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**Composition, abundance and biomass of microplanktonic unicellular
eukaryotic communities during bloom periods in Thessaloniki Bay**

MASTER DISSERTATION THESIS

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Abstract

Human pressures such as the increase of human population, the industrialization, and the intensification of agriculture, especially, in coastal areas increase the inputs of nutrients to the sea through industrial and urban waste water (Garnier *et al.*, 2010). Thus, eutrophication phenomena have stimulated the proliferation of potentially harmful algal taxa resulting in the prevalence of frequent and intense harmful algal blooms (HABs) in coastal areas. The purpose of this study was to investigate the composition and dynamics of planktonic communities during bloom events in two different seasons in Thessaloniki Bay along spatial gradients. Furthermore, causative species of the observed HABs were identified and are discussed.

Water samples were taken, in spring and summer, from Thessaloniki Bay in order to examine the unicellular planktonic communities. During spring and summer of 2022, 31 surface water samples were collected from 3 sampling sites, Music Hall (MH), White Tower (WT), and Harbor (HB) in Thessaloniki Bay. A total of 83 unicellular eukaryotic plankton species were identified in the 3 sampling sites. Dinoflagellates were more diverse accounting for 38 taxa, followed by diatoms with 34 taxa. According to the official IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae, 7 potentially toxic taxa were recorded in the study area. During spring, dinoflagellates dominated with bloom-forming *Prorocentrum micans* being the most abundant taxon. Also, during March a macroscopically visible *Noctiluca scintillans* blooms was detected in the White Tower sampling point forming a red tide event. In summer diatoms outcompeted all the other taxonomic groups, with *Chaetoceros* sp. dominating in all 3 sampling sites. The complementary to the *in-situ* observations 23-year satellite data of Chla-a were analyzed and compared with the unicellular planktonic biomass that was calculated with the inverted microscope method but the match-ups were not enough to have a reliable statistical relationship. There is a need for continuous monitoring of target bioindicators of nutrient pollution because of the occurrence of the harmful events and the presence of harmful algae.

1. Introduction

1.1 Anthropogenic eutrophication in coastal areas

More than 50% of the world's population lives in urban areas, mostly located in coastal areas or areas strongly affected by coastal systems (Von Glasow *et al.*, 2013). It has been predicted that the increase in the global population will coincide with a rise in coastal urbanization (Rabalais *et al.*, 2009). The increase of the human population, the industrialization, and the intensification of agriculture directly affect river inputs, land runoff, and industrial or urban wastewater (Davidson *et al.*, 2012; Guillaud *et al.*, 1992). Human pressures close to the coast involve intensive agriculture, partially treated domestic and industrial sewage, fish and mussel farming and fishing (Dimitriou *et al.*, 2015). These activities also constitute a basic risk, which concerns the reduction of the biodiversity of the coastal areas which is often related to the health and functionality of the ecosystems (Basset *et al.*, 2008; Kominoski, 2009). Human pressures have contributed to a 3-fold increase of nitrogen and phosphorus in their global delivery to the ocean (Smith *et al.*, 2003).

Nutrient fluxes control the distribution and succession of phytoplankton communities (Souchu *et al.*, 2010), which is the basis of marine ecosystems. In addition to producing oxygen and controlling carbon levels, they are also the base of the food webs since they are a direct or indirect source of food, for many marine organisms of higher trophic levels (Van Dolah, 2000). Nearly 50% of the atmospheric O₂ comes from phytoplankton's primary production, and one of the major functions of phytoplankton is to draw CO₂ from the atmosphere and introduce it into ocean ecosystems. Thus, phytoplankton contributes to climate change mitigation by absorbing 25-30% of CO₂ emissions annually (Tweddle, 2018).

Phytoplankton abundance is enhanced by the presence of nutrients mainly nitrogen (N), and phosphorus (P) which are fundamental nutrients for primary production. Thus, eutrophication, one of the major threats to the health of marine environments, is directly related to human activities in coastal areas, which increase the input of nutrients, especially the supply of nitrogen and phosphorus to the sea (Billen & Garnier, 2007). According to, the global Indicator for Coastal Eutrophication Potential (ICEP) analysis, coastal eutrophication has the potential to continue to increase (Garnier *et al.*, 2010). Eutrophication combined with the results of climate

change in global warming and sea temperatures which affect the hydrological cycles, and wind patterns, leads to the proliferation of phytoplankton biomass causing conspicuous and frequent algal blooms (Glibert *et al.*, 2001; Sadeghi Mazidi *et al.*, 2011; Glibert *et al.*, 2018; Glibert, 2020).

Thus, the unicellular plankton abundance and biomass have increased globally due to eutrophication, while harmful algal blooms (HABs) accompanied by toxic species have been observed along coastal areas worldwide (Tsikoti & Genitsaris, 2021). Phytoplankton blooms can be toxic or non-toxic. This depends on the involved species, their biological and ecological characteristics, and the state of the respective environment where they are (Bahrami, 2013). A typical example of unicellular eukaryotic plankton blooms is red tides which are used to describe blooms that can cause discoloration of seawater (Turkoglu, 2010).

Task Group 5 of the Marine Strategy Framework Directive reports that there are three types of harmful algal blooms. The first type includes microalgal species that produce potent toxins which can cause serious gastrointestinal and neurological illnesses and death to humans, after contaminated seafood consumption and deaths to other organisms (fish, marine mammals, birds) (Scholin *et al.*, 2000). The occurrence of toxic microalgae represents a significant threat to human health, aquaculture, fishery resources and marine ecosystems throughout the world (Hallegraeff *et al.*, 2003). Secondly, there are HABs that are caused by likely toxic algae such as some species of the genus *Pseudonitzschia* sp. And finally, here are also the high-biomass blooms. Of the ≥ 5000 unicellular phytoplanktonic species described worldwide, nearly 300 species can generate high-biomass blooms including red tides. These species mainly belong to dinoflagellates, diatoms, and haptophytes while some of them have been recognized to be toxic to fish, shellfish, marine mammals, and humans (Zohdi & Abbaspour, 2019).

Diatoms and species dinoflagellates are typical groups of primary producers in eutrophic systems, while they are less abundant in oligotrophic tropical environments (Calbet & Landry, 1999; Gaudy & Youssara, 2003). There is evidence that these taxonomic groups flourish in coastal areas that are enriched by anthropogenic activities (Ciavatta *et al.*, 2019). For example, if silicon is limited in coastal ecosystems, non-siliceous phytoplankton proliferates, such as flagellates and dinoflagellates, which can gradually dominate and often cause HABs. Dinoflagellates stand out in their species

richness and broad nutritional diversity (Smayda & Reynolds, 2003). According to the literature, dinoflagellates are classified according to their feeding mechanisms as autotrophic photosynthetic species, mixotrophic organisms obtaining their nutrition by photosynthesis and the uptake of dissolved organic matter (osmotrophy) and/or particulate organic matter (phagotrophy), and heterotrophic species whose feeding is phagotrophic (Jeong *et al.*, 2010; Burkholder *et al.* 2008; Gaines & Elbrächter, 1987).

It is likely that mixotrophic and heterotrophic dinoflagellates blooms, will have significant effects on marine ecosystems since their nutritional mode has important ecological implications. They are key grazers, feeding on prey ranging in size from bacteria to fish tissues (Jeong *et al.*, 2010), and at the same time they constitute a significant food source for mesozooplankton (Sherr & Sherr, 2007). Thus, they may play a crucial role in the food web because they reticulate a heterotrophic link between the microbial loop and the classic food chain (Sherr *et al.*, 1986). Even if non-toxic, bloom-forming mixotrophic and heterotrophic dinoflagellates, have the potential to dominate the unicellular planktonic community, and subsequently have ecological consequences, related to the degradation of water quality. The impacts depend on the life cycle of the exact species which cause the blooms (Ignatiades & Gotsis -Skretas, 2010).

1.2 HABs at a global scale

Eutrophication remains a global, common problem in coastal areas such as in Netherlands (de Jonge, 2000), Brazil (Braga *et al.*, 2000; Reis-Filho *et al.*, 2012; De Jesus Affe *et al.* 2021), the Baltic Sea (Yurkovskis *et al.*, 1993; Karlson *et al.*, 2021; Rene *et al.*, 2022; Konik *et al.*, 2023), the Northern Adriatic Sea (Barmawidjaja *et al.*, 1995; Facca *et al.*, 2014; Accoroni *et al.*, 2015), and China (Lee & Arega, 1999; Fang *et al.*, 2018; Yu *et al.*, 2023). In China several toxic blooms have been recorded and especially red tides forming by *Noctiluca scintillans* in spring to summer often resulting in a strong pinkish red or orange discoloration of the water (Huang & Qi 1997). In South China Sea, also, *Scrippsiella trochoidea* was forming blooms annually from 1998-2004 in Daya Bay (Wang *et al.*, 2007). The industrial sewage and the inputs from the polluted river of Mississippi have contributed to the bloom formation of many causative species such as *Karenia brevis*, *Aureoumbra lagunensis*, *Chattonella subsala*, *N. scintillans*, *Ceratium furca*, *Scirpsiella* sp., and *Pseudonitzschia* sp. at the Gulf of Mexico (Ulloa *et al.*, 2017; Xiao *et al.*, 2018). Extensive toxic blooms have been reported in the west

coast of India (Turkoglu, 2013), in France (Guillou *et al.*, 2002), North America and Japan, as well as in Chile, South Africa and Australia (Pitcher *et al.*, 2007; Krock *et al.*, 2007; Robertson *et al.*, 2004).

1.3 HABs at the Mediterranean region

During the past years, an increasing number of studies, in the Mediterranean Sea, indicates harmful species proliferations, particularly along the northern coasts cause by the morphology of its coastline and nutrient-rich freshwater inputs from coastal urbanization and intensification of agriculture (Collos *et al.*, 2004; Spatharis *et al.*, 2007a; Vila *et al.*, 2005; Barale *et al.*, 2008). For example, *Dinophysis* blooms have been documented in the western Mediterranean Sea (Vila *et al.*, 2001). Several greek coastal areas characterized by high pollution levels, restricted water mass circulation, and riverine inflows, are hotspots of frequently HABs occurrence (Gotsis-Skretas *et al.*, 1999; Ignatiades *et al.*, 2009; Siokou-Frangou *et al.*, 2002). Most Greek gulf with restricted circulation exhibit eutrophication problems, such as the Elefsis Bay and the northern part of the inner Saronikos Gulf (Friligos & Barbetseas, 1990; Theodorou, 1997; Pagou *et al.*, 2002; Pavlidou *et al.*, 2004), the Patraikos Gulf (Friligos *et al.*, 1985), the Gulf of Kalloni in Lesbos Island (Panayotidis *et al.*, 1999), and the Thermaikos Gulf (Gotsis-Skretas & Friligos, 1990; Pagou, 2000; Genitsaris *et al.*, 2019).

Among the most common unicellular planktonic eukaryotes found to cause blooms in Thermaikos Gulf are the genera *Pseudo-nitzshia* sp., *Skeletonema* sp., *Chaetoceros* sp., *Alexandrium* sp., *Ceratium* sp., *Scripsiella* sp., *Prorocentrum* sp., *N. scintillans*, *Spatulodinium pseudonociluca*, *Gymnodinium breve*, *Mesodinium* sp., and species of the genus *Prorocentrum* sp. (Xiao *et al.*, 2018). Specifically, it was found that *P. micans*, *Protoperidinium* sp. *Gymnodinium* sp. and *Ceratium pulchellum*, flourish in polluted waters such as Thessaloniki Bay (Friligos & Koussouris, 1984; Nikolaidis & Moustaka – Gouni, 1990). From 2000 to 2004, the dinoflagellates *N. scintillans*, and some species from the genus of *Prorocentrum* such as the species *P. micans* were responsible for the discoloration of the water in Thermaikos Gulf and the anoxia conditions caused in the ecosystem (Ignatiades & Gotsis-Skretas, 2010). Also, HABs and recurrent red tides caused by dinoflagellates such as *Protoperidinium* sp. and *N. scintillans* have been witnessed before in Thessaloniki Bay (Evagelopoulos, 2002). Furthermore, from March to June 2017 diatom blooms, caused by *L. danicus*

and *L. minimus* were detected in Thessaloniki Bay (Genitsaris *et al.*, 2019), even though biological water treatment has been implemented since 2001, aiming to reduce the effects of anthropogenic eutrophication (Krestenitis *et al.*, 2012). Even though citizens and authorities are increasingly concerned about the water quality of the Bay and particularly the urban area, extensive studies on red tides and mucilage aggregate phenomena are lacking for Thermaikos Gulf with few exceptions (Genitsaris *et al.*, 2021; Androulidakis *et al.*, 2021; Patoucheas & Savvidis, 2010; Nikolaidis *et al.*, 2006).

1.4 Use of remote sensing towards Chl-a estimation in coastal areas

The Marine Strategy Framework Directive requires that European Union Member States achieve Good Environmental Status (GES) in marine waters by 2020 (MSFD, 2008/56/EC). Phytoplankton is the biological element most closely related to eutrophication, and a primary indicator for the assessment of water quality, as registering high reproduction rates and rapidly responding to environmental changes. According to the Water Framework Directive (WFD), different phytoplankton attributes are considered essential for the appraisal of ecological status, including species composition, abundance and biomass, as well as frequency and intensity of phytoplankton blooms (WFD, 2000/60/EC). Furthermore, the composition of phytoplankton communities, the dominance of taxonomic groups (e.g., diatoms or dinoflagellates) and the frequency of HABs are also considered for overall assessment of coastal eutrophication status (EC 2005). A frequently used parameter for monitoring eutrophication at spatial and temporal scales is Chl-a concentrations which can be an indicator of phytoplankton biomass (Cullen, 1982; Jeffrey & Vesik, 1997) and, in turn, a primary indicator of ecosystem functioning (Boyce *et al.*, 2010; Roemmich & McGowan, 1995).

One of the most common methods used to estimate Chl-a concentrations is satellite remote sensing. In the detection of phytoplankton blooms, the amount and extent of the bloom can be estimated using Chl-a estimation algorithm, phytoplankton absorption assay, turbidity control, and light penetration changes in seawater and spectral study of different wavelengths. Steidinger and Haddad (1979) first used Chl-a estimated from the color satellite pattern sensor, the “Coast Zone Color Scanner” (CZCS, 1978–1986) to observe a significant bloom of the toxic dinoflagellate *Karenia*. Since then, several sensors have been launched and put into operation for stronger and more effective monitoring. These sensors include the “Sea-viewing Wide Field-of-view

Sensor” (SeaWiFS, 1997–2010), the “Moderate Resolution Imaging Spectroradiometer” (MODIS, 2000–present on Terra and 2002–present on Aqua), and the “Medium Resolution Imaging Spectrometer” (MERIS, 2002–2012).

Most detection algorithms are based on Chl concentration (Stumpf, 2001; Cannizzaro *et al.*, 2008). Also, in solar-stimulated fluorescence using the MODIS fluorescence line height (FLH) tool (Hu *et al.*, 2005). Now, however, MODIS nFLH (normalized FLH) provides a better measure of phytoplankton abundance (Mckee *et al.*, 2007) because it is based on blue-green reflectance ratios, which are more sensitive to perturbations from dissolved organic matter (CDOM) than usual, due to river runoff.

Most studies using remote sensing to examine bloom occurrence and dynamics have been done in the areas of the Gulf of Mexico but also in the East China Sea and Japan. In addition, a large percentage of research was aimed at the toxic dinoflagellate *Karenia brevis* (Poli *et al.*, 2000). Ishizaka *et al.* (2006) successfully used the Chl-a concentration product derived from the SeaWiFS sensor to detect spatial and temporal variations of red tide in Japan. However, red tide delineation using satellite Chl-a cannot differentiate phytoplankton blooms (e.g., seasonal blooms) from red tides and/or HABs (Tomlinson *et al.*, 2004). To overcome this problem, satellite Chl-a anomaly extraction was introduced and adopted as one of the most functional techniques for monitoring blooms (Stumpf *et al.*, 2003; Tomlinson *et al.*, 2004; Gokul *et al.*, 2023; Gokul *et al.*, 2020; Gokul *et al.*, 2019).

Satellite observation of ocean color provides an estimate of Chl-a concentration in near surface waters down to 20–25 m in the clearest waters (Kemp & Villareal, 2013), although the availability of information is limited by clouds. The dependence on Chl-a makes this information more important when biomass is high, such as in cases of red tides (e.g., Japan) (Ishizaka *et al.*, 2006) and blooms of the toxic diatom *Pseudo-nitzschia* spp. (e.g., Spain) (Spyrakos *et al.*, 2011).

Also, although few in number, there are operational coupled toxic bloom observation/modeling systems. NOAA's operational forecast of harmful algal blooms (HAB-OFS) operating in Florida and Texas in the USA (Stumpf *et al.*, 2008). This system is based on a number of approaches including satellite images, field observations and mathematical models. It also incorporates public health reports and data from existing buoys to predict blooms and their course. However, it is limited to

mono-specific high-biomass blooms that are detectable by satellites using ocean color algorithms, and actual predictions are based on passive particle transport.

The aim of this study was to examine planktonic unicellular eukaryotic communities' structure and abundance in three sites of close proximity in Thessaloniki Bay, during different bloom periods, recorded in spring and summer. At the same time satellite remote sensing data were analyzed and were integrated with the *in-situ* results. The main questions were: i) What is the composition of unicellular planktonic eukaryotic communities during spring and summer blooms in Thessaloniki Bay?, ii) Which species dominated in terms of abundance and biomass? iii) Were there differences in the composition of blooming unicellular eukaryotic communities in the three sites and the two sampling seasons?, iv) How remote sensing data can be integrated to the investigation of coastal bloom events of the study area?.

2. Materials and Methods

2.1 Study Area

Thessaloniki Bay in the inner Thermaikos Gulf, is a semi-closed embayment of the northwest Aegean Sea with a maximum depth of 45 m (Balopoulos & Friligos, 1993) (Fig. 1). Thermaikos Gulf is defined to the north by the coastline of the city of Thessaloniki, to the west by the coastline of Nea Pieria, to the east by the coastline of the Kassandra peninsula, and to the south by the 200 m isobath (Krestenitis *et al.*, 1997). The Gulf is divided into inner and outer parts. The Northern part of the Gulf is compressed into two shallow and successive bays with a maximum depth of 25 m, which are subjected to urban and industrial pollution. Thessaloniki Bay, and its commercial harbor are considered as the human-affected coastal area in Greece and, on a broader basis, in the Eastern Mediterranean (Dimiza *et al.*, 2020). On the other hand, the Southern part of Thermaikos, with a maximum depth of 40 m, communicates with the open sea receiving seawater from the Aegean Sea (Robles *et al.*, 1983; Balopoulos, 1986).



Figure 1. Map of Thermaikos Gulf, indicating the locations of the three sampling sites in Thessaloniki Bay. HB: Harbor, WT: White Tower, MH: Music Hall.

There is a strong correlation between the eutrophic status of the Gulf and river discharges. Thermaikos Gulf, the western part in particular, receives significant amounts of water from the Axios, Aliakmon, Loudias, and Gallikos Rivers, with the

Axios and Aliakmon contributing the biggest percentage of nutrients (Androulakis *et al.*, 2021). The waters of the large river systems and especially of the river Axios are heavily polluted by industrial effluents and domestic waste. Due to the continental inputs, the Gulf is rather fertile and is one of the major sites for trawling activities in Greece (Stergiou *et al.*, 1997).

Also, Thermaikos Gulf receives the municipal sewage of the metropolitan city of Thessaloniki and the Atherida Coast, as well as the 2 pumping sites (Malgaron and Kleidi). For decades, there has been a large volume of industrial and domestic waste from Thessaloniki being discharges with primary treatment only in Thessaloniki Bay. The Thessaloniki Bay, in particular, has been recognized as being quite affected by nutrient enrichment, often resulting in the appearance of red tides with significant socio-economic implications in the area (Karageorgis *et al.*, 2005; Pagou, 2005; Genitsaris *et al.*, 2019).

2.2 Sample collection

Water samples from Thessaloniki Bay were collected during April 2022 and weekly from July 2022 to August 2022, from three inshore sites along the urban front of the Bay (Table 1). In total, 9 surface samples of 500 mL were collected from the harbor area of Thessaloniki (HB), 11 from White Tower (WT), and 11 from Music Hall (MH). After samplings, water samples were preserved in Lugol's solution for microscopic analysis.

Table 1. Sampling sites at Thessaloniki Bay, and the total number of samples collected in each sites.

Sampling sites	Latitude	Longitude	Number of samples
HB (HARBOR)	40.63155° N	22.93454° E	9
WT (WHITE TOWER)	40.62542° N	22.94776° E	11
MH (MUSIC HALL)	40.59631° N	22.94878° E	11

2.3 Satellite Data Collection

Satellite data for Thessaloniki Bay, were obtained from the EU Copernicus Marine Environment Monitoring Service (CMEMS) at <https://marine.copernicus.eu/> using multisensor Chl-a (mg/m³) daily products at 1 km resolution, which are a mashup of MODIS-Aqua, NOAA-20-VIIRS, NPP-VIIRS, and Sentinel3A-OLCI data, covering

the period from September 1997 to September 2022. The sampling locations were matched up with the corresponding pixels in the images. To get a Chl-a value, the average of the pixels was calculated (window 2*4 in Thermaikos Gulf). Finally, the corresponding Chl-a values were extracted.

2.4 Microscopic analysis

Before the microscopic examination, all samples were gently homogenized in the 0.5 L plastic container, with careful mixing to avoid destroying the microorganisms, and were examined in 2 mL and 25 mL sedimentation chambers. The required settling time of the sample in the chambers was 24 hours. The quantitative and qualitative measurements of planktonic communities were performed with an inverted microscope (Zeiss AxioVert 100 Inverted Microscope), with phase contrast. The identification of the planktonic unicellular eukaryotes was based on the taxonomic keys and relevant papers of Schiller (1937), Dodge (1985), Round et al. (1990), Tomas (1993, 1996), and also the database of Kuylenstierna & Karlson (1996-2006).

The cells of the planktonic unicellular eukaryotic microbes were counted at 100X and 400X magnification according to the inverted microscope method (Utermöhl, 1958). Larger cells were counted by scanning the chambers with 100X magnification. For the smaller cells, visual fields were examined at the 400X magnification to reach 100 cells counts of the most dominant taxa and 400 cells counts of the total community. The threshold of 400 counted cells from all species was set in order to achieve a statistically accepted estimation of the unicellular eukaryotic abundance (Katsiapi *et al.*, 2011). Mean cell volume estimates were calculated using appropriate geometric formulae according to Hillebrand et al. (1999). Biovolumes were converted to biomass assuming a density equal to water, i.e 1 g cm^{-3} (Rott, 1981).

2.5 Phytoplankton blooms and Red Tides

In the present study, water samples were analyzed to describe seasonal planktonic blooms. According to the ecology of marine microorganisms and some extent the incorporation and implementation by European countries of the Water Framework Directive (2000/60/EC) and as well as the Marine Strategy Directive (2008/56/EC) the definition of what is a bloom varies from species to species and from region to region. In the present study, phytoplankton and protozooplankton organisms were divided into size classes based on their cell biovolume, and for each class was established the limit

of phytoplankton and protozooplankton abundance per unit volume of water corresponding to a bloom or red tide. More specifically, seven size categories were created to define abundances follows:

- a) cells with size $< 100 \mu\text{m}^3$ and abundance $> 10000 \text{ cells mL}^{-1}$
- b) cells with size $100 - 250 \mu\text{m}^3$ and abundance $10000 - 5000 \text{ cells mL}^{-1}$
- c) cells with size $250 - 1000 \mu\text{m}^3$ and abundance $5000 - 1000 \text{ cells mL}^{-1}$
- d) cells with size $1000 - 2500 \mu\text{m}^3$ and abundance $1000 - 400 \text{ cells mL}^{-1}$
- e) cells with size $2500 - 5000 \mu\text{m}^3$ and abundance $400 - 200 \text{ cells mL}^{-1}$
- f) cells with size $5000 - 10000 \mu\text{m}^3$ and abundance $200 - 100 \text{ cells mL}^{-1}$
- g) cells with size $> 10000 \mu\text{m}^3$ and abundance of $25 - 100 \text{ cells mL}^{-1}$

3. Results

3.1 Environmental Parameters

The surface temperature recorded, during the study period in Thessaloniki Bay, ranged from 19°C to 32°C. In spring the highest was observed on 25-Apr. In summer the highest values were recorded on the 10th and 17th of August 2022 (fig. 2).

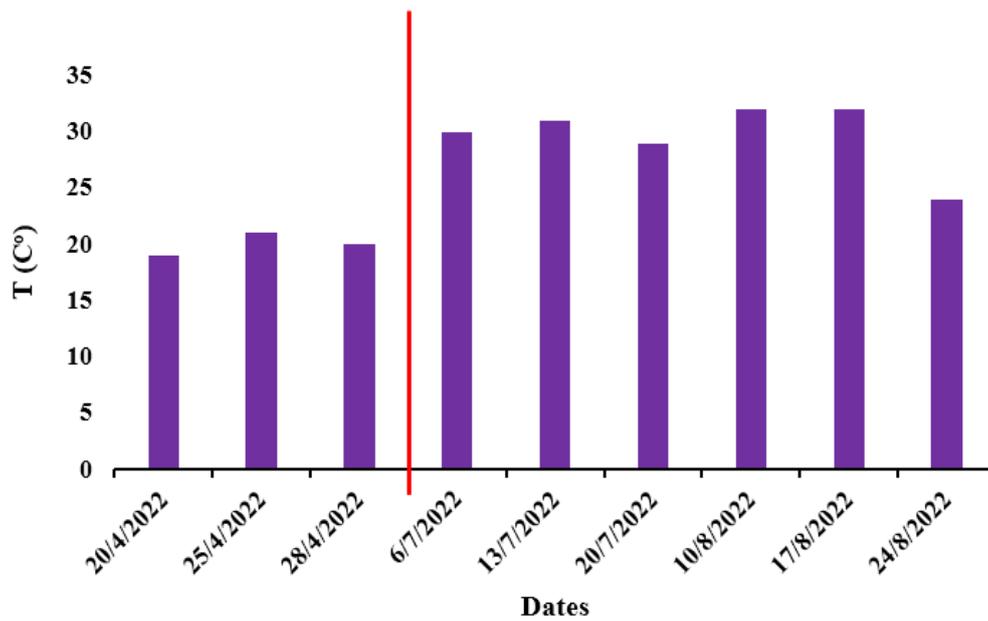


Figure 2. Temperature range in Thessaloniki Bay during the period of the study. Two bloom periods are separated with the vertical red line.

3.2 Satellite Data

The results of satellite observations of Chl-a concentrations in Thessaloniki Bay area and the adjacent offshore region, are presented in Figure 3. Monthly climatology of Chl-a concentrations from 1998 to 2021 reveal a single seasonal repetitive peak during the period of March (5.82 mg m^{-3}) which then drop. The lowest value recorded in December with 2.07 mg m^{-3} . Chl-a concentrations of 2022 have lower values than the 23-year climatology.

During the study period, the satellite data values differed from the *in-situ* measurements. The climatology of Thessaloniki Bay in 2022 has two peaks, the first occurring on 16 – 31 of January (6.51 mg m^{-3}) and a second peak on 16 – 31 of March with 6.70 mg m^{-3} . After one month there is a pronounced decrease until 16 – 31 of April, where the Chl-a concentration has the lowest value (2.19 mg m^{-3}), still very high for marine environment.

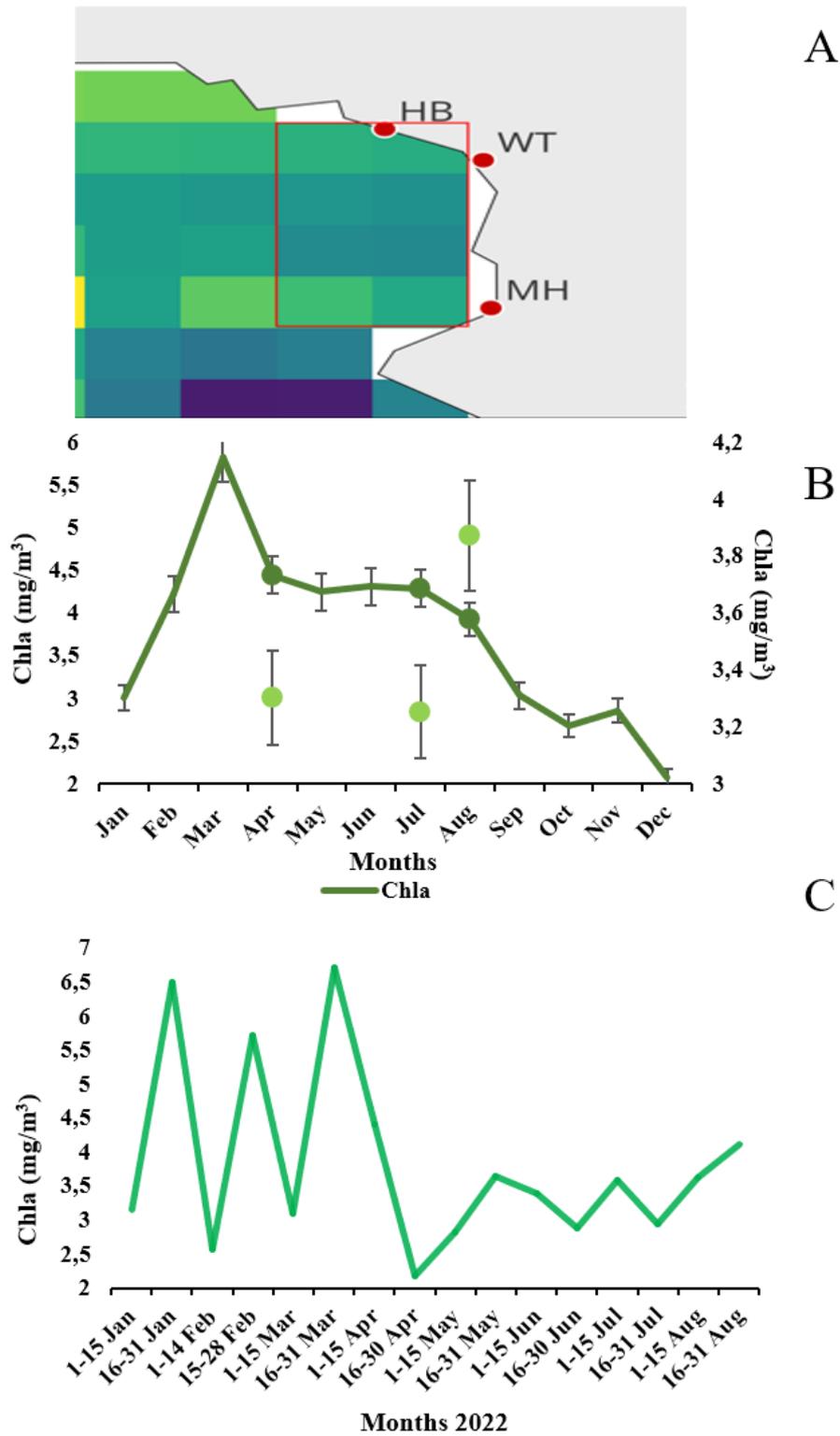


Figure 3. A) Map showing satellite-derived Chl-a concentration in Thessaloniki Bay. B) Monthly Climatology of Chl-a in Thessaloniki Bay for the period 1998 – 2021 with the climatology values of April, July and August of 2022. C) Fortnight Climatology of Chl-a in Thessaloniki Bay for 2022 (until 31th of August).

In addition to the monthly climatology, the interannual Chl-a climatology was calculated. As shown in Figure 5, 2003 was the year in which Chl-a reached the highest values with an average of 5.54 mg m^{-3} . From 1998 onwards the Chl-a values did not fall below 2.5 mg m^{-3} , a very high concentration for marine environment, but in 2019 counted the lowest concentrations of Chl-a (2.79 mg m^{-3}). From the satellite observations the anomaly of Chl-a concentrations was calculated. The biggest positive deviation was on April of 2013 with an average of 17.63 mg m^{-3} , while on February of 2011 the highest negative value was noted (-3.61 mg m^{-3}) (Fig 4).

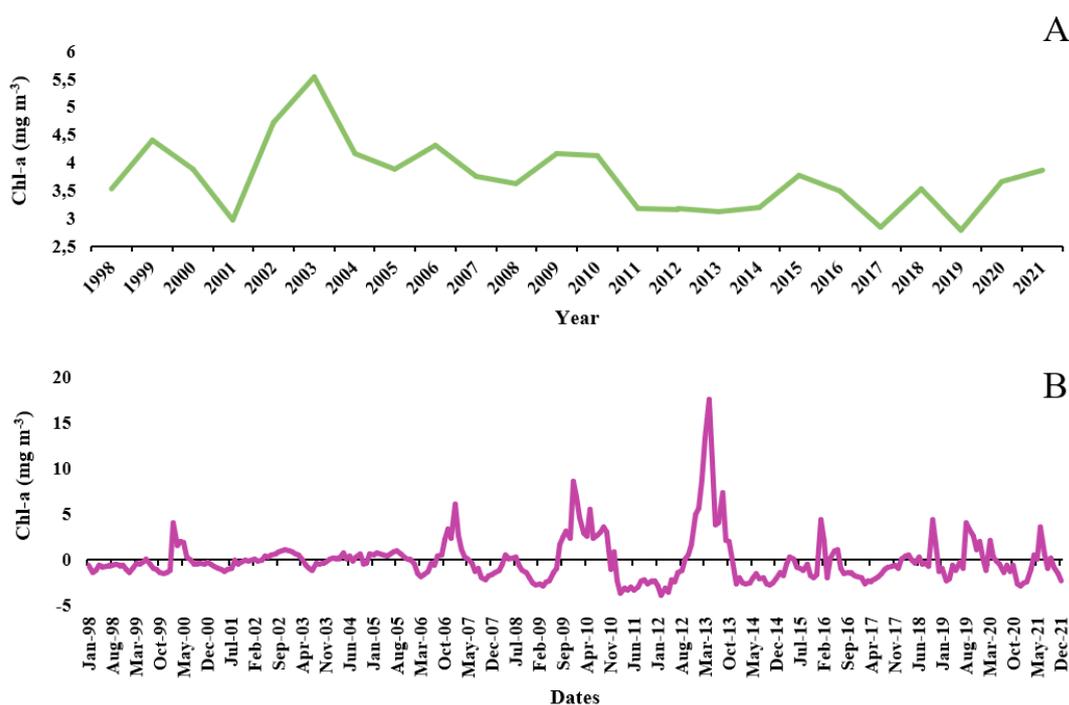


Figure 4. A) Interannual Climatology of Chl-a in Thessaloniki Bay for the period 1998-2021. B) Anomaly Climatology of Chl-a in Thessaloniki Bay for the period 1998-2021.

3.3 Composition of the planktonic unicellular eukaryotes

Overall, 9 high-level taxonomic groups were identified in Thessaloniki Bay in all 3 sampling sites during the study period (Fig. 5). Dinophyceae had the highest total species richness with 46% of the total number of species, including also mixotrophic and heterotrophic dinoflagellates. Bacillariophyceae followed with 41%, while other groups like Chlorodendrophyceae, Chlorophyceae, Cryptophyceae, Dictyochophyceae, Euglenophyceae, Prasinophyceae, and Prymnesiophyceae contributed with < 5% of the total number of taxa.

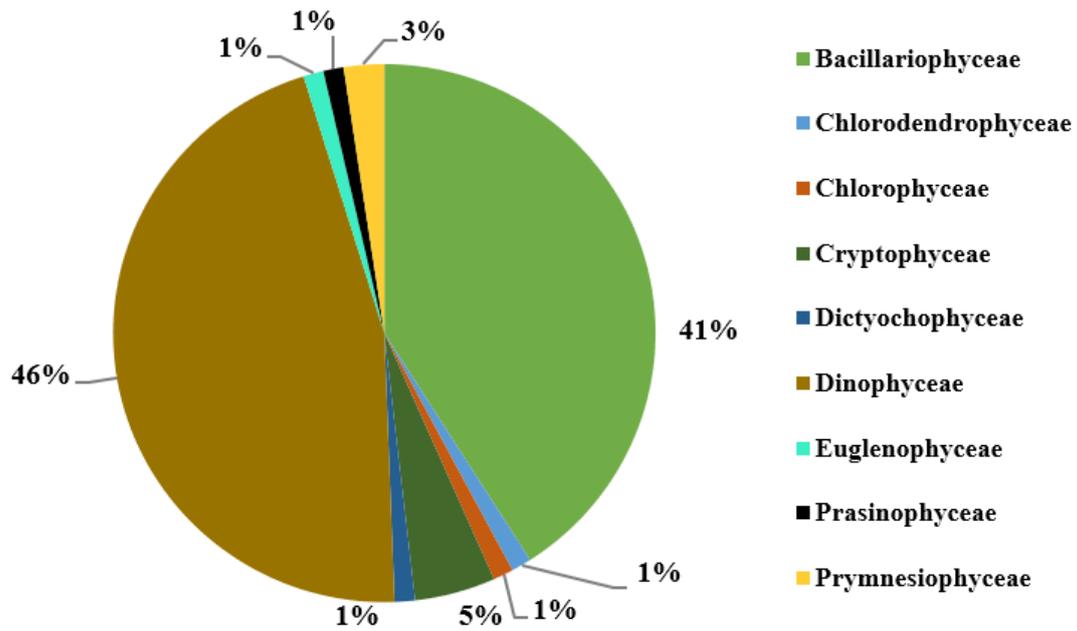


Figure 5. Percentage (%) of taxa per taxonomic group of the planktonic unicellular eukaryotic microorganisms of Thessaloniki Bay.

A total of 83 plankton species were identified in the 3 sampling sites (Table 2). During the study period, Dinophyceae were more diverse in terms of their species richness in the 3 sampling sites accounting for 38 taxa in total followed by Bacillariophyceae with 34 taxa. In spring, more species of Dinophyceae were counted than in the summer samplings (Fig. 6). Cryptophyceae and Prymnesiophyceae comprised of 4 and 2 taxa, respectively, while the other taxonomic groups were represented by only 1 taxon. The presence of each unicellular eukaryotes in the samplings is given in Supplementary Tables 1 - 3 (Appendix).

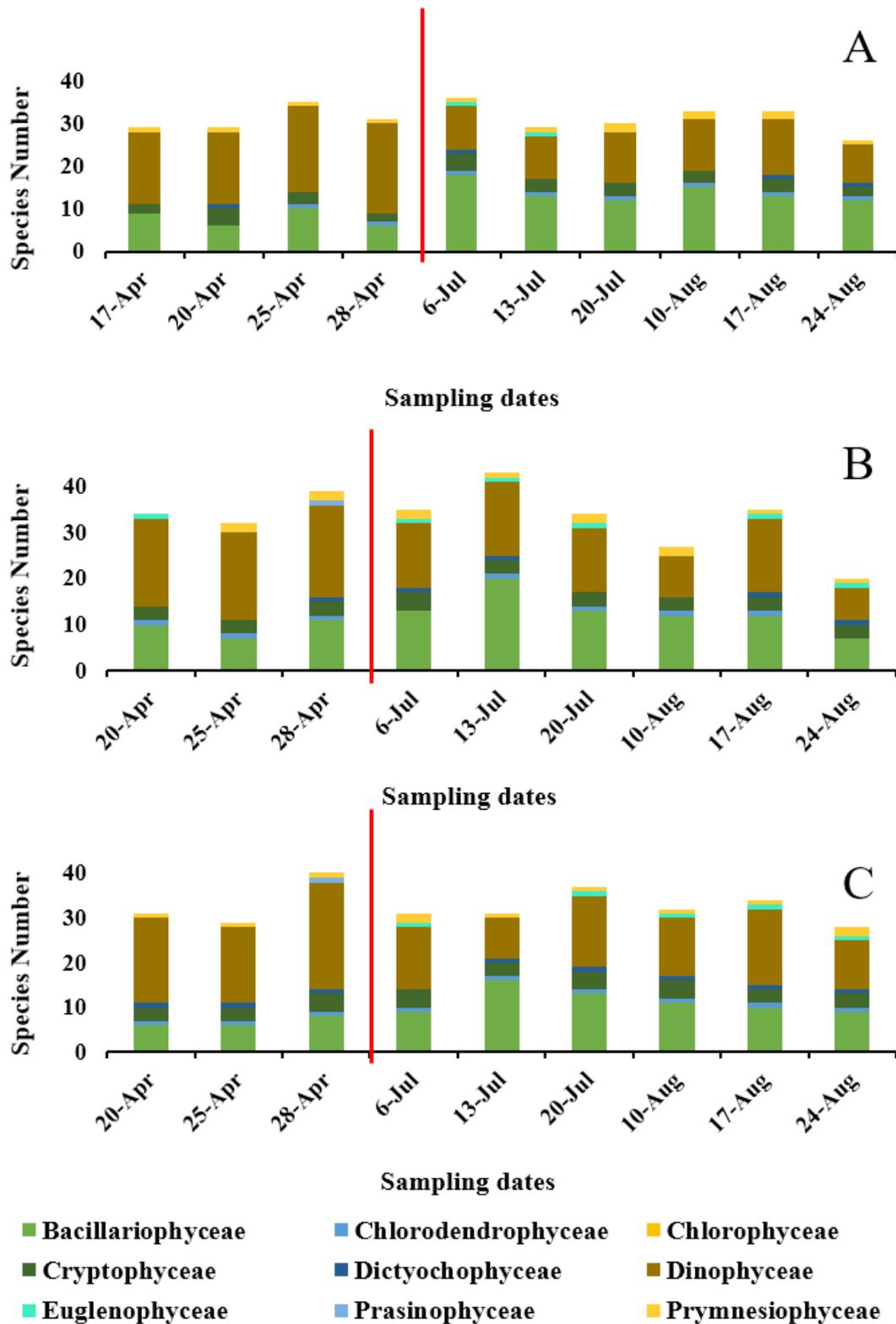


Figure 6. Number of taxa per taxonomic group of the planktonic unicellular eukaryotic microorganisms in Thessaloniki Bay: A) Music Hall (MH), B) White Tower (WT), C) Harbor (HB). Two bloom periods are separated with the vertical red line.

Table 2. List of planktonic unicellular eukaryotic taxa that have been identified in the samples of seawater from Thessaloniki Bay during the period April 2022 – August 2022. (*) indicates heterotrophic species. (**) indicates mixotrophic species.

Bacillariophyceae		
<i>Asterionellopsis glacialis</i>	<i>Hemiaulus hauckii</i>	<i>Pseudo-nitzschia seriata</i> sf.
<i>Bacteriastrium furcatum</i>	<i>Hemiaulus sinensis</i>	<i>Rhizosolenia imbricata</i>
<i>Chaetoceros affinis</i>	<i>Leptocylindrus danicus</i>	<i>Rhizosolenia setigera</i>
<i>Chaetoceros lorenzianus</i>	<i>Leptocylindrus minimus</i>	<i>Rhizosolenia pungens</i>
<i>Chaetoceros</i> sp.	<i>Lioloma pacificum</i>	<i>Rhizosolenia</i> sp.
<i>Chaetoceros tenuissimus</i>	<i>Lithodesmium undulatum</i>	<i>Skeletonema costatum</i>
<i>Chaetocheros socialis</i>	<i>Meuniera membranacea</i>	<i>Thalassionema nitzschoides</i>
<i>Coscinodiscus</i> sp.	<i>Navicula</i> sp.	<i>Thalassiosira oceanica</i>
<i>Cylindrotheca closterium</i>	<i>Nitzschia longissima</i>	<i>Thalassiosira</i> sp.
<i>Dactyliosolen fragillissimus</i>	<i>Pleurosigma normanii</i> .	
<i>Eucampia zodiacus</i>	<i>Proboscia alata</i>	
<i>Guinardia striata</i>	<i>Pseudo-nitzschia australis</i>	
Chlorodendrophyceae		
<i>Tetraselmis</i> sp.		
Chlorophyceae		
<i>Chlamydomonas coccoides</i>		
Cryptophyceae		
<i>Chryptomonas</i> sp.**		
<i>Hillea fusiformis</i> **		
<i>Plagioselmis</i> sp.**		
<i>Teleaulax acuta</i> **		
Dictyochophyceae		
<i>Dictyocha fibula</i> **		
Dinophyceae		
<i>Ceratium candelabrum</i> **	<i>Gymnodinium elongatum</i> * sf.	<i>Prorocentrum gracile</i> **
<i>Ceratium furca</i> **	<i>Gyrodinium</i> sp.*	<i>Prorocentrum lima</i> **
<i>Ceratium fusus</i> **	<i>Heterocapsa triquetra</i> **	<i>Prorocentrum micans</i> **
<i>Ceratium lineatum</i> **	<i>Karenia</i> sp.**	<i>Prorocentrum minimum</i> **
<i>Ceratium macroceros</i> **	<i>Katodinium</i> sp.**	<i>Proto-peridinium brevipes</i> *
<i>Ceratium trichoceros</i> **	<i>Noctiluca scintillans</i> *	<i>Proto-peridinium claudicans</i> *
<i>Ceratium tripos</i> **	<i>Oxytoxum glacile</i> *	<i>Proto-peridinium steinii</i> *
<i>Diplopsalis</i> sp.*	<i>Oxytoxum longiceps</i> *	<i>Proto-peridinium oceanicum</i> *
<i>Dinophysis acunimata</i> **	<i>Podolampas palmipes</i> *	<i>Pyrophacus hologonium</i> **
<i>Dinophysis dens</i> **	<i>Polykrikos Schwartzii</i> **	<i>Scripsiella trochoidea</i> **
<i>Gonyaulax fragilis</i> **	<i>Prorocentrum compressum</i> **	<i>Spatulodinium pseudonoctiluca</i> *
Euglenophyceae		
<i>Euglena</i> sp.**		
Prasinophyceae		

Pyramimonas octopus

Prymnesiophyceae

Chrysochromulina sp.**

*Phaeocystis pouchetii***

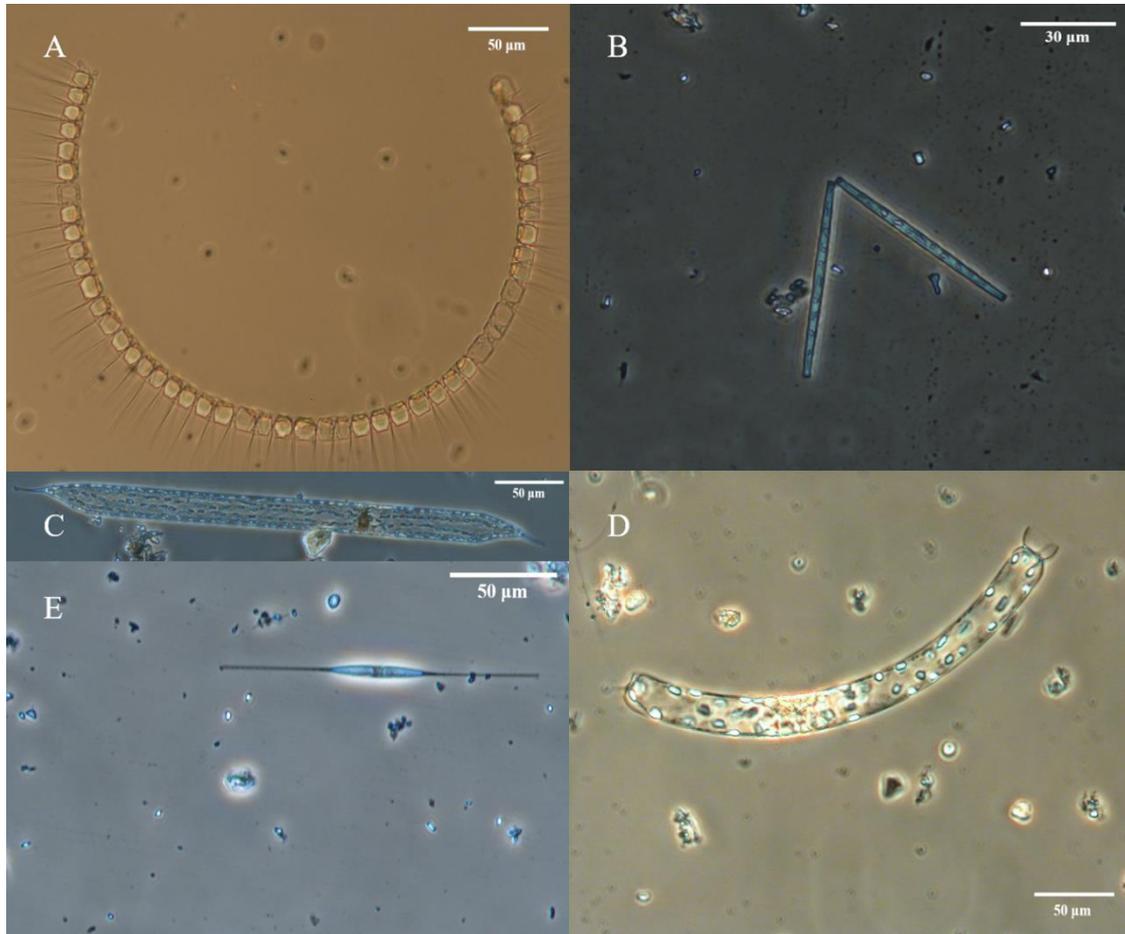


Figure 7. Microphotographs of diatoms in the study area of Thessaloniki Bay. A) *Chaetoceros* sp., B) *Thalassionema nitzschoides*, C) *Rhizosolenia imbricata*, D) *Guinardia striata*, E) *Cylindrotheca closterium*.

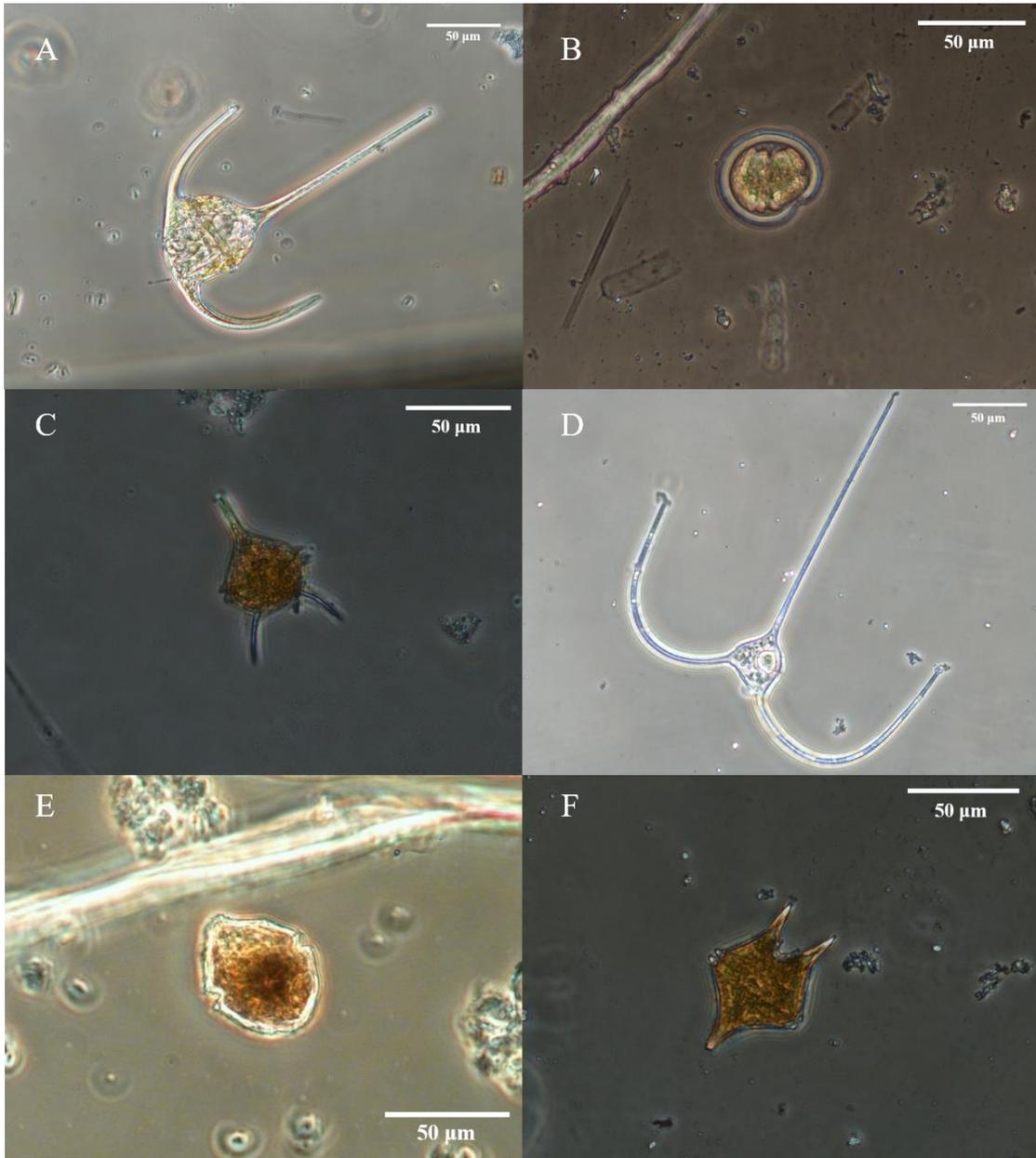


Figure 8. Microphotographs of Dinophyceae species in the study area of Thessaloniki Bay. A) *Ceratium tripos*, B) *Pyrophacus holorogium*, C) *Protoperidinium steinii*, D) *Ceratium trichoceros*, E) *Gonyaulax fragilis*, F) *Protoperidinium claudicans*.

3.4 Abundance

Overall, the cell abundance of the planktonic unicellular eukaryotes in the 3 sampling sites, recorded high variability. The lowest abundance value was recorded on 28-Apr in WT (377.68 cells mL⁻¹). The highest abundance was recorded on 20-Jul with 1816.35 cells mL⁻¹ (Fig. 9). The highest values of total cell numbers were observed at MH, followed by WT and then HB (MH>WT>HB).

In the spring samples, MH was the site with the highest microeukaryotic abundance, with the highest value recorded on 28-Apr (1454.43 cells mL⁻¹), while WT had the lowest abundance on the exact same date with 377.68 cells mL⁻¹. MH had, also, the highest abundance of unicellular eukaryotes in the summer samples compared to the other two sampling sites.

Regarding the values of the total abundance of unicellular eukaryotes compared to the limits of the trophic state proposed for Greece, all 3 sampling sites exceeded the limit for the upper mesotrophic state (960 cells mL⁻¹, UNEP/MAP 2015). Especially, in spring only MH exceeded 960 cells mL⁻¹ on 20-Apr, 25-Apr and 28-Apr. On the same dates WT and HB had an average abundance value of 664.71 cells mL⁻¹ and 517.76 cells mL⁻¹, respectively. In summer, all 3 sites exceeded the Greek eutrophication scale for water quality.

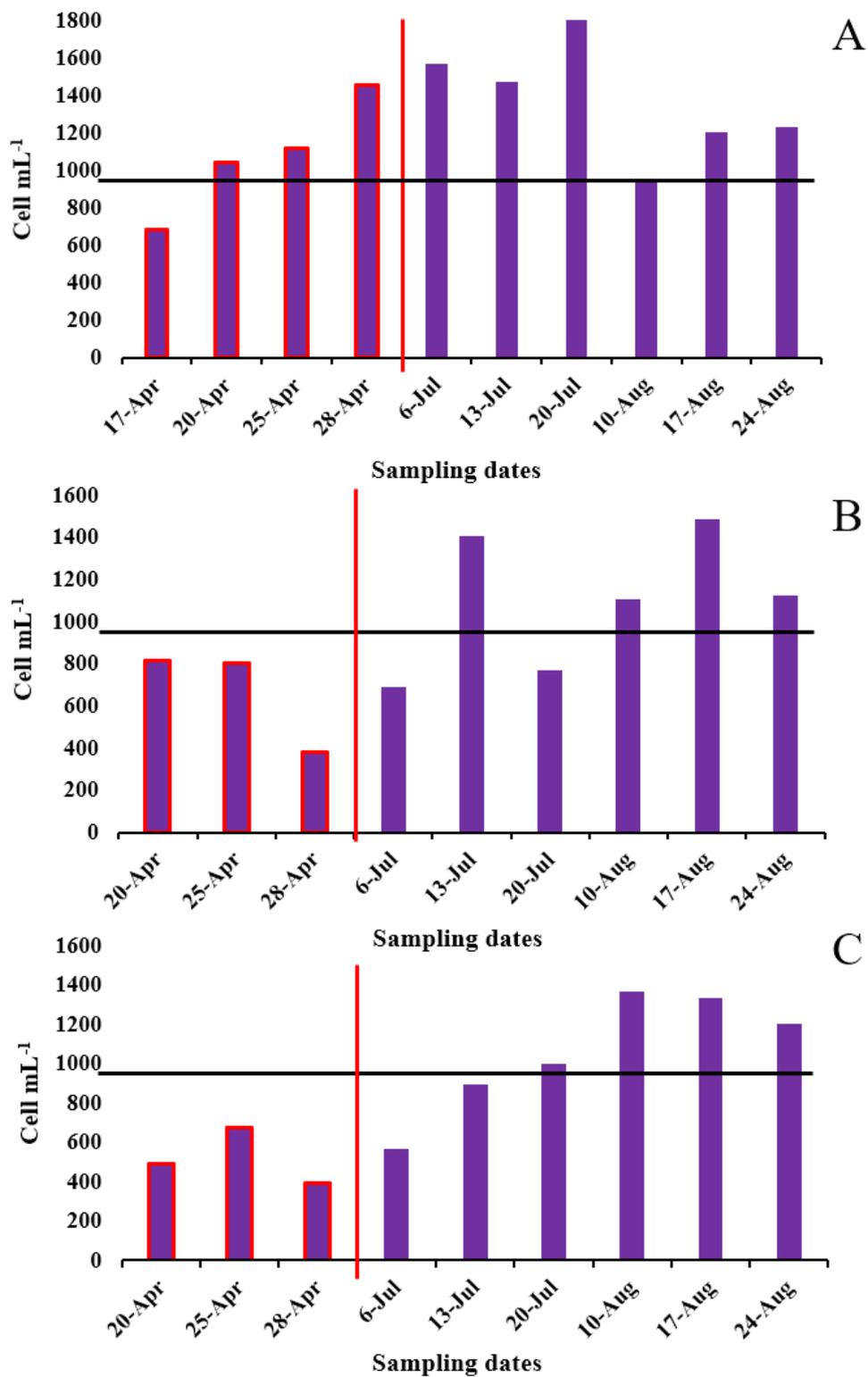


Figure 9. Total abundance of the unicellular eukaryotic communities at the 3 sampling sites of Thessaloniki Bay: A) Music Hall (MH), B) White Tower (WT), C) Harbor (HB). Two bloom periods are separated with the vertical red line. The black line corresponds to the upper mesotrophic state according to UNEP/MAP 2015.

Overall, Bacillariophyceae was the most abundant unicellular eukaryotic group. They reached 70% of the total abundance in most of the sampling sites (Fig. 10). The lowest diatom participation was observed in the spring period, on 20-Apr (~ 10%). In the summer period Bacillariophyceae reached two peaks (13-Jul and 17-Aug) exceeding 80% > of total abundance. Dinophyceae was the second most abundant unicellular eukaryotic group. According to Figure 10, Dinophyceae dominated in the spring period, with their highest contribution, to the total abundance, on 25-Apr. Chlorodendrophyceae, Chlorophyceae, Cryptophyceae, Prymnesiophyceae and HNFs contributed less than 9% of total abundance each. Other taxonomic groups (Dictyochophyceae, Euglenophyceae and Prasinophyceae) comprised less than 1% of total abundance.

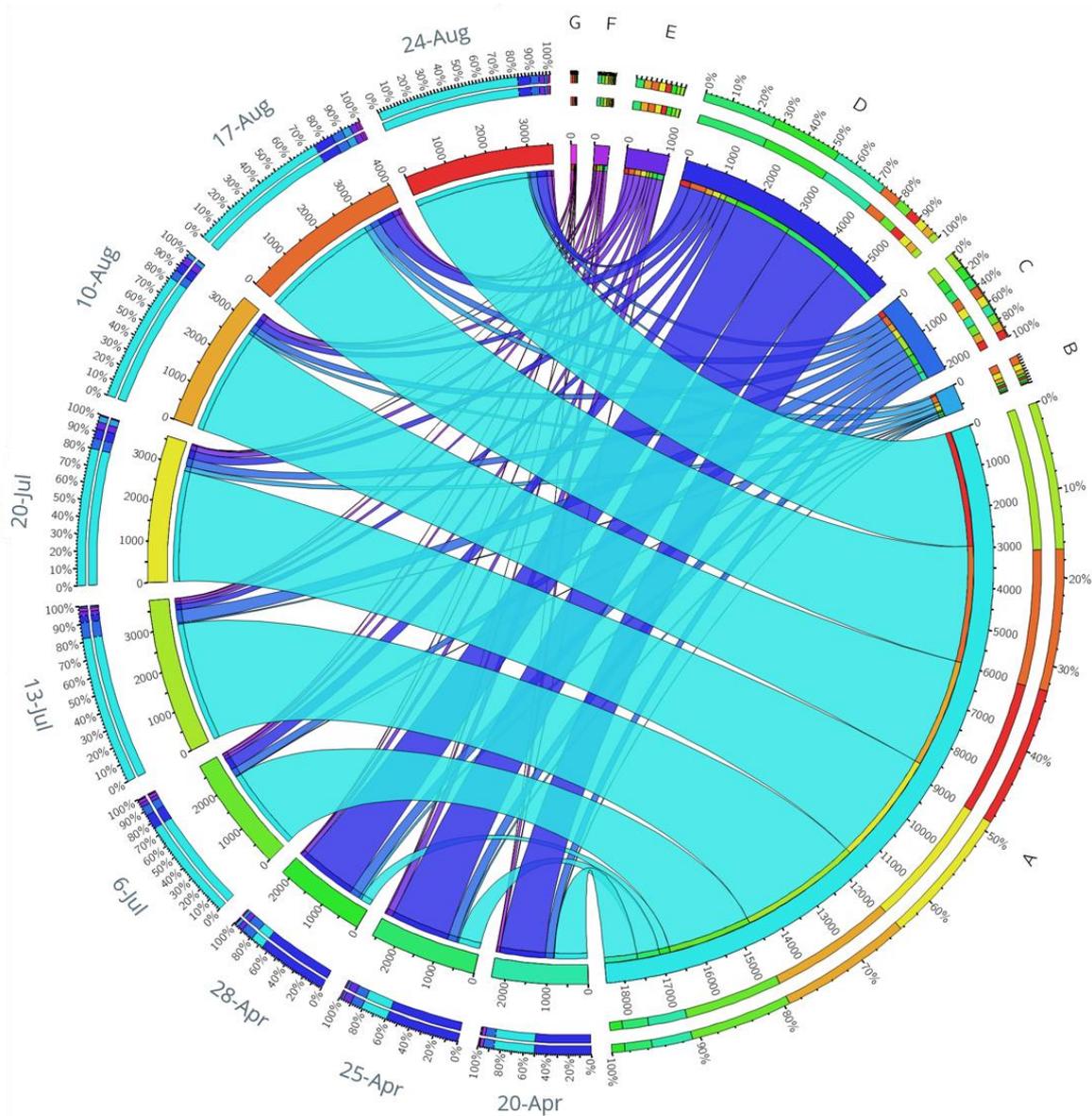


Figure 10. Circular representation of the contribution of each planktonic unicellular eukaryotic group to the total abundance in Thessaloniki Bay. A: Bacillariophyceae, B: Chlorodendrophyceae, C: Cryptophyceae, D: Dinophyceae, E: Prymnesiophyceae, F: HNF, G: Other groups (Dictyochophyceae, Euglenophyceae Prasinophyceae).

In general, Bacillariophyceae and Dinophyceae dominated in all 3 sites compared to the other taxonomic groups. In spring, Dinophyceae contributed approximately ~ 52% to the total abundance of unicellular eukaryotes, in all three sampling sites. As it is shown in Figure 11I, the total abundance of Bacillariophyceae varied from 16% in MH to 38% in WT. In summer, from 6-Jul to 24-Aug the percentage of their relative abundance was very low (< 11%) compared to Bacillariophyceae (Fig. 11II). The percentage of Cryptophyceae didn't exceed 15% in HB, while

Chlorodendrophyceae, Chlorophyceae, Prymnesiophyceae, HNFs contributed with less than 6% to the total abundance in each sampling site.

There was a big variability in the summer abundance of Bacillariophyceae and Dinophyceae. The abundance of Bacillariophyceae covered approximately 80% of total abundance in each sampling sites A big decline was observed in Dinophyceae, close to 40%, compared to the spring abundance. Chlorodendrophyceae, Chlorophyceae, Cryptophyceae, Prymnesiophyceae and HNFs contributed less than 4% in the total abundance of summer samples. Even if the spring and the summer abundance were close, different unicellular planktonic eukaryotic taxa dominated in the two different seasons.

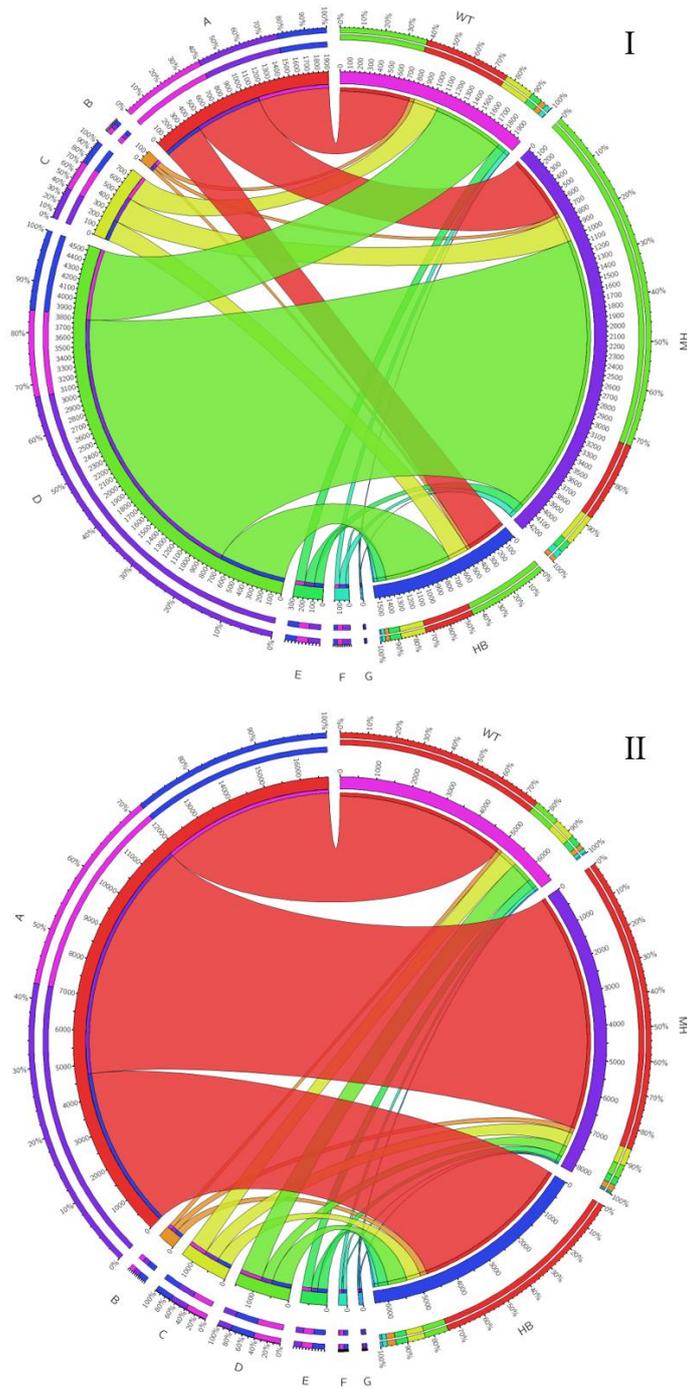


Figure 11. Circular representation of the contribution of each unicellular plankton group to: I) Total spring abundance, II) Total summer abundance, based on the 3 sampling sites of Thessaloniki Bay. A: Bacillariophyceae, B: Chlorodendrophyceae, C: Cryptophyceae, D: Dinophyceae, E: Prymnesiophyceae, F: HNF, G: Other groups (Dictyochophyceae, Euglenophyceae Prasinophyceae).

3.5 Plankton Biomass

Biovolumes (μm^3) of the species identified during the study period, in Thessaloniki Bay, are given in the Supplementary Table 4 (Appendix). The maximum unicellular plankton biomass, during the study period, varied from 1.43 mg L^{-1} at MH in 17-Apr to 27.61 mg L^{-1} at WT in 20-Apr. WT and HB had both their lowest biomass values on 28-Apr with 1.46 mg L^{-1} and 1.66 mg L^{-1} , respectively (Fig. 12).

Both MH and WT (MH on 28-Apr [20.36 mg L^{-1}] and WT on 20-Apr [27.62 mg L^{-1}]) exhibited peaks biomass of unicellular planktonic eukaryotes on the same date in spring. In comparison with the spring samples, only HB's peak was in summer, on 10-Aug (5.87 mg L^{-1}). In general, WT had the highest planktonic unicellular eukaryotes biomass, followed by MH. From 20-Apr to 24-Aug, the total unicellular biomass at HB remained very low in comparison with the other two sampling sites. The biomass of heterotrophs and mixotrophs was higher in the spring period in all three sampling sites. The mixotrophs (mixotrophic species of Dinophyceae, Cryptophyceae, Haptophyceae) were almost absent in the summer samples while autotrophs dominated at the same period.

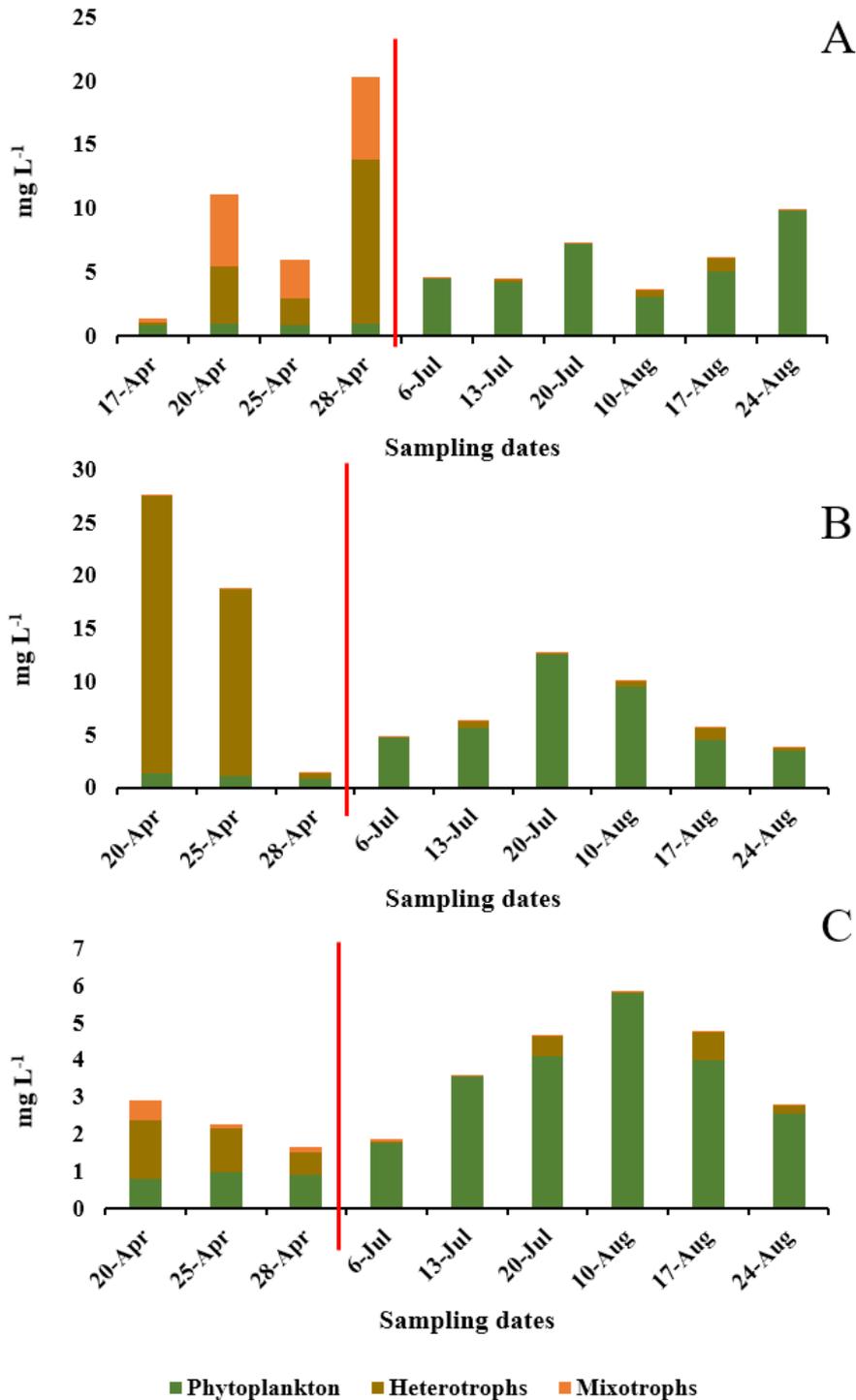


Figure 12. Total unicellular plankton biomass at 3 sampling sites of Thessaloniki Bay: A) Music Hall (MH), B) White Tower (WT), C) Harbor (HB). Two bloom periods are separated with the vertical red line.

The biomass values of each unicellular eukaryotic taxon generally followed the pattern of abundance, except for HB on 20-Apr. Even if Bacillariophyceae had recorded higher abundance values, Dinophyceae reached higher biomass value, in spring.

During the summer period, Bacillariophyceae were dominant in all 3 sampling sites. The highest value was recorded at WT on 20-Jul with 12.41 mg L⁻¹, while MH and HB reached their peaks on 20-Jul (7.13 mg L⁻¹) and 10-Aug (5.59 mg L⁻¹), respectively. Total unicellular biomass of Dinophyceae varied from 0.085 mg L⁻¹ on 13-Jul (HB) up to a pronounced peak reaching approximately 26.59 mg L⁻¹ on 20-Apr (WT) (Fig. 13). Chlorodendrophyceae, Chlorophyceae, Cryptophyceae, Dictyochophyceae, Euglenophyceae, Prasinophyceae, Prymnesiophyceae and HNFs were not dominant in any sample. Phytoplankton (i.e. without taking into account heterotrophs and mixotrophs) is given in Supplementary Figures 1 - 2 (Appendix).

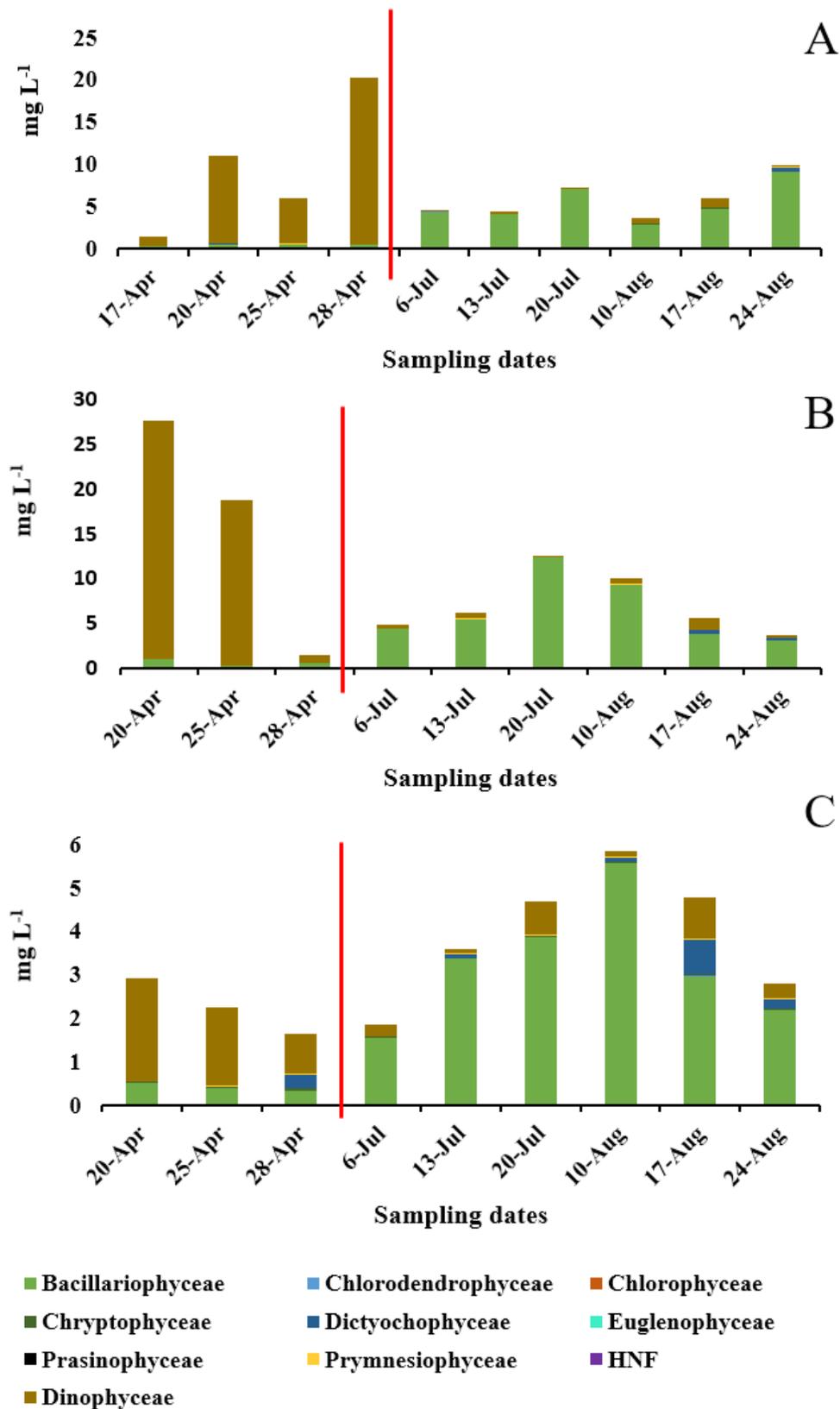


Figure 13. Biomass of each unicellular eukaryotic planktonic group at 3 sampling sites of Thessaloniki Bay: A) Music Hall (MH), B) White Tower (WT), C) Harbor (HB). Two bloom periods are separated with the vertical red line.

3.6 Phytoplankton Blooms – Red tides

Regarding the abundances of the most dominant unicellular planktonic taxa, Bacillariophyceae dominated in all 3 sampling sites of Thessaloniki Bay, during the summer period. On the contrary, Dinophyceae were the most abundant group blooming in spring except from two samples at WT (20-Apr and 28-Apr) (Figures 14). Their peak was on 28-Apr at MH with 1207.77 cells mL⁻¹. During the summer period, there was a big decline in Dinophyceae abundance in all 3 sampling sites with the lowest abundance value recorded on 13-Jul in MH (31.11 cells mL⁻¹).

The dominant species in spring were *P. micans* and *C. closterium*, and in summer were *Chaetoceros* sp., and *C. closterium*. According to their biovolumes (Supplementary table 4 – Appendix) the limits of *P. micans* is 100 cells mL⁻¹, *Chaetoceros* sp. 200 cells mL⁻¹ and *C. closterium* 10000 cells mL⁻¹. *C. closterium* didn't exceed the bloom limit according to WFD (2000/60/EC) and MSD (2008/56/EC) (see methods, section 2.5). Also, in summer *Chaetoceros* sp. exceeded the abundance threshold indicating bloom formation, according to its biovolume.

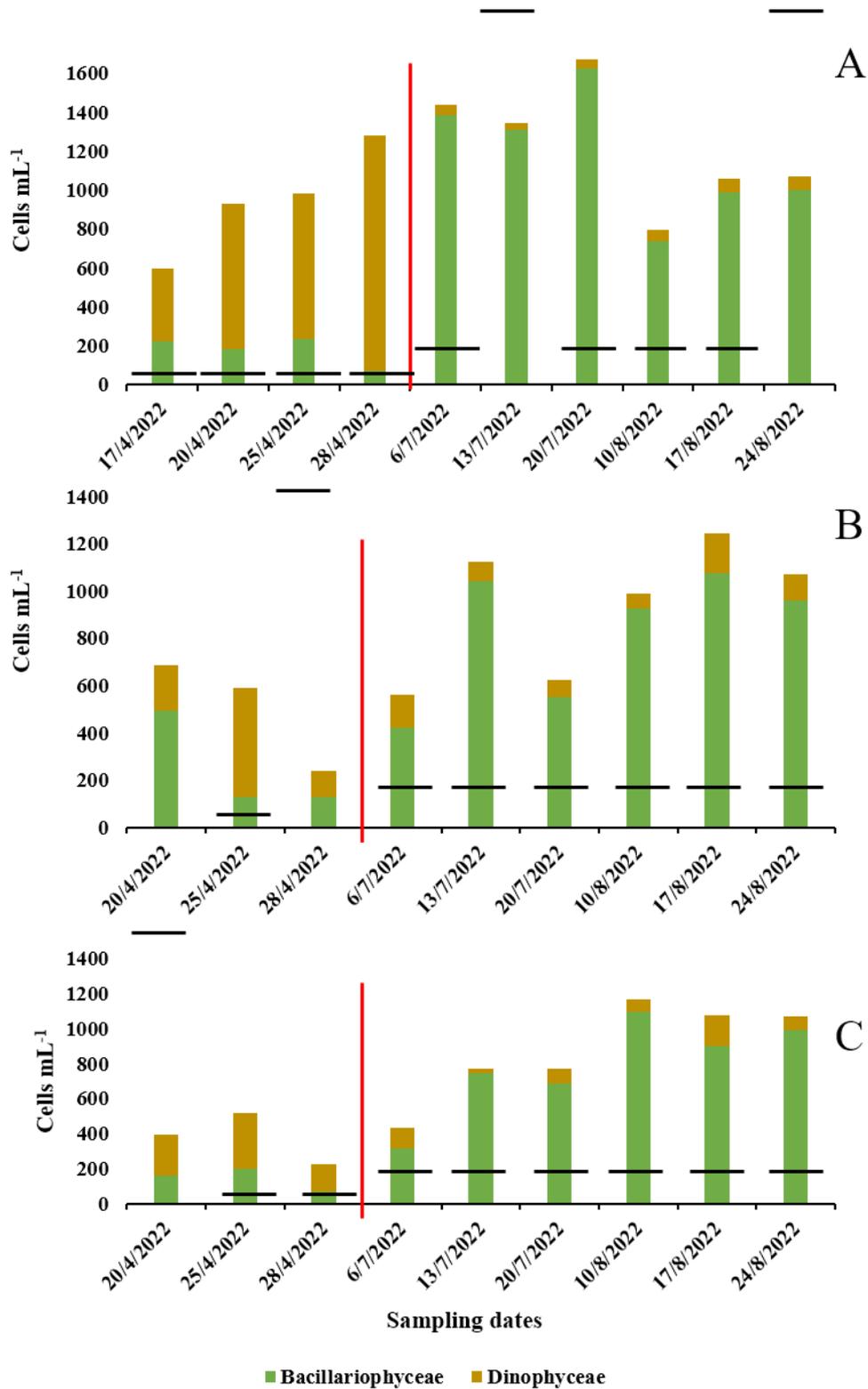


Figure 14. Abundance of Bacillariophyceae and Dinophyceae at 3 sampling sites of Thessaloniki Bay: A) Music Hall (MH), B) White Tower (WT), C) Harbor (HB). The spring samples are separated from the summer samples with a red line. The black lines indicate to the bloom thresholds of phytoplankton and protozooplankton abundance according to WFD (2000/60/EC) and MSD (2008/56/EC).

During the study period, *Chaetoceros* sp., *C. closterium*, *C. furca*, *N. scintillans* and *P. micans* had higher abundance, and biomass, than the other unicellular eukaryotes. Figure 15, represents the heat map, based on their abundance values. During the study period, only *Chaetoceros* sp., exceeded the limit for the upper mesotrophic state (960 cells mL⁻¹, UNEP/MAP 2015), with 1064.97 cells mL⁻¹ on 20-Jul. According to WFD (2000/60/EC) and MSD (2008/56/EC) only *Chaetoceros* sp. and *P. micans* exceeded the limit of abundance per unit volume of water corresponding to a bloom or red tide.

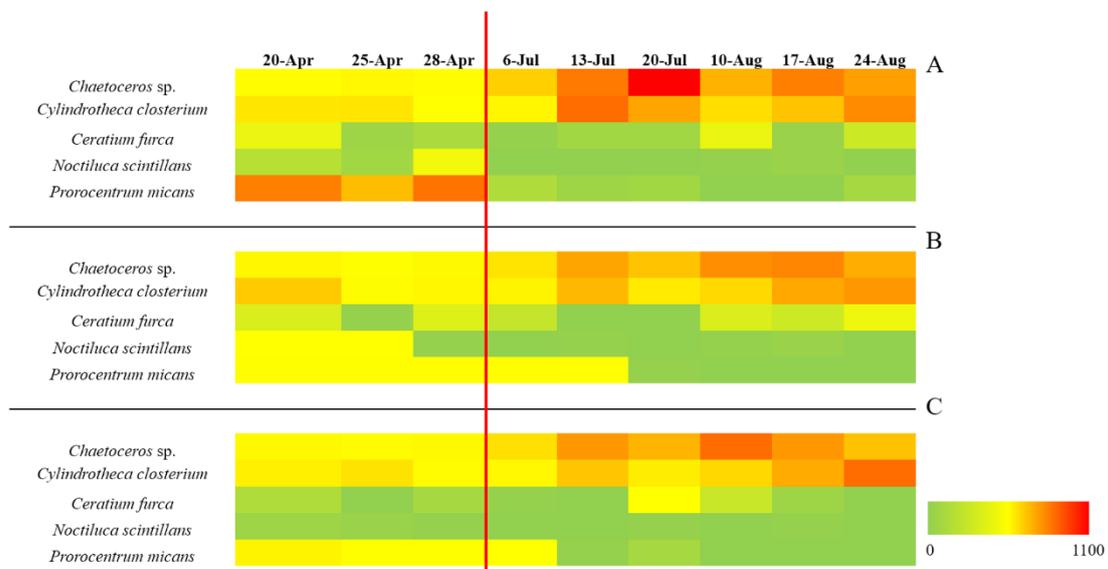


Figure 15. Heat map of 4 unicellular eukaryotes based on their abundance values (cells mL⁻¹). A) Music Hall, B) White Tower, C) Harbor. Two bloom periods are separated with the vertical red line.

The biomass heat map represents a different pattern for these dominant species, because of their different biovolume values and their contribution in total biomass (Fig. 16). Although *N. scintillans* was observed in low density through the study period, due to its large size (Supplementary Table 6, Appendix) it contributed to most of the biomass on 20-Apr in WT.

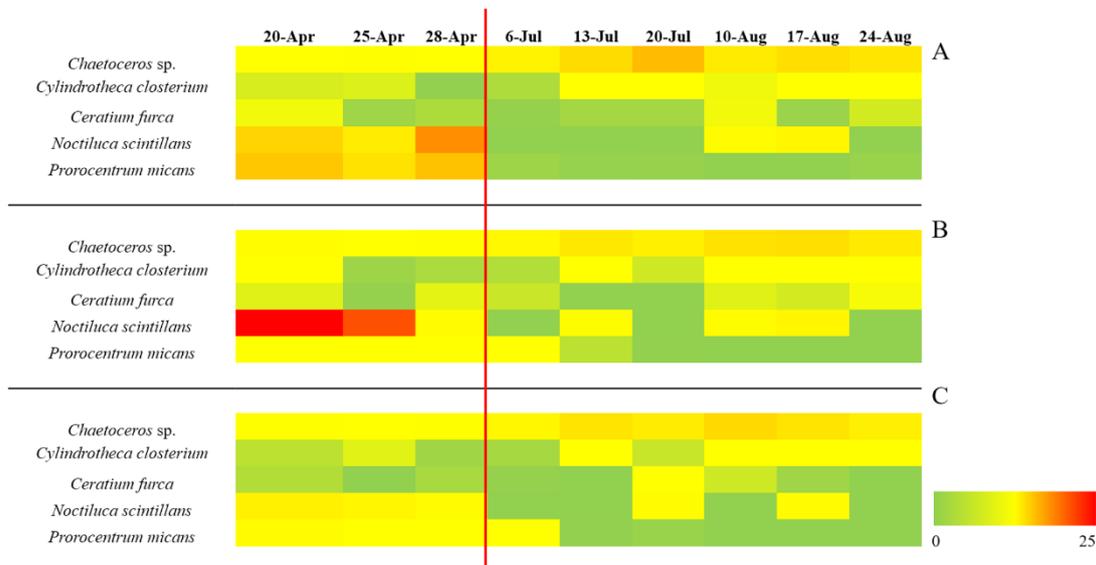


Figure 16. Heat map of 4 unicellular eukaryotes based on their biomass values (mg L^{-1}). A) Music Hall, B) White Tower, C) Harbor. Two bloom periods are separated with the vertical red line.

In March 2022 a macroscopically visible, red tide was detected in WT, consisting of the known red tide forming dinoflagellates *N. scintillans* and its close relative *Spatulodinium pseudonoclituca*. Also, on the 17th of April, mucus was macroscopically detected in MH. Overall, three samples were taken for research. The peak of the red tides appeared to be on 24-Mar with $497.00 \text{ cells mL}^{-1}$ (Fig. 17). There was a great decrease, in total abundance, on 20-Mar and on 17-Apr compared to the abundance of the 24-Mar sample, with $211.5 \text{ cells mL}^{-1}$ and $88.5 \text{ cells mL}^{-1}$, respectively. The total biomass followed the pattern of the abundance, recording the highest value on 24-Mar with $6156.92 \text{ mg L}^{-1}$. MH had the lowest biomass value ($1069.15 \text{ mg L}^{-1}$) on 17-Apr (Fig. 18).

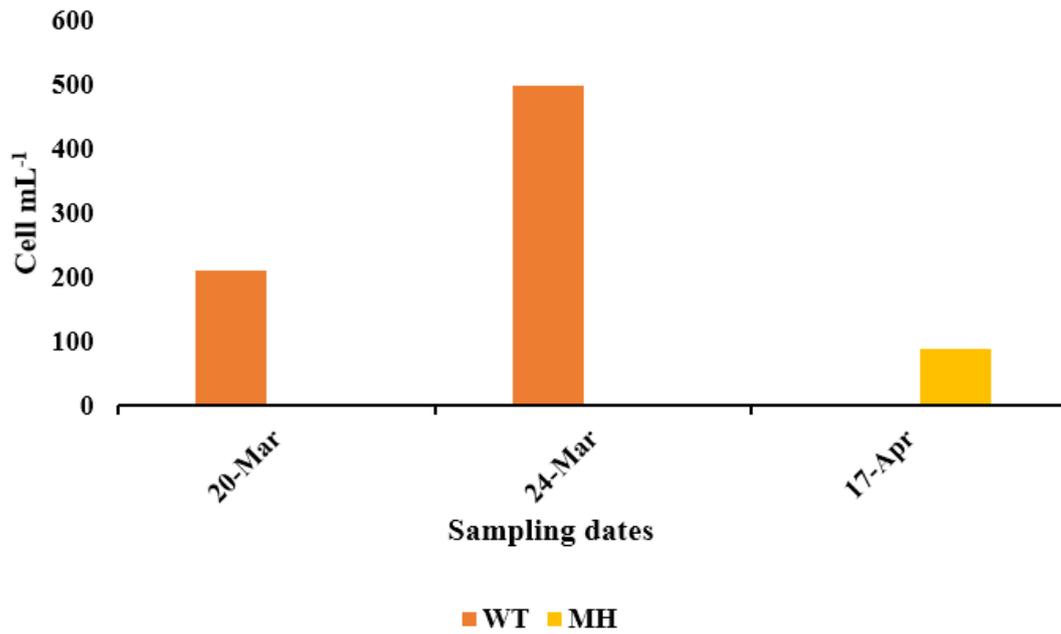


Figure 17. Total abundance of *Noctiluca scintillans* and *Spatulodinium pseudonoclituca* bloom at 2 sites of Thessaloniki Bay: White Tower (WT) and Music Hall (MH).

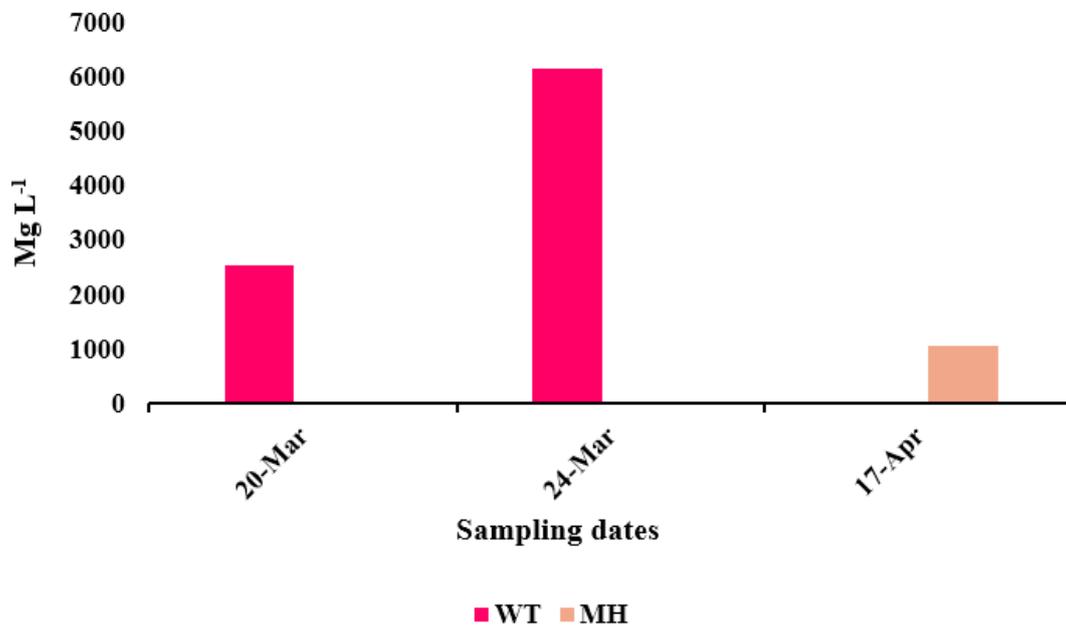


Figure 18. Total biomass of *Noctiluca scintillans* and *Spatulodinium pseudonoclituca* bloom at 2 sites of Thessaloniki Bay: White Tower (WT) and Music Hall (MH).

4. Discussion

4.1 Bloom dynamics

Diatoms and dinoflagellates were the most dominant taxonomic groups in Thessaloniki Bay during the study period in 2022. Observations from previous studies have shown that the dominance of diatoms and dinoflagellates is common in the Aegean Sea (Nikolaidis & Moustaka – Gouni, 1990; Mihalatou & Moustaka-Gouni, 2002; Pirreda *et al.*, 2018; Genitsaris *et al.*, 2019; Ignatiades, 2012). Petala *et al.* (2018), similarly recorded dominant diatom and dinoflagellate communities in Thermaikos Gulf.

During the present study, two seasonal unicellular eukaryotic assemblages have been distinguished in the planktonic community of the area. The first assemblage in April, consisted of the dinoflagellates *Scropsiella trochoida*, *Pyrophacus hologonium*, *P. micans*, *P. minimum*, *P. gracile* and *P. compressum*. The second, in July – August comprised of the diatoms, *Chaetoceros* sp., *C. closterium*, *L. minimus* and *Dactyliosolen fragilissimus*. These species dominated in all three sampling sites. These species were also identified dominating in the inner Thermaikos Gulf, in weekly samples during 2017 – 2018 (Genitsaris *et al.*, 2019), when diatoms had the highest contribution, followed by dinoflagellates which included mixotrophic and heterotrophic taxa.

In spring samples, a bloom of the dinoflagellate *P. micans* was detected in all three sampling sites. From 20-Apr to 28-Apr the abundance of alive cells of *P. micans* declined, while the occurrence of dead *P. micans*, increased indicating the dynamics of the blooms till its senescence. *P. micans* has been recorded causing HABs also in Pagassitikos and Saronikos Gulfs (Tsikoti & Genitsaris, 2021). Furthermore, in all sites heterotrophic and mixotrophic dinoflagellates contributed significantly to the total biomass in April. *P. micans* has been recorded before in Thermaikos Gulf to increase its biomass during spring (Ignatiades, 2012). Species of the genus *Prorocentrum* have caused water discoloration in different regions of Greece. For example, *P. cf. obtusidens* occurring in the inner part of Thermaikos Bay during the winter 2000-2001 was responsible for water discoloration in the area (Nikolaidis *et al.*, 2005). Previous studies have shown that mixotrophic dinoflagellates such as *Ceratium furca*, *P. micans* and heterotrophic dinoflagellates such as *N. scintillans*, *Gyrodinium* sp., *Gymnodinium* sp. often dominate in high densities in the study area (Ignatiades, 2012).

In contrast to spring, diatoms were the most dominant planktonic group in the summer bloom. Previous studies have shown that mostly diatoms have a strong dominance in Thermaikos Gulf (Genitsaris et al., 2020; Zapounidou, 2020). *Chaetoceros* sp. and *C. closterium* were the most dominant species (Azov et al., 1986). These species were also observed in Adriatic Sea (Cerino et al., 2019), and in Aegean Sea (Bizsel & Bizsel, 2015). *C. closterium* has been observed again in the coastal area of Thessaloniki Bay and Thermaikos Gulf (Genitsaris et al., 2019; Zapounidou, 2020). According to the results of a study conducted in several gulfs of Greece, including Thermaikos, diatoms predominate over dinoflagellates (Ignatiades, 2012). Compared to the moderate/good quality thresholds (0.1 - 0.6 µg L⁻¹) suggested by UNEP/MAP (2015), Thessaloniki Bay exceeded by far these values.

Eutrophic marine ecosystems can produce highly dominant diatom communities within phytoplankton assemblages (Le Moal et al., 2019). Under various turbulent and stratification conditions diatoms can cause persistent growth and bloom formations (Bužančić et al., 2016; Sommer et al., 2017). In previous studies, the diatom spring blooms in Thermaikos Gulf (Nikolaides & Moustaka – Gouni, 1990; Genitsaris et al., 2019; Tsikoti & Genitsaris, 2021) have been attributed to their ability to utilize nutrients inputs more efficiently, together with ecological traits that provide advantages in well-mixed conditions (Karageorgis et al., 2005). However, the late March - April samplings of 2022 showed a high dominance of a consortium of dinoflagellate taxa, with mainly heterotrophic or mixotrophic strategies. It is possible that this dinoflagellate bloom followed a previous proliferation of diatoms, which can constitute a food source of dinoflagellate grazers (Sherr & Sherr, 2007), such as *N. scintillans*.

It is known from the literature that the trigger mechanisms governing bloom events and seasonality probably have changed substantially in response to climate change (Radach, 1998). Analogous to earlier blooming in terrestrial plants, a warming of aquatic systems could be expected to cause an earlier timing of the spring phytoplankton bloom. The bloom starts when nutrient/ light conditions are sufficient for growth of the bloom-forming species and terminates through the onset of nutrient limitation (Smetacek 1999), after which large amounts of organic material sediment out. Algal blooms do not last indefinitely and the cells comprising a bloom will inevitably senesce and die, or enter a state of metabolic dormancy. For example, *Pheocystis globosa* bloom decay in the eastern English Channel has been associated

to pressures from parasitic unicellular eukaryotes, such as taxa belonging to Syndiniales (Genitsaris *et al.*, 2015). Thus, the dynamics of a bloom depends on the interplay of multiple factors, including light and nutrient availability as well as grazing and parasitic pressures and species composition of both the grazing and the grazed communities (Irigoién *et al.* 2005).

4.2 HABs

Harmful Algal Blooms HABs have been observed frequently in Thessaloniki Bay and Thermaikos Gulf. The first record of a HAB in the area was a *P. micans* bloom, in 1994; three during 2000 – 2001 caused by species of genus *Prorocentrum* sp., four during 2000 – 2004 caused by *Dinophysis acuminata*, one in 2003 caused by *Chatonella globose*, three during 2013 – 2017 caused by *Gonyaulax spinifera*, one in 2018 caused by *Vicicitus globosus*, and seven during the period 2017 – 2019 caused by *N. scintillans*. Except for *N. scintillans* and *P. micans* that were dominant in spring, the rest of the species that have been previously proliferated in the bay were identified in the samples during the study period in very low abundances, suggesting a large pool of potential HAB forming taxa.

In March 2022, a macroscopically visible bloom forming a red tide was observed in WT. The causative species were the heterotrophic dinoflagellates *N. scintillans* and close relative *S. pseudonociluca*. Red tides caused by *N. scintillans* have been linked to eutrophication in several areas of East Mediterranean and especially in the Sea of Marmara (Turkoglu & Koray, 2002), the Aegean Sea (Koray *et al.*, 1996) and the Adriatic Sea (Umani *et al.*, 2004). In contrast, its relative, the dinoflagellate *S. pseudonociluca* has been rarely reported in the international literature, although there are records suggesting a cosmopolitan distribution. Its distribution has been underestimated due to its complex life cycle, morphological variability, and taxonomic problems in its recognition (Gómez, 2010). Previous studies have shown that, among other smaller taxa, *N. scintillans* is feeding on harmful *Dinophysis* sp. and *Pseudonitzschia* sp cells. As a result, *N. scintillans* containing the toxins of these species may act as a vector of toxins by toxigenic algae to higher trophic levels or transport to shellfish aquaculture (Escalera *et al.*, 2007). On the other hand, *N. scintillans* grazing on toxic dinoflagellates should be considered as a possible regulator of toxic phytoplankton production (Frangópulos, 2011).

Especially the presence of *N. scintillans* has been linked to high concentrations of nutrients, mainly, nitrogen and phosphorus (Genitsaris *et al.*, 2019). Genitsaris *et al.* (2019) have detected several events of macroscopically visible red tides over a temperature range of 10 to 25 °C and a salinity variation of 36 to 38.5 psu in the survey area. Overgrowth of *N. scintillans* is indirectly related to nutrients NH⁴ and PO⁴, which may lead to phytoplankton proliferation, prey to the phagotrophic *Noctiluca*. *N. scintillans* is one of the most important organisms that form red tides worldwide in a water temperature range of 10-25 °C and a salinity range of 28 to 36 psu in fertile areas that are dominated by diatoms like the Thessaloniki Bay (Genitsaris *et al.*, 2019).

Based on the IOC-UNESCO Taxonomic Reference List of Harmful Microalgae at least 7 out of 83 plankton taxa found in the present study have been characterized as potentially harmful. These taxa are *Pseudo-nitzschia australis*, *Pseudo-nitzschia seriata*, *Phaeocystis* sp., *Chrysochromulina* sp., *D. acuminata*, *Alexandrium catenella* and *Prorocentrum lima*. It is important to note that *P. micans* is not included in the IOC UNESCO Taxonomic Reference List of Harmful Microalgae, yet it has caused discoloration of the water in many areas of Greece (Nikolaidis *et al.*, 2005), and it was dominant in the spring red tide.

Species of *Dinophysis* sp. have been observed in the past in the Eastern Mediterranean and especially in the coastal waters of the Thermaikos Gulf (Nikolaidis & Moustaka-Gouni, 1990; Koukaras & Nikolaidis, 2004). According to local authorities, the first record of *Dinophysis* sp. was in 2000, and caused cases of Diarrheic Shellfish Poisoning (DSP), in humans (200 people were hospitalized) (Koukaras & Nikolaidis, 2004). In particular, the massive bloom of the toxic *D. acuminata* in January 2000 in Thermaikos Gulf (Koukaras & Nikolaidis, 2004), was the first documented toxic outbreak in Greece (Mouratidou *et al.*, 2004) resulting in the loss of approximately 5 million euros in the shellfish industry (Nikolaidis *et al.*, 2005). These features together with the co-occurrence of several harmful organisms (e.g., *Alexandrium* sp.), demonstrate the eutrophic nature of Thermaikos Gulf, and illustrate the need for continuous monitoring of the ecological quality of the water and the environmental situation of Thermaikos Gulf (Petala *et al.*, 2018).

Also, species of the genus *Pseudo-nitzschia* sp. have been associated with amnesic shellfish poisoning (ASP, Amnesic Shellfish Poisoning) and the production of

domoic acid (DA, Domoic Acid) (Nikolaidis *et al.*, 2005). Fifteen of the 26 species of the genus *Pseudo-nitzschia* sp. that produce domoic acid have been found in the Mediterranean Sea (Zingone *et al.*, 2020). Another harmful unicellular planktonic taxon identified in the present study was the genus *Alexandrium* sp. Species of the genus *Alexandrium* sp. have been associated with Paralytic Shellfish Poisoning (PSP) (Nikolaidis *et al.*, 2005), causing both neurological and gastrointestinal symptoms. In the Mediterranean Sea, 6 species of the genus *Alexandrium* sp., are associated with PSP events (Zingone *et al.*, 2020). All the above were recorded in the present study but in very low abundance and biomass values. *Phaeocystis* sp. which has also been identified in the present study, has been associated with mucilage production (Noordkamp *et al.*, 1998). *Phaeocystis* sp. is a colony-forming microalgae that has a global distribution and can develop HABs (Noordkamp *et al.*, 1998).

4.3 Remote sensing

The 23-year monthly climatology of Chl-a in Thermaikos Gulf showed one peak of biomass in March. Also, in 2022 there were 2 biomass peaks, the first in January and the second in March. The general seasonality pattern of the phytoplankton blooms in Thermaikos Gulf suggests increased biomass in winter and spring and a decrease in the summer months (Price *et al.*, 2005; Nikolaidis *et al.*, 2006). There was an attempt to convert the *in-situ* phytoplankton biomass into Chl-a values according to the paper of Alvarez *et al.* (1999). The comparison of the *in-situ* values and the data values was reliable, but the *in-situ* data is too scarce to have a reliable statistical relationship. At least 150 matchups are needed, during every season, to capture the low medium and high concentrations.

In two samples, 20-Jul and 10-Aug, there was a big divergence between *in-situ* values and satellite data. For example, in 10-Aug the projected chl-a based on phytoplankton biomass values, would be 3 times higher than the chl-a calculated from satellite data. Also, Stramska *et al.* (2021) tried to compare *in-situ* values and satellite data but, the satellite Chl-a did not always agree with *in-situ* measurements as well, in particular when *in-situ* values were high. Satellite ocean color algorithms do not provide satisfactory results in many coastal areas, where some optically significant water components can be present in larger concentrations than in the open ocean, and can vary independently from one another (Mobley, 1994). More *in-situ* biomass measurements are necessary in order to have trusted results. Satellite and *in-situ*

observation systems are therefore complementary and merging their products for an operational and long-term monitoring of coastal waters is a major challenge for the next years (Gohan *et al.*, 2020).

Although satellite remote sensing is one of the most widespread methods, it is limited by certain characteristics. Initially, satellite remote sensing is limited to near surfaces and cannot detect what may be below the first optical depth (Tomlinson *et al.*, 2004). Sensor detection levels are not sensitive enough to low Chl-a densities. There are ultra-oligotrophic Mediterranean-tuned algorithms that capture low densities. Clouds also play a key role in the collection and monitoring of data. In addition to the collection of satellite data, some degree of *in-situ* validation is required to verify the composition of a remotely sensed bloom (Moita *et al.*, 2006).

5. Conclusions

During this study, weekly water samples were taken from 3 sites in Thessaloniki Bay (Thermaikos Gulf). Also, satellite data of Chl-a, for Thermaikos Gulf, were analyzed. The *in-situ* samples were not enough in order to develop a statistical robust relationship between the *in-situ* Ch-a with the satellite observations. Overall, 83 plankton species were identified in Thessaloniki Bay. In the unicellular planktonic composition, two different seasonal dynamics were observed. In the spring samples, dinoflagellates dominated in all 3 sites (Music Hall, White Tower, Harbor) in terms of diversity, abundance, and biomass. *P. micans* dominated in all three sampling sites during the spring period. During the period 20 Apr-28 Apr there was a decline in *P. micans* alive cells, while the number of dead *P. micans* increased, indicating the progression of the bloom to its senescence. In contrast, in summer, the phytoplankton bloom was dominated by the diatom species *C. Closterium* and *Chaetoceros* sp.

The total abundance of unicellular eukaryotes, compared to the limits of the trophic state, exceeded the limit for the upper mesotrophic state (960 cells mL⁻¹, UNEP/MAP 2015). In March, a macroscopically visible red tide was observed caused by *N. scintillans*. According to the IOC-UNESCO Taxonomic Reference List of Harmful Microalgae, in total, 7 harmful species were observed but in very low abundances, which indicates a large pool of potential HAB forming taxa. The harmful events, along with the presence of harmful algae illustrate the need for continuous monitoring of target indicators of nutrient pollution.

6. References

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7. Appendix

Supplementary Table 1. Presence of unicellular eukaryotes during the study period at MH (Music Hall). (*) indicates heterotrophic species. (**) indicates mixotrophic species.

	MH									
	17-Apr	20-Apr	25-Apr	28-Apr	6-Jul	13-Jul	20-Jul	10-Aug	17-Aug	24-Aug
Bacillariophyceae										
<i>Asterionellopsis glacialis</i>										
<i>Bacteriastrum furcatum</i>						+	+	+		
<i>Chaetoceros affinis</i>										
<i>Chaetoceros lorenzianus</i>					+		+			
<i>Chaetoceros</i> sp.	+	+	+	+	+	+	+	+	+	+
<i>Chaetoceros tenuissimus</i>										
<i>Chaetocheros socialis</i>										
<i>Coscinodiscus</i> sp.	+		+						+	+
<i>Cylindrotheca closterium</i>	+	+	+	+	+	+	+	+	+	+
<i>Ditylum brightwelli</i>						+				
<i>Dactyliosolen fragillissimus</i>	+				+	+	+	+	+	+
<i>Eucampia zodiacus</i>										
<i>Guinardia striata</i>					+	+	+	+	+	
<i>Hemiaulus hauckii</i>					+			+		+
<i>Hemiaulus sinensis</i>	+				+					
<i>Leptocylindrus danicus</i>			+	+	+	+	+	+		
<i>Leptocylindrus minimus</i>		+	+	+	+	+	+	+	+	+
<i>Lioloma pacificum</i>					+					
<i>Lithodesmium undulatum</i>										
<i>Meuniera membranacea</i>					+	+		+		
<i>Navicula</i> sp.	+	+	+	+	+	+		+	+	+
<i>Nitzschia longissima</i>		+							+	+
<i>Pleurosigma normanii</i>				+		+		+	+	+
<i>Proboscia alata</i>	+	+	+		+		+	+	+	
<i>Pseudo-nitzschia australis</i>										
<i>Pseudo-nitzschia seriata</i>			+		+		+	+	+	+
<i>Rhizosolenia imbricata</i>					+		+			+
<i>Rhizosolenia setigera</i>										
<i>Rizosolenia pungens</i>					+		+	+	+	+
<i>Rizosolenia</i> sp.	+		+		+	+				
<i>Skeletonema costatum</i>										
<i>Thalassionema nitzschoides</i>										
<i>Thalassiosira oceanica</i>										
<i>Thalassiosira</i> sp.	+		+		+	+		+	+	

Chlorodendrophyceae									
<i>Tetraselmis gracile</i>		+	+	+	+	+	+	+	+
Chlorophyceae									
<i>Chlamydomonas coccoides</i>									
Cryptophyceae									
<i>Chrytomonas</i> sp.**		+	+	+	+	+	+	+	+
<i>Hillea fusiformis</i> **		+			+		+		
<i>Plagioselmis</i> sp.**	+	+	+		+	+		+	+
<i>Teleaulax acuta</i> **	+	+	+	+	+	+	+	+	+
Dictyochophyceae									
<i>Dictyocha fibula</i> **		+			+				+
Dinophyceae									
<i>Alexandrium catenella</i>					+				
<i>Ceratium candelabrum</i> **									
<i>Ceratium furca</i> **	+	+	+	+	+	+	+	+	+
<i>Ceratium fusus</i> **		+	+						
<i>Ceratium lineatum</i> **									
<i>Ceratium macroceros</i> **									
<i>Ceratium trichoceros</i> **							+	+	+
<i>Ceratium tripos</i> **									
<i>Cochlodinium polykikoides</i> cf.					+				
<i>Diplopsalis</i> sp.*									
<i>Dinophysis acunimata</i> **	+	+		+		+			
<i>Dinophysis dens</i> **	+	+	+	+					
<i>Gonyaulax fragilis</i>	+	+	+			+	+	+	+
<i>Gymnodinium elongatum</i> *						+			
<i>Gymnodinium</i> sp.*	+	+	+	+				+	+
<i>Gyrodinium</i> sp.*	+	+	+	+	+		+		+
<i>Heterocapsa triquetra</i> **									
<i>Karenia</i> sp.	+	+	+	+	+			+	+
<i>Katodinium</i> sp.				+	+		+	+	+
<i>Noctiluca scintillans</i> *			+	+	+			+	+
<i>Oxytoxum glacile</i> *									
<i>Oxytoxum longiceps</i> *	+	+	+	+			+		
<i>Podolampas palmipes</i> *									
<i>Polykrikos Schwartzii</i>									
<i>Prorocentrum compressum</i>	+		+	+		+		+	+
<i>Prorocentrum gracile</i>	+		+	+	+		+	+	+
<i>Prorocentrum lima</i>	+	+		+					
<i>Prorocentrum micans</i> **	+	+	+	+	+	+	+		+
<i>Prorocentrum minimum</i> **	+		+		+			+	+
<i>Proto-peridinium brevipes</i> *		+	+						

<i>Protoperidinium claudicans</i> *	+	+		+		+	+		+
<i>Protoperidinium sp.</i> *			+						
<i>Protoperidinium steinii</i> *	+	+	+	+	+	+		+	+
<i>Protoperidinium oceanicum</i> *					+				
<i>Pyrophacus holorogium</i>	+	+	+	+			+		+
<i>Scripsiella trochoidea</i>	+	+	+	+	+	+	+	+	+
<i>Spatulodinium pseudonoclituca</i> *				+					
Euglenophyceae									
<i>Euglena sp.</i> **					+	+			
Prasinophyceae									
<i>Pyramimonas octopus</i>									
Prymnesiophyceae									
<i>Chrysochromulina sp.</i> **		+	+	+	+	+	+	+	+
<i>Phaeocystis pouchetii</i> **	+						+	+	+
HNF	+	+	+	+	+		+		+

Supplementary Table 2. Presence of unicellular eukaryotes during the study period at WT (White Tower). (*) indicates heterotrophic species. (**) indicates mixotrophic species.

	WT									
	20-Apr	25-Apr	28-Apr	6-Jul	13-Jul	20-Jul	10-Aug	17-Aug	24-Aug	
Bacillariophyceae										
<i>Asterionellopsis glacialis</i>									+	
<i>Bacteriastrum furcatum</i>					+		+			
<i>Chaetoceros affinis</i>										
<i>Chaetoceros lorenzianus</i>	+				+					
<i>Chaetoceros sp.</i>	+	+	+	+	+	+	+	+	+	
<i>Chaetoceros tenuissimus</i>										
<i>Chaetocheros socialis</i>										
<i>Coscinodiscus sp.</i>			+	+	+	+	+			
<i>Cylindrotheca closterium</i>	+	+	+	+	+	+	+	+	+	
<i>Ditylum brightwelli</i>				+	+	+	+	+	+	
<i>Dactyliosolen fragillissimus</i>					+					
<i>Eucampia zodiacus</i>										
<i>Guinardia striata</i>				+	+	+	+	+		
<i>Hemiaulus hauckii</i>				+						
<i>Hemiaulus sinensis</i>					+					
<i>Leptocylindrus danicus</i>			+	+	+	+				

<i>Leptocylindrus minimus</i>	+	+	+	+	+	+	+	+	+
<i>Lioloma pacificum</i>					+				
<i>Lithodesmium undulatum</i>									
<i>Meuniera membranacea</i>	+				+	+	+		
<i>Navicula</i> sp.	+	+	+		+	+	+	+	
<i>Nitzschia longissima</i>				+	+	+			
<i>Pleurosigma normanii</i>				+	+	+		+	+
<i>Proboscia alata</i>	+	+	+	+	+	+	+	+	+
<i>Pseudo-nitzschia australis</i>					+				
<i>Pseudo-nitzschia seriata</i>	+	+				+	+	+	
<i>Rhizosolenia imbricata</i>				+					
<i>Rhizosolenia setigera</i>									
<i>Rizosolenia pungens</i>	+		+			+		+	
<i>Rizosolenia</i> sp.	+			+	+				
<i>Skeletonema costatum</i>									
<i>Thalassionema nitzschoides</i>									
<i>Thalassiosira oceanica</i>									
<i>Thalassiosira</i> sp.		+	+		+	+	+	+	+

Chlorodendrophyceae

<i>Tetraselmis gracile</i>	+	+	+		+	+	+	+	
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Chlorophyceae

<i>Chlamydomonas coccoides</i>									
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Cryptophyceae

<i>Chryptomonas</i> sp. **	+	+	+	+		+		+	
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<i>Hillea fusiformis</i> **				+	+	+		+	+
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<i>Plagioselmis</i> sp.**	+	+		+	+	+	+		+
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<i>Teleaulax acuta</i> **	+	+	+	+	+	+	+	+	+
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Dictyochophyceae

<i>Dictyocha fibula</i> **				+	+	+		+	+
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Dinophyceae

<i>Alexandrium catenella</i>							+		+
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<i>Ceratium candelabrum</i> **									
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<i>Ceratium furca</i> **	+	+	+	+			+	+	+
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<i>Ceratium fusus</i> **						+			
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<i>Ceratium lineatum</i> **									
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<i>Ceratium macroceros</i> **									
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<i>Ceratium trichoceros</i> **							+	+	+
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<i>Ceratium tripos</i> **									
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<i>Cochlodinium polykikoides</i>									
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<i>Diplopsalis</i> sp.*									
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<i>Dinophysis acunimata</i> *	+		+		+	+			
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<i>Dinophysis dens</i> **	+	+	+					+	
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<i>Gonyaulax fragilis</i>	+	+	+	+		+	+		+
<i>Gymnodinium elongatum</i> *									
<i>Gymnodinium sp.</i> *	+	+	+	+	+			+	+
<i>Gyrodinium sp.</i> *	+	+	+		+	+	+	+	+
<i>Heterocapsa triquetra</i> **									
<i>Karenia sp.</i>	+	+		+	+	+		+	
<i>Katodinium sp.</i>	+	+	+	+		+	+	+	
<i>Noctiluca scintillans</i> *	+	+	+		+		+	+	
<i>Oxytoxum glacile</i> *				+					
<i>Oxytoxum longiceps</i> *			+	+	+		+	+	
<i>Podolampas palmipes</i> *									
<i>Polykrikos Schwartzii</i>	+								
<i>Prorocentrum compressum</i>	+	+	+	+	+	+		+	
<i>Prorocentrum gracile</i>	+	+	+	+	+		+		+
<i>Prorocentrum lima</i>			+	+	+				+
<i>Prorocentrum micans</i> **	+	+	+	+	+	+			
<i>Prorocentrum minimum</i> **	+	+	+	+	+	+		+	
<i>Protoberidinium brevipes</i> *									
<i>Protoberidinium claudicans</i> *	+		+		+	+		+	
<i>Protoberidinium sp.</i> *			+						
<i>Protoberidinium steinii</i> *	+	+	+	+	+	+		+	
<i>Protoberidinium oceanicum</i> *	+				+				
<i>Pyrophacus holorogium</i>	+	+	+		+	+		+	
<i>Scripsiella trochoidea</i>			+	+	+	+	+	+	
<i>Spatulodinium pseudonoclituca</i> *	+								
Euglenophyceae									
<i>Euglena sp.</i> **	+			+	+	+		+	+
Prasinophyceae									
<i>Pyramimonas octopus</i>				+					
Prymnesiophyceae									
<i>Chrysochromulina sp.</i> **			+	+	+	+	+	+	+
<i>Phaeocystis pouchetii</i> **			+	+	+		+	+	
HNF	+	+		+	+	+		+	

Supplementary Table 3. Presence of unicellular eukaryotes during the study period at HB (Harbor). (*) indicates heterotrophic species. (**) indicates mixotrophic species.

	HB								
	20-Apr	25-Apr	28-Apr	6-Jul	13-Jul	20-Jul	10-Aug	17-Aug	24-Aug
Bacillariophyceae									
<i>Asterionellopsis glacialis</i>									
<i>Bacteriastrum furcatum</i>			+		+				
<i>Chaetoceros affinis</i>									
<i>Chaetoceros lorenzianus</i>									
<i>Chaetoceros</i> sp.	+	+	+	+	+	+	+	+	+
<i>Chaetoceros tenuissimus</i>									
<i>Chaetocheros socialis</i>									
<i>Coscinodiscus</i> sp.			+		+	+	+		
<i>Cylindrotheca closterium</i>	+	+	+	+	+	+	+	+	+
<i>Ditylum brightwelli</i>				+	+	+	+	+	+
<i>Dactyliosolen fragillissimus</i>					+				
<i>Eucampia zodiacus</i>									
<i>Guinardia striata</i>				+	+	+	+	+	
<i>Hemiaulus hauckii</i>									
<i>Hemiaulus sinensis</i>									
<i>Leptocylindrus danicus</i>			+	+	+	+		+	
<i>Leptocylindrus minimus</i>	+	+	+	+	+	+	+	+	+
<i>Lioloma pacificum</i>									
<i>Lithodesmium undulatum</i>									
<i>Meuniera membranacea</i>					+				+
<i>Navicula</i> sp.	+	+			+	+	+		+
<i>Nitzschia longissima</i>									
<i>Pleurosigma normanii</i>			+		+	+		+	+
<i>Proboscia alata</i>	+	+	+	+		+	+		+
<i>Pseudo-nitzschia australis</i>					+				
<i>Pseudo-nitzschia seriata</i>	+	+				+	+	+	
<i>Rhizosolenia imbricata</i>				+	+				
<i>Rhizosolenia setigera</i>									
<i>Rizosolenia pungens</i>					+		+	+	
<i>Rizosolenia</i> sp.		+		+		+			
<i>Skeletonema costatum</i>									
<i>Thalassionema nitzschoides</i>									
<i>Thalassiosira oceanica</i>									
<i>Thalassiosira</i> sp.					+	+	+	+	+
Chlorodendrophyceae									

<i>Tetraselmis gracile</i>	+	+	+	+	+	+	+	+	+
Chlorophyceae									
<i>Chlamydomonas coccoides</i>									
Cryptophyceae									
<i>Chrytomonas</i> sp.**	+	+	+	+	+	+	+	+	
<i>Hillea fusiformis</i> **	+		+	+		+	+		+
<i>Plagioselmis</i> sp.**	+	+	+	+	+	+	+	+	+
<i>Teleaulax acuta</i> **	+	+	+	+	+	+	+	+	+
Dictyochophyceae									
<i>Dictyocha fibula</i> **	+	+	+		+	+	+	+	+
Dinophyceae									
<i>Alexandrium catenella</i>							+	+	
<i>Ceratium candelabrum</i> **									
<i>Ceratium furca</i> **	+		+	+		+	+	+	
<i>Ceratium fusus</i> **						+			
<i>Ceratium lineatum</i> **									
<i>Ceratium macroceros</i> **									
<i>Ceratium trichoceros</i> **			+						+
<i>Ceratium tripos</i> **									
<i>Cochlodinium polykikoides</i>			+						
<i>Diplopsalis</i> sp.*									
<i>Dinophysis acunimata</i> **	+	+	+		+				
<i>Dinophysis dens</i> **	+	+	+						
<i>Gonyaulax fragilis</i>	+	+	+	+			+	+	+
<i>Gymnodinium elongatum</i> *									
<i>Gymnodinium</i> sp.*	+	+	+	+		+	+	+	+
<i>Gyrodinium</i> sp.*	+	+	+		+	+	+	+	+
<i>Heterocapsa triquetra</i> *	+								
<i>Karenia</i> sp.	+	+	+			+		+	+
<i>Katodinium</i> sp.		+	+	+	+	+		+	
<i>Noctiluca scintillans</i> *	+	+	+			+		+	
<i>Oxytoxum glacile</i> *			+				+		
<i>Oxytoxum longiceps</i> *	+	+	+		+		+	+	+
<i>Podolampas palmipes</i> *									
<i>Polykrikos Schwartzii</i>				+		+		+	
<i>Prorocentrum compressum</i>	+	+	+	+	+	+	+	+	+
<i>Prorocentrum gracile</i>	+	+	+	+		+	+	+	
<i>Prorocentrum lima</i>		+	+	+		+		+	+
<i>Prorocentrum micans</i> **	+	+	+	+	+	+			
<i>Prorocentrum minimum</i> **	+		+	+			+	+	+
<i>Protoperidinium brevipes</i> *			+						
<i>Protoperidinium claudicans</i> *	+	+	+	+	+	+		+	

<i>Protoperidinium</i> sp.*									
<i>Protoperidinium steinii</i> *	+	+	+	+	+	+	+	+	
<i>Protoperidinium oceanicum</i> *	+								+
<i>Pyrophacus holrogium</i>	+		+	+			+		
<i>Scripsiella trochoidea</i>	+	+	+	+	+	+	+	+	+
<i>Spatulodinium pseudonoclituca</i> *									
Euglenophyceae									
<i>Euglena</i> sp.**				+		+	+	+	+
Prasinophyceae									
<i>Pyramimonas octopus</i>				+					
Prymnesiophyceae									
<i>Chrysochromulina</i> sp.**	+	+	+	+	+	+	+	+	+
<i>Phaeocystis pouchetii</i> **			+	+					+
HNF	+	+	+	+	+	+	+	+	+

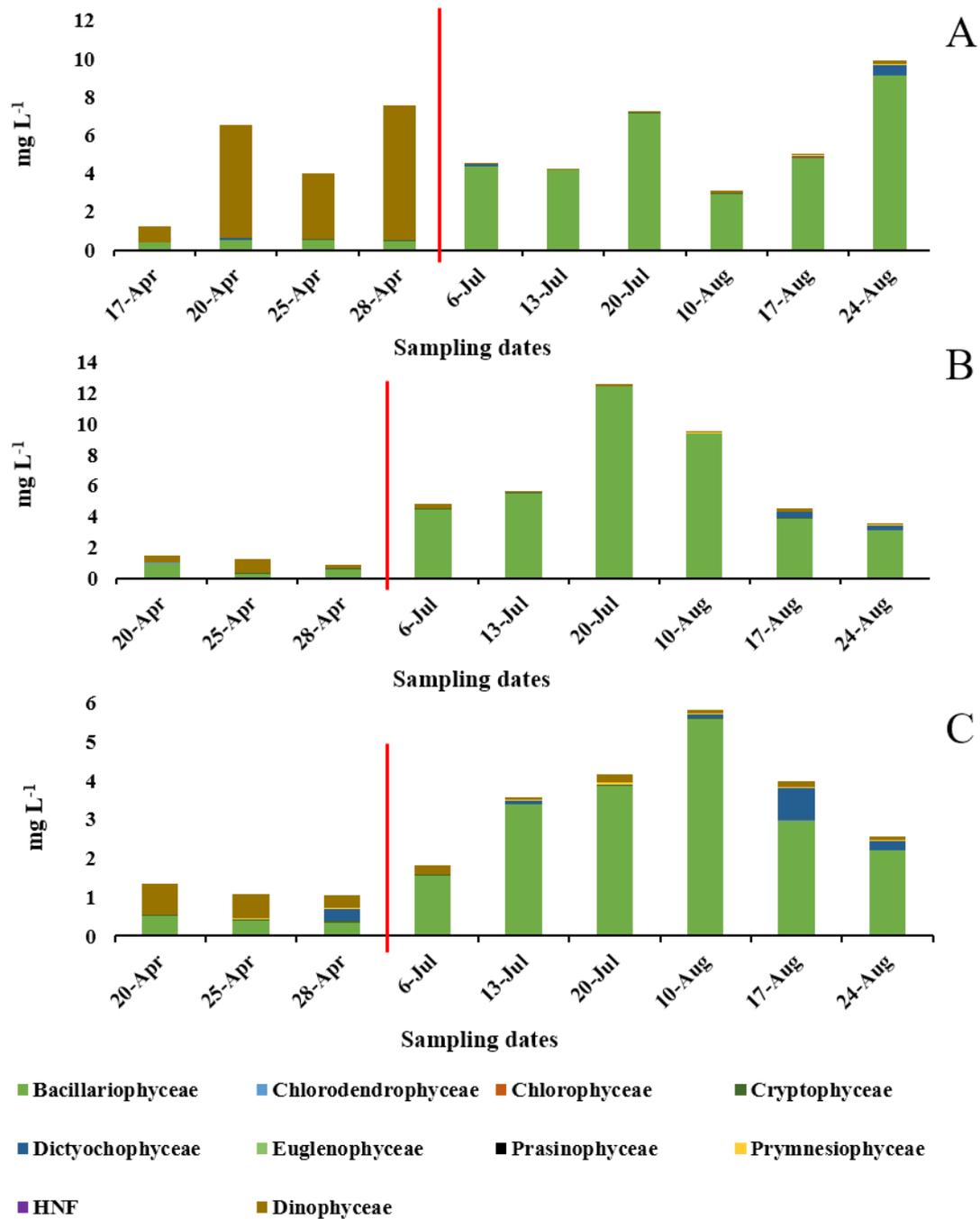
Supplementary Table 4. Used biovolumes for the estimation of biomass of Thessaloniki Bay.

(*) indicates heterotrophic species. (**) indicates mixotrophic species.

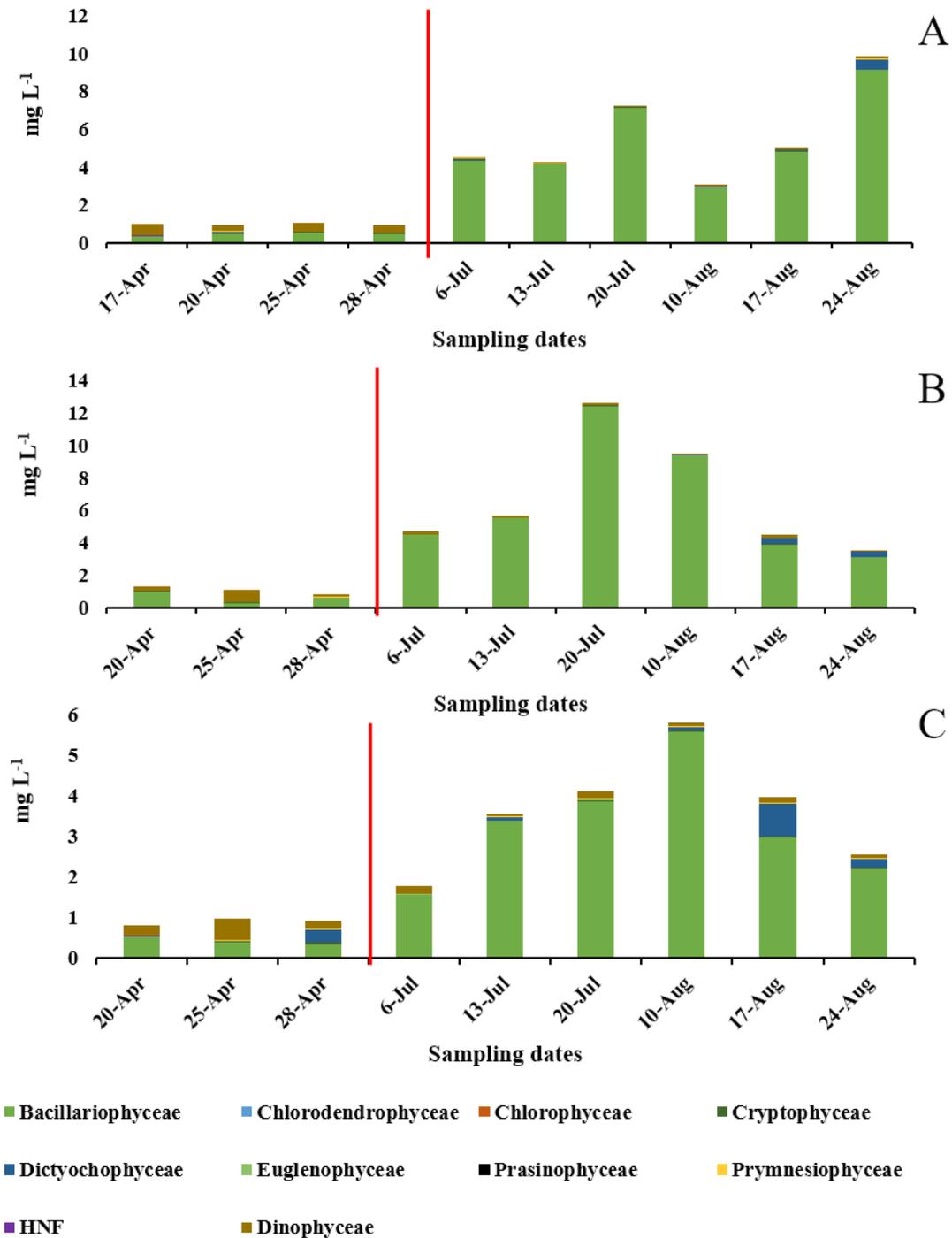
	$\mu\text{m}^3/\text{cell}$
Bacillariophyceae	
<i>Asterionellopsis glacialis</i>	2538
<i>Bacteriastrum furcatum</i>	4708
<i>Chaetoceros affinis</i>	3096
<i>Chaetoceros lorenzianus</i>	6500
<i>Chaetoceros</i> sp.	6230
<i>Chaetoceros tenuissimus</i>	68
<i>Coscinodiscus</i> sp.	425293
<i>Cylindrotheca closterium</i>	175
<i>Dactyliosolen fragillissimus</i>	2484
<i>Ditylum brightwelli</i>	27235
<i>Eucampia zodiacus</i>	12191
<i>Guinardia striata</i>	14942
<i>Hemiaulus hauckii</i>	344
<i>Hemiaulus sinensis</i>	1981
<i>Leptocylindrus danicus</i>	3146
<i>Leptocylindrus minimus</i>	109
<i>Lioloma pacificum</i>	1200
<i>Meuniera membranacea</i>	11197
<i>Navicula</i> sp.	118
<i>Nitzschia longissima</i>	734
<i>Pleurosigma normanii</i>	20040
<i>Proboscia alata</i>	7850
<i>Pseudo-nitzschia australis</i>	314
<i>Pseudo-nitzschia seriata</i> sf.	314
<i>Rhizosolenia imbricata</i>	65600
<i>Rhizosolenia setigera</i>	11200
<i>Rizosolenia pungens</i>	1016
<i>Rizosolenia</i> sp.	2064
<i>Skeletonema costatum</i>	42
<i>Thalassionema nitzschoides</i>	179
<i>Thalassiosira</i> sp.	8373
Chlorodendrophyceae	
<i>Tetraselmis gracile</i>	236
Chlorophyceae	

<i>Chlamydomonas coccoides</i>	90
Cryptophyceae	
<i>Chrytomonas</i> sp.**	706
<i>Hillea fusiformis</i> **	150
<i>Plagioselmis</i> sp.**	67
<i>Teleaulax acuta</i> **	146
Dictyochophyceae	
<i>Dictyocha fibula</i> **	29955
Dinophyceae	
<i>Alexandrium catenella</i>	8182
<i>Ceratium furca</i> **	30000
<i>Ceratium fusus</i> **	90000
<i>Ceratium lineatum</i> **	17000
<i>Ceratium macroceros</i> **	41000
<i>Ceratium trichoceros</i> **	77580
<i>Ceratium tripos</i> **	40300
<i>Cochlodinium polykikoides</i> cf.	20000
<i>Diplopsalis</i> sp.*	47700
<i>Dinophysis acunimata</i> **	11340
<i>Dinophysis dens</i> **	10024
<i>Gonyaulax fragilis</i>	1417
<i>Gymnodinium elongatum</i> * sf.	23000
<i>Gymnodinium</i> sp.*	3779
<i>Gyrodinium</i> sp.*	1110
<i>Heterocapsa triquetra</i>	570
<i>Karenia</i> sp.	2068
<i>Katodinium</i> sp.	3245
<i>Noctiluca scintillans</i> *	12000049
<i>Oxytoxum glacile</i> *	15093
<i>Oxytoxum longiceps</i> *	8232
<i>Podolampas palmipes</i> *	16212
<i>Polykrikos Schwartzii</i>	100480
<i>Prorocentrum compressum</i>	2381
<i>Prorocentrum gracile</i>	935
<i>Prorocentrum lima</i>	920
<i>Prorocentrum micans</i> **	10366
<i>Prorocentrum minimum</i> **	810
<i>Proto-peridinium brevipes</i> *	6900
<i>Proto-peridinium claudicans</i> *	54808
<i>Proto-peridinium</i> sp.*	11650
<i>Proto-peridinium steinii</i> *	19666
<i>Proto-peridinium oceanicum</i> *	61740
<i>Pyrophacus holrogium</i>	2308

<i>Scripsiella trochoidea</i>	2153
<i>Spatulodinium pseudonoclituca</i> *	26288372
<hr/>	
Euglenophyceae	
<hr/>	
<i>Euglena</i> sp.**	392
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Prasinophyceae	
<hr/>	
<i>Pyramimonas octopus</i>	78
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Prymnesiophyceae	
<hr/>	
<i>Chrysochromulina</i> sp.**	592
<i>Phaeocystis pouchetii</i> **	110
<hr/>	
HNF	39
<hr/>	



Supplementary Figure 1. Biomass without heterotrophs of each phytoplankton taxon at 3 sampling sites of Thessaloniki Bay: A) Music Hall (MH), B) White Tower (WT), C) Harbor (HB). The spring samples are separated from the summer samples with a red line.



Supplementary Figure 2. Biomass without heterotrophs and mixotrophs each phytoplankton taxon at 3 sampling sites of Thessaloniki Bay: A) Music Hall (MH), B) White Tower (WT), C) Harbor (HB). The spring samples are separated from the summer samples with a red line.