.0	0.3	0.0
<i>Pyxidinospis reticulata</i>	0.0	1.5	0.7	1.0	0.0	0.3	1.4
<i>Spiniferites cruciformis</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Spiniferites hyperachantus</i>	0.0	0.0	0.0	1.0	0.0	0.0	0.3
<i>Spiniferites</i> cf. <i>lazus</i>	0.0	0.0	0.0	0.0	0.6	0.7	0.6
<i>Spiniferites</i> cf. <i>multisphaerus</i>	0.0	0.3	0.0	0.0	0.0	0.0	0.0
<i>Spiniferites mirabilis</i>	7.5	5.0	4.2	8.5	5.4	1.4	2.9
<i>Spiniferites ramosus</i>	9.5	1.5	4.2	2.3	1.9	0.7	1.1
<i>Spiniferites</i> cf. <i>ristingensis</i>	0.0	1.2	0.4	0.0	1.3	0.3	0.9
<i>Spiniferites</i> spp	22.5	16.0	23.3	16.9	15.4	5.7	17.8
Heterotrophic species							
<i>Brigantedinium cariacense</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Brigantedinium irregulare</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Brigantedinium simplex</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Brigantedinium</i> spp.	11.0	8.0	8.8	12.7	4.5	20.3	12.3
<i>Dubridinium</i> spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Echinidinium</i> spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Echinidinium</i> cf. <i>delicatum</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.3
<i>Gymnodinium nolleri</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Gymnodinium</i> spp.	0.0	0.0	0.4	0.0	0.0	0.0	0.3
<i>Lejeunecysta marieae</i>	0.5	0.0	0.0	0.0	0.0	0.0	0.0
<i>Peridinium ponticum</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Protoperidinium oblongum</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Protoperidinium</i> type F	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Protoperidinium</i> spp.	0.5	0.0	0.4	0.0	0.0	0.3	0.0
<i>Quinquecuspsis concreta</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Selenopemphix nephroides</i>	0.5	0.0	0.0	0.3	0.6	0.0	0.3
<i>Selenopemphix undulata</i>	0.0	0.3	0.0	0.3	0.0	0.0	0.0
<i>Selenopemphix quanta</i>	0.5	0.0	0.4	0.0	0.6	0.0	0.3
<i>Stelladinium stellatum</i>	1.5	0.0	0.0	1.0	0.3	0.0	0.0
<i>Stelladinium</i> cf. <i>robustum</i>	0.0	0.0	0.0	0.3	0.0	0.0	0.0
<i>Stelladinium</i> spp.	0.0	0.3	0.0	0.0	0.0	0.0	0.3
<i>Trinovantedinium applanatum</i>	0.0	0.3	0.4	0.0	1.0	1.0	0.0
<i>Votadinium</i> cf. <i>calvum</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Spiny brown	0.0	0.0	0.0	0.0	0.3	0.0	0.0
Unknown cyst	0.0	0.0	0.0	0.0	0.0	0.7	0.0

Dinoflagellate assemblages in the Aegean Sea are dominated by *Operculodinium centrocarpum*, *Spiniferites* spp., *Spiniferites ramosus*, *Brigantedinium* spp., *Impagidinium aculeatum* and *Operculodinium israelinum*. Based on the visual examination of the dataset, *Operculodinium centrocarpum* dominance in the northern stations has been replaced by *Impagidinium aculeatum* dominance in the southern stations. Relative abundances of the individual species, total counted cysts and total cyst concentrations in each station and depth of the stations are given in the Table 3.4. First part of the table includes northern stations in the Aegean Sea while second part is including southern stations in the Aegean Sea and EastMed Sea. *Brigantedinium* spp. has a wide distribution in the Aegean basin, no spatial difference (north-south) has been observed. Relative abundance values of *Brigantedinium* spp. ranged between 0.9-20.3 (%) and it reached the maximum value in the southernmost deepest station (26G, <4000m). *Impagidinium aculeatum* species have an increasing trend towards south. *Pentapaharsodinium dalei* and *Alexandrium* spp. were the autotrophic species that distributions restricted in the northernmost stations. All the peridinoid cysts generally showed higher relative abundances in the northern Aegean Sea.

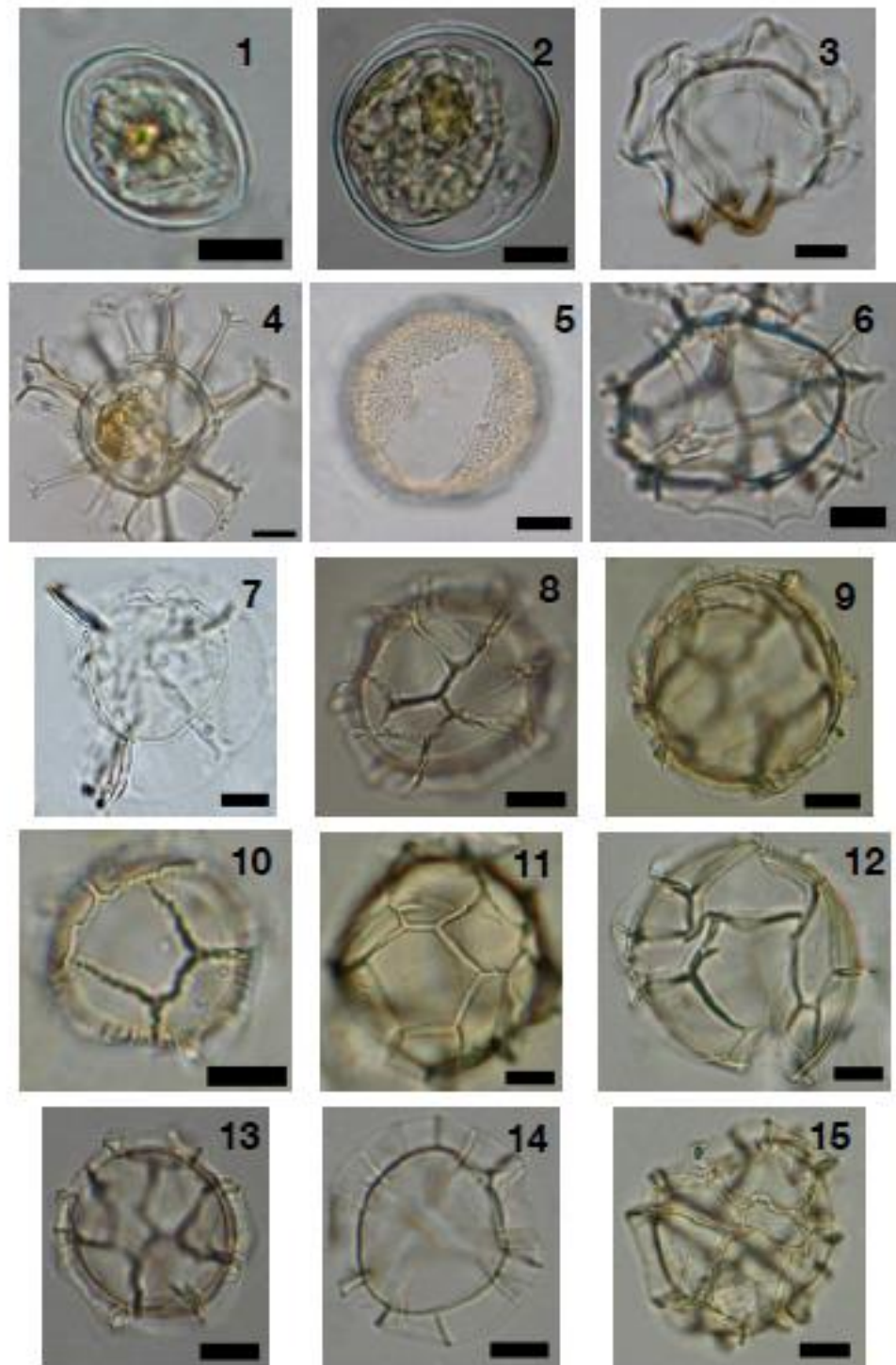


Figure 3.28 Bright field micrographs of the identified species_1 (1-2. *Alexandrium* sp. 3. cf. *Ataxiodinium choane* 4. *Achomosphaera andalousiense* 5. *Bitectatodinium tepikiense* 6. *Impagidinium aculeatum* 7. *Impagidinium velorum* 8-9. *Impagidinium* cf. *paradoxum* 10. *Impagidinium* cf. *plicatum* 11-12. *Impagidinium* cf. *patulum* 13. *Impagidinium strialatatum* 14. *Impagidinium* sp. T1 15. *Impagidinium* sp. T2) (photos by Vera Pospelova, Janset Kayaalp, Nihayet Bizsel)

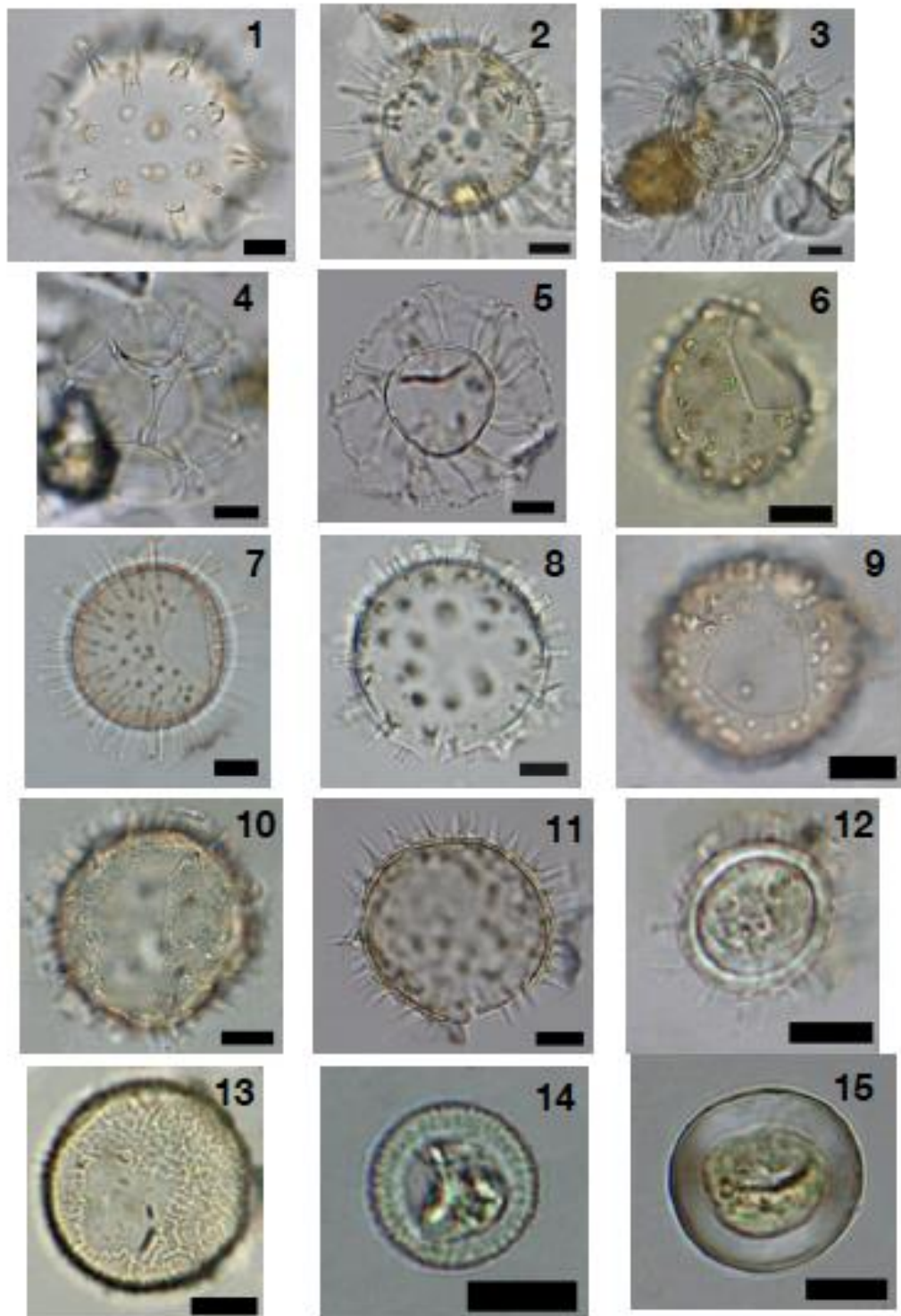


Figure 3.29 Bright field micrographs of the identified species_2 (1-3. *Lingulodinium machaerophorum* (3. long process) 4-5. *Nematosphaeropsis labyrinthus* 6. *Operculodinium janduchenei* 7-9. *Operculodinium centrocarpum* (8-9 short process) 10-11. *Operculodinium israelinum* 12. *Pentapharsodinium dalei* 13. *Pyxidinosopsis reticulata* 14-15. *Biecheleria* spp.) (15 without outer spiny layer) (photos by Vera Pospelova, Janset Kayaalp, Nihayet Bizsel)

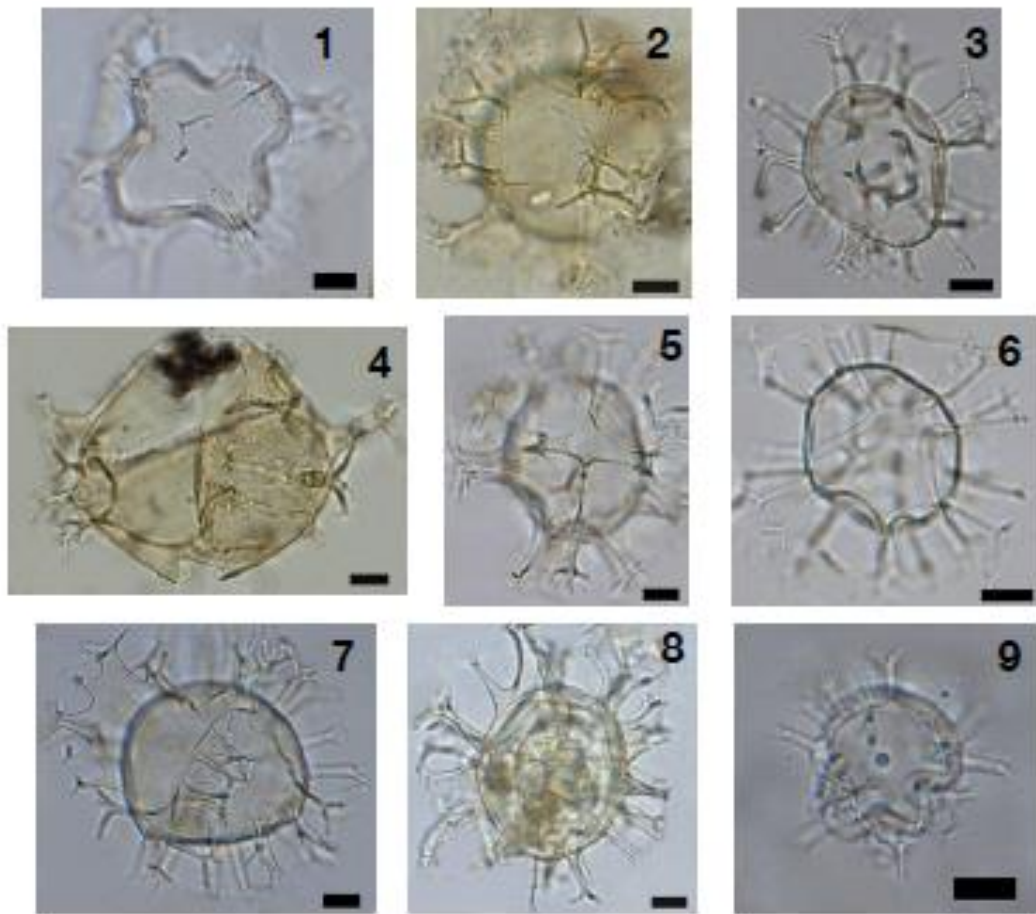


Figure 3.30 Bright field micrographs of the identified species_3 (1. *Spiniferites cruciformis* 2. *Spiniferites* cf. *hyperachantus* 3. *Spiniferites* cf. *lazus* 4. *Spiniferites* cf. *multisphaerus* 5. *Spiniferites* cf. *ristingensis* 6. *Spiniferites ramosus* 7-8. *Spiniferites mirabilis* 9. *Spiniferites* sp. T1) (photos by Vera Pospelova, Janset Kayaalp, Nihayet Bizsel)

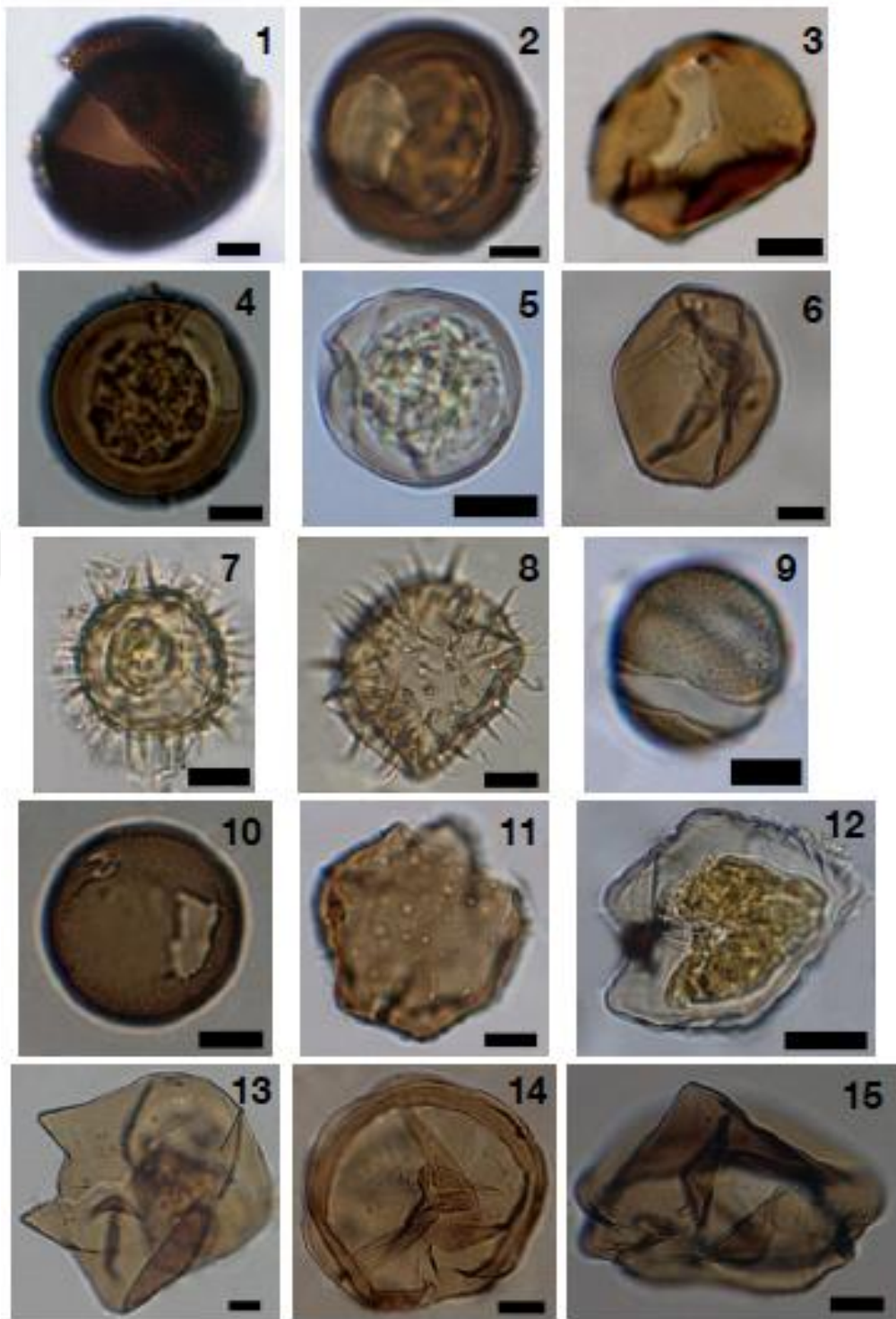


Figure 3.31 Bright field micrographs of the identified species (1. *Dubridinium* sp. 2. *Brigantedinium carioense* 3. *Brigantedinium irregulare* 4. *Brigantedinium simplex* 5-6. *Brigantedinium* spp. 7-8. *Echinidinium* spp. 9-10. *Gymnodinium nolleri* 11. *Peridinium ponticum* 12. cyst of *Protoperidinium oblongum* 13. *Quinquecusps concreta* 14-15. *Selenopemphix nephroides*) (photos by Vera Pospelova, Janset Kayaalp, Nihayet Bizsel)

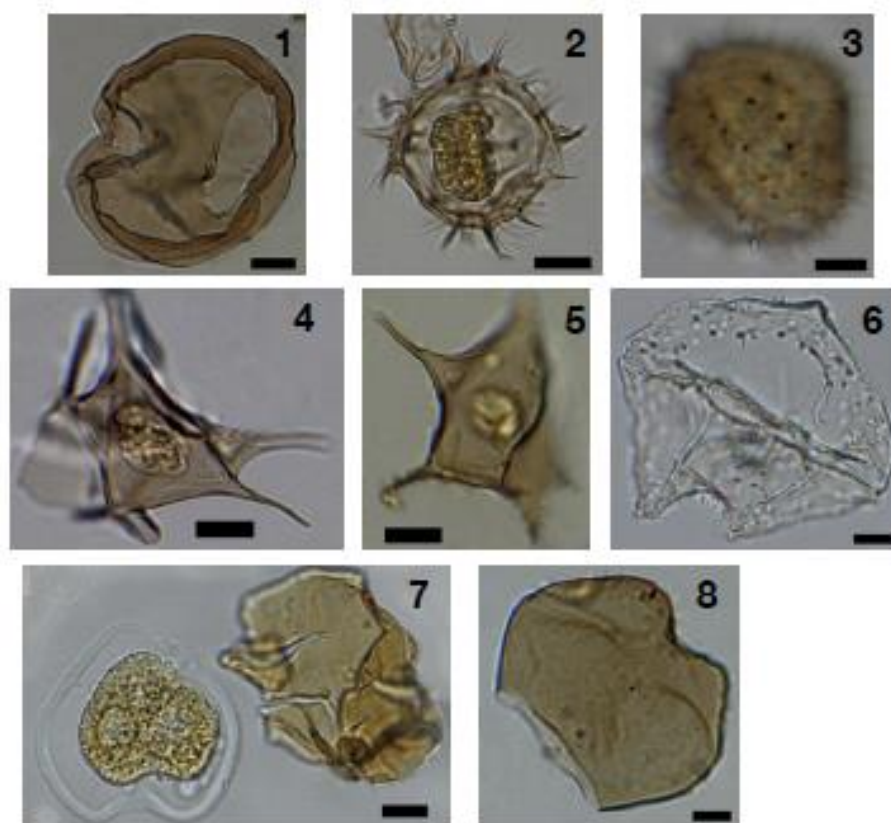


Figure 3.32 Bright field micrographs of the identified species (1. *Selenopemphix undulata* 2-3. *Selenopemphix quanta* 4. *Stelladinium stellatum* 5. *Stelladinium* cf. *robustum* 6. *Trinovantedinium applanatum* 7-8. *Votadinium* cf. *calvum*) (photos by Vera Pospelova, Janset Kayaalp, Nihayet Bizsel)

3.2.3 Statistical Analysis in the Sediment

The Shannon-Weaver diversity index, Pielou evenness index values of each station are given in Table 3.5. The highest number of species was found at St 2C and the highest diversity index value was found at St 5C. The lowest number of species, evenness and diversity index values were found at 4C station. At St 4C, high concentration of *Gymnodinium nolleri* compared to other species, reduced the evenness index and therefore caused to lower diversity index value. As stated above, this high concentration of *Gymnodinium nolleri* is related with deep water oxygen concentrations in the area. A similar situation was observed at St 32C. High concentration of *Operculodinium centrocarpum* and *Spiniferites* spp. caused to lower evenness index value (Table 3.5).

Table 3.5 Diversity index values of the species in the area

	S	J'	H'(loge)
1C	23	0.76	2.37
2C	30	0.74	2.50
3C	22	0.74	2.28
4C	18	0.66	1.91
5C	21	0.83	2.52
6C	19	0.81	2.40
7C	20	0.77	2.32
8C	21	0.75	2.29
9C	20	0.81	2.42
10C	20	0.77	2.30
26G	21	0.73	2.21
28G	25	0.75	2.42
31G	24	0.74	2.35
32G	27	0.68	2.23

PCA were performed to assess the nature of variability of the dinoflagellate cyst assemblages within the samples. Rare taxa were excluded from all statistical analyses. Abbreviations of the species names used in the visualization of the statistical analysis, can be checked from Table 3.6. The first three principal component axis explains 89.2% of the total variance; first principal component (PC1), second principal component (PC2) and third principal component (PC3) explains 66.1%, 17.8% and 5.3% of the variance, respectively. PCA diagram showing that the autotrophic taxa can be clearly distinguished from heterotrophic taxa according to PC1, except *Brigantedinium* spp. which were the only heterotrophic taxa that have a general distribution in the area in high abundances in each station. The other heterotrophic species ordinated at the left side of the first axis with the autotrophic species that generally showing a random distribution or occurring in low values. PC2 separates the species according to spatial distributions of the stations. The species ordinated at the bottom side of the second axis were the autotrophic group that characterized with higher abundances in the Eastern Mediterranean off-shore stations. Additionally, the species ordinated at the upper and more positive side of the second axis were organisms that showed higher abundances in the northern Aegean Sea.

Table 3.6 Abbreviation and nutrition types of the species used in statistical analysis

Species name	Abbreviations	Nutrition type
<i>Alexandrium</i> spp.	A.spp	A
cf. <i>Ataxodinium choane</i>	A.and	A
<i>Biechleria</i> spp.	Bie.spp	A
<i>Bitectatodinium tepikiense</i>	B.tep	A
<i>Impagidinium aculeatum</i>	I.ac	A
<i>Impagidinium</i> cf. <i>paradoxum</i>	I.cf.par	A
<i>Impagidinium</i> cf. <i>patulum</i>	I.cf.pat	A
<i>Impagidinium</i> cf. <i>plicatum</i>	I.cf.pli	A
<i>Impagidinium</i> spp.	I.spp	A
<i>Lingulodinium machaerophorum</i>	L.mac	A
<i>Nematosphaeropsis labyrinthus</i>	N.lab	A
<i>Operculodinium centrocarpum</i>	O.cen	A
<i>Operculodinium israelianum</i>	O.isr	A
<i>Operculodinium janduchenei</i>	O.jan	A
<i>Pentaparthodinium dalei</i>	P.dal	A
<i>Pyxidinospis reticulata</i>	P.ret	A
<i>Spiniferites mirabilis</i>	S.mir	A
<i>Spiniferites ramosus</i>	S.ram	A
<i>Spiniferites</i> spp	Spi.spp	A
<i>Brigantedinium</i> spp.	Bri.spp	H
<i>Gymnodinium nolleri</i>	G.nol	H
<i>Peridinium ponticum</i>	P.pon	H
<i>Quinquecupis concreta</i>	Q.con	H
<i>Selenopemphix nephroides</i>	S.nep	H
<i>Selenopemphix undulata</i>	S.und	H
<i>Selenopemphix quanta</i>	S.qua	H
<i>Stelladinium stellatum</i>	S.ste	H
<i>Trinovantedinium applanatum</i>	T.app	H
<i>Votadinium</i> cf. <i>calvum</i>	V.cf.cal	H

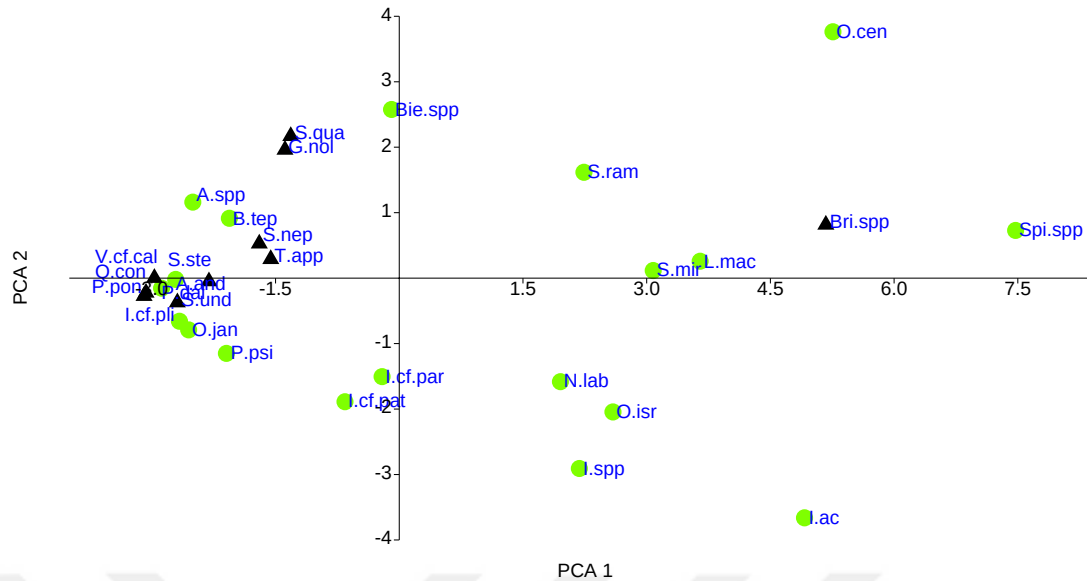


Figure 3.33 PCA diagram showing variance of the modern dinoflagellate species distribution in the sediment (Circles represent phototrophic taxa and triangles represent heterotrophic taxa)

To determine the most important environmental parameters that influencing dinocyst distributions, CCA performed. Figure 3.34 shows the resulting diagram of the first CCA analysis. The first three CCA axes have eigenvalues of 0.236, 0.072 and 0.044 and explain 57%, 17% and 11% (86% in total) of the species–environment relation, respectively. Because of the lowest number of species, evenness and diversity index values found at St 4C and the dominant species, *Gymnodinium nolleri* (39%), at St 4C, first CCA result was not distinguishable. To achieve more explicit image to assess species distribution CCA has implemented without St 4C, again (Figure 3.35). For the second CCA analysis, the first three CCA axes have eigenvalues of 0.211, 0.046 and 0.025 and explain 64.5%, 13.9% and 7.5% (85.9% in total) of the species–environment relation, respectively. Sea surface temperature and sea surface salinity gradients pointed into the same direction, opposite to the bottom water oxygen and sea surface nitrate, phosphate and Chl a. *Impagidinium aculeatum*, *Impagidinium cf. paradoxum*, *Impagidinium cf. patulum*, *Impagidinium cf. plicatum*, *Impagidinium spp.*, *Operculodinium israelianum*, *Operculodinium janduchenei*, *Pyxidinospis reticulata* and *Nematosphaeropsis labyrinthus* were ordinated at the positive side of the salinity and temperature gradients. This group is the one ordinated bottom side of the second axis in the PCA diagram and belongs to Eastern Mediterranean off-shore

organisms. *Selenopemphix nephroides*, *Selenopemphix undulata*, *Achomosphaera andalousiense*, *Bitectatodinium tepikiense*, *Pentapharsodinium dalei* and *Quinquecuspis concreta* are ordinated at the positive side of the bottom water oxygen. *Gymnodinium nolleri* was also in this grouping according to first CCA results.

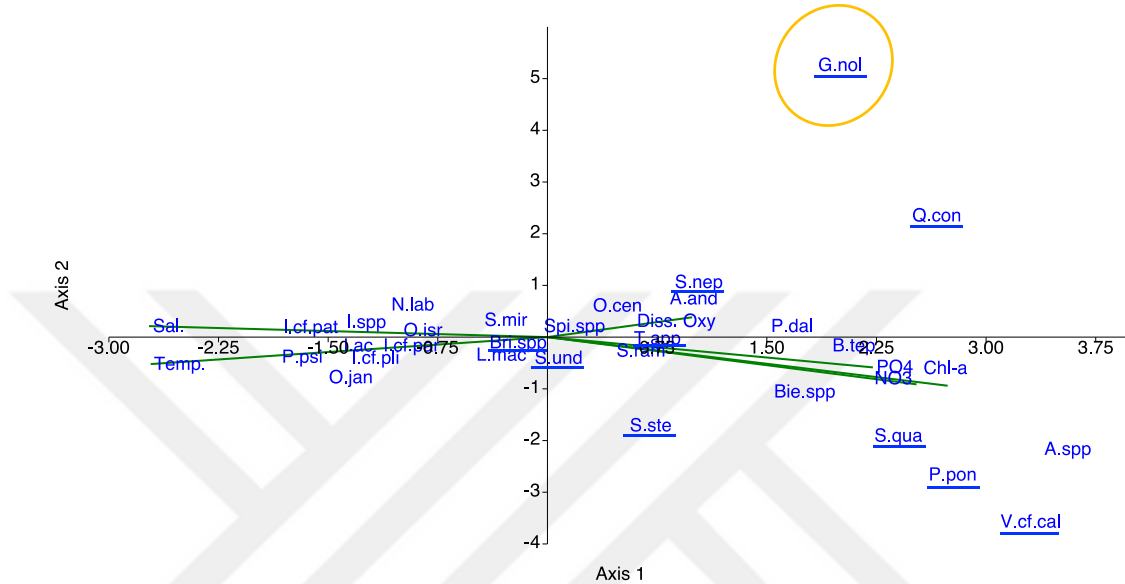


Figure 3.34 Ordination diagram of the CCA analysis (Arrows represent environmental parameters. Heterotrophic species are underlined)

Selenopemphix quanta, *Biecheleria* spp., *Peridinium ponticum*, *Alexandrium* spp. and *Votadinium cf. calvum* were ordinated at the more positive side of the phosphate, nitrate and Chl a gradients. *Brigantedinium* spp., *Stelladinium stellatum*, *Spiniferites ramosus*, *Spiniferites* spp., *Spiniferites mirabilis*, *Lingulodinium machaerophorum*, *Trinovantedinium applanatum* and *Operculodinium centrocarpum* were ordinated in the central part of the diagram. These species have a random distribution and generally seen at all stations in high concentrations.

To determine the spatial structure in the Aegean Sea sediments, Moran's I values and correlograms were used for Spatial Autocorrelation Analysis. Resulted correlograms for the environmental variables in Aegean Sea sediments in March 2012 are given in Figure 3.36. Both total and organic fractions of carbon and nitrogen percentages in the sediment showed a patchy structure and especially the organic

fraction parameters have highly large oscillations. Importantly, no significant spatial autocorrelation was observed in the sediment chemical data.

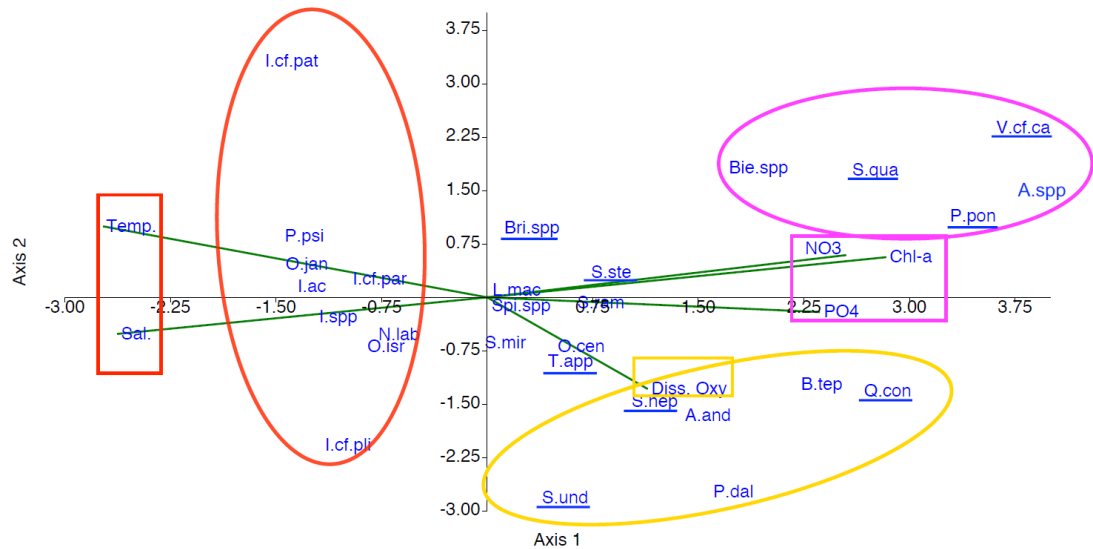


Figure 3.35 Ordination diagram of the CCA analysis except St 4C (Arrows represent environmental parameters. Heterotrophic species are underlined)

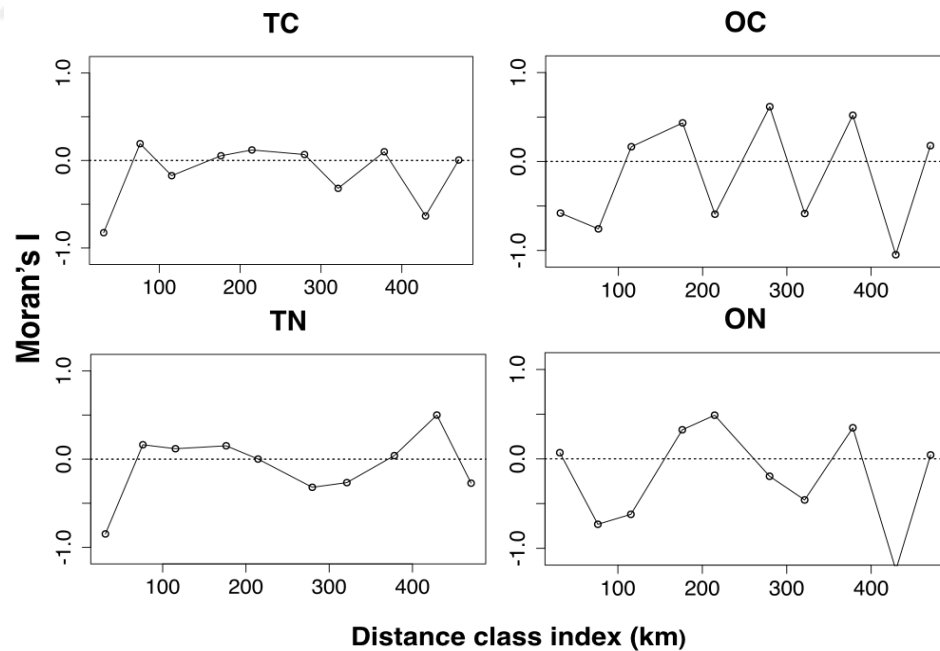


Figure 3.36 Spatial autocorrelation of total/organic carbon and nitrogen based on Moran's I with 999 permutations. The solid circles indicate the significantly autocorrelated ($p < 0.05$)

Percentage values of heterotroph/autotroph species spatial autocorrelation was not significant and did not show remarkable oscillations. On the other hand, concentration values of total, heterotrophic and autotrophic dinoflagellate cysts significantly autocorrelated and indicated slight gradient-like structure where correlation values were positive at shorter distances than 300-350 km, but negative at longer distances.

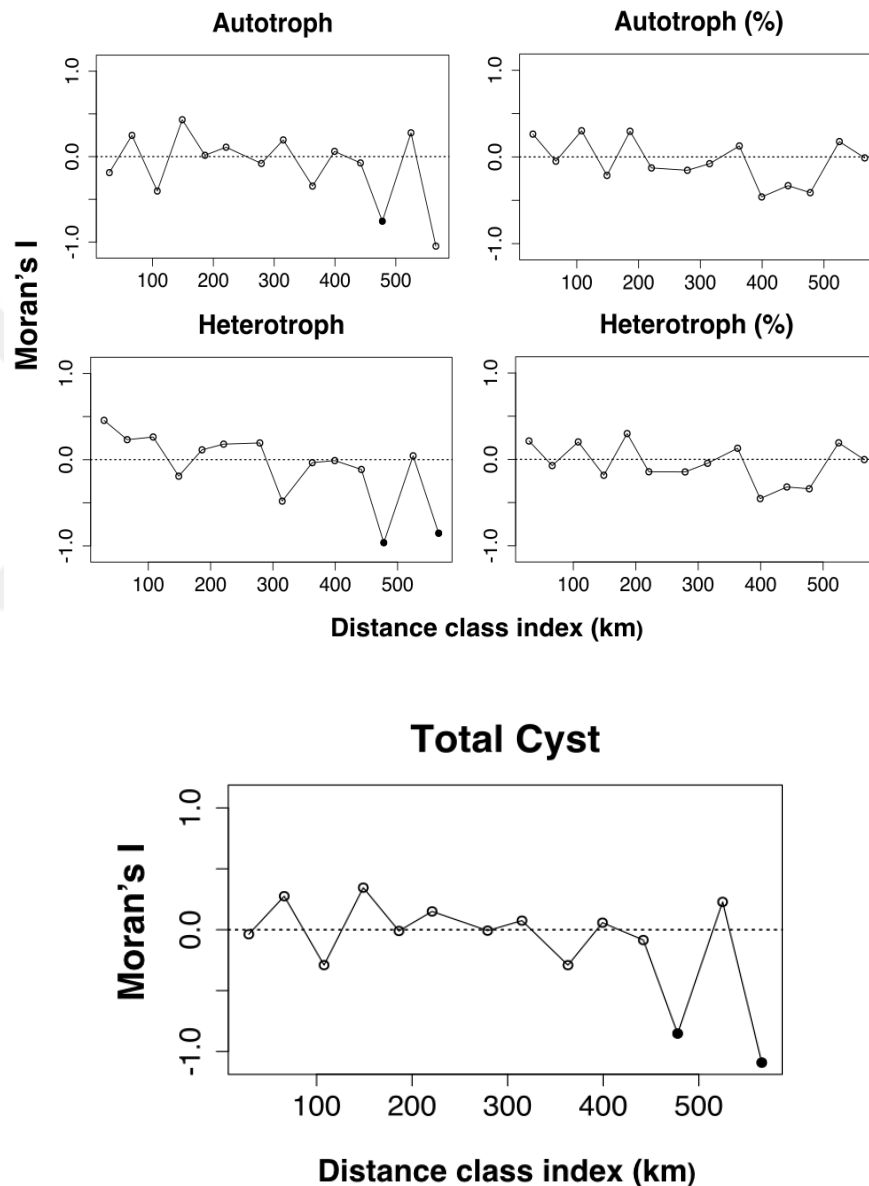


Figure 3.37 Spatial autocorrelation of total cyst concentrations and heterotroph/autotroph species distribution based on Moran's I with 999 permutations. The solid circles indicate the significantly autocorrelated ($p < 0.05$)

CHAPTER FOUR

DISCUSSION AND CONCLUSION

Med Sea is the largest European semi-enclosed sea. Within the Med Sea, a west–east gradient exists because of the eutrophic character of the western part and one of the most oligotrophic area of the world in the eastern part (Siokou-Frangou et al., 2010). In the concept of nutrients and chl_lorophyll a (Chl a), such gradient from eutrophy to oligotrophy can be observed in the direction of both west-east and north-south. Aegean Sea has a unique structure in the Mediterranean basin with its horizontal trophic gradient due to Black Sea entrance in the north and poor EastMed Sea waters in the south.

The Aegean Sea is known to have a complex hydrographic and ecological structure due to its geographical position between the Black Sea, the Levantine and Ionian seas (Varkitzi et al., 2019). During the late winter cruise, Aegean Sea demonstrated three separate sub-regions according to physical parameters. North Aegean where surface waters are cooler and less salty with the effect of the Black Sea originated waters, Middle Aegean with the highest density in the upper layers due to lower temperatures and higher salinity than its surroundings and South Aegean where typical Eastern Mediterranean water type observed with its salty and warmer waters. Among these regions, the most striking area is Middle Aegean. T-S diagram showed that water masses in the Middle Aegean are denser than other regions due to the lower temperatures and higher salinity.

Previous studies have documented that the North Aegean can support high plankton stocks and productivity due to the enrichment from inflowing Black Sea originated waters (Ignatiades et al., 2002; Lykousis et al., 2002; Lagaria et al., 2017; Varkitzi, 2019). In the thermohaline front of the North Aegean, modified BSW masses with lower salinity and temperature outflow through the Dardanelles straits and meet the warmer more saline Levantine water (Vervatis et al., 2011, Beşiktepe, 2015; Georgiou et al., 2015). The less dense modified BSW remains mostly close to the surface layer

and therefore maxima of Chl a, phytoplankton productivity and abundances are mostly constrained within the surface. Our findings further support this feature of high Chl a values (0.38-1.99 µg/l) in the North Aegean stations 1C, 2C and 3C within the upper 25m layer.

The concentrations of nutrients were relatively similar in the North, Middle and South Aegean Sea within the photic zone (till euphotic depth -Z_{eu}-, derived from Tot Chl a values according to Morel, 1988), the integrated values of nitrate were 0.51, 0.55, 0.75, phosphate were 0.05, 0.03, 0.07 and silicate were 2.17, 1.40, 1.17 in North, Middle and South Aegean Sea, respectively. The similar values between in northern and southern Aegean Sea support the previous studies (Ignatiades et al, 2002; Lykousis et al., 2002; Varkitzi et al., 2019) in late winter and spring.

Table 4.1 Nutrient ratios in the Aegean Sea in March 2012. Significant regressions (p>0.05) were highlighted

Nutrient Ratios				
		r	n	Si: NO ₃ :PO ₄
Whole Area	Si=23 PO₄+0.69	0.6	115	23:24:1
	NO₃=24 PO₄+0.03	0.4	115	
	Si=0.15 NO₃+2.2	0.2	115	
Sub regions		r	n	Si: NO ₃ :PO ₄
North	Si=7PO ₄ +2.2	0.21	33	7:13:1
	NO₃=13PO₄+0.13	0.77	33	
	Si=0.43 NO₃+2.2	0.23	33	
Middle	Si=55PO₄-0.5	0.48	32	55:42:1
	NO ₃ =42PO ₄ -0.37	0.28	32	
	Si=0.006NO ₃ +2.2	0.008	32	
South	Si=30 PO₄-0.58	0.87	50	30:24:1
	NO₃=24PO₄-0.11	0.42	50	
	Si=0.22NO₃+2.02	0.38	50	

Nutrient ratios in the whole Aegean Sea and in the sub-regions were calculated for the whole water column. The regression equations, sample size and R^2 values were summarized in Table 4.1. Phosphorus is the limiting nutrient in the whole area. In the sub-regions, middle and southern parts showed the phosphate limitation either while nitrogen was the relatively limiting nutrient in the eutrophic northern part. Phosphorus limitation in the Aegean and EMed Sea up to 60 was reported previously (Siokou-Frangou et al., 2010; Varkitzi et al., 2019).

Vertical distribution of chemical parameters in the Aegean Sea showed different trends within sub-regional areas. In the northern part first 25 m (8 m for station 1C) were showed classical features of Black Sea originated waters and after this depth, Aegean Sea water type was observed. In the Middle Aegean Sea, homogenous vertical trends were observed for chemical parameters while South Aegean Sea showed typical Levantine water type.

Dissolved Oxygen values in the northern part were relatively high with a mean value of 6.06 ml/l in the photic zone, values were relatively lower in the aphotic zone but vertically homogenous till the bottom. Middle part showed similar vertical trend with the mean value of 5.70 ml/l in the photic zone and vertically homogenous through the bottom except a peak value range at 600 m depth in station 5C. DO values in the southern part were higher in the first 50 meters and vertically decreasing through the bottom. Vertical nitrate, phosphate and silicate concentrations showed more or less the same trend within each sub-region, the most striking common feature was the relatively lower nutrient ranges in the upper layers than aphotic zones. In the northern part, nutrients were showing “complex shape” vertical profile which is a phenomenon with several peaks through the water column. In the upmost layer which is under effect of BSW, values were not that much high as expected, after 25 meters till approximately 100 meters in the photic zone values oscillated in a range lower than aphotic zone range but both layers showed several peaks and declines. In the middle part, nutrient vertical profiles differed between sub-regions unlike the northern and southern part. Nitrate and phosphate showed comparable complex shaped vertical trends in the middle part that both nutrients were oscillated in the upper layers in a lower range than

aphotic zone oscillation range except the extreme nitrate value (St 5C 200m: 23 μM) in comparison with this study range (BDL-12 μM) and previous reports (Ignatiades et al, 2002; Lykousis et al., 2002; Varkitzi et al., 2019). Silicate vertical profiles in the middle part were quite different. Stations 4C and 5C showed vertically homogenous profiles without a significant photic and aphotic difference but station 10C showed a complex shape vertical profile with several peaks same as the other nutrients. In the southern part all three nutrients showed similar vertical profiles as a typical Levantine water type with vertically increasing values. An extreme nitrate value (St 7C 300m: 28 μM) was observed in southern deeper layers. Highest values of all three nutrients were observed in deeper aphotic zone of southern part where nitrite have also reached maximum values and the dissolved oxygen was relatively low. The denser waters that originated from middle region apparently the source of the southern deep waters in March 2012 cruise. Vertical POC and Tot Chl-a values showed similar trends within each sub-region. In the northern part higher values in BSW affected waters (Chl a: 0.04-1.99 $\mu\text{g/l}$) with subsurface peaks were decreasing till 100 meters and continued with minimum levels (Chl a around 0.02 $\mu\text{g/l}$) through the bottom except a slight increase at station 3C after 500 meters (Chl a: >0.2 $\mu\text{g/l}$) for both parameters. In the middle part, at station 10C there was a vertical homogenous trend for both parameters till the bottom depth at minimum levels (Chl a : 0.02-0.11 $\mu\text{g/l}$) except a slight Chl a increase after 500 meters (~0.2 $\mu\text{g/l}$). Vertical profiles of POC and Chl a differ at stations 5C and 4C, values higher in the uppermost 100 meters (Chl a: 0.21-1.12 $\mu\text{g/l}$) with subsurface peaks and vertically decreasing through bottom (Chl a: <0.1 $\mu\text{g/l}$) except a minor peak at 500 meters at 5C for both parameters (Chl a: 0.14 $\mu\text{g/l}$). Exact values for POC measurements were not given in this discussion but trends were mentioned because of the expected similar instrumental bias. The minor increases of particulate matter in the middle part aphotic zone (there were higher values of TSS in the aphotic zones at 5C and 10C also) may be a result of the vertical mixing due to cyclonic feature of the area but it is hard to explain the mechanism of phytoplankton biomass increase because of the absence of nitrate and phosphate measurements of this depth but no increase observed for silicate. POC and Chl a vertical profiles in southern Aegean Sea were similar with each other as well. Values were higher in first 100 meters with a complex shape with subsurface peaks and DCM peaks (Chl a range:

0.02-0.33 $\mu\text{g/l}$) and decreasing through bottom depth as a typical Levantine water type. DCM was observed at 50 m depth at stations 7C, 8C and 9C and at 100 meters at 6C. A deep chlorophyll maximum (DCM) is a permanent feature for the whole Mediterranean basin over the entire annual cycle (Siokou-Frangou et al., 2010) and observed down to 50-100 meters in the Levantine basin in late winter conditions (Ediger&Yilmaz, 1996; Varkitzi et al., 2019). In the aphotic zone of station 9C, a particulate matter increase was observed that POC values were increasing after 200 meters, a minor peak of Chl a (0.04 $\mu\text{g/l}$) was observed in the bottom depth and additionally TSS values were increasing after 200 meters (>3 mg/l). Station 9C was the northernmost station in the southern part that closest one to middle part so most probably at this station deeper peaks were related with vertical mixing too.

Previously pronounced north-south trophic gradient in terms of Chl a and primary production (Latasa et al., 2010; Lykousis et al, 2002; Ignatiades et al, 1992; Ignatiades et al., 2009) was observed in March 2012 cruise in Aegean Sea. The integrated values of Tot Chl a in the photic zone were 0.78, 0.40, 0.19 $\mu\text{g/l}$ in North, Middle and South Aegean Sea, respectively. Based on the pigment analysis, DV Chl a, key pigment of the Prochlorophyceae, and Zea, key pigment of the Cyanobacteria, have higher percentages at southern stations while Per, key pigment of dinoflagellates, and Fuco, the key pigment of the diatoms, is lower higher in the north. Size classification of the phytoplankton community according to Uitz, 2006 confirmed that the pigments (Hex, Allo and Chl b) related with nano-sized phytoplankton cells of various groups (Prymnesiophytes, Chlorophytes, Prasinophytes, Pelagophytes and Cryptophytes) were generally did not changed between the sub-regions (23%, 33%, 27% in north, middle and south, respectively. While picoplankton size class (represented with DV Chl a and Zea) have been prevailed over microplankton size class (represented with Per and Fuco) in the southern part and the opposite situation was observed in the northern part. The average percentages were 56%, 26%, 12% for microplankton and 21%, 41%, 61% for picoplankton in north, middle and south, respectively. Picoplankton increase in the southern part, confirming the more pronounced oligotrophic feature of this region. Siokou-Frangou et al. (2010) reported that larger diatoms and dinoflagellates become abundant after intermittent nutrient pulses

associated with upwelling, fronts and gyres and Lagaria et al. (2017) concluded that larger organisms, particularly diatoms were closely associated with the surface Black Sea masses outflowing from the Straits in the Northern Aegean Sea. To date, the phytoplankton size classification (nano: 2-20 μm ; micro: 20-200 μm ; macro: >200 μm) introduced by Sieburth et al. (1978), has met wide acceptance as a standard procedure but today it is sometimes really complicated to compare the studies that there are various accepted size limits to define classes (ultra, pico, nano, micro). Over 85% of Chl a in the Eastern Mediterranean basin is found in ultraplankton (<10 μm), that comprises cyanobacteria (*Synechococcus* spp. are dominant), chlorophytes, prasinophytes and haptophytes (Denis et al., 2009). Yılmaz & Beşiktepe (2010) reported that picoplankton fraction (0.7-2.7 μm) contributes more than 50% to the total chlorophyll-a concentration during the year in the open waters in the Gulf of Mersin. Polat (2006) have reported that pico+ultraplankton (<5 μm) is an important part of the phytoplankton biomass in the Iskenderun Bay and likewise in the North and South Aegean. Ignatiades et al., (2002) have shown that the picoplankton fraction (0.2–1.2 μm) predominated and accounted from %49 to 56% of total chl a and 41% to 51% of total primary production in the North and South Aegean Sea, respectively. Size fractionated Chl a analysis at 2-5 meters depth in offshore surface waters in the Aegean Sea in March, 2012 resulted that the stations that under the effect of Black Sea originated water outflow has %44, %33 and %23 of micro, nano and pico sized distribution, respectively and middle and southern part of the area showed an oligotrophic character with %1, %44 and %54 of micro, nano and pico sized distribution, respectively (Bizsel, 2014). Pigment based classification in the surface waters was %58, %18 and %12 of micro, nano and pico sized in the northern part, %22, %38 and %25 of micro, nano and pico sized in the middle part and %19, %44 and %62 of micro, nano and pico sized in the southern part. Although the same trend was observed more or less, this result obviously shows the difference of the aspect of size classification. Previously conducted pigment-based size classification researches on remotely sensed Chl a data (Basterretxea et al., 2018; D'ortenzio & d'alcala, 2009; Navarro et al., 2014; 2017; Sammartino et al., 2015; Uitz et al., 2012) based on the similar algorithms used in this study concluded with comparable results that

picoplankton playing a larger role than microphytoplankton in the oligotrophic areas especially in the Levantine waters.

Another methodological comparison between instruments have been mentioned in this study that spectrophotometric measurement of Chl a overestimates the values. As concluded by Jeffrey and Humphrey (1975) and Neveux et al. (1999), spectrophotometric measurements of Chl a generally have higher values than HPLC measurements because the Chl a degradation products (pheophorbide, chlorophyllide and Chl a epimers and/or allomers) are absorbing light in the same spectral region as Chl a so they interfere with the measurements and a bias is being observed in the positive direction. In addition to the obvious overestimation in the more productive northern waters, the spectrophotometric method is less sensitive at low concentrations in southern part and creates an inconsistency that reduces correlation.

In Rhodes Gyre, overall Chl a concentrations (BDL-0.47 µg/l) for border and core of the eddy was found close to previous winter or early spring Rhodes gyre researches (Ediger & Yilmaz, 1996; Ediger et al., 2005, Vidussi et al., 2001). Vidussi et al. (2001) investigated the phytoplankton community structure by size in whole Mediterranean and have found that nanoplankton is the main component of phytoplankton in all area. Prochlorophyte contribution, estimated using DV Chl a accounted for only 11% of Tot Chl a. These results completely overlap with our results in March, 2016 that nanoplankton dominated the sampling area with 55% mean value and Prochlorophyceae contribution to Tot Chl a was 10%. At the data points where DV Chl a was reasonably high, DV Chl b was qualitatively observed, as expected. For eddy scale, in the Rhodes gyre Vidussi et al. (2001) have found that microplankton and nanoplankton in the core of the gyre and nanoplankton and picoplankton in the border of the gyre (determined from pigment data) were dominant. Same results observed in the study area and nanoplankton dominance in the vicinity of the Rhodes gyre have similar conditions with whole Mediterranean Sea. But these results differ from typical picoplankton dominated open ocean oligotrophic areas. Uysal et al. (2014), has observed higher proportions of pico (as size fractionated Chl a, primary productivity and size derived pigments) in the vicinity of the Rhodes gyre in March, 2013. Denis

et al. (2009), found picophytoplankton dominance in the Mediterranean, *Synechococcus* contributed 19.3%, *Prochlorococcus* contributed 1.01% to the overall phytoplanktonic biomass in the Rhodes gyre scale. These results differ from 9% of *Prochlorococcus* and 12% of *Synechococcus* contribution to the Tot Chl a in this study. These discrepancies can be explained by: (1) seasonal or interannual variability, (2) method difference for percentages: Denis et al. (2009) used flow cytometry counts and converted it to C biomass with a conversion factor, then pigment derived Chl a results converted to C biomass with another conversion factor and percentages given. But still Cyanophyceae prevailed over Prochlorophyceae in both studies, and this differs from oligotrophic oceans. This situation has been explained by adaptation capability of *Synechococcus* to the hydrodynamic and nutrient conditions of the eastern Mediterranean, nitrogen fixation capacity of a strain present in the eastern Mediterranean and winter mixing (Denis et al., 2009).

The pigment analysis in the Rhodes Gyre showed that Prochlorophyceae, *Synechococcus*, Prymnesiophyceae and Chlorophyceae were the major contributors for those small sized phytoplankton in the periphery of the gyre and Diatom related pigment (fuco) concentrations were increasing towards the core same as the light microscopy (LM) results (Beşiktepe et al, 2017) concluded with a small sized (<10 µm) phytoplankton dominance in the vicinity of the gyre while Diatom abundances increasing towards the core. In WestMed Sea and EastMed Sea, colonial bloom forming diatoms have been seen in fronts and gyres which are the “oasis” of the Mediterranean desert (Claustre et al., 1994). The high signal for Prymnesiophyceae in the pigment results was not observed in the light microscopy for the same cruise (Beşiktepe et al, 2017) but in the literature, Prymnesiophyceae reported with LM in Eastern Mediterranean and in Rhodes Gyre (Uysal et al., 2014; Yılmaz et al., 2002) with HEX, Prymnesiophyceae related pigment (Uysal et al., 2014, Yücel et al, 2017). A study conducted in Mersin bay in 2017 (Yücel et al., 2017) discussed the difference of the phytoplankton pigment differences in coastal and off-shore waters and prymnesiophytes was the cosmopolitan class of phytoplankton in Mersin Bay. The dominant species of the Prymnesiophyceae in the Levantine Sea is known for *Emiliana huxleyi* (Pagou & Gotsis-Skretas, 1990; Uysal et al, 2008; Uysal et al, 2014).

Degradation products occurrence can be related to <lots of chemical activities and biodegradation. In the study are, 3 different degradation products of Chl a separated and determined: (1) Chlide a, related process: diatom senescence (2) Phide a, related processes: protozoa grazing; (3) Phytin a, related process: protozoa grazing (Jeffrey et al, 2005). Phide a and Phytin a are generally found in intestinal tracts of grazers and in their fecal material (Roy, 1989; Strom, 1993). Chlide a is associated with collection of chlorophyllase-containing senescent diatoms and the extraction process (Trees et al., 2000, Jeffrey & Hallegraeff, 1987). Generally, all three degradation products were low in the Rhodes Gyre area (0.001-0.009 $\mu\text{g/l}$). An increase of Chlide a and Phide a (0.01-0.08 $\mu\text{g/l}$) at the stations 9, 10, 11, 12 that are in the core of the eddy have been observed. These results indicated that with increasing phytoplankton abundance and Diatom abundance, senescence and grazing tracers started to occur. Phytin a concentrations are generally low in all area and any increase couldn't be observed like other degradation products in the core of the eddy which can be explained by grazers in the core was zooplankton with increasing Diatom not protozoan. The degradation product values in the Aegean Sea in March 2012 were in higher ranges (Phytin a: BDL-0.09; Phorbide a: BDL-0.03 $\mu\text{g/l}$) where the highest values observed in the surface waters of northern part which has the highest Chl a values (1.99 $\mu\text{g/l}$) and highest Fuco (diatom key pigment) percentages (%50). Chlide a was not measured in Aegean Sea cruise. Both two degradation products were increased in the northern part that grazers included both zooplankton and protozoa.

The power to characterise phytoplankton samples quickly and rapidly, to reach such a precision of 1% and to catch the smallest fraction of the community in one measurement with the other fractions makes pigment analysis one of the best technique for mapping phytoplankton populations and monitoring their abundance and composition (Wright, 2005). Despite these advantages, pigments also suffer several disadvantages as markers. Special condition needs to preserve them, as they are sensitive to light, heat, oxygen, acids and alkalis. Their expression is variable, even within a particular class, and their content per cell varies with environmental factors such as irradiance, depth in the water column and nutrient availability. Finally and most importantly their distribution is complex, with few unambiguous markers. Some

pigments span many algal classes, making interpretation of pigment data difficult. It is important that other techniques (e.g. microscopy or genetic analysis) are used to support identifying the algal types. Pigment content spans most widely in dinoflagellates and especially for the classification of the micro size class it can cause underestimated results.

In Rhodes Gyre, it is found that there was not any significant correlation between Dinoflagellate Carbon ($\mu\text{g/l}$) (which is derived from microscopic measurements and volume calculations) and dinoflagellate marker pigment, peridinin (Per) values ($\mu\text{g/l}$) ($R^2 = 0,006$, $n=41$). Dinoflagellate abundance (cells/l) and Per did not show any significant correlations as well ($R^2 = 0,003$, $n=41$). Dinoflagellates has higher proportions in light microscopy (2-46 % with a mean value of 15%) than in pigment analysis (0-20% with a mean value of 3%). Light microscopy data for the Rhodes Gyre cruise were taken from Beşiktepe et al., 2017 and personal communication with Nihayet Bizsel. In the Aegean Sea, between Dinoflagellate Carbon ($\mu\text{g/l}$) and Per ($\mu\text{g/l}$) ($R^2 = 0,41$, $n=116$) and dinoflagellate abundance (cells/l) and Per ($\mu\text{g/l}$) ($R^2 = 0,46$, $n=116$) significant correlations have been found. The results were more satisfying than Rhodes Gyre because of the higher number of observation (n) but still dinoflagellates have higher proportions in light microscopy (0-61 % with a mean value of 15%) than in pigment analysis (0-46% with a mean value of 0.07%). Light microscopy data for the Aegean Sea were taken from Bizsel et al., 2014 and personal communication with Tuba Kısacık. The main causes behind the difference between light microscopy and HPLC pigment analysis were the precision (1%) reached by pigment analysis does not that low for single cell counting method and nano- and pico-planktonic organisms are normally unrecognizable by light microscopy. Additionally, tracking dinoflagellates is a limitation for HPLC derived phytoplankton community composition. Dinoflagellates could be underestimated due mainly to multiple endosymbiotic events (Zapata et al., 2012). It is now generally accepted that living dinoflagellates in modern oceans may be found either with no pigments (heterotrophs), with their own unique plastid (containing the dinoflagellate carotenoid, peridinin), or with plastids derived from smaller taxa that obtained through tertiary endosymbiosis (Roy et al, 2011). Caution is a fundamental need when only peridinin is used for

tracking dinoflagellates that it represents only the photosynthetic proportion these complicated eukaryotes. These limitations can be eliminated by future investigations on specific pigment contents by experiments and applying these taxa related pigment results to run CHEMTAX analysis properly.

Trophic strategy diversity in dinoflagellates is remarkable and puts them in an important position in the food web dynamics. Over the past few decades, dinoflagellates have aroused considerable attention primarily due to their contribution to Harmful Algal Blooms (HAB) and their potential to serve as a source of possible bloom events in the surface sediment and as environmental indicators or signals to track cultural eutrophication (e.g., Dale, 1996; Hallegraeff, 1993, Bizsel & Bizsel; 2015). Modern dinoflagellate cyst assemblages in the Aegean and Eastern Mediterranean Seas have been investigated in relation with environmental parameters. A dinoflagellate cyst community with high diversity has been observed with 56 different taxa belonging to 25 genera and 5 families were identified in the area. Modern dinoflagellate cyst abundances ranged from 70 to 2222 cysts per gram dried sediment (max: St2C, min: St1C) with an average value of 723 cysts/g in the Aegean Sea. St 1C showed different characteristics of the grain size that 93% of the sediment was bigger than 120 μm and content of the coarse material was mainly sand and shells. This may be associated with the position of the northernmost station in the entrance of the Çanakkale Strait. Except the low values observed at the station 1C (70 cysts/g), abundances were found higher in the Black Sea influenced northern waters than the oligotrophic Eastern Mediterranean waters. Based on the visual examination of the dataset, dinoflagellate assemblages in the Aegean Sea are dominated by *Operculodinium centrocarpum*, *Spiniferites* spp., *Spiniferites ramosus*, *Brigantedinium* spp., *Impagidinium aculeatum* and *Operculodinium israelinum*. *Operculodinium centrocarpum* dominance in the northern stations have replaced by *Impagidinium aculeatum* dominance in the southern stations. *Pentapaharsodinium dalei* and *Alexandrium* spp. were the autotrophic species that their distribution is restricted in the northernmost stations. All the peridinoid cysts generally showed higher relative abundances in the northern Aegean Sea.

The nutrition or trophic modes of the biological affinities of the dinoflagellate cysts (autotrophy/heterotrophy) have been widely used to obtain information for the ecological preferences of dinoflagellate species. Studies from the Aegean Sea and Black Sea show that the autotrophic gonyaulacoids form an association characteristic for the hypersaline Aegean and Eastern Mediterranean water, whereas heterotrophic protoperidinioids are observed in relative high abundances in areas influenced by low-salinity Marmara-Black Sea water (Elshanawany et al., 2010; Marret et al; 2008, Mudie et al, 2004, 2017). Our results supported this difference between northern and southern parts of the Aegean Sea that the relative abundance of cysts produced by autotrophic dinoflagellates were predominant in the whole basin, ranging from 73 to 98%, except St 4C (45%). While the dominance of the autotrophic cysts is obvious, the heterotrophic cyst proportion in the dinoflagellate cyst assemblages was slightly decreasing through the south. The PCA analysis clearly separates the heterotrophic taxa from the autotrophic taxa except *Brigantedinium* spp. which were the only heterotrophic taxon that have a general distribution at all stations in high abundances (0.9-20.3%). *Brigantedinium* spp. have been found to be in high amounts (51%) and have wide distribution in the basin in a west-east gradient Mediterranean Sea modern dinoflagellate cyst research (Elshanawany et al., 2010). Highest relative abundances (app. 50%) have been found at the western area but remarkable values (25%) have also been recorded around the Rhodes island (Eastern Mediterranean) where the maxima (20%) for this study has observed exactly in the deepest station (26G, >4000 m) around Rhodes island. PCA analysis separated all the other heterotrophic species ordinated at the left side of the first axis and the autotrophic species with high abundances were ordinated at the very positive side of the first axis. Some autotrophic group were classified with heterotrophic species in the negative side of the first axis, which would have been explained by having a random distribution or occurring in low values.

Apart from the trophic status related distribution of dinoflagellate cysts, it is important to determine the possible effect of physicochemical mechanisms that might have altered the assemblage. Dinoflagellate cyst studies have been used by micropalaeontologists for decades because of the retrospective data up to millions of years to explore the evolutionary processes or large-scale climatic changes. The term

“modern dinoflagellate cysts” corresponds to recently deposited cysts in a period of approximately last 100 years. Hence, in recent research, dinoflagellate cysts have been preferred in ecological evaluations of climate change and cultural eutrophication tracking signals because they contain both planktonic and benthic phases in life cycle. The motile forms of cysts in the photic zone are closely related to their surroundings so long-term physicochemical data (World Ocean Atlas provided by U.S National Oceanic and Atmospheric Administration and COPERNICUS provided by Marine Environment Monitoring Service of European Commission) have started to be used widely to determine the relationship between environmental data and cysts have being deposited for decades (Bringué et al., 2014; de Vernal et al., in press; Ellegaard et al.; 2017; Elshanawany, 2010; Li et al., 2018; Pospelova et al., 2006, 2008; Pospelova and Kim, 2010; Radi et al., 2007; Radi & de Vernal, 2004; Ribeiro et al., 2016; Zonneveld et al., 2013). World Ocean Atlas 2013 for long term environmental data (1955-2012) couldn't be used for this research because of the enormous number of islands in the Aegean Sea and 1.0 degree resolution was not enough for half of the stations that sampled closer than 111 kilometres. Long-term environmental data that extracted from Copernicus was found more suitable for the area with better resolution (0.06x0.06 degrees). The deviations between the datasets are quantified in terms of Root Mean Square Error (RMSE) in the quality control document (Fратиanni et al, 2015) and this model works quite well for the Aegean Sea except the entrance of Çanakkale Strait in the north where St 1C located from our sampling stations. The comparison of field data in March 2012 with extracted data in that period supported the suitability and the exception of northernmost station.

Canonical Correspondence Analysis was used to assess the relation between the chosen environmental data and dinoflagellate cyst relative abundances. CCA were conducted two times because of the special situation of station 4C. Because of the high relative abundance of *Gymnodinium nolleri* (39.2%) compared to other species first diagram was not distinguishable. In order to achieve more explicit image to assess the most important environmental parameters that influencing dinocyst distribution, CCA has repeated without 4C. Sea surface temperature and sea surface salinity gradients pointed into the same direction, opposite to the bottom water oxygen and sea surface

nitrate, phosphate and Chl a. According to CCA results four different cyst associations observed that can be related to different oceanographic regimes

Impagidinium aculeatum, *Impagidinium* cf. *paradoxum*, *Impagidinium* cf. *patulum*, *Impagidinium* cf. *plicatum*, *Impagidinium* spp., *Operculodinium israelianum*, *Operculodinium janduchenei*, *Pyxidinosia reticulata* and *Nematosphaeropsis labyrinthus* were the Eastern Med off-shore group related with saline and warmer waters. Salinity and temperature found the most important environmental factors influencing the Mediterranean cyst distribution (Elshanawany et al., 2010).

Selenopemphix nephroides, *Selenopemphix undulata*, *Achomosphaera andalousiense*, *Bitectatodinium tepikiense*, *Pentaparsodinium dalei* and *Quinquecuspidata concreta* were the northern group which was related mostly to the bottom water oxygen concentrations. *Gymnodinium nolleri* was also in this grouping according to first CCA results. In a global assessment of dinoflagellate cyst distributions, Cysts of *Gymnodinium nolleri* were observed in coastal sediments from temperate to sub-tropical regions of the eastern Atlantic Ocean and such the Mediterranean Sea, and the eastern Pacific. Highest relative abundances were observed oligotrophic/mesotrophic regions where bottom waters are moderately to well ventilated (Zonneveld et al., 2013).

Selenopemphix quanta, *Biecheleria* spp., *Peridinium ponticum*, *Alexandrium* spp. and *Votadinium* cf. *calvum* were the northern group strongly related with phosphate, nitrate and Chl a gradients.

Brigantedinium spp., *Stelladinium stellatum*, *Spiniferites ramosus*, *Spiniferites* spp., *Spiniferites mirabilis*, *Lingulodinium machaerophorum*, *Trinovantedinium applanatum* and *Operculodinium centrocarpum* were ordinated in the central part of the diagram. This group has a random distribution and is generally seen at all stations in high concentrations

Focusing on individual species we found that globally distributed species had a general distribution in the Aegean Sea also. These were the world-wide distributed *Operculodinium centrocarpum*, *Lingulodinium machaerophorum* and *Nematosphaeropsis labyrinthus* (Zonneveld et al., 2013). *Spiniferites mirabilis*, *Spiniferites ramosus*, *Brigantedinium* spp., *Impagidinium aculeatum* (except St1) and *Operculodinium israelinum* (except St1) have been observed almost at all stations and they have a general distribution except polar areas in the world (Zonneveld et al., 2013). *Operculodinium israelinum* has a wide distribution in equatorial and subtropical areas in the world (Elshanawany et al., 2010; Zonneveld et al., 2013) and it reaches its maximum concentrations in the Eastern Mediterranean waters. The same trend has been observed for the species *Impagidinium aculeatum*, *Impagidinium patulum*, *Impagidinium paradoxum*, *Impagidinium* spp., *Impagidinium plicatum*, *Pyxidinosia reticulata* and *Nematosphaeropsis labyrinthus*. The dominance of these species in oligotrophic regions is in accordance with the previous studies in the Mediterranean (Rubino et al. 2000; Mudie et al. 2004; Giannakourou et al. 2005; Sangiorgi et al. 2005; Marret et al. 2008; Zonneveld et al. 2009). Studies from the Aegean Sea and Black Sea show that the autotrophic gonyaulacoids form an association characteristic for the hypersaline Aegean Mediterranean water, whereas heterotrophic protoperidinioids are observed in relative high abundances in areas influenced by low-salinity Marmara-Black Sea originated water. In a survey of modern dinoflagellates in the eastern North Atlantic, Dodge (1994) noted that: (1) only the gonyaulacoids show a clear influence of temperature, and (2) protoperidinioids are rare in the warm temperate region. This dominance of gonyaulacoids over protoperidinioids is evident in the study area. *Peridinium ponticum* which is endemic to Black Sea (Mudie et al., 2004; 2017) have been observed at St 2C and St 3C, located in the north and influenced by the entrance of Black Sea originated waters through the Dardanelles. Potential toxic/nuisance species that have been observed in all area are *Operculodinium centrocarpum* (cyst of *Protoceratium reticulatum*) ve *Lingulodinium machaerophorum* (cyst of *Lingulodinium polyedrum*). Another toxic species, *Alexandrium* spp., have been observed in the Black Sea affected northernmost stations.

To understand the broad ecological roles of the dinoflagellates in the food web, lots of studies have been conducted. Heterotrophic flagellates have a leading role in the Med Sea food web (Braleska & Witek, 1999; Siokou Frangou et al, 2010; Unrein et al., 2014) and one of the most important group in the microplankton size is dinoflagellates. The researches which explored the potential effect of trophic status on the requirements for organic compound and nutrient resources as driving factors of food-web dynamics used either fluorescence microscopy or literature based information to classify the nutrition of dinoflagellate species. In recent years, similar species in the studies have been assessed as strictly heterotrophs as they have no chloroplast (Ignatiades, 2012; Lagaria et al., 2017; Naustvoll, 2000) and pigmented dinoflagellates started to be considered as mixotrophs because no dinoflagellates are purely photoautotrophic all of the time (Barton et al., 2013, Skovgaard, 1998). The role of the pigmented dinoflagellates were found to be least in the oligotrophic Eastern Mediterranean Sea after prymnesiophytes, haptophytes and cryptophytes (Unrein et al., 2014). Heterotrophic dinoflagellate ratios were found dominant in off-shore (Kısacık, 2018) and coastal (Ignatiades, 2012) waters of the Aegean Sea. According to sediment assemblages of dinoflagellates it was obvious that autotroph (or mixotroph in the new classification) cyst dominance was clear for the oligotrophic waters of Aegean Sea and Eastern Mediterranean Sea. In this matter, the difference of dominant groups of dinoflagellates in the water column and sediment may be the cause of preservation conditions and transportation of the cells without ability of movement. Preservation of dinoflagellates cysts are strongly related with oxygenated bottom waters and post-depositional degradation is species-selective (Elshanawany et al., 2010; Zonneveld et al. 2007, 2008). On the other hand, dinoflagellate cysts can be laterally displaced during or after settling to the sea floor. Bottom topography appears to be of great importance that inter basin transport by currents is mainly limited (Elshanawany et al., 2010). It has been proved in our study that CCA clearly separated the groups related with deep water oxygen and the central and southern deep basins from each other with different community composition. The general idea that higher heterotrophic dinoflagellate cyst abundance in relation with cultural/industrial eutrophication or upwelling conditions in the upper water column can change with depth. The limits of trophic modes of dinoflagellates and assessment of the roles are

still a ‘black box’ (Unrein, 2014). There has been evidence of experimental studies that mixotrophic dinoflagellates may evolve to heterotrophs to increase the growth and ingestion rates (Jeong et al., 2010) or heterotrophic dinoflagellates may evolve to mixotrophs for short period of times by phagotrophy under limited prey conditions (Skovgaard, 1998). Finally feeding on mixotrophic species and having various plastids supports the hypothesis of tertiary endosymbiosis (ancestor of dinoflagellates engulfing a eukaryotic cell with a secondary plastid) (Jeong et al., 2010).

Statistical analyses were performed to explain the spatial structures in the water column and sediment of the Aegean Sea. PCA was performed to determine the parameters that have the largest variation in photic and aphotic layers. The results can be summarized as in the photic zone stations were separated into two groups (north: 1C, 2C, 3C, 5C and south: 6C, 7C, 8C, 9C, 10C) according to first component axis and temperature, salinity and particulate matter (Chl a, POC, TSS) were related with the 48% of the variance. High temperature and salinity values in the southern photic zone and the high particulate matter content in the northern were the driven factors of the spatial separation of the stations. In the aphotic zone stations were separated into three groups (north: 1C, 2C, 3C; middle: 4C, 5C, 9C and south: 6C, 7C, 8C) according to both first and second component axes. 50% of the variance were related with particulate matter, DO and nutrient values while 21% of the variance were related with temperature and salinity. High nutrient values in the southern aphotic zone, high particulate matter values in the northern aphotic zone and the high temperature, salinity and DO values in the middle aphotic zone were the driven factors of the spatial separation of the stations. Spatial Autocorrelation were also used to determine the spatial structure of the Aegean Sea photic zone. Temperature, salinity and DO typically showed gradient-like structure, where the differentiation starts at the 250-300 km distance class. Phosphate, silicate and density showed almost gradient-like structures except the positive autocorrelation in the last distance class (around 500km) but still differentiation start at the 250-300 km distance class. As a result, spatial distribution according to physical parameters and nutrients Aegean Sea were separated in two groups as north and south. Particulate matter (Chl a and POC) showed significant spatial autocorrelations indicating a patchy structure (large oscillations) at

the levels of positive spatial autocorrelation at small spatial scales (i.e., till 150 km), most positive spatial autocorrelation at around 220 km and negative autocorrelation increasing from 280 km with a sharp decrease at around 320 km and oscillated at negative side at largest spatial scales. So, these oscillations and patchiness of the particulate matter resulted in three groups in the Aegean Sea which can be explained by the relatively higher values in the station 5C, located in the middle part, as a result of strong vertical mixing. PCA and CCA results in the sediment were discussed in detail above but as a general summary, Aegean Sea is mostly separated into two groups as north and south. It is noteworthy to emphasize that there were four extra stations in the sediment analysis that made the separation stronger especially with the effect of the stations close to Rhodes island that can be considered as in the Eastern Mediterranean. Spatial Autocorrelation were used to determine the spatial structure of the Aegean Sea sediments in the means of chemical and biological parameter. Both total and organic fractions of carbon and nitrogen percentages in the sediment showed a patchy structure and especially the organic fraction parameters have highly large oscillations so that was hard to explain a clear cut separation according to chemical variables in the sediment in the Aegean Sea. On the other hand, concentration values of total, heterotrophic and autotrophic dinoflagellate cysts showed a slight gradient-like structure, significantly. Correlation values were stronger at the negative side at longer distances than shorter distances. As a result, the differentiation starts around 300 km distance class and Aegean Sea separated in two groups as north and south.

Phytoplankton community size structure is the base of the understanding the carbon cycle by controlling the efficiency of the biological pump (Mousing et al., 2014). Cell size estimation of phytoplankton using various new types of measurements, including flow cytometry, High Performance Liquid Chromatography (HPLC) analysis of marker pigments and DNA sequencing and using an all-aspect approach would certainly provide better evaluation based on the complex dynamics. Researchers have so far failed to reach consensus on the individual effects of temperature and nutrient effect on phytoplankton community size structure but this concept has a crucial importance for predicting the effect of climate change in the future, exclusively in the Mediterranean region as a laboratory-type environment or a hot-spot for potential

climate response studies. The composition shifts of phytoplankton in the concept of changing trophic status for the two special area in the Med Sea were discussed but it is obvious that, complexity of the system and the current understanding of the processes controlling the system ranging from small to global scales is still inadequate to understand the system, as a whole (Siokou-Frangou et al., 2010). Considering the small scale and the enclosed nature of this basin, this “miniature ocean” under an intense anthropogenic pressure (pollution, overfishing, habitat destruction, the emergence of new species), hosting a surprising and still largely underestimated variety of planktonic organisms linked together by dynamic trophic pathways. It is the latter aspect that Aegean Sea dynamics, which has a few open water researches, would be less paradoxical in the light of the up to date details of this study. The scarcity of such research on a national scale emphasized the importance of the study in order to provide sufficient and reliable data in international waters in the Aegean Sea

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APPENDICES

APPENDIX 1: List Of Abbreviations

AgIW: Aegean Sea Intermediate Water

Allo: Alloxanthin

B-car: Beta-carotene

BSW: Black Sea Water

But: 19-Butanoyloxyfucoxanthin

CAGDW: Central Aegean Deep Water

CDW: Cretan Deep Water

Chl a: Chllorophyll a

Chl b: Chllorophyll b

Chl c3: Chlorophyll c3

Chlide a: Chlorophyllide a

DCM: Deep Chlorophyll Maximum

Diadino: Diadinoxanthin

Dinocyst: Dinoflagellate Cyst

DP: Diagnostic Pigments

DSP: Diarrhetic Shellfish Poisoning

DV Chl a Divinyl Chlorophyll a

EastMed Sea: Eastern Mediterranean Sea

EMDW: Eastern Mediterranean Deep Water

EMT: Eastern Mediterranean Transient

f: Pigment index

Fuco: Fucoxanthin

HAB: Harmful Algal Bloom

Hex: 19-Hexanoyloxyfucoxanthin

HPLC: High Pressure Liquid Chromatography

LSW: Levantine Sea Water

Lut: Lutein

Med Sea: Mediterranean Sea

MV Chl a Monovinyl Chlorophyll a
NAdw: North Aegean Deep Water
NEastMed Sea: North Eastern Mediterranean Sea
OC: Organic Carbon
ON: Organic Nitrogen
Per: Peridinin
Phide a: Pheophorbide a
Phytin a: Pheophytin a
POC: Particulate Organic Carbon
PON: Particulate Organic Nitrogen
PP:Primary Production
SSS: Sea Surface Salinity
SST: Sea Surface Temperature
TC: Total Carbon
TMW: Transient Mediterranean Water
TN: Total Nitrogen
Tot Chl a: Total Chlorophyll a
TSS: Total Suspended Solids
Viola: Violaxanthin
Zea: Zeaxanthin