IMPACTS OF EUTROPHICATION AND CLIMATE CHANGE ON PHYTOPLANKTON COMMUNITY STRUCTURE, SIZE DIVERSITY, AND PHYTOPLANKTON BASED ECOLOGICAL STATUS

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

ŞEYDA ERDOĞAN

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY
IN
BIOLOGY

SEPTEMBER 2016

Approval of the thesis:

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ABSTRACT

IMPACTS OF EUTROPHICATION AND CLIMATE CHANGE ON PHYTOPLANKTON COMMUNITY STRUCTURE, SIZE DIVERSITY, AND PHYTOPLANKTON BASED ECOLOGICAL STATUS

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September 2016, 176 pages

The aim of this thesis is to investigate the effects of climate change and eutrophication on phytoplankton; community structure, mean size, mean variance in size and water quality with two main approaches: i) Space-for-time substitute approach by using snap-shot sampling in 47 from 42 N to 32 N latitude of Turkey. ii) Mesocosm experiment through latitudinal gradient in six countries (Sweden, Estonia, Germany, Czech Republic, Greece and Turkey) using nutrient temperature and depth as main parameters.

Mean phytoplankton size and variance in size structure was mainly regulated by top down control (zooplanktivorous fish and zooplankton) however nutrient also has indirect effect via regulating fish and zooplankton community composition. Especially rotifer species has positive significant effect on mean phytoplankton size increase probably due to their selective grazing on small size phytoplankton species. Mesocosm experiment results showed that phytoplankton biovolume increase mainly driven by temperature and nutrient increase. Moreover,

cyanobacteria contribution also increased with nutrient and temperature interaction. While severe water level decrease leads to macrophyte death, low water level decrease promoted macrophye growth and suppressed phytoplankton increase likely due to nutrient competition and allelopathic effects.

Phytoplankton quality indice that was developed for Mediterranean reservoirs seem practicable for Turkish reservoirs. However, for the shallow lake ecosystems applied indice results showed high variability, to develop a reliable phytoplankton indice Turkey should start regular monitoring studies, based on national typologies and data a national phytoplankton indice should be developed.

Keywords: phytoplankton, eutrophication, size, water frame work directive, climate change

ÖZ

ÖTROFİKASYON VE İKLİM DEĞİŞİKLİĞİNİN FİTOPLANKTON KOMÜNİTE YAPISI, BOYUT ÇEŞİTLİLİĞİ VE FİTOPLANKTON TEMELLİ EKOLOJİK DURUM ÜZERİNE ETKİLERİ

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Bu tez iklim değişikliği ve ötrofikasyonun fitoplankton komunite yapısı, ortalama fitoplankton büyüklüğü, boyuttaki varyans ve su kalitesi üzerindeki etkilerini iki farklı yaklaşımla araştırmayı amaçlamıştır: 1) Türkiye'deki 36-42 kuzey enlemleri arasındaki 47 gölün örneklemesini içeren zaman yerine mekân yaklaşımı 2) Farklı enlemlerde bulunan 6 ülkede (İsveç, Estonya, Almanya, Çek Cumhuriyeti, Yunanistan ve Türkiye) gerçekleştirilen, besin tuzu, sıcaklık ve su seviyesinin test edildiği mezokozm deneyi.

Ortalama fitoplankton büyüklüğü ve boyuttaki varyansın genel olarak yukarıdan aşağıya kontrol ile düzenlendiği (zooplanktivor balık ve zooplankton), bununla birlikte besin tuzunun da dolaylı bir biçimde balık ve zooplankton komünite yapısını etkilediği bulunmuştur. Özellikle rotifer türlerinin muhtemel küçük boylu fitoplaktonlar üzerindeki seçici avlanmalarının ortalama fitoplankton büyüklüğü

üzerinde pozitif etki yarattığı kaydedilmiştir. Mezokozm deneyi sonuçları ise fitoplankton biyo-hacmindeki artışın besin tuzu ve sıcaklıkla ilişkili olduğunu göstermiştir. Ek olarak besin tuzu ve sıcaklık etkileşiminin siyonabakter oranını arttırdığı görülmüştür. Su seviyesindeki düşüş su içi bitki gelişimini arttırırken fitoplankton büyümesini baskılamıştır ancak su seviyesinin aşırı düşmesi su içi bitkilerinin gelişimini tamamen ortadan kaldırmış ve fitoplankton artmıştır.

Akdeniz iklim kulağından bulunan barajlar için geliştirilen fitoplankton kalite indeksinin Türkiye'deki barajlar için de uygulanabilir olduğu gözlenmiştir. Bununla birlikte bu indeks kalite sınıflandırmaları sığ göl ekosistemlerine uygulandığında sonuçlarda çok fazla değişkenlik gözlenmiştir, güvenilebilir bir indeks geliştirmek için Türkiye'de düzenli izleme çalışmalarının başlanması ve ulusal tipolojilere bağlı olarak fitoplankton indekslerinin geliştirilmesi gerekmektedir.

Anahtar kelimeler: fitoplankton, ötrofikasyon, boyut, su çerçeve direktifi, iklim değişikliği

To my mom,

ACKNOWLEDGEMENTS

I am grateful to my supervisor Prof. Dr. Meryem Beklioğlu for her great support, valuable guidance, inspiration and supervision throughout in this study.

I also would like to thank my co-supervisor Prof. Dr. Ayşe Nilsun Demir and Prof Dr. Elena Litchman for their valuable suggestions and encouragement.

I want to express my gratitude to previous and current members of METU Limnology Laboratory Yrd. Doç. Dr. Arda Özen, Dr. Ayşe İdil Çakıroğlu, Gizem Bezirci, Ali Serhan Çağan, Esra Kuzyaka, Zeynep Ersoy, Jan Coppens, Peren Tuzkaya, Duygu Tolunay, Uğur Işkın and Saygın Sual.

Eti Levi, my "library partner", I am very lucky to have a friend like you. You are the most warm-hearted person that I have ever met. This work would not have been possible without your advices and cheer up. I am highly indebted to Doç. Dr. Nihan Yazgan Tavşanoglu for her constant guidance and encouragement throughout my study period. I am extremely thankful to our 'golden girl' Tuba Bucak for her keen interest and enthusiasm on me at every stage of my research. I have no valuable words to express my gratitude to Nur Filiz. Thank you for tolerating my idiosyncrasies over the last several years. I will always remember our fun moments and laughs shared. Emel Kunduz thank you for being there every single part of this thesis.

I would like to express my heartfelt appreciation to my friend Aylin, who inspire me to be a better person. Thank you for always being there and answering my endless questions with a wisdom, courtesy and endless patience.

And to my beloved family for their endless love, encouragement and support.

This study was supported by the METU-DPT ÖYP programme of Turkey (DPT-2011-1786), Turkish Scientific and Technological Council (TÜBİTAK) under the project ÇAYDAG 105Y332, 110Y125, Theme 6 (Environment including Climate Change) projects REFRESH (Contract No.: 244121).

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INTRODUCTION

1.1 Phytoplankton in Shallow Lakes

Plankton means free living, seston, weakly swimming or floating organism and - *phyto* means plant (Moss, 2010). However, phytoplankton are different than plants, they do not have root leaves and stem structures (Figure 1.1). Phytoplankton also comprises both prokaryotic and eukaryotic organisms. Moreover, they are physiologically diverse group. Although phytoplankton are mainly autotroph (producer), it also includes heterotrophic (consumer) and mixotrophic (capable of both autotrophic and heterotrophic) organisms (Wehr *et al*., 2003).

Figure 1.1 Green algae

Phytoplankton species can also be categorized as planktonic and substrate associated according to their habitat preference. While planktonic species float in water column, substrate associated ones can attach to plants, stones or can live in benthic fauna and are referred to be benthic phytoplankton (Bellinger *et al*., 2010). As they are mainly photosynthetic organisms they need sun light to survive thus they can only survive in euphotic zone, which includes the depth of water column that is exposed to more than 1% of the incoming light and usually it can be roughly as 2,5 times Secchi disc depth (Moss, 2010). Beside light, nutrient availability is also important for phytoplankton growth. Living organisms require some elements H, O, C, N, P, Na, K, Ca, Mg, S, Cl, Fe, Mn, Cu, Zn, B.

Shallow lakes constitute nearly 80 percent of surface freshwater sources and one of the main sources for drinking and agricultural activities and they are sensitive to physical and environmental conditions, like nutrient concentration, temperature, water level fluctuations (Moss 2010). Increased eutrophication due to nutrient enrichment became an important problem during last decades for lake ecosystems. Hence ecosystems and their restoration became important due to increased drinking and agricultural water need (Jeppesen *et. al*., 2009; Beklioğlu *et al*., 2011).

Aquatic eutrophication is inorganic nutrient enrichment of water bodies, especially with phosphorus and nitrogen (Moss, 2010). Agricultural and industrial wastewater and untreated domestic sewage effluent increase nutrient input to lakes from catchment area (Jeppesen *et al*., 2009; Beklioğlu *et al*., 2011). According to Alternative Stable States Theory shallow lakes possess in two different states: macrophyte dominated clear state and phytoplankton dominated turbid state (Moss, 1973). Nutrient concentrations and light conditions are the main factors affecting primary production. Nutrient is one of the growth limiting factors in nutrient rich water bodies as certain phytoplankton groups may cause “algal blooms” as a result of eutrophication (Bellinger *et al*., 2010). Especially cyanobacteria blooms come into prominence in recent decades and anthropogenic nutrient enrichment is the main reason of these blooms (Paerl *et al*., 2011).

Climate change and increased temperature also effect phytoplankton seasonality community structure or size distribution (Sheridan *et al*., 2011). Understanding phytoplankton community structure and response to environmental variables will help to improve restoration studies.

1.2 Climate change

Climate change refers changes in temperature, precipitation and wind speed average for long time period. Solar radiation is the main source of climate and there are three ways to change radiation equilibrium of the Earth; changes in both Earth's or Sun's orbit, decrease of reflected radiation percentage 'albedo effect' and increase of trapped radiation in atmosphere 'greenhouse effect' (IPCC 2007).

Certain gases in atmosphere like carbon-dioxide, methane, nitrous-oxide, and fluorinated absorb solar radiation which reflected from Earth surface and keep Earth temperature stable. Increases amount of these gases also increase trapped radiation and result increased temperature. After the industrial revolution greenhouse gases amount in atmosphere started to increase and still continue. Global average temperature increased 0.55 °C during last 30 years (IPCC 2007) (Figure 1.2). Temperature increase cause ice melting in polar regions normally ice has high albedo effect as a result of decreased ice cover albedo effect also decrease and cause decreased reflected solar radiation amount and increase absorption of more solar radiation (Dyurgerov *et al*., 1999). After the industrial revolution emission of CO_2 started to increase when CO_2 concentration was 315. 9 ppm in 1959, 2011 average was 391. 6 ppm and the upper safety limit is 350 ppm (Hansen *et al*., 2008).

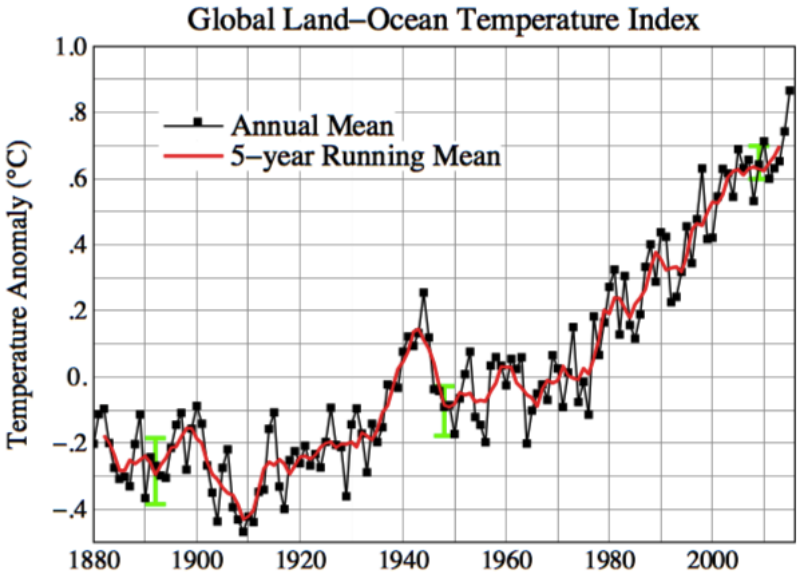

Figure 1.2 Average global temperature between 1880-2010. (source: http://data.giss.nasa.gov/gistemp/graphs).

1.3 Ecological consequences of climate change

Main consequences of climate change include; shift in phenology, geographic range shift towards higher latitudes and reduced body size (Penuelas *et al*., 2002; Daufresne *et al*., 2009; Sebastian *et al*., 2012). Phenological shift means that time changes in breeding and growth events across the life span of organisms (Walther *et al*., 2002). Seasonal timing is important for organism's life cycles like breeding, flowering, resting, leafing times. These periods are highly depending on temperature and environmental variables (Roy *et. al*., 2000; Frank *et al*., 2001; Badeck *et al*., 2004). For instance, flowering starts earlier if spring temperature increases earlier (Rafferty *et al*., 2011). Many species have specialized in different environmental conditions like temperature, humidity. Their niche preferences are different that affect their geographical distribution range, and changes in environmental differences force them to change their distribution to relatively suitable environments (Thomas *et al*., 2004; Foden *et al*., 2007) (Figure 1.3). Due

to climate change increased temperature had severe effects on highly sensitive species that have narrow niche preferences.

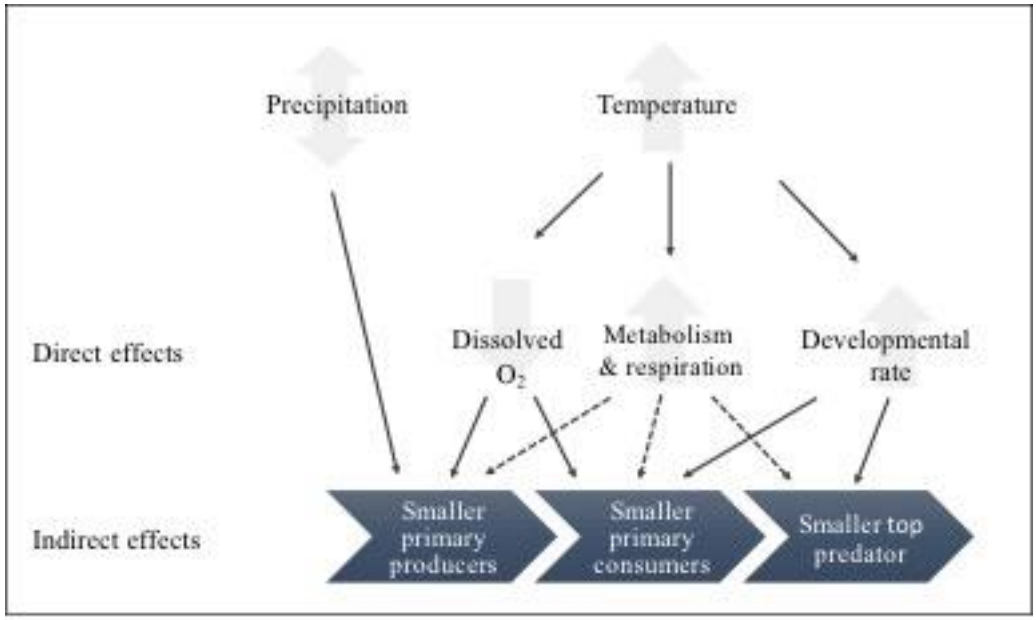

Figure 1.3 Direct and indirect effects of temperature and precipitation lead to changes in organism size (modified from Sheridan *et al*., 2011).

Body size is one of the basic characteristics of organisms and effects organisms' physiological and morphological performance like fecundity, population growth rate, and competitive interactions related with body size (Kingsolver *et al*., 2008; Daufresne *et al*., 2009). There are three main rules that define body size decrease; Bergman's rule, James' rule and Temperature Size Rule (TSR). According to Bergman's rule races of one species live in cold regions have larger body size than the race lives in warmer regions. This rule originally stated genus of endothermic vertebrates (Bergmann *et al*., 1847; Mayr *et al*., 1956, 1963). Size of a population decrease as a result of mean size decrease according to James' rule. Further, this could be possible in two ways; firstly, decrease in size at age (TSR) and secondly, increase in juvenile proportion should increase with increased temperature (Daufresne *et al*., 2009). According to the Bickford *et al*., (2010) if

all other variables equal only temperature increase cause %10-75 rise of metabolic rate of ectotherms. Organisms need to allocate the energy for growing reproduction and physiological maintenance. They might limit growing energy to favor maintenance and reproduction (Bickford *et al*., 2010). Moreover, less precipitation due to climate change may result small sized primary producers may lead to decrease body size of primary and secondary consumer as a result of food scarcity (Sheridan *et al*., 2011).

1.4 Phytoplankton size structure

Trait based information like physiology, behavior or life strategy about phytoplankton species are still limited due to excessively high diversity of phytoplankton. Size of phytoplankton cell is a key trait because it has impacts on metabolism, growth and access to resources (Litchman *et al*., 2008) (Figure 1.4). Natural phytoplankton communities compose highly variable size spectra and it changes with temporally and spatially as a response to impacts (Gaedke *et al*., 2004). Different sizes have advantageous under different environmental conditions. Small size phytoplankton (10-20 μm) species have advantage under nutrient deficient conditions due to high surface volume ratio. Another advantage of small size is smaller diffusive boundary layer for spherical cells its proportion increases with volume and negatively affects nutrient diffusion (Sherwood et al., 1975, Ploug *et al*., 1999). Moreover, grazer resistance, light and nutrient utilization are significantly correlated with size (Shuter *et al*., 1978; Sterner *et al*., 1989; Finkel *et al*., 2001; Litchman *et al*., 2007). Larger cells have several traits to mitigate nutrient transport and uptake limitations, for example sinking or swimming distort diffusive boundary layer and enhance nutrient uptake ratio, also cell elongation is another way to increase uptake ratio because elongated cells surface volume ratio is higher than spherical cells (Karp *et al*., 1996; Pahlow *et al*., 1997). Nutrient fluctuation also favors large cells because they have bigger

vacuoles than smaller cells and can store more nutrients (Grover *et al*., 1991; Stolte *et al*., 1996; Litchman *et al*., 2009).

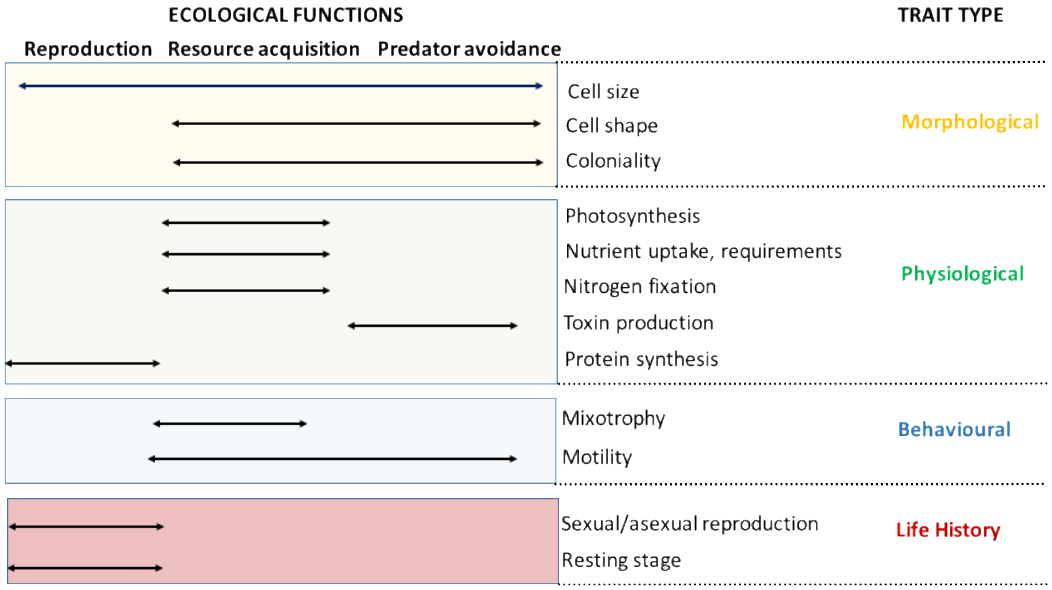

Figure 1.4 Typology of phytoplankton functional traits (Litchman *et al*., 2008).

Temperature increase also effect mixing regimes increased stratification prevent upward flux of nutrients and reduces available nutrient concentration at upper layer. These effect was studied both lake (Adrian *et al*., 2009) and ocean ecosystems (Finkel *et al*., 2005). Diatom community structure was changed with increased temperature in Lake Tahoe, temperature increase induced lake stratification and small diatom species like *Cyclotella* became dominant because their small size and high surface volume ratio their nutrient uptake ratio is higher than large cells (Adrian *et al*., 2009). Moreover, paleoecological studies also indicate increase in small sized individuals (Sarvori *et al*., 2002). Rühland *et al*., (2008) studied 200 Northern Arctic and temperate lakes and found that sharp decline large *Aulacoseria* and *Fragilaria* species and increase in *Cyclotella* species in both temperate and arctic regions. According to Finkel (2005) increased temperature and diatom frustle size highly correlated, when temperature increases

diatom frustle size become smaller over the Cenozoic (Finkel *et al.*, 2005). In addition, the ocean and lake studies mesocosm experiments also showed size decrease of phytoplankton species related with temperature. Smaller phytoplankton species became dominant in the +4 °C warmed mesocosms (Drocher *et al*., 2011).

1.5 Phytoplankton Community Structure and Its Relation with Environmental Variables

Phytoplankton community structure mainly driven by light, productivity and nutrient uptake ratios in local range, geographical differences among these variables also cause variability in different regions (Leibold *et al*., 1996, Stomp *et al*., 2007). According to the competitive exclusion principle species that occupy same niche and compete for same resources cannot coexist (Hardin, 1960). Due to non-equilibrated nutrient limited aquatic conditions, coexistence of many phytoplankton species known as "paradox of plankton" proposed by Hutchinson (Hutchinson, 1961). Latitudinal phytoplankton diversity pattern was studied by Barton *et al*., (2010). They found that phytoplankton diversity decreased with increased latitude because seasonal differences in sub-polar regions prevent phytoplankton species increase that have slow growth rate, nevertheless in trophic and sub-trophic regions weak seasonality enhance phytoplankton diversity (Barton *et al*., 2010). However, this idea not widely accepted according to Huisman (2010) intermediate temporal variation may enhance diversity. Phytoplankton diversity and their distribution pattern were studied across United States that phytoplankton diversity increased with temperature, lake area and decreased with lake depth. In addition, phytoplankton diversity decreased with altitude and latitude however increased with longitude. Chlorophyll-*a* concentration and phytoplankton diversity showed unimodal distribution (Stomp, *et al*., 2011).

1.5.1 Nutrient

Generally, phosphorus is considered as main limiting element for the growth of primary producers in freshwater ecosystems (Schindler, 1974 &1977). According to whole lake experiment results, Schindler (1974) suggested that most of the freshwater lakes are P limited because C and N can be exploited from atmosphere if they are limited in the system. However, phosphorus cycle does not include atmosphere and there is no biological mechanism like nitrogen fixation (cyanobacteria) like the N cycle. Schindler (2006) showed that when the lake was N limited Nitrogen fixer cyanobacteria can become dominant. However, there are still some controversies (Elser *et al*., 2007; Scott *et al*., 2010; Paterson *et al*., 2011). Large scale meta analyses showed that P and N enrichment shows positive synergistic response in marine, freshwater and terrestrial ecosystems and both N and P co-limitation is much more frequent in freshwater ecosystem (Elser *et al*., 2007; Allgeier *et al*., 2011).

Phytoplankton species richness shows unimodal distribution to increased TP gradient in temperate region. Moreover, community structure also changed with increased TP gradient, at low TP concentration dinophytes, chlorophytes, diatoms and chrysophytes, at intermediate TP concentration diatoms and cyanophytes, at high TP concentration chlorophytes and cyanophytes became dominant (Jeppesen *et al*., 2000). They also found that increased TP concentration positively correlated with planktivorus fish density.

1.5.2 Temperature

Photosynthesis, resource acquisition, respiration also depend on temperature. Different phytoplankton species have different temperature optima and temperature play important role in phytoplankton species succession. Phytoplankton seasonal growth usually starts with early spring diatom bloom and during the summer period green algae became dominant, late summer period cyanobacteria follows green algae and dinoflagellate or diatom species become dominant during fall period in temperate region (Litchman *et a*l., 2010). Many diatom species generally have low optimum growth temperature (15-25 °C), however cyanobacteria species optimum growth temperature is usually higher than other groups (25- 35°C) (Robarts *et al*., 1987) (Figure 1.5). Temperature increase also enhances stratification increased density difference between epilimnion and hypolimnion. Many cyanobacteria species have gas vesicles and they are able to exploit stratified conditions (Paerl *et al*., 2009). Cyanobacterial bloom on surface water also locally increase water temperature due to high light absorption by photosynthetic pigments in turn enhance their competitive advantage over other phytoplankton groups (Hense *et a*l., 2006).

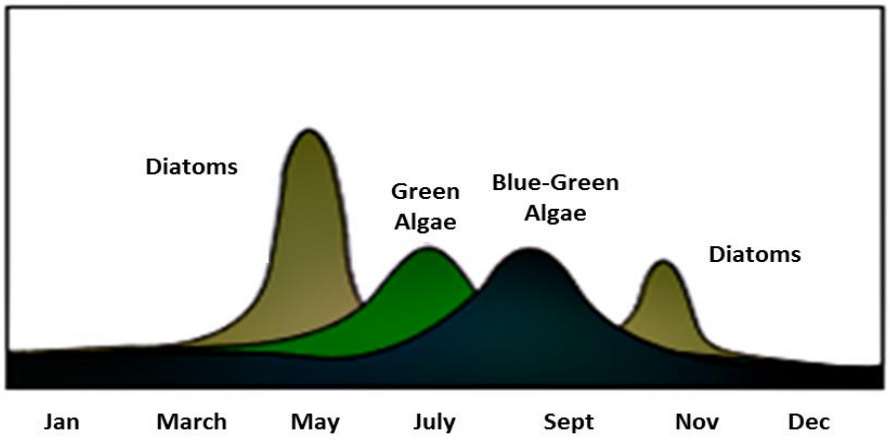

Figure 1.5 Phytoplankton seasonal succession (source: http://www.waterontheweb.org/under/lakeecology/14_algalsuccession.html)

1.5.3 Hydrology

Many lakes show water level fluctuation as response to temperature increase and precipitation regime shallow lakes are more sensitive than other water bodies and effects of water level fluctuation is more severe at shallow lake ecosystems (Coops *et al*., 2003). Moreover, anthropogenic water usage like irrigation, drinking water supply or hydroelectric power needs also affect hydrology (Pimental *et al*., 2004; Beklioglu *et al*., 2007). As a result of climate change high precipitation rate and high run off from catchment area may result high nutrient input to the lakes expected in temperate region (IPCC 2007, Jeppesen *et al*., 2009) however, in south Europe decreased precipitation may increase water retention time and result decreased run off from catchment area (IPCC 2007, Özen *et a*l., 2010).

Mediterranean climate is characterized by rainy winter and dry summer periods (Bolle *et al*., 2003). Reduced precipitation and high evaporation rate alter water level fluctuations in Mediterranean region also long dry periods may cause dry out of shallow lakes (Özen *et al*., 2010). Hydrological changes also affect lake structure and functioning. Seasonal differences among dry and wet periods directly affect lake water level and retention time (Beklioğlu *et al*., 2008; Jeppesen *et al*., 2009). During the dry periods inflow rates decrease and nutrient intake from catchment also decrease but internal loading may cause high nutrient concentration and may result eutrophication (Beklioğlu *et al*., 2008; Özen *et al*., 2010). Omnivorous and planktivorous fish species are dominant in Mediterranean region and lead to high predation pressure on large bodied zooplankton species (Beklioğlu *et al*., 2011). Furthermore, salinity increase due to low precipitation and high evaporation negatively affect large zooplankton species like Cladocera species (Brucet *et al*., 2010). High salinity (0.05 %) favor Copepod species and limit phytoplankton predation pressure (Beklioğlu *et al*., 2007). Moreover, high evaporation rate increase salinity and Copepod species gain advantage above 0.5% salinity lead to in effective predation pressure on phytoplankton species

(Jeppesen *et al*., 1994). At eutrophic condition with high phytoplankton abundance, aquatic plants may stay resilient though their stabilizing buffer mechanisms for maintaining clear water conditions appeared to be weak compared to the northern shallow lakes (Özkan *et al*., 2010; Beklioğlu *et al*., 2011; Özen *et al*., 2010; Bucak *et al*., 2012).

1.6 Water Framework Directive and Phytoplankton

Water frame directive entered into force in 2000. The directive called as Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for the Community action in the field of water policy″.
The main objectives of the directive;

- Protection, improvement and prevention of further degradation of water resources.
- Long-term protection of water resources to support sustainable water use.
- Protection and enhancement of aquatic ecosystems quality.
- Reducing pollution of underground water, prevention of further pollution.
- Flood and drought mitigation.

The directive includes inland surface waters, transitional waters, coastal waters and groundwater. The directive includes lake ecosystems as one of the surface water body type. According to the directive reference (undisturbed) and current conditions of water bodies should be determined via biological and physico-chemical elements. There are five ecological classes high, good, moderate, poor, and bad. It aims to achieve at least "good water status" all over EU waters by 2015.
Reference conditions and current status of lakes will be evaluated and Ecological Quality Ratio will be calculated (EQR) (Fig 1.6). according to the EQR value current status of lake will be determined, If the results are high or good it's

acceptable but if the EQR ratio shows moderate, bad or poor status the system should be restore to achieve good or high status. The EQR value should be determined both for biological and physico-chemical variables.

$$EQR = \frac{\text{Observed biological value}}{\text{Reference biological value}}$$

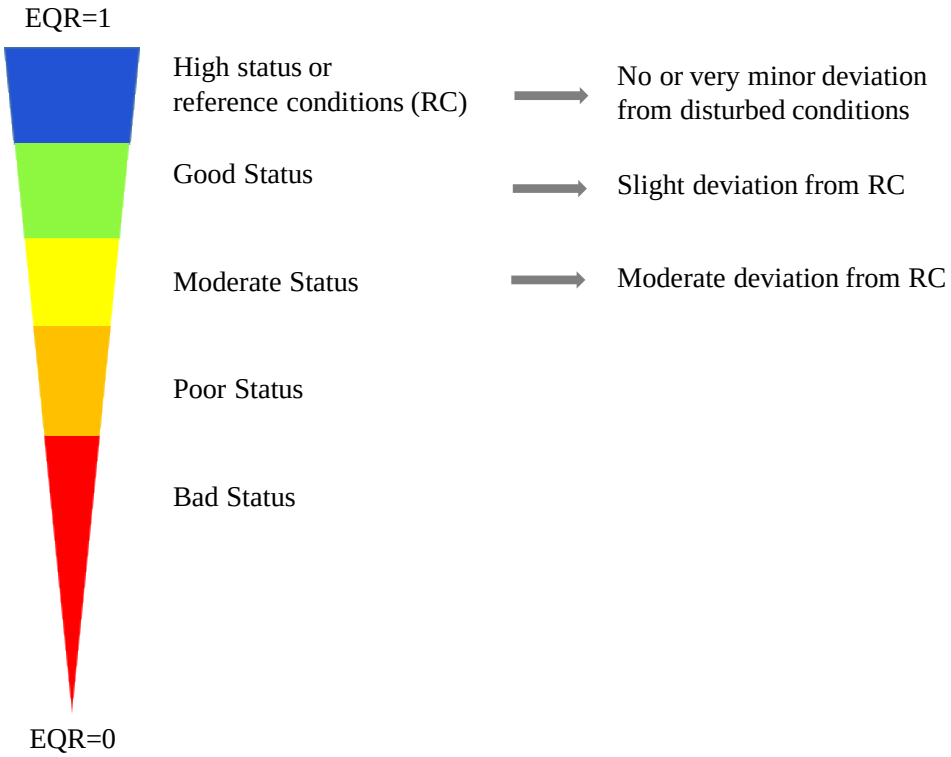

Figure 1.6 Ecologic Quality Ratio (EQR) classes and calculation.

1.6.1 Implementation of Water Framework Directive in Turkey

Turkey was introduced to the project as a candidate country in December 1999, therefore it needs to adapt water frame directive implementations. Turkey has a larger surface area than most of the other candidate and EU countries where also 25 river basins are occurred (Figure 1.7). Considering the geographical and physical conditions of Turkey, it would take more time and effort to achieve all the goals of the directive for the country.

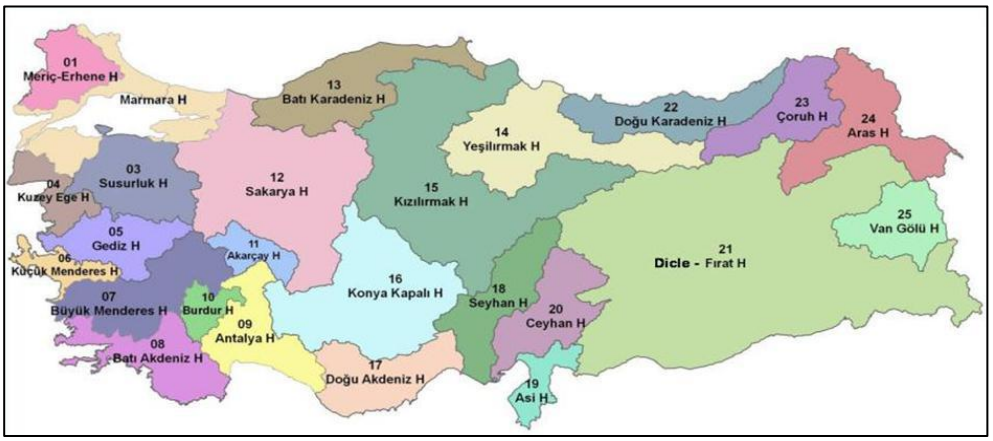

Figure 1.7 River basins of Turkey (Çiçek & Sahtiyancı)

First project was conducted in Büyük Menderes River basin and supported by Dutch government between 2007 and 2010. Büyük Menderes river basin management draft was prepared. Main challenges were determined some of them were in sufficient data, qualified personal, and laboratory equipment. Soil and Water Quality Monitoring Department was founded in 2010. The department started monitoring in 11 river basins. Moreover, in 2010 "TR 09 IB EN 03" project has started and was supported by Holland, Spain and France. The project will finish in 2013. They made final management plan for Büyük Menderes river basin. Surface and ground water monitoring program will be made at 52 point in the basin. Moreover, they will try to make a monitoring network along whole Turkey. Till now they made monitoring in 11 river basins. However, their

monitoring program does not include biological elements only chlorophyll-a measurements were made.

1.6.2 Phytoplankton and Water Framework Directive

According to Water Framework Directive (WFD) there are five main ecological quality elements are going to be used to determine lake ecological status; phytoplankton, macrophyte, phytobenthos, benthic invertebrate and fish fauna, for lakes (WFD, 2000). Phytoplankton are short lived organisms and nutrient concentration in water directly affect their composition and intensity hence they are good and early indicator of deterioration in lake ecosystems. According to WFD 25 ecological regions were determined among which Mediterranean climatic region includes Italy, Spain, Greece, Portugal, Slovenia, Cyprus, Malta, and Romania.

Directive outlines three features of phytoplankton (WFD, 2000);

- Phytoplankton composition
- Phytoplankton abundance
- Bloom occurrence frequency and intensity

Different countries have developed their own phytoplankton indices however comparison should be done between countries through inter-calibration which have already been done for some of them (Poikane *et al.,* 2011). The aims of inter-calibration studies are not to harmonize the assessment systems but their results or evaluation. The results must be comparable between countries especially for the “high-good” and the “good-moderate” boundaries. Because it decides whether precautions required or not. However inter-calibration studies were done mainly for reservoirs in Mediterranean countries. Lake inter-calibration studies were started only for Spain, France, Greece and Italy and have not been finished for whole Mediterranean countries yet. Mediterranean countries also use different indices. The indices used by Italy, Cyprus, Spain and Portugal are

specific indices for reservoirs (Table 1.1) accessible at: http://www.wiser.eu/programme-and-results/data-and-guidelines/method-database/. Turkey has still not developed any phytoplankton indices yet.

Table 1.1 Phytoplankton indices used in Mediterranean countries. Taken from: http://www.wiser.eu/results/method-database/

Country	**Method**
Italy	Mediterranean Phytoplankton Trophic Index
Cyprus	Mediterranean Assessment System for Reservoirs Phytoplankton
France	Lake phytoplankton index
Spain	Mediterranean Assessment System for Reservoirs Phytoplankton
Portugal	Lakes and Reservoirs Biological Quality Assessment Method
Greece	Greek Assessment System for Reservoirs and Lakes

Developing an approach to understand the relationship between phytoplankton communities and ecological quality of the water is crucial. Many phytoplankton indices were developed in different countries based on their environmental features and further adopting these indices not always give actual conditions of lakes in different regions (Salmaso, 2006; Spatharis, 2010). Although many studies were done to determine environmental preferences of species there is no general consensus or generalization (Reynolds, *et al*., 2000).

For Turkish lakes modification of already used indices need to be modified or new indices needs to be developed using like size classification. However, this poses a huge challenge as Turkey being very large and presenting features of continents due to climatic regions and still lack of monitoring studies.

1.7 Objectives

The main aim of this thesis to investigate effects of climate change and eutrophication on phytoplankton community structure and size diversity. Furthermore, to investigate ecological status and classifications of the Turkish Shallow lakes and reservoirs. For these reasons in total 48 lakes and 12 reservoirs were sampled in Turkey. Moreover, a mesocosm experiment along latitudinal gradient (Sweden, Estonia, Czech Republic, Germany, Turkey, Greece) was conducted. Therefore, the objectives of this thesis are;

i. To elucidate the effects of biotic and abiotic factors on phytoplankton community size structure (Chapter 2).

ii. To investigate temperature nutrient and depth and their interaction effects phytoplankton community structure (Chapter 3).

iii. To determine the possibility of employing phytoplankton-based WFD indices developed by various countries for defining the ecological quality classes of the lakes and reservoirs in Turkey (Chapter 4-5).

CHAPTER 2

DETERMINANTS OF PHYTOPLANKTON SIZE STRUCTURE IN WARM, SHALLOW LAKES

2.1 Introduction

As a result of climate change, temperature increase has been observed all around world during the last decades (Parmesan & Yohe, 2003; Parmesan, 2006; Salazar *et al*., 2007). Main ecological consequences of climate change for many taxa include shift in phenology and geographic range towards higher latitudes, and reduced body size (Walther *et al.,* 2002; Penuelas *et al.,* 2002; Daufresne *et al.,* 2009; Sebastian *et al.,* 2012; Durocher *et al.,* 2012). Therefore, during the last decades, the large number of research on direct or indirect effects of temperature or effect of other abiotic/biotic interactions on body size (Peter & Sommer, 2012; Rüger &Sommer, 2012; Winder *et al*., 2009; Quintana *et al*., 2015) has increased (Finkel *et al*., 2005; Sommer & Lengfellner, 2008; Gardner *et al*., 2011).

Body size is an important trait of any organism because it affects physiological and morphological performances e.g. fertility, population growth rate and competitive interactions (Damuth, 1981; Emmerson & Raffaelli, 2004; Kingsolver *et al.,* 2008; Daufresne *et al.,* 2009). According to the Bergmans' and James' rules, both inter and intraspecific size of organisms' decrease (Bergmann, 1847; James 1970) at higher temperatures. Temperature Size Rule (TSR) also states that organisms of the same age or developmental stage are smaller at high temperature because of rapid maturation (Atkinson, 1994). However, current knowledge about physiology of size shrinkage is limited particularly for the microscopic organisms as phytoplankton.

Phytoplankton are the main primary producers in most aquatic ecosystems and responsible for nearly half of primary production on Earth (Field *et al*., 1998). Because they form the base of aquatic food webs, phytoplankton productivity has repercussions through all levels of the food web (Moss, 2010). Moreover, they are a highly diverse group and range in size from picoplankton with cell dimensions around 1-5 μm to some colonial or filamentous species that can be visible to the naked eye (Moss, 2010). Cell size is a key trait for the phytoplankton, because it affects fundamental survival functions like nutrient uptake (Aksnes & Egge, 1991; Litchman & Klausmeier, 2008), sinking rate (Smayda, 1970) and grazer resistance (Burns 1968; Porter, 1973). Nutrients (Likens, 1972), temperature and light (Karentz & Smayda, 1984) are the main abiotic drivers for controlling directly or indirectly phytoplankton size and community structure (Watson *et al*., 1997; Mooij *et al.,* 2005; Jeppesen, 2009). For instance, small phytoplankton (10-20 μm) species have an advantage under nutrient-deficient conditions due to high surface-to-volume ratio (Chisholm *et al.,* 1992). Furthermore, prolonged thermal stratification prevents an upward flux of nutrient and reduces available nutrient concentration in the epilimnion, thus small size species again have advantages due to high surface-to-volume ratio (Smayda, 1970). Large cells have several traits like distorting diffusive boundary layer by swimming, sinking or cell elongation to increase nutrient uptake (Karp-Boss *et al.,* 1996) and they can have bigger vacuoles to store more nutrients (Litchman *et al.,* 2009). Moreover, high temperature and TP conditions generally favor cyanobacteria species (Paerl & Huisman 2008, 2009). Based on these knowledge, we assumed that mean variance in unit size should increase with increasing TP concentration, thus leading to low mean community size variance in oligotrophic conditions with abundant small sized species and an increasing variance in mesotrophic and eutrophic conditions due to higher diversity and large cyanobacteria abundance.

Not only abiotic variables (bottom-up) but also predation pressure (top-down) has effects on phytoplankton size through trophic cascade in lake ecosystems (Carpenter *et al.,* 1985). For instance, large Cladocera species like *Daphnia* generally prefer large size single cell phytoplankton species, however rotifer or copepod species usually prefer small sized phytoplankton. On the other hand, large size filamentous or colonial phytoplankton species are grazing resistant (Lynch & Shapiro, 1981). Zooplankton community is expected to be dominated by small zooplankton species as a result of selective predation pressure on large zooplankton species (Jeppesen *et al.,* 1997; Vadadi-Fülöp *et al.,* 2012; Tavşanoğlu *et al.,* 2015) by fish, which is predominated by small omnivorous fish in warm lakes (Meerfhoff *et al*., 2012). Consequently, grazing pressure on small size phytoplankton expected to be more intense in warm regions. However, these interaction pathways and their effect on phytoplankton size structure is not completely known.

Most of the body size studies in aquatic ecosystems were done in the ocean, and there are few studies from freshwater lakes (Daufresne *et al.,* 2009; Winder *et al*., 2009). Moreover, many studies use average sizes from the literature or from coarse filter separation. Here, we investigated the effects of major abiotic and biotic drivers, in 46 Turkish lakes, to further our understanding of drivers that control phytoplankton cell/unit size and variance structure in warm regions. In the present study, we used direct microscopic measurements, which allowed us to make more accurate predictions. Additionally, we sampled most of the biotic, physical and chemical parameters and these detailed ecosystem data allowed us to explore driver interactions and effects of them on phytoplankton size structure. We hypothesized that:

i) While bottom-up control on phytoplankton mean size is more pronounced under low nutrient conditions, top down control on phytoplankton mean size is stronger under eutrophic and hyper-eutrophic conditions.

ii) Mean phytoplankton cell/unit size in a community is smaller under high temperature conditions.

iii) Variance in phytoplankton unit size is highest in eutrophic and hypereutrophic lakes.

2.2 Materials and Methods

Turkey is located between 36-42°N latitudes and 26-45°E longitudes, with highly mountainous topography and having multiple climates. Arid, cold steppe, hot Mediterranean and temperate climates are the most prevalent ones according to Köppen-Geiger climate classification (Peel *et al.,* 2007). The lakes in this study are located in Western Anatolian Plateau, with an altitude range of sea-level to 1423 m.a.s.l. (Figure 2.1, Table 2.1). We sampled 46 mostly shallow and permanent lakes between 2006 and 2012. Samples were collected during the peak growing season (July- August) following a snapshot sampling methodology that is widely used for sampling of shallow lakes (Kruk *et al.,* 2009; Kosten *et al.,* 2012).

Samples were taken from the deepest point of each lake. Temperature (°C), conductivity (±1 µS cm^{-1}), salinity (‰), pH and total dissolved solid (TDS) were measured *in situ* by YSI multiprobe field meter at the same time. Water samples (40 L) were retrieved from the entire water column by KC Denmark water sampler, and sub-sampled for phytoplankton, zooplankton and chemical analyses were taken. Water samples were stored frozen until analyzed for total phosphorus (TP), soluble reactive phosphorus (SRP) (Mackereth *et al.,* 1978), silicate (Si), chlorophyll *a* (Chl-*a*) (Jespersen & Christoffersen, 1987) and total nitrogen (TN) (using a Scalar Auto-analyzer, San++ Automated Wet Chemistry Analyzer, Skalar Analytical, B.V. Breda, The Netherlands) (Table 2.1).

Phytoplankton samples, fixed with 2% Lugols' solution, were stored in 50 ml dark glass bottles and counted according to Utermöhl technique (1958). Samples were shaken at least 100 times, then, depending of the sample volume, were settled in

Utermöhl chambers for 16-24 hours. Subsequently, countings were conducted in horizontal transects under an inverted microscope (Leica DMI 4000B), until reaching 400 individuals of the most abundant species. Filamentous and colonial species were counted as one unit, while organisms smaller than 2 µm were not counted. Identification of phytoplankton species were done by the same person by using taxonomic reference books (Whitton *et al.*, 2002; Prescott *et al.*, 1973; Cox, 1996; Komarek, 1983, 1999; Popovski, 1990). Dimensions of at least 10 individuals from each phytoplankton species were measured by Leica image analysis program, and biovolume was calculated according to Hillebrand *et al.* (1999) and Wetzel (1991), by using mean volume of each (Appendix-E Table 1).

The mean phytoplankton cell (only single cell species) and unit (single cell, colony, filament) size for each lake was calculated based on the following formula:

$$Cell\ (Unit)\ Size = \frac{\sum(Cell\ volume * Cell\ density)}{\sum Cell\ density}$$

Based on calculated size data, variance of the size (average squared deviation from the mean) for each lake (only for unit size) was calculated. Consequently, we obtained following 3 main variables for each lake *i*) mean cell size *ii*) mean unit size and *iii*) variance in unit size.

Carlson's trophic state index (TSI) was used to determine trophic status of the lakes (Carlson, 1977; Carlson & Simpson, 1996). TP, Chl-*a* and Secchi depth measurements were used to calculate TSI index as follows:

$$TSI\ (Chla) = 9.81 * \ln(Chla) + 30.6$$

$$TSI\ (SD) = 60 - 14.41 * \ln(SD)$$

$$TSI\ (TP) = 14.42 * \ln(TP) + 4.15$$

Average of these three equations was calculated as a final TSI value for each lake.

Zooplankton data were taken from Tavşanoğlu *et al.,* in prep. For the zooplankton analyses, 20 L of water sample was filtered through a 20 μm mesh size. Zooplankton samples stored in 50 ml dark glass bottles and preserved in 4% Lugol's iodine solution. Zooplankton counts were done at the genus or species level, where possible. Body sizes of about 25 individuals of each taxon were measured, when possible, and body weight was calculated from length-weight allometric relationships (Dumont *et al.,* 1975; Bottrell *et al.,* 1976; McCauley 1984; Michaloudi, 2005). Biomass of each species or genus was calculated and dry weight conversion was done according to Dumont *et al.* (1975), Ruttner and Kolisko (1977) and Malley *et al.* (1989).

Percentage of plant volume inhabited (PVI) data of the each of the study lakes, were taken from Levi *et al*., (2014) and it was calculated based on the formula of plant coverage×average plant height/water depth (Canfield *et al.,* 1984). Fish data on community structure and abundance were taken from Boll *et al.,* (2016) and were determined with the multimesh gillnets, covering 12 mesh sizes (5, 6.5, 8, 10, 12.5, 15.5, 19.5, 24.5, 29, 35, 43, 55 mm). Nets were cast in both littoral and pelagic zones for 12 hours and the net number was determined according to lake area (0-2 ha: 2 nets, 2-20 ha: 4 nets, 20-100 ha: 6 nets, >100ha: 8 nets).

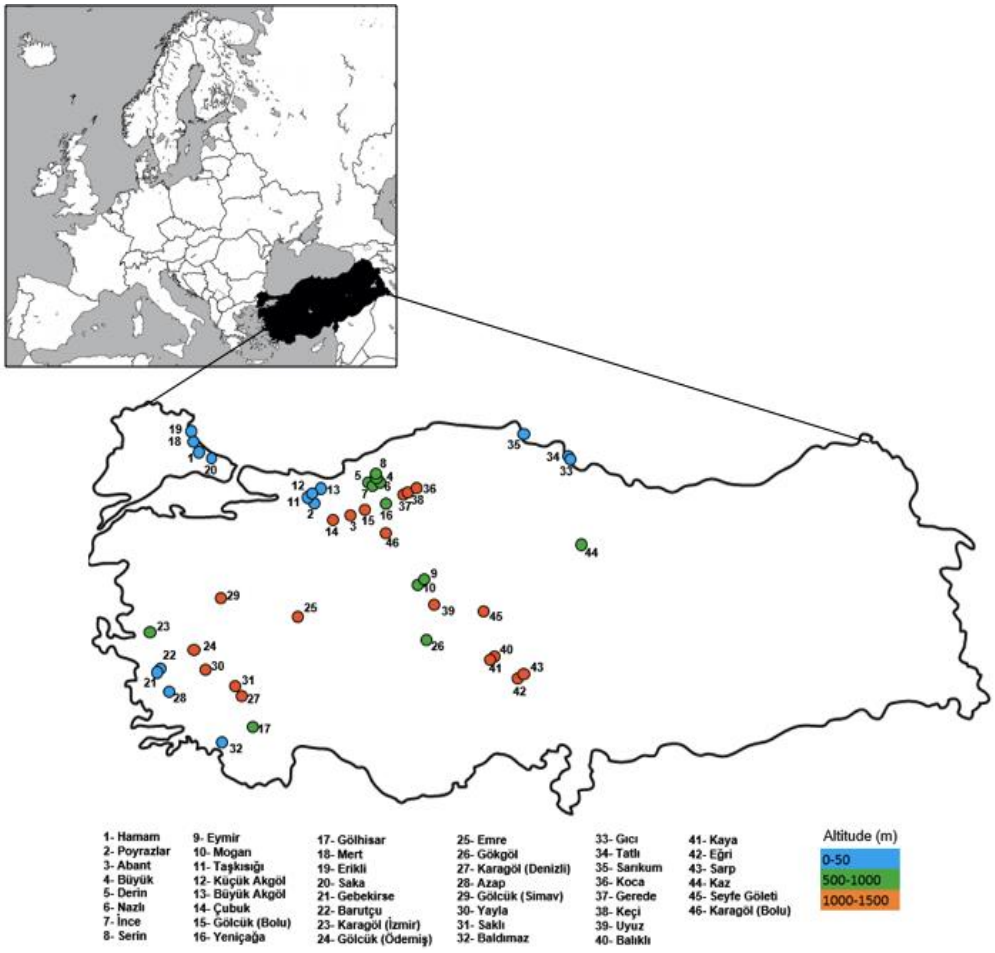

Figure 2.1 Study lakes on the map of Turkey given each with a number. The color coding indicates the altitude of the lake; blue: 0-50 m, green:500-1000 and orange:1000-1500.

Table 2.1 Main physical chemical and biological characteristics of the study sites (n = 46)

Variables	Range	Mean	Median
Altitude (m)	0-1423	716.9	950
Area (ha)	0.1-635	74.5	20
Mean depth (cm)	71.7-1740	399.6	340
Secchi Depth (cm)	20-900	135.6	97.5
Average Temperature (°C)	8.3-32.2	22.4	23.3
Surface Temperature (°C)	16-32.4	24.4	25
TP (μg L^{-1})	15.-632	120.8	85.6
SRP (μg L^{-1})	3.4-120	27.7	17.9
TN (μg L^{-1})	238.8-2340	1075.2	972.9
Silicate (μg L^{-1})	1096-15650	5153.7	4642
pH	6.3-9.5	8.1	8
Salinity (‰)	0.05-14.5	1.1	0.3
TDS	0.06-15.5	1.3	0.3
Chlorophyll-*a* (μg L^{-1})	1.9-181.1	30.5	15.9
TSI	38.9 - 83.7	62.1	63.6
Total phytoplankton biovolume (mm^3 L^{-1})	0.1-76.7	14.7	6.5
Zooplankton (μg L^{-1})	0.1-678.3	59.9	12.7
Cladocerans (μg L^{-1})	0.02-131.1	12.1	3.2
Copepods (μg L^{-1})	0.11-623.8	45.2	3.3
Rotifers (μg L^{-1})	0.002.-133.3	9.6	1.8
Zooplanktivorous fish (number of fish net^{-1} $night^{-1}$)	0 - 1210	100.5	2
PVI (%)	0-79.9	20.5	7.2

2.2.1 Statistical analysis

Bivariate regression analysis was used for both mean phytoplankton size and variance to test statistical significance of each predictor variable. Values were log transformed and standardized to meet the normality and homoscedasticity assumptions of the analysis. All the assumptions were met for the mean unit/cell size data, however, for the Lakes Küçük Akgöl, Büyük Akgöl and Yeniçağa, the

normality assumption was not met for the variance in unit analysis. While Lakes Yeniçağa and Büyük Akgöl were mostly dominated by colonial cyanobacteria, Lake Küçük Akgöl was dominated by both *Euglena* and cyanobacteria, and the variance was higher than in other lakes. As the high variance was not due to sampling or counting error, we did not exclude these three lakes from the analyses but also conducted the same analyses without these 3 lakes and presented the results in supplementary material.

We also used structural equation modelling (SEM), a multivariate statistical analysis method that detects interaction pathways, also direct and indirect effects among numerous variables (Anderson & Gerbing, 1988; Grace, 2006). It can untangle direct effects of temperature on cell size from those that work through temperature effect on nutrient availability and grazer communities. SEM fits were evaluated using chi-square statistic, also upper and lower root mean square error of approximation (RMSEA). Based on these values, a convincing SEM model should have non-significant chi-square, lower RMSEA of <0.05 and upper RSMEA of <0.1. If the fit measurements were not satisfactory, the initial model was modified according to the reasonable biological interactions. Analyses were repeated until the best fit measures and significant interactions among all the remaining variables were obtained.

SEM analyses were carried out to understand how much variation (SEM-R^2) of the mean phytoplankton cell/unit size and of the variance in unit size could be explained by significant environmental or biological variables that were identified in the bivariate regression analyses. In the current study, sample size was small (46 lakes) so we could only use four environmental variables in SEM analysis to achieve an adequate statistical power. These variables were chosen from the significant bivariate regression results. If none of the bivariate regression result was significant, SEM analysis was conducted with TP, temperature, zooplankton and zooplanktivorous fish since they are assumed as primary determinants of phytoplankton size structure.

R version 3.1.3 (R Development Core Team, 2015) was used to conduct all statistical analyses.

2.3 Results

2.3.1 Phytoplankton and zooplankton taxonomic composition

Overall, total phytoplankton biovolume and cyanobacteria contribution were high in high nutrient lakes (Figure 2.2). However, there were some lakes with low nutrients but high cyanobacteria contribution (Lakes Baldımaz, Poyrazlar, Gebekirse) (Figure 2.2). According to the TSI classification, there were 6 mesotrophic, 32 eutrophic and 8 hypereutrophic lakes (Figure 2.3a). Bacillariophyta, Dinophyta and Chlorophyta contributions were high in mesotrophic lakes, while in eutrophic and hypereutrophic ones the higher abundance belonged to cyanobacteria, Chlorophyta and Cryptophyta groups (Figure 2.3b).

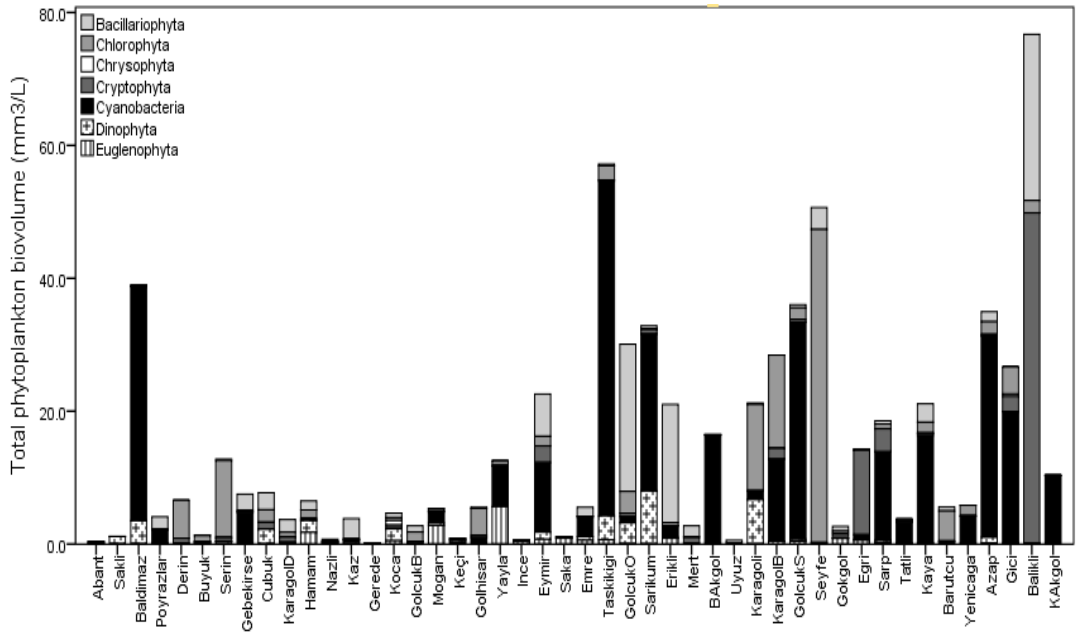

Figure 2.2 Total phytoplankton biovolume (mm^3 L^{-1}) for each study site (TP concentration increase from left to right).

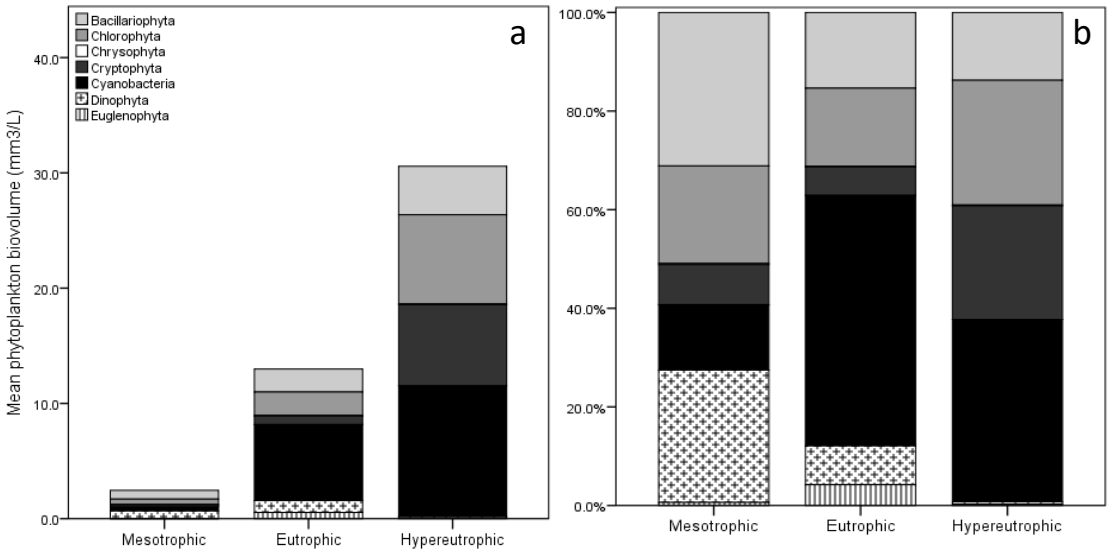

Figure 2.3 Phytoplankton community composition in the study lakes grouped based on the TSI classification a) mean phytoplankton biovolume b) percent contribution of phytoplankton groups.

Total zooplankton biomass increased along the nutrient gradient and the community composition changed (Figure 2.4a). While the Cladocera percentage was high in mesotrophic lakes, in eutrophic and hypereutrophic ones copepods and rotifers constituted the dominant groups (Figure 2.4b).

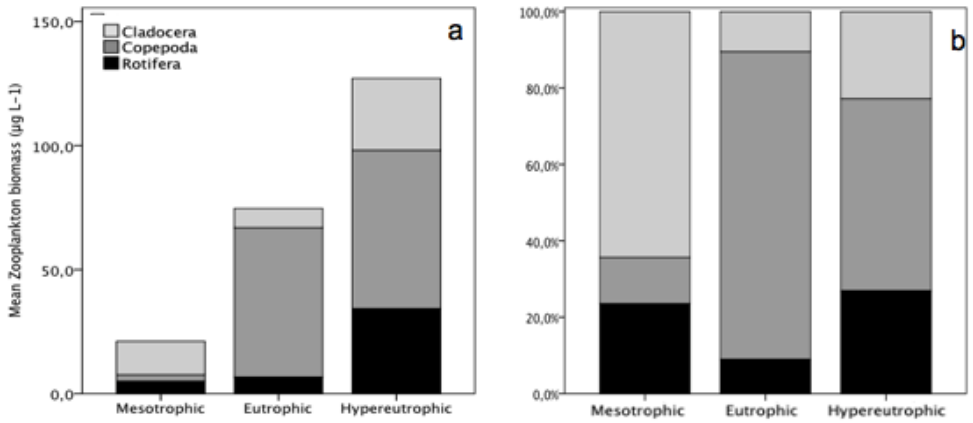

Figure 2.4 Zooplankton taxonomic composition in lakes grouped by the TSI classification a) mean zooplankton biomass b) zooplankton biomass percentage.

2.3.2 Mean size and variance in size analysis

In total twenty-two environmental and biological variables (Table 2.1) were tested by bivariate regression analysis to determine the most important environmental and biological predictors for mean phytoplankton size and variance. According to our analysis results, only rotifers (p<0.01) and zooplanktivorous fish (p<0.05) had positive significant effects on mean phytoplankton unit size. Moreover, salinity was also close to significance (p<0.07). The regression analyses were also conducted with the cell size data but we did not find any significant relationship or trend for those species (Table 2.2, Figure 2.5). TP (p=0.001), SRP (p<0.01), TN (p<0.05), zooplankton (p<0.01), Cladocera (p<0.05), Copepoda (p<0.01), rotifers (p<0.01), zooplanktivorous fish (p=0.001), Secchi depth (p<0.01) and TSI (p<0.01) had significant effects on variance in unit size (Table 2.2, Figure 2.6).

Variance in unit size and TP had a significant correlation (p<0.001) (Figure 2.6).

Table 2.2 Bivariate regression results for environmental variables and mean unit size, variance in unit size.

	Unit			
Size				
	Coefficient	**R^2**	***p* value**	**F value**
Rotifers	0.3168	0.19	<0.01	$F_{1.44}$=10.63
Zooplanktivorous fish	0.2171	0.09	<0.05	$F_{1.44}$=4.42
Variance				
TP	0.2673	0.2	0.001	$F_{1.44}$=11.06
SRP	0.2433	0.17	<0.01	$F_{1.44}$=8.79
TN	0.2172	0.13	<0.05	$F_{1.44}$=6.73
Zooplankton	0.2303	0.15	<0.01	$F_{1.44}$=7.71
Cladocerans	0.2191	0.14	<0.05	$F_{1.44}$=6.86
Copepods	0.2451	0.17	<0.01	$F_{1.44}$=8.94
Rotifers	0.2501	0.18	<0.01	$F_{1.44}$=9.38
Zooplanktivorous fish	0.2789	0.22	0.001	$F_{1.44}$=12.31
Secchi depth	-0.288	0.23	<0.01	$F_{1.44}$=13.34
TSI	0.256	0.18	<0.01	$F_{1.44}$=9.93
Phytoplankton richness	0.1599	0.05	<0.1	$F_{1.44}$=3.41

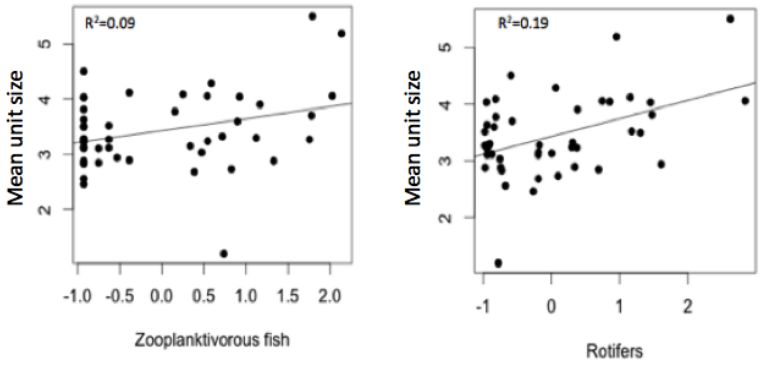

Figure 2.5 Bivariate regression plots for unit size and zooplanktivorous fish (left), rotifers (right).

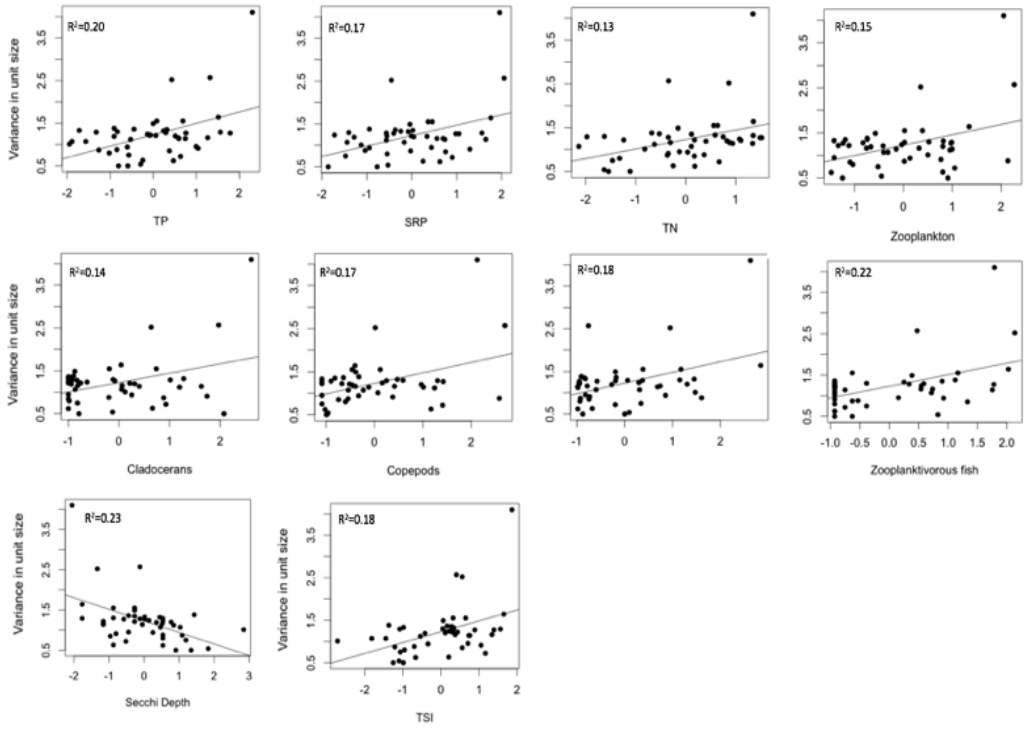

Figure 2.6 Bivariate regression plots for variance in unit/cell size. Environmental variables were given in x axis

2.3.3 SEM Analysis

SEM analyses were carried out to understand how much variance of mean phytoplankton cell/unit size and the variance in unit size could be explained by significant environmental or biological variables that were identified in the

bivariate regression analyses. Because our sample size was small (46 lakes) we could use only four environmental variables in SEM analysis to achieve an adequate statistical power. These 4 variables were chosen from the significant bivariate regression results (Table 2.2). If none of the bivariate regression result was significant, SEM analysis was conducted with TP, temperature, zooplankton and zooplanktivorous fish since they were assumed as primary determinants of phytoplankton size structure.

2.3.3.1 Unit and Cell Size SEM Analysis

According to bivariate regression results no significant correlation was found between mean cell size end environmental variables. Therefore, SEM analysis was conducted with TP, temperature, zooplankton and zooplanktivorous fish. Mean unit size analysis included TP, salinity, zooplanktivorous fish and rotifer as independent variables (Figure 2.7). SEM did not reveal direct effects of TP, salinity and zooplanktivorous fish on phytoplankton size, however their interaction with rotifer abundance and the rotifer biomass direct effect on phytoplankton size structure were statistically significant. Overall SEM results explained 20% of total variance (RMSEA 95% CI = (0, 0.223), X^2 = 0.310, df = 5) (Figure 2.7).

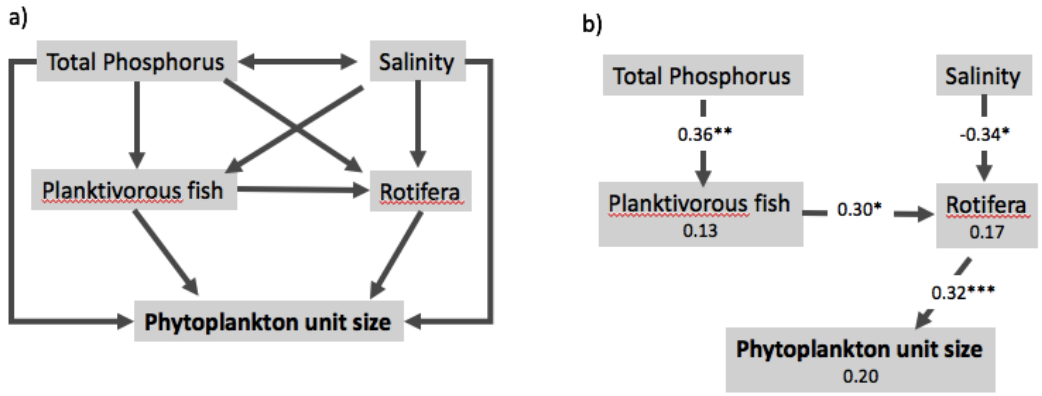

Figure 2.7 Phytoplankton unit size SEM analysis results a) initial SEM diagram, b) final SEM results. Arrows represent casual positive relationship, coefficients and significance values were presented on arrow lines. R^2 values were given under variable names. p<0.05*; 0.01**; 0.001***

2.3.3.2 Variance in Unit Size SEM Analysis

The SEM analysis of variance in unit size included TP, TN, zooplankton and zooplanktivorous fish data as predictors (Figure 2.8). While zooplankton and zooplanktivorous fish had a direct effect on phytoplankton unit size variance, TP had an indirect effect. Overall SEM result explained 40% of total in phytoplankton unit size variance (RMSEA 95% CI = (0, 0.335), X^2 = 0.203, df = 5 2).

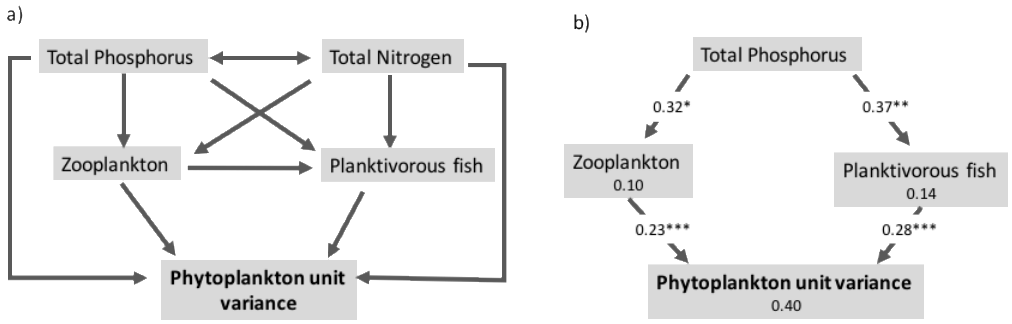

Figure 2.8 Variance in unit size SEM analysis results a) SEM diagram, b) final SEM results. Arrows represent casual positive relationship, coefficients and significance values were presented on arrow lines. R^2 values were given under variable names. p<0.05*; 0.01**; 0.001***

Regressions and SEM analysis of the variance in unit size were also conducted without 3 outlier lakes (Appendix-A Table 1, Figure 1). Regression analysis carried out without 3 lakes showed that zooplanktivorous fish, Secchi depth, TSI and TN were significant drivers for variance in unit size, similar to the results of analyses including outlier lakes. However, unlike the previous analyses (with outlier lakes), zooplankton (all groups), TP and SRP were not significant. Instead, altitude (-), total phytoplankton biovolume (+) and Chl-*a* (+) were significant (Appendix-A Table 1). Since significant variables were different between the two analyses, we conducted a second SEM analysis with the environmental variables that were significant: altitude, TN, total phytoplankton biovolume and zooplanktivorous fish. We found that only TN had a positive effect on total phytoplankton biovolume (Appendix-A Figure 2).

2.4 Discussion

2.4.1 Mean Cell / Unit size

According to SEM analysis, only rotifer biomass had direct positive significant effect on phytoplankton unit size and no significant relationship was found for cell size (Figure 2.7). The reason was probably due to the selective rotifer predation pressure on small size phytoplankton species (Figure 2.7). Owing to their small size, rotifers generally are not considered as a potential phytoplankton biomass regulator, however when present in high densities rotifers have a strong grazing impact on phytoplankton biomass (Lionard *et al*., 2005). Since most of our lakes were eutrophic and hypereutrophic this result was in accordance with our first hypothesis which stated that top down regulation on phytoplankton size would be more pronounced under eutrophic and hypereutrophic conditions. Moreover, instead of direct effect of TP on phytoplankton size, we found indirect of TP effect via zooplanktivorous fish and through rotifer (Figure 2.7). Selective fish predation on large zooplankton species (e.g. *Daphnia*) could be the main reason of positive correlation between zooplanktivorous fish and rotifer. Accordingly, total rotifer biomass was positively correlated with eutrophication in our lakes and while it was highest in hypereutrophic lakes, mesotrophic lakes had the lowest biomass (Figure 2.4). This is also in accordance with previous studies as high fish predation leads to small sized grazers to predominate (Strecker *et al*., 2004). The significant negative impact of salinity on rotifer biomass found in the current study (Figure 2.7) is likely related to the decrease in zooplankton species richness with increasing salinity, owing to their limited osmoregulation capacity as it was stated in Sarma (2006) and Blinn (2004). Moreover, higher salinities can also cause community composition shifts from large bodied zooplankton species, especially *Daphnia* spp., which are very sensitive to salinity changes (except *D. magna*), to small sized species (Brucet *et al*., 2009; Jensen *et al*., 2010; Tavşanoğlu *et al*., 2015).

Temperature differed considerably among the study lakes (Table 2.1) and it was hypothesized (second hypothesis) to be an important driver of phytoplankton size, nevertheless we did not observe a significant temperature effect. Moreover, Rüger and Sommer (2011) found only one out of seven phytoplankton species, showing significant size shrinkage in response to temperature increase. On the other hand, many studies support species replacement hypothesis for the mean phytoplankton community size decrease (Winder *et al*., 2009; Daufresne *et al*., 2009). Additionally, indirect temperature effects on phytoplankton cell size were also mentioned as a causal size shrinkage mechanism (Winder *et al*., 2009). One of the well-studied indirect temperature effect is that due to the prolonged stratification periods, leading to nutrient scarcity at upper water layers, non-motile large phytoplankton species (e.g. large diatoms) usually cannot survive, but for small or motile species (e.g. small, centric diatoms) survival is much easier (Winder *et al*., 2009). Most of our lakes were shallow and non-stratified, thus this could not be applicable though in very warm and calm days even shallow lakes may have impermanent stratification. Instead we assumed that small sized species should be more abundant in oligotrophic and mesotrophic lakes. Nevertheless, we did not find any significant relationship between TSI values and phytoplankton unit or cell size. Our lakes were mostly eutrophic and hypereutrophic according to TSI classification and we do not have any oligotrophic lakes in our data set and it could be the reason of non-significant trophic state size relationship.

2.4.2 Variance in unit size

We found positively significant correlation between variance in unit size and TP, however this significant pattern was most probably due to 3 outlier lakes (Figure 2.9), since we did not find any significant correlation after excluding these outliers (Appendix-A Figure 1). Nevertheless, large size and colonial/filamentous phytoplankton species was abundant in these outlier lakes, therefore our results support our third hypothesis.

Besides TP, TN, zooplankton (all sub groups), zooplanktivorous fish and TSI had positive significant effect on variance in unit size, while negative correlation was only observed with Secchi depth (Table 2.2). According to SEM analysis results TP does not have any direct effect on variance in unit size, instead it had an indirect effect via zooplankton and zooplanktivorous fish (Figure 2.9), leading to the pronounced top-down effect in our lakes. Moreover, variance in unit size and zooplanktivorous fish correlation could be as a result of selective predation pressure on zooplankton. As abundance of planktivorous fish increases, grazing pressure on cladocerans also increases especially in eutrophic and hypereutrophic ecosystems (Brooks, 1968; Vadadi-Fülöp *et al*., 2012). Even though large bodied zooplankton species, especially cladocerans, generally known as the most effective phytoplankton grazers (Leibold, 1989), they could only graze on phytoplankton species which are smaller than 50 μm. Other zooplankton groups, like copepods and especially rotifers, can only graze on relatively smaller phytoplankton species. Since colonial, filamentous or other large species, like euglena, are grazing resistant, thus intense grazing on mid- and small size phytoplankton species probably led to an increase in variance in unit size (Lynch & Shapiro, 1981). Moreover, in second SEM analysis (without 3 outlier lakes) only TN had a positive effect on the total phytoplankton biovolume, which, in turn, had a significant positive effect on variance in unit size (Appendix-A Figure 2). Different than the first SEM analysis we found that bottom up effect was more important than top down effect in second SEM analysis. However, the lack of significant zooplankton and fish effect in the second SEM results may also be related to the excluded outlier lakes, which were all hypereutrophic.

On the other hand, we did not find any significant correlation or hump shape relationship between species richness and TP, neither when all the lakes were included, nor when outliers were excluded. Even though the correlation was not significant, species richness tends to decrease with increasing TP. Our sample size is small and mostly cover eutrophic and hypereutrophic lakes, probably causing the lack of significant correlations. Overall results support the idea that variance

in size could be used as a simple parameter to predict the current status of shallow lake ecosystems.

2.4.3 Conclusion

In summary, our results highlight the sensitivity of cell size structure to biotic and abiotic variables, like nutrient increase and zooplankton grazing. Moreover, our results suggest that trait-based approaches, cell size in particular, can be used as a tool to assess ecological responses to climate change in aquatic ecosystems (Litchman & Klausmeier 2008; Durocher *et al.*, 2011). We found that the top-down regulation is more pronounced in eutrophic and hypereutrophic lakes than in oligotrophic lakes. Planktivorous fish had an especially strong effect on phytoplankton, probably due to enhanced predation on zooplankton. Climate change scenarios predict increased drought periods, higher evaporation rate such conditions likely to lead to intensified irrigation, and consequently, salinization as well as intensifies eutrophication of already nutrient rich lakes in semi-arid to arid Mediterranean (Christensen *et al.*, 2013; Jeppesen *et al.*, 2009). To better understand lake ecosystem responses to environmental parameters, shallow lake ecosystems should be monitored regularly. However, taxonomic identification of phytoplankton is time consuming and requires expertise, but size data collection is simpler and does not require much taxonomic background and according to our results can give insights about ecosystem functioning, however more detailed researches are needed to clarify main mechanisms.

CHAPTER 3

EFFECT OF CLIMATE, WATER LEVEL AND NUTRIENTS, ON PHYTOPLANKTON COMMUNITY STRUCTURE AND DIVERSITY: PAN-EUROPEAN MESOCOSM EXPERIMENTS

3.1 Introduction

Concentration of greenhouse gases on atmosphere has been gradually increasing since the industrial revolution (Hougton *et al.,* 2001). Accordingly, average global temperature also increases, having direct or in direct effects on both terrestrial and aquatic ecosystems (Vitousek *et al.,* 1997; Jeppesen *et al.,* 2009; Franks *et al.,* 2014). As a consequence of climate change, temperature increase and seasonal precipitation regime changes are projected all over the world (IPCC, 2013) with intensified and frequent droughts in the Mediterranean and flooding in the North Temperate regions. Consequently, lakes are expected to have higher external and internal nutrient loadings and more pronounced water level fluctuations (IPCC, 2007; Beklioğlu *et al.,* 2007; Jeppesen *et al.,* 2009; Jeppesen *et al.,* 2015).

As a major primary producer, phytoplankton play a critical role in all aquatic ecosystems (Moss, 2010). Phytoplankton community composition and seasonality are highly sensitive to total nutrient, temperature and hydrology, having a direct or indirect impact on biovolume, taxonomic and functional phytoplankton community structure (Prairie *et al*., 1989; Falkowski, 2007; Jeppesen *et al*., 2005; Salmaso *et al*., 2015). Phytoplankton species diversity generally shows unimodal distribution with increasing TP concentration in temperate regions (Jeppesen *et al*., 2000). However, in the subtropics despite the wide total phosphorus range of sampling sites (24-413 $\mu g\ L^{-1}$), phytoplankton species diversity did not follow a unimodal distribution (Kruk *et al*., 2009). Accordingly, Muylaert *et al*. (2010) did

also not find any relationship between phytoplankton generic richness and total phosphorus in a set of 98 shallow lakes from Spain to Denmark and Belgium/The Netherlands. Therefore, rather than only nutrient availability, more complicated biotic and abiotic interactions might be the determinants of phytoplankton diversity patterns. Since phytoplankton is an extremely diverse group and taxonomic identification requires high level of expertise, diversity studies may not appropriately reflect the real diversity trends. On the other hand, phytoplankton functional classification which is generally based on both basic taxonomic and functional properties may give insights about the ecological situation of shallow lake ecosystems (Salmaso & Padisak, 2007). However, as of current time, there has been no investigation, which experimentally focuses on the direct effects of climate change on phytoplankton functional groups.

In many places all around the world, increased harmful cyanobacteria blooms has been causing fish kills, also deteriorating drinking and irrigation water leading to a severe shortage during the last decades (Havens *et al*., 2001; Qin *et al*., 2010; Paerl *et al*., 2011). Large number of studies covering lakes with wide range of trophic conditions attributed this recent increase to direct and indirect effects of global warming (Wagner *et al.*, 2009; Anneville *et al.*, 2005; Winder *et al.*, 2012; Pearl *et al.*, 2008), since higher temperatures favors cyanobacteria species, due to their high optimum growth temperature (25-35 °C) (Robart & Zohary, 1987), while negatively affecting other phytoplankton groups with low optimum growth temperature (e.g. diatom species) (Pearl, 2011; Litchman *et al.*, 2010). Furthermore, synergistic effects of temperature and nutrient may also promote cyanobacteria blooms (Elliot *et al.*, 2012), either by a major increase in total biomass or by a proportional increase (Kosten *et al.*, 2012). Recent meta-analysis of >1000 U.S. lakes showed that interaction between temperature and nutrient was mostly depended on the trophic state of the lakes (Rigosi *et al.*, 2014). While nutrient had primary effect in oligotrophic lakes, temperature effect was more significant in mesotrophic ones, and temperature-nutrient interaction was the main determinant in eutrophic and hypertrophic lake ecosystems (Rigosi *et al.*, 2014).

Hydrology is also an important factor for determining phytoplankton community structure. Even though phytoplankton studies mostly focus on nutrient and temperature parameters, since which are known as the main drivers, the effect of hydrological changes and its interaction with temperature and nutrient are hardly investigated. As a consequence of climate change, expected more intense precipitation in the future may cause high loading of nutrients from the catchment and may lead to increase in-lake concentration in northern countries, while prolonged drought periods may lead to nutrient up concentration through evaporative water loss as well as internal loading in southern countries, resulting in eutrophication (Bolle *et al*., 2003; Özen *et al*., 2010; Coppens *et al*., 2016). Moreover, due to projected high evaporation rates and water level decrease, salinity increase may also become a problem (osmotic stress, toxicity) for southern countries (Beklioğlu *et al*., 2008; Jeppesen *et al*., 2009).

There is no consensus on the effects of temperature, nutrient, hydrology and their interactions on phytoplankton biovolume, taxonomic and functional structures, or diversity patterns. Mesocosm experiments can give an opportunity to abstract local ecological differences, providing an understanding of the main influential effects of tested variables. In present study, we conducted synchronized mesocosm experiments along a latitudinal gradient, from Sweden to Greece in six countries, to investigate the effects of water level, nutrient concentration and temperature changes on phytoplankton taxonomic and functional groups biomass, also on diversity patterns. We hypothesized that i) total phytoplankton biovolume would be highest in warm (southern countries) especially in the high nutrient treatments, having a high cyanobacteria contribution, while in colder (northern) countries Chrysophyta and Bacillariophyta species would be dominant, ii) in the shallow mesocosms with warm and high nutrient treatments, high macrophyte coverage would suppress phytoplankton biovolume increase, and iii) in southern countries richness and diversity would decrease though the biovolume was expected to be the opposite.

3.2 Material and Methods

3.2.1 Experimental set-up and sites

The strictly synchronized mesocosm experiments were carried out in Sweden (SE), Estonia (ES), Czech Republic (CZ), Germany (GE), Turkey (TR), and Greece (GR) from May to November 2011 (Figure 3.1). Experimental lakes were selected according to their depth (< 4 m), total alkalinity (1< TA < 4 meq L^{-1}), salinity (<1‰) and colour (<50 mg Pt L^{-1}). The experimental set up consisted of 16 mesocosms in total, having two nutrient concentrations (low:L, high:H) crossed with two water levels (deep:D, shallow:S) and each treatment represented by four replicates. Cylindrical mesocosms made from fiberglass plastic with a diameter of 1.2 m and 4 mm thickness were used and they were all constructed in the same firm and were transported to each country. For low nutrient treatments, nutrient levels of 25 µg L^{-1} total phosphorous (TP) and 0.5 mg L^{-1} total nitrogen (TN), while for high nutrient treatments 200 µg L^{-1} TP and 2.0 mg L^{-1} TN concentrations were used. Phosphorus (Na_2HPO_4) and nitrogen ($Ca(NO_3)_2$) were added to each mesocosm every month to diminish the effect of natural removal (sedimentation, nitrification) of nutrient concentrations. The ratio of TP and TN was kept as 1:20 and monthly additions assumed that 40% of TP and TN were removed from the system. Each mesocosm was filled with 10 cm (10% mud, 90% sand) sediment. Sediment was collected from oligo-mesotrophic lakes and was equilibrated to the TP levels that were used as nutrient treatments prior to experiment. Mesocosm tanks with 1m and 2m heights were used for shallow and high water levels, respectively. A detailed description of the experimental set-up has been given in Landkildehus *et al.* (2014).

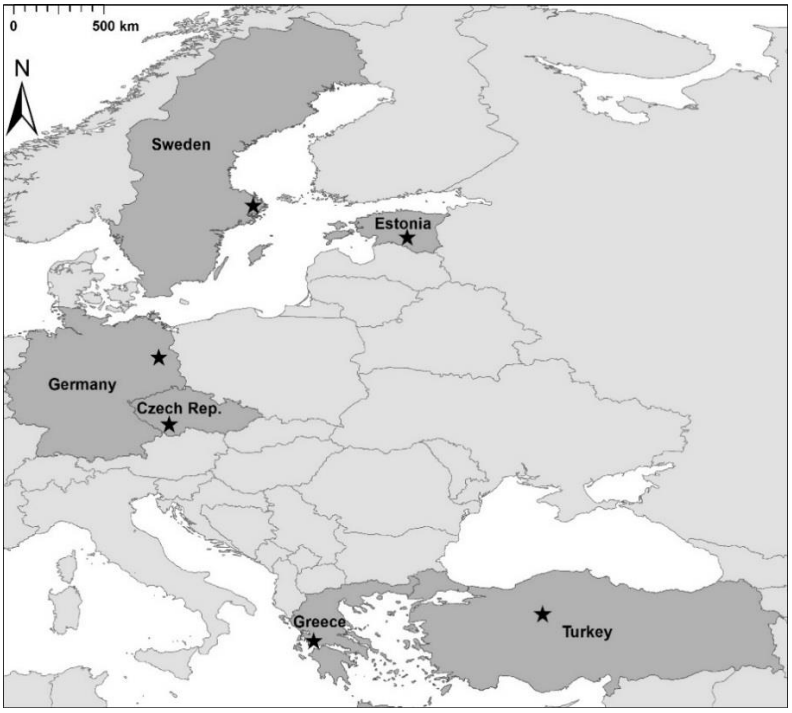

Figure 3.1 Study sites

Plankton samples were collected by vertical hauling with a 50 μm mesh size plankton net from five different local lakes (TP 25-200 μg L^{-1}), where from each 5 L water sample were collected and mixed, and 1 L of this mixed plankton inoculum was added to each mesocosm. Afterwards fish and macrophyte inoculations were carried out, eight *Myriophyllum spicatum* shoots (shoot length 5-10 cm) were planted to each mesocosm. Shoots were anchored with small stones to increase their growing chance and to be sure that the shoots were evenly distributed in the tanks (Landkildehus *et al*., 2014). As fish species *Gasterosteus aculeatus* L. (tree spined stickleback), ranging in size between 3-4 cm, was chosen because of its cosmopolitan distribution. However, instead of sticklebacks *Rutilus rutilus* L. and *Gambusia affinis* Baird and Girard were added to the mesocosms in Sweden and Greece, respectively. Six fish were introduced to each enclosure (3 females - 3 male).

Physico-chemical and biological sampling was conducted once a month. Water samples were taken with a plastic tube from the entire water column, approximately 5 cm above the sediment and they were kept frozen prior to analysis. Water depth was measured with a depthmeter and water temperature, salinity, pH, conductivity and total dissolved solid (TDS) were measured with a standard multiprobe field meter. Also, alkalinity, total phosphorus (TP), soluble reactive phosphate (SRP) (Mackereth *et al.,* 1978) and total nitrogen (TN) (Scalar Autoanalyzer Method was used (San++ Automated Wet Chemistry Analyzer, Skalar Analytical, B.V., Breda, Netherlands) analysis were conducted according to standard procedures in all countries. Chlorophyll-a concentration was determined through ethanol extraction method (Jespersen & Christoffersen, 1987).

Percent plant coverage and plant height were measured to calculate percent plant volume inhabited (PVI) (Canfield *et al.,* 1984). Photosynthetically active radiation (PAR) was measured monthly in each mesocosm and Euphotic depth (%1 light penetration) was calculated according to the Beer Lambert law, by using the following formula;

$$E_{d(z)} = E_d(0) \mathrm{x} e^{-kz}$$

Where; $E_{d(z)}$ is PAR measurement at depth z, $E_d(0)$ is the PAR measurement just under the water surface, k is the attenuation coefficient and z is the depth (Kirk, 1996).

Phytoplankton samples were taken monthly from the entire water column, and preserved in Lugols' iodine solution (1%) in 50 ml brown glass bottles. Twenty-five percent of monthly phytoplankton samples from each mesocosm (except the sample from first month) were subsampled and mixed to form the bulk samples, which represented the whole experimental period. Therefore, instead of conducting the counting for each month, only the first month and the bulk samples for the rest of the sampling period were counted. First month samples were counted to determine the initial phytoplankton community compositions in each

country. Utermöhl technique was used for counting the phytoplankton species (Utermöhl, 1958). Organisms were settled for 24h and identified and counted usually to genus level by using an inverted microscope at 40X magnification by an expert of each country. At least 100 individual of the most abundant species were counted in random fields, and at least 10 individuals from each species were measured to calculate the biovolume of each species (mm^3 L^{-1}) according to the closest geometrical shapes (Hillebrand *et al.,* 1999). To prevent the discrepancies that could originate from different levels of taxonomic expertise, genus level identification data was used for diversity and evenness (Shannon Weaver) calculations (Shannon and Weaver, 1963) and genus richness was calculated as number of identified genus for each country. Moreover, Morpho-Functional (MF) groups were identified according to Salmaso and Padisak (2007) and Tolotti *et al*. (2012) (Table 3.1).

Table 3.1 Phytoplankton Morpho-Functional classification (Salmaso & Padisak, 2007).

			Functional Group	Code
Flagella	Potential mixotroph	Large (colonial or unicellular)	Large Chrysophytes/Haptophytes	1a
			Large Euglenophytes	1c
		Small (unicellular)	Small Chrysophytes/Haptophytes	2a
			Small Dinophytes	2b
			Small Euglenophytes	2c
			Cryptophytes	2d
	Mostly autotrophs	Phytomonadia	Unicellular Phytomonadina	3a
Without Flagella	Cyanobacteria	Unicellular	Unicellular cyanobacteria	4
		Colonial	Thin filaments (Oscillatoriales)	5a
			Large vacuolated Chroococcales	5b
			Other large colonies, mostly non-vacuolated Chroococcales	5c
			Small colonies, Chroococcales	5d
	Diatom	Large	Large Centrics	6a
			Large unicellular pennate	6b
			Large colony forming pennate	6c
		Small	Small Centrics	7a
			Small Pennates	7b
	Other unicellular	Large	Large unicells—Unicellular Conjugatophytes/Chlorophytes	8a
		Small	Small unicells—Conjugatophytes	9a
			Small unicells—Chlorococcales	9b
			Small Chrysophytes	9c
			Small unicells—Other groups	9d
	Other colonial	Non filament Colonies	Filaments—Chlorophytes	10a
			Chlorococcales—Naked colonies	11a
			Chlorococcales—Gelatinous colonies	11b

3.2.2 Statistical Analyses

The effect of water depth and nutrient levels along with temperature on phytoplankton biovolume were determined by analysis of covariance (ANCOVA), by using the air temperature as a covariate, and nutrient and depth as fixed factors for the bulk samples (2002-2008 by SAS Institute Inc., Cary, NC, USA). ANCOVA analyses was also carried out with MF classification data. TP and TN differences among high and low nutrient treatments and temperature differences among countries were tested with one-way ANOVA in R version 2.15.1. Non-metric multi-dimensional (nMDS) scaling with Bray-Curtis dissimilarity ordination method and analysis of similarity (ANOSIM, 999 permutations) used to calculate differences among countries based on phytoplankton biovolume and environmental data. (Clarke, 1993, R version 2.15.1, R Development Core Team, 2012). Then, pairwise comparison test was performed among countries. In order to achieve normality assumptions of statistical analysis $\log_{10}$ and $\log_{10}(x+1)$ transformations were done for sub data sets.

3.3 Results

3.3.1 Physical and Chemical Variables

Air temperature differences between countries was significant and was correlated with water temperature, with low temperatures in northern countries (Sweden mean air temperature: 15.5 ºC) and high in southern ones (Greece mean air temperature: 25.1 ºC) (Figure 3.2). Moreover, water level change was more prominent in southern countries and it decreased ca. 40 cm and 93 cm in Turkey and Greece, respectively (Figure 3.2). Average TP and TN concentrations were significantly different (One-way ANOVA, $p<0.001$) in high and low nutrient treatments (high: 94±31 P μgL^{-1} and 1.6±0.5 N mgL^{-1}, low: 31±17 P μgL^{-1} and 0.9±0.3 N mg L^{-1}). PAR at the deepest points of the mesocosms were higher than 1% of the surface PAR measurements in most of the treatments and countries.

However, there were some exceptions with PAR being <1% at the bottom of all the DH treatments (except Turkey) and DL treatment of Germany (Table 3.2).

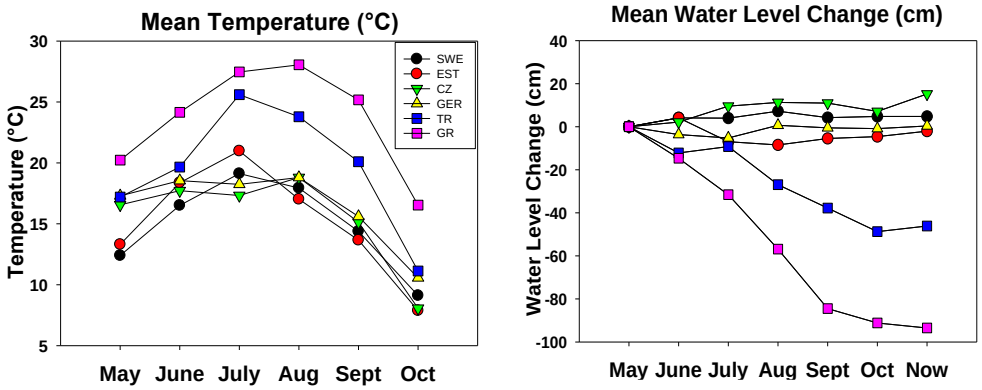

Figure 3.2 Monthly mean air temperature (left) and water level change (right). (SE: Sweden, ES: Estonia, CZ: Czech Rep., GE: Germany, TR: Turkey, GR: Greece)

Table 3.2 Light penetration percentages to sediment for each mesocosm treatment. Mesocosm order: Shallow Low (SL), Shallow High (SH), Deep Low (DL), Deep High (DH).

PAR % at deepest point	**SL**	**SH**	**DL**	**DH**
Sweden	9.6	7.8	1.4	0.6
Estonia	7.2	7.5	1.9	0.8
Czech	28.9	13.7	8.1	0.4
Germany	7.9	1.6	0.8	0.9
Turkey	50.6	65.8	18.2	15.6
Greece	29.2	10.7	9.7	0.1

3.3.2 Phytoplankton Community Structure

Initial phytoplankton community structure was different among countries, but with mostly similar total phytoplankton biovolume and species compositions among treatments in each country (Figures 3.3 and 3.4). Initial phytoplankton composition mostly dominated by Bacillariophyta species in Estonia, Czech,

Germany and Greece, while it was mostly dominated with Dinophyta and Chlorophyta species in Turkey and Sweden, respectively (Appendix-B Figure 1, Table 1).

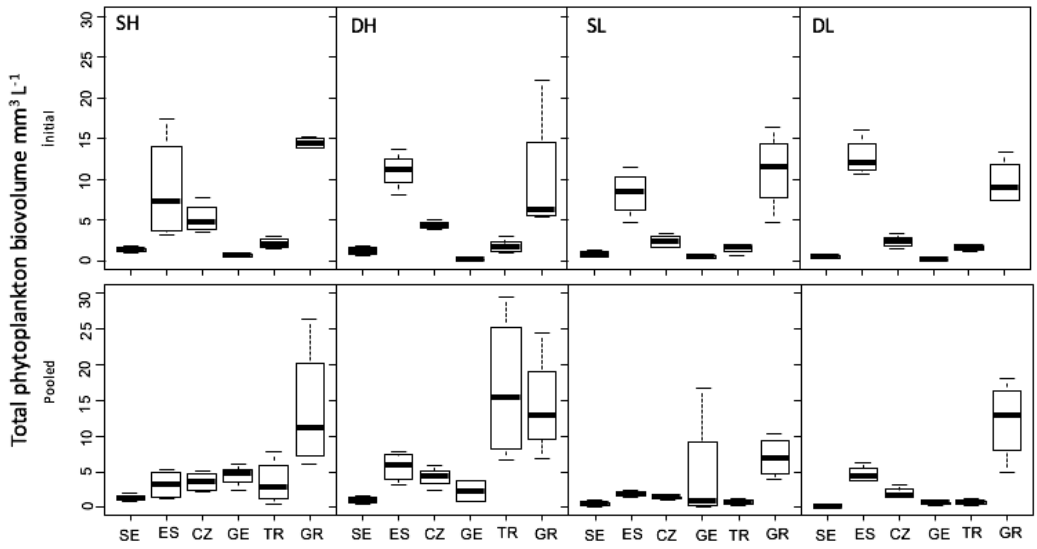

Figure 3.3 Initial (top) and bulk (bottom) total phytoplankton biovolume (mm^3 L^{-1}). Sweden (SE), Estonia (ES), Germany (GE), Czech Republic (CZ), Greece (GR) and Turkey (TR), Along temperature gradient.

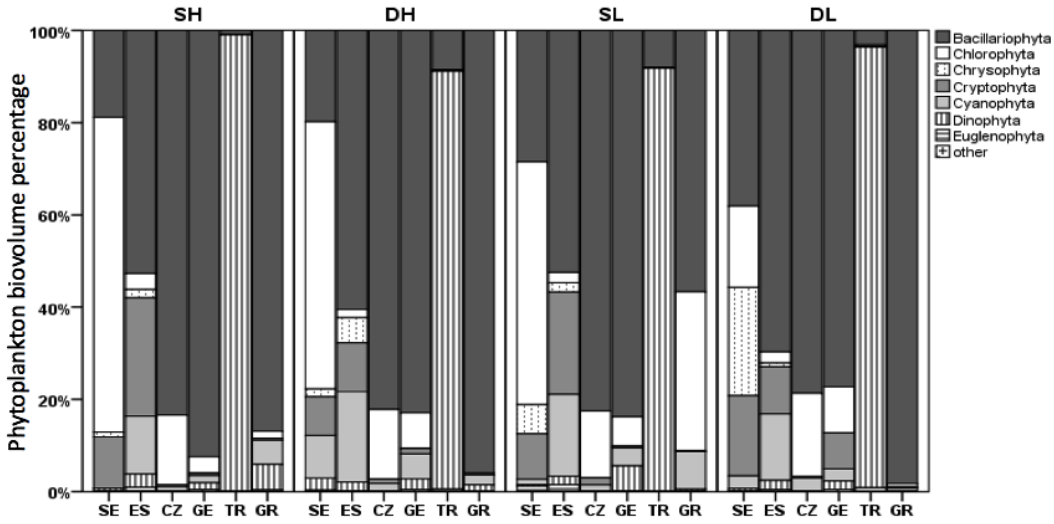

Figure 3.4 Initial total phytoplankton biovolume percentage.

Total phytoplankton biovolume increased and the community composition changed during the experimental period in all countries (Figures 3.3-3.5, Appendix-B Table 1, Figure 1). According to ANOSIM results all countries were significantly different from each other based on phytoplankton biovolume data (p<0.001). With respect to nMDS results Greece, Turkey and Estonia were much more similar, however Czech Republic, Germany and Sweden did not overlap with any other country (Appendix-B Figure 2). Total phytoplankton biovolume was lowest in all the treatments of Sweden and mostly dominated by Chlorophyta and Chrysophyta species, however it was highest in Greece (except DH treatment) and cyanobacteria and Chlorophyta species were dominant. Cryptophyta species contributions was higher in northern countries (SE, ES, CZ) whereas it was low in southern countries (GE, TR, GR). Total Cyanobacteria biovolume was highest in Turkey and Greece in DH treatments. Although total phytoplankton biovolume was low in Estonia and Germany, the contribution by cyanobacteria was high as much as Greece and Turkey both in low and high nutrient treatments (Figure. 3.5).

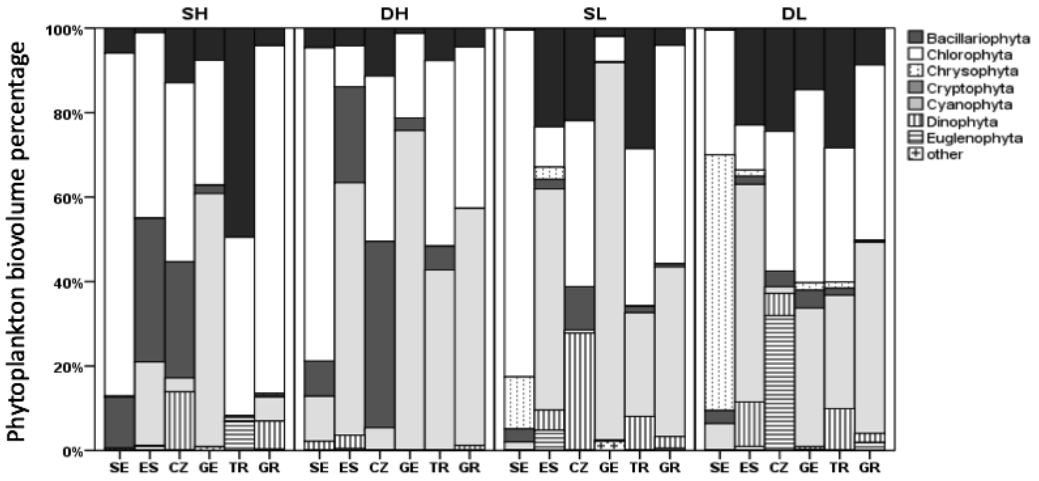

Figure 3.5 Bulk phytoplankton biovolume percentage for each phytoplankton phylum.

ANCOVA results showed that temperature ($p< 0.001$) and nutrient ($p< 0.001$) had positive significant effect on total phytoplankton and Chlorophyta biovolumes. While cyanobacteria ($p<0.05$), Bacillariophyta ($p<0.05$) and Chrysophyta ($p<0.05$) biovolumes increased by the temperature-nutrient interaction, whereas Cryptophyta biovolume ($p<0.05$) significantly decreased. On the other hand, Dinophyta was increased only with temperature ($p<0.05$) and no significant result was observed for the Euglenophyta species (Table 3.3).

Phytoplankton genus diversity was generally low in southern countries excluding shallow low nutrient treatments. ANCOVA revealed that temperature-nutrient-depth interaction negatively affected both genus diversity ($p<0.01$) and richness ($p<0.05$). On the other hand, genus evenness was decreased only with temperature ($p<0.001$) and depth ($p<0.01$) (Table 3.3) (Fig. 3.6).

Table 3.3 Analysis of covariance (ANCOVA) results (depth and nutrients: fixed factors; temperature: covariate) for the taxonomic classification.

	Total Biovolume	Chlorophyta	Cyanobacteria	Bacillariophyta	Chrysophyta	Cryptophyta	Dinophyta	Euglenophyta	Diversity	Evenness	Richness
Nutrient	<0,001 ↑	<0,001 ↑	<0,05 ↑	<0,05 ↑	<0,05	<0,01 ↓	NS	NS	NS	NS	NS
Depth	NS	NS	NS	NS	NS	NS	NS	NS	NS	<0,01 ↓	NS
Temperature	<0,001 ↑	<0,001 ↑	<0,001 ↑	NS	NS	<0,05 ↓	<0,05 ↑	NS	<0,001 ↓	0,001 ↓	<0,001 ↓
Nutrient*depth	NS	NS	NS	NS	NS	NS	NS	NS	<0,05 ↓	NS	<0,05 ↓
Nutrient*tmep	NS	NS	0,05 ↑	<0,05 ↑	0,04 ↑	<0,05 ↓	NS	NS	NS	NS	NS
Temp*depth	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Nutrient*depth*temp	NS	NS	NS	NS	NS	NS	NS	NS	0,015 ↓	NS	<0,05 ↓
R^2	0,32	0,54	0,27	0,11	0,21	0,31	0,19	0,09	0,36	0,18	0,33

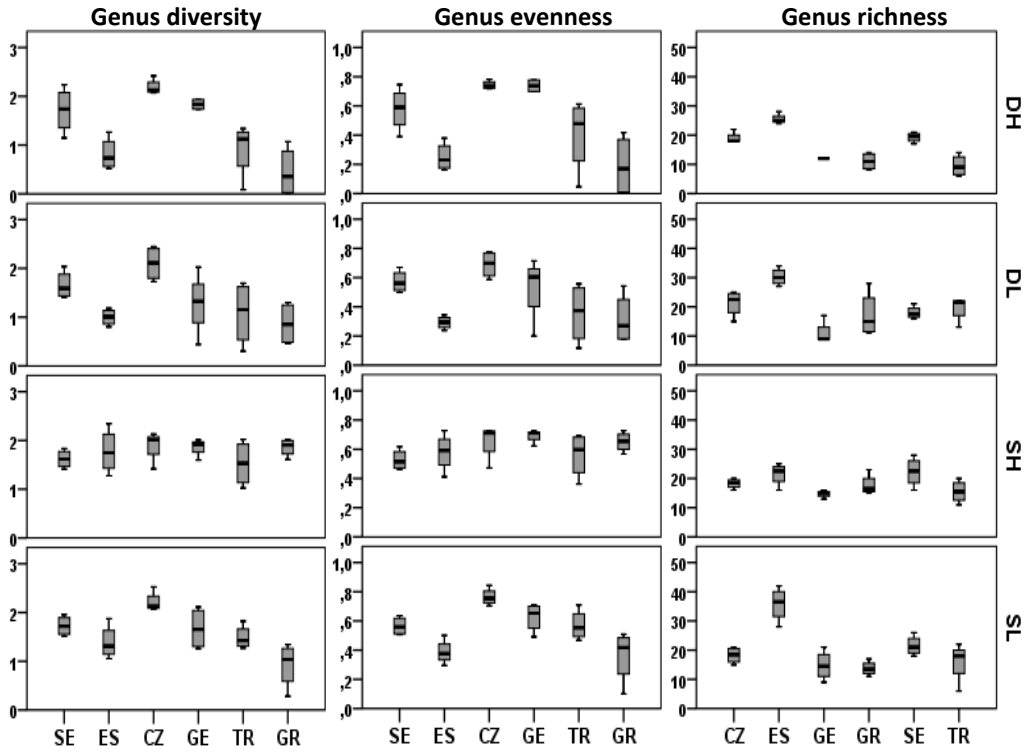

Figure 3.6 Genus diversity, evenness and richness results.

According to Morpho-Functional (MF) classification ANCOVA analysis results were significant for 7 MF groups (Table 3.4). Nutrient-depth-temperature interaction was significant ($p< 0.05$) for 2c (small Euglenophyta) group. While all functional group biovolume increased with tested variables only 2d (Cryptophyta) ($p< 0.05$) group biovolume was decreased with nutrient-temperature interaction ($p< 0.05$). Nutrient-temperature and nutrient-depth interactions were significant for 5b (large vacuolated Chroococcales) ($p< 0.05$) and 5d (Small colonies, Chroococcales) ($p< 0.05$) groups. Only temperature was significant ($p< 0.01$) for 6b (Large unicellular pennate) and 8a (unicellular Conjugatophytes/Chlorophytes) ($p< 0.001$) groups. Lastly nutrient-temperature was significant for 11a (Chlorococcales-Naked colonies) ($p< 0.05$) group.

Table 3.4 Analysis of covariance (ANCOVA) results for Morpho-functional classification (depth and nutrients: fixed factors; temperature: covariate).2c (Small Euglenophytes) ,2d (Cryptophytes), 5b (Large vacuolated Chroococcales), 5d (Small colonies, Chroococcales), 6b (Large colony forming pennate diatom), 8a (Large unicells—Unicellular Conjugatophytes/Chlorophytes), 11a (Chlorococcales — Naked colonies).

	Functional Group						
Treatment	**2c**	**2d**	**5b**	**5d**	**6b**	**8a**	**11a**
Nurtient	NS	<0,01 ↓	NS	NS	NS	NS	NS
Depth	NS	NS	NS	NS	NS	NS	NS
Temperature	<0,05 ↑	<0,05 ↓	<0,01 ↑	<0,01 ↑	<0,01 ↑	<0.001 ↑	<0.001 ↑
Nurtient*depth	<0,05 ↑	NS	NS	<0,05 ↑	NS	NS	NS
Nutrient*temp	NS	<0.05 ↓	<0,05 ↑	NS	NS	NS	0,05 ↑
Temp*Depth	NS	NS	NS	NS	NS	NS	NS
Nutrient*depth*temp	<0,05 ↑	NS	NS	NS	NS	NS	NS
R^2	0.20	0.31	0.19	0.24	0.15	0.34	0.42

3.4 Discussion

Our synchronized and standardized mesocosm experiment results revealed strong positive temperature and nutrient effect on total phytoplankton biovolume, however for different taxonomic and functional groups effect of tested treatments (nutrient, temperature, depth) showed high variability. Instead of singular nutrient temperature and depth effect, interactions between these variables were more pronounced for taxonomic and functional classification, and also for diversity and richness parameters (Tables 3.3 and 3.4).

Phytoplankton community compositions changed through the course of the experiment in all countries and total biovolume showed an increasing trend with temperature and nutrient, leading to the lowest and the highest phytoplankton biovolumes in the coldest (Sweden) and the warmest (Greece) countries, respectively. Our results suggested that the effect of temperature overrode the nutrient enrichment impact, since even low nutrient treatments in Greece had higher phytoplankton biovolumes compared to high nutrient treatments in

Sweden. Accordingly, the strong impact of temperature on phytoplankton biovolume increase has been shown by several studies (e.g. Elliott *et al*., 2006; Deng *et al*., 2014). Non nuisance phytoplankton groups like Chlorophyta, Cryptophyta and Chrysophyta were mostly dominated in Sweden and Czech Republic, however unexpectedly we found high cyanobacteria contribution in Estonia, which could be as a result of the comparatively high cyanobacteria biovolume of initial conditions (Figure 3.4). Furthermore, as expected we found relatively high cyanobacteria contribution in warmer countries especially in high nutrient treatments (Germany, Turkey and Greece). These results confirm our first and second hypothesis. This is also in accordance with several previous studies that revealed phytoplankton biovolume and especially cyanobacteria contribution increased with warmer temperature (Weyhenmeyer, 2001; Pearl, 2008; Wagner & Adrian, 2009). A study conducted by Kosten *et al*. (2012) also found a synergistic effect of both nutrient and temperature on phytoplankton biovolume increase in 143 South American lakes. However, unlike other low nutrient treatments, in two of the SL treatments in Germany phytoplankton biovolumes were high, with high cyanobacteria contribution, likely as a result of high TP concentrations in these mesocosms (Figure 3.5).

Only 7 out of 25 morpho-functional groups were significantly increased with at least one of the tested parameter. Different than taxonomic classification, only colonial small and large vacuolated cyanobacteria species were significantly increased with nutrient-depth and nutrient-temperature, respectively. Moreover, there were 3 other functional groups, being large (6b, 8a) or colonial (11a), which showed a significant increase with environmental parameters (Table 3.4). The reason for the increase of large or colonial species may be related to their grazing resistance. On the other hand, small euglenophyta species (2c) were also increased with nutrient-depth-temperature interaction, pointing to their mobility advantage, which allows them to cope with the changes in temperature nutrient and depth better than other groups (Bellinger, 2010).

We did not find any depth effect on neither total phytoplankton nor cyanobacteria biovolumes. Also our light measurements indicated that sufficient light penetrated till the sediment in all mesocosms, with the exception of most of the DH treatments (excluding Turkey and Germany DL treatment.) Stratified conditions, thus increasing/higher depths, promote cyanobacteria increase owing to their buoyancy capabilities (Diehl *et al*., 2002; Huisman *et al*., 2004; Salmaso, 2005). However, in our experiment water pumps were used in order to prevent stratification, possibly preventing the effect of depth in our experiment. Light limitation can also indirectly effect phytoplankton abundance by affecting macrophyte growth and except Turkey and Estonia macrophyte coverage was low in all DH treatments.

Even though, temperature and nutrient concentration were possibly the main factors controlling the composition and biovolume of phytoplankton species, excluding the mesocosms where macrophyte growth was high e.g. the SH treatments of Turkey, despite high temperature and nutrient concentrations, low phytoplankton biovolume was observed (Ersoy *et al*., in prep). As aquatic plants may have negative impacts on phytoplankton through allelopathic interactions and competition for nutrients (Körner & Nicklisch, 2002; Mulderij *et al*., 2005). This observation also confirms our third hypothesis. However, in another warm country, such as in Greece, high phytoplankton biovolume was observed in both nutrient treatments of shallow mesocosms. During the course of the experiment severe water level decrease was observed in their mesocosms, leading to turbid conditions and the die back of macrophytes early in the season (Ersoy *et al*., in prep) and possibly causing an increase in the phytoplankton biovolumes (Figure 3.2). These results suggest that presence of macrophytes at high nutrient availability due to water level drop may have a potential to suppress phytoplankton growth (Özkan *et al*., 2010; Bucak *et al*., 2012).

Large-sized zooplankton species (e.g. *Daphnia magna*) are the main grazers of phytoplankton species and therefore they can suppress phytoplankton abundance (Moss, 2010), while small bodied zooplankton are not efficient grazers (Jeppesen *et al*., 1999). In our experiment large bodied zooplankton species were observed in Sweden to Germany, however they were not found in Turkey and Greece (Tavşanoğlu *et al*., submitted) Therefore, absence of large bodied zooplankton and their grazing potential may be another reason favoring phytoplankton increase in Turkey and Greece.

Usually species diversity increase from pole to equator and high altitude to low altitude (Fischer 1960; Pianka 1966; Gaston 2000). Nevertheless, not all organisms follow the same pattern (Kindlmann, 2007). Phytoplankton diversity pattern studies revealed controversial results and thus the change in their diversity is still not fully understood neither in freshwater nor in ocean ecosystems (Muylaert *et al*., 2010; Barton, 2010; Huisman, 2010; Ptacnik, *et al*., 2008). In our experiment total number of identified phytoplankton genera was higher in northern countries, compared to southern ones, confirming our fourth hypothesis. We found that phytoplankton diversity and richness were negatively affected by temperature, nutrient and depth interactions in all treatments (except SH). According to competitive exclusion principle long term stable environmental conditions may cause diversity decrease due to long term growing season, being in accordance with the observations from our southern countries (Sommer *et al*., 1993; Naselli-Flores, 2005).

As a conclusion in our mesocosm experiment we found that temperature and nutrient are the main forces that positively affect phytoplankton biovolume increase and that Cyanobacteria abundance was mainly driven by nutrient-temperature interaction. Therefore, our results suggest that to diminish possible phytoplankton bloom formations as a consequence of climate change induced temperature increase, critical nutrient loading concentrations should be reduced in shallow lake ecosystems, especially in warm southern countries. We did not find a

direct depth effect on phytoplankton biovolume. On the other hand, phytoplankton diversity and richness patterns were not only dependent on temperature increase, but also on the interaction between nutrient-depth-temperature, which had a negative effect. However, similar to other studies (e.g. Kruk *et al*., 2009; Muylaert *et al*., 2010; McCauley *et al*., 1979) our results also pointed out that other biological parameters, like macrophyte abundance or selective zooplankton grazing likely causes variation in phytoplankton abundance and diversity, suggesting that microorganism diversity patterns were not only dependent on temperature increase or latitudinal gradient. Nevertheless, to better understand microorganism diversity patterns more detailed studies are needed.

CHAPTER 4

INVESTIGATING THE SUITABILITY OF PHYTOPLANKTON BASED WATER FRAMEWORK DIRECTIVE INDICES FOR TURKISH LAKES

4.1 Introduction

Freshwaters are crucial natural resources for living communities, since water is the major component of all living organisms and the medium in which all biochemical reactions take place. Of all the water in the world, only 2.5% is freshwater and only 0.02% of this amount comprises of rivers and lakes. Therefore, lakes and rivers are notably important components of the landscape for supporting human life, due to their usage as drinking water supply, and in agriculture, industry, fishing and recreational activities (Moss, 2010).

Lakes are highly sensitive to activities in their catchment areas. During the past decades intensified agricultural activities, animal farming, industrialization and urbanization have led to increased nutrient input especially phosphorous (P) and nitrogen (N) to lake ecosystems (Moss, 1998; Jeppesen *et al*., 2009). Consequently, eutrophication of lake ecosystems has become a serious world-wide problem (Smith, 2003). To achieve sustainable water management, European Union Water Framework Directive (WFD 2000/60/EC) was established in 2000 with the purpose of reaching at least "good ecological and chemical status" in all European waters (EC, 2000). Thereafter, member states (MS) have been responsible for determining current and original ecological status of the water bodies, based on Ecological Quality Ratios (EQR) calculated by using biological and chemical parameters (EC, 2000).

Directive includes phytoplankton as one of the five (macrophytes, macroinvertebrates, phytobenthos, fish) biological quality elements (BQE) (EC, 2000). Since phytoplankton are fast growing organisms and sensitive to environmental fluctuations, especially to nutrient loading, they are fast and early BQEs. Total phytoplankton biomass, species composition and bloom frequency-intensity are the main phytoplankton parameters used to determine the ecological status of the water bodies (Bellinger, 2010).

Chlorophyll-*a* (Chl-*a*) concentration is also mostly used as total phytoplankton biomass predictor (Heinonen 1980; OECD 1982; Hillbricht-Ilkowska and Kajak, 1986). Phytoplankton taxonomic indices are based on different requirements of the species, since some phytoplankton groups generally prefer oligotrophic conditions (desmids), while some others would become dominant under eutrophic conditions (cyanobacteria). Besides biomass and composition, excessive increase of a certain phytoplankton species is another way to make ecological quality predictions based on phytoplankton (Thunmark 1945; Nygaard 1949). This kind of excessive increase is known as phytoplankton bloom formation and usually cyanobacteria species cause massive increase under high nutrient and temperature conditions (Paerl *et al*., 2011). Since some bloom forming cyanobacteria species are harmful for human and other organisms, they also cause drinking and irrigational water shortage in many places around the world during the last decades (e.g. Lake Taihu, Lake Erie) (Wang *et al*., 2008; Smith *et al*., 2015). In order for each member state to develop metrics that are comparable to each other, intercalibration studies has been carried out among Geographical Intercalibration Groups (GIGs). However, due to the lack of sufficient data intercalibration could not be conducted for all the member states. Moreover, a common phytoplankton index named Plankton Taxonomic Index (PTI) was developed based on trophic scores of phytoplankton species from 1795 lakes, covering 20 European countries in the Water bodies in Europe: Integrative Systems to assess Ecological status and Recovery (WISER) EU FP7 project (Phillips *et al*., 2012).

Similar to European lakes, Turkish lakes are also under pressure of intensified human activities during the last decades (e.g. Girgin, 2000). Even though, Turkey has a highly mountainous landscape and cannot be characterized by a single climatic pattern, the predominant climate is semi-arid Mediterranean, with hot-dry summers and warm-wet winters (Peel *et al.*, 2007). Due to highly variable annual and inter-annual precipitation regimes water level fluctuations also have an impact on lake ecosystems (Naselli-Flores & Barone, 2005; Beklioğlu *et al*., 2007). According to climate change scenarios arid and semi-arid regions will become drier (IPCC, 2013; Bates *et al*., 2008) and through global warming 25-30% decrease in precipitation is predicted for Mediterranean region (Giorgi, 2006; Giorgi and Lionello, 2008). This amount of water reduction may exacerbate eutrophication symptoms and could cause water shortage for society (EEA Report No 2/2009). Additionally, during the dry years pumping water from lakes would also increase and intensify the effect of climate on lake ecosystems (Erol & Randhir, 2012). Besides these factors, Turkey is not a water rich country and according to expectations its population will reach to 84 million in 2023 (TUIK), causing an increase in water demand. Therefore, it is crucial for Turkey to take precautions in order to protect and improve the current freshwater resources. In this respect several twinning projects had been carried out between member states and Turkey. The first twinning project, MATRA, was supported by Dutch government between 2002 and 2004 and as a result a draft basin management plan was developed for Büyük Menderes River Basin. Furthermore, another project, EU-TWINNING (2008-2009), enabled the determination of river basins of Turkey. Following this, "Technical Assistance for Capacity Building on Water Quality Monitoring (TR2009/0327.02-02/001)" Project was completed in 2015 (Chapter 5). Within the scope of this project, reservoirs in Büyük Menderes River basin were sampled during spring and summer months and a multimetric phytoplankton quality indice, namely New Mediterranean Assessment System for Reservoirs Phytoplankton (NMASRP), was applied to determine the EQRs based on phytoplankton data (Hoyos *et al*., 2014).

Nutrient and temperature are generally known as main determinants of phytoplankton community structure and abundance. Semi-arid Mediterranean climate characterized by wet winters and arid summers, resulting with water level decreases and nutrient-up concentrations during summer months. In parallel with this nutrient increase phytoplankton community composition may also differ due to temperature difference among Central Baltic and Mediterranean countries. Since study sites in this current research mostly cover semi-arid Mediterranean climate, temperature effect on production and composition of phytoplankton may not be detected by Central Baltic indices. Even though, phytoplankton is the one of the main BQEs in WFD, national or intercalibrated phytoplankton quality indices are not available neither for Turkey nor for natural lakes of Mediterranean regions. In this study, 40 freshwater mostly shallow lakes from the north the south in the western part of Turkey were sampled. Five different indices including three from Central Baltic (CB), one Mediterranean reservoir index (NMASRP) and the intercalibration common metric index (PTI), that was developed in the WISER EU FP7 project were employed in order to investigate the applicability of these indices to Turkish lakes. We hypothesized that Mediterranean (NMASRP) and PTI indices should give more reliable results than Central Baltic ones as the former ones would be able to capture climatic differences.

4.2 Methods

4.2.1 Study sites and Field Sampling

Turkey is located between 36-42°N latitudes and 26-45°E longitudes and has a highly mountainous landscape. Study sites are located in the mid- to western part of Turkey between ca. 10 and 1400 m altitude, comprising hot/warm-summer Mediterranean, cold semi-arid and oceanic climatic zones (Köppen-Geiger climate classification; Peel *et al*., 2007).

In total 40 lakes were sampled between 2006 and 2012 (Figure 4.1), with half of the lakes being sampled in both spring (April) and summer (July-August) periods, while the rest only in summer period. Samples for various biological, chemical and physical parameters were retrieved following a snap-shot methodology (Kruk *et al.*, 2009; Kosten *et al.*, 2012). Water samples, covering the entire water column from the deepest points of each lake were gathered using a KC Denmark Ruttner sampler. Phytoplankton samples were obtained from these depth-integrated water samples and preserved in dark in 2% Lugol's solution. Water chemistry samples were stored frozen until the analysis of total phosphorus (TP), soluble reactive phosphorus (SRP) (Mackereth *et al.*, 1978), chlorophyll-*a* (Chl-*a*) (Jespersen and Christoffersen 1987) and total nitrogen (TN) (using a Scalar Auto-analyzer, San++ Automated Wet Chemistry Analyzer, Skalar Analytical, B.V. Breda, The Netherlands). Secchi depth was also measured from the deepest point by employing a white disc with 20 cm diameter and YSI 556 MPS multiprobe field meter (YSI Incorporated, OH, USA) was employed to measure the water temperature.

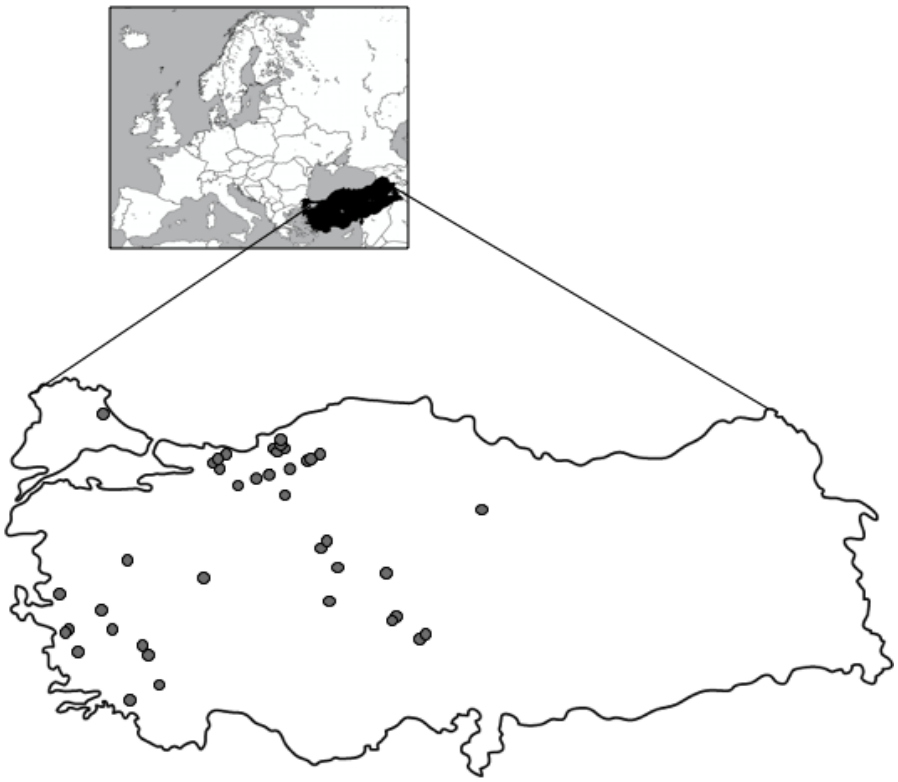

Figure 4.1 Location of the study sites.

4.2.2 Phytoplankton Identification and Counting

Phytoplankton samples were counted according to Utermöhl technique (1958). Prior to counting, to obtain a homogenous species distribution, samples were stirred by gently turning the sample bottles upside-down (100 times). Depending on the samples' abundance, different volumes were settled for 16-24 hours and subsequently the counting were conducted along horizontal transects under an inverted microscope (Leica DMI 4000B). Samples were counted until reached to 400 individuals of the most abundant species and organisms smaller than 2µm were not counted. Phytoplankton dimensions of at least 10 individuals from each species were measured by Leica image analysis program and mean measurements were used for biovolume calculations (Hillebrand *et al*., 1999; Wetzel, 2001).

Filamentous species counted as one unit, thus their biovolumes were calculated with unit size measurements. The identification of phytoplankton species was carried out by using reference books (Appendix-E Table 1). (Whitton *et al*., 2002; Prescott *et al*., 1973; Cox, 1996; Komarek, 1999; Popovski, 1990).

4.2.3 Lake Ecological Potential Evaluation

European Union, member states have been divided into geographical intercalibration groups (GIG) according to an ecoregion map adapted from Illies' (1967/1978). However, Turkey was not included to these processes and even though it can partly be included in the Mediterranean GIG, due to its diverse climatic characteristics and landscape structures, it could not be categorized under only one GIG. Furthermore, another important point is that intercalibration studies were completed only for Mediterranean reservoirs, but not for natural lakes. Therefore, in this study various indices, developed for the natural lakes of Central/Baltic (CB) GIG (*Danish Lake Phytoplankton Index-DLPI, Netherlands Phytoplankton Indice and Phytoplankton Metrics for Polish Lakes-PMPL*) and for the reservoirs in Mediterranean GIG (*Mediterranean Reservoir intercalibration-NMASRP*), were applied to our data set. CB-GIG was chosen since it covers a wider climatic region and has similar typologies to Turkey compared to other GIG. Moreover, Phytoplankton Trophic Index (PTI), that was developed as a common metric for CB and Northern GIG, based on 1795 lakes from 20 countries located in Europe, was also applied to our data set.

4.2.4 Typology and Reference Sites

Central-Baltic typology determination were based on lake altitude, alkalinity and depth (Table 4.1). According to alkalinity and depth parameters our dataset comprises of 9 sites belonging to LCB-1 type and 31 sites belonging to LCB-2 type. For PTI index, typology includes alkalinity and depth parameters and class boundary values were the same with CB boundary values (Philips *et al.,* 2012). Thus same typology classification results were applied to PTI indice.

Table 4.1 Description of Central Baltic GIG LCB-1 and LCB-2 water body types.

Determinant	**Unit**	**Value classes**	**Boundary**
Altitude	m	low	<200
Alkalinity	$meqL^{-1}$	calcareous	>1
Mean depth	m	shallow	<3
		very shallow	3-15
Residence time	year	shallow	1-10
		very shallow	0.1-1.0

Mediterranean GIG typology classification system uses data on mean depth, alkalinity, altitude and catchment area (Table 4.2). Since Mediterranean calibration studies carried out only for reservoirs, depth parameter was not taken into account. Considering alkalinity, our data set comprises mostly high alkaline lakes, therefore study lakes fell only for LM-8 type. However, lakes were divided into two categories according to their mean depth same as CB typology thus comparison between CB and Mediterranean would be possible.

Table 4.2 Description of Mediterranean GIG LCB-1 and LCB-2 water body types.

Type	Lake characterization	Altitude (m)	Annual mean precipitation (mm) and T (°C)	Mean depth (m)	Area (km^2)	Catchment (km^2)	Alkalinity (meq/l)
L-M5/7	Reservoirs, deep, large, siliceous "wet" areas	< 1000	> 800 and/or<15	> 15	0.5-50	< 20.000	< 1
L-M8	Reservoirs, deep, large, calcareous	< 1000	–	> 15	0.5-50	< 20.000	> 1

For each typology, reference lakes should also be defined to be able to understand the difference between natural conditions and current ecological status of the lakes. These reference sites are determined according to long term historical data, modelling or expert judgement (Hoyos *et al*., 2014). In the current study, criteria for reference lakes were determined based on total phosphorous (TP<12 μgL^{-1}), percent area of artificial land use (ALU, 1 %), total percentage of agricultural land use (IA, 10 %) and natural-semi natural land use percentage (NASN-20%) (Hoyos *et al*., 2014). None of our study site achieved to pass reference site threshold criterions Thus we choose closest two lakes (LCB-1 (Lake Abant) and LCB-2 (Lake Poyrazlar) as a control sites and expected these lakes were in high or good condition.

4.2.5 Indice Calculations

Phytoplankton quality indices include phytoplankton biomass, taxonomic composition, bloom frequency and intensity parameters. Phytoplankton counting by each member country was carried out according to the Utermöhl technique, nevertheless, phytoplankton sampling period and frequency differed for each country varied from late summer to growing season. In the current study peak growing season or summer/spring (one sample from each season) average

phytoplankton data was used. Phytoplankton bloom frequency and intensity parameters were not included. In the current study five different indices given in Table 4.3 were applied to our data set.

Table 4.3 Indice details.

Indice	Abundance Metric	Taxonomy metric
DLPI	Chl-*a*	Nutrient poor/rich indicator species
NETHERLANDS	Chl-*a*	Bloom forming species, cyanobacteria %, Chrysophyta %
PMPL	Chl-*a*, total biovolume	Cyanobacteria total biomass
NMASRP	Chl-*a*, total biovolume	IGA, cyanobacteria biovolume
PTI	Chl-*a*	Indicator species

4.2.5.1 Denmark: Danish lake phytoplankton Index (DLPI)

This index comprises both abundance and composition metrics.

Abundance: Calculations were carried out based on summer mean phytoplankton data (1 May - 31 September). Phytoplankton abundance metrics were calculated using;

- Chl-*a*
- cyanobacteria %
- Chrysophyta %

Taxonomic composition: Phytoplankton compositional metric includes indicator species for nutrient rich and nutrient poor conditions (Appendix-C Table 1). Metric is based on number of species indicating nutrient poor conditions minus the number of species indicating nutrient rich conditions.

Final index value, which was calculated according to Appendix-C Table 2, could be between 0 and 12. While 0 represent bad ecological condition, 12 means highest ecological quality. These index values were converted to EQR that ranges between 1-0 by using Appendix-C Table 3.

4.2.5.2 Netherlands Phytoplankton Indice

Abundance metric: was calculated using average Chl-*a* concentration measured during growing season (1 April -1 October). Chl-*a* class boundaries were given in Appendix-C Table 4 for both LCB1 and LCB2 types.

Bloom Metric: The indice does not consider taxonomic composition it only uses bloom abundance metric which is derived from the abundance of bloom forming species (e.g. *Planktothrix agardhii*, *Scenedesmus*, *Anabaena*, *Botryococcus*, *Dinobryon* and *Peridinium)* as filament, colony or cell number. For each bloom type specific EQR value was assigned ranging from 0.1 to 0.7 depending on the species relation to eutrophication. If more than one bloom is observed in a lake, the lowest EQR value is decisive (Phillips *et al*., 2014).

4.2.5.3 Phytoplankton Metrics for Polish Lakes (PMPL)

PMPL includes concentration of Chl-*a*, total phytoplankton and cyanobacteria biomasses.

In the current study, samplings were only conducted during the March and September, though requirement of this metric was to have samples from the four seasons (spring water mixing, early summer, late summer, and autumn). PMPL also divides the lakes into two subclasses considering the ratio of the lake volume to catchment area (VQ, Schindler`s ratio) and mixing regimes (polimictic and stratified). Due to lack of data on the catchment area for 7 lakes, PMPL index was calculated only for 33 lakes and the lakes were divided into two, being polimictic (LCB2) and stratified (LCB1). Final index value ranges between 0 and 5, indicating the best and worst status, respectively.

Chlorophyll-a Metric: Spectrophotometric Chl-*a* concentration measurement was used to determine the ecological class boundaries, which were calculated by using the equations given below (Appendix-C Table 5):

$$\text{Stratified lakes; } Y_{Chl-a} = k + z * Chl\text{-}a_{obs} + m * \ln(Chl\text{-}a_{obs})$$

$$\text{Polimictic lakes; } Y_{Chl-a} = k + z * Chl\text{-}a_{obs} + m * Chl\text{-}a_{obs} * \ln(Chl\text{-}a_{obs})$$

Where, $Y_{Chl\text{-}a}$ is Chl-*a* metric, and k, z and m are coefficients, which are specific for each lake type (Appendix-C Table 6).

Total Biomass Metric: Total phytoplankton biomass was used as a second metric by Polish system. Class boundaries were given in Appendix-C Table 7. This metric is calculated by using the equation given below:

$$\text{Stratified lakes; } Y_{Bm} = k + m * \ln(B_{obs})$$

$$\text{Polimictic lakes; } Y_{Bm} = k + m * \ln(B_{obs}) + z * B_{obs} + o\sqrt{B_{obs}}$$

Where,

Y_{Bm} is total biomass, and k, z and m are coefficients, which are specific for each lake type (Appendix-C Table 8).

Cyanobacteria Metric: Calculation of this indice requires total cyanobacteria biomass. Class boundaries were determined using 133 lake-years of data from Polish lakes. Ecological quality boundaries were determined using cyanobacteria and total phosphorous correlation. Class boundary values were given in Appendix-C Table 9. Metric values were determined between 5 and 0, therefore for further PMPL calculations values higher than 5 was truncated to 5 and values smaller than 0 to 0. The metric was calculated by using the equation given below:

$$\text{Stratified lakes; } Y_{Cy} = m * \ln\left[\frac{B_{cyobs} + B_{cyobs} * \left(B_{cyobs}/B_{phobs}\right)}{2}\right] + k$$

$$\text{Polimictic lakes; } Y_{Cy} = m * \ln B_{Cy} + k$$

Where,

Y_{Cy} is the Cyanobacteria metric, and k, and m are coefficients, which are specific for each lake type. B_{cyobs} is the mean biovolume of Cyanobacteria and B_{phobs} is the mean total phytoplankton biovolume (Appendix-C Table 10).

Calculation of PMPL index and EQR: Average of three phytoplankton metrics should be taken into account to calculate the final PMPL indice according to the following equations;

$$\text{Stratified lakes; } PMPL = \left[Y_{Ch} + Y_{Bm} + Y_{Cy}\right]/3$$

$$\text{Polimictic lakes; } PMPL = \left[Y_{Ch} + Y_{Bm} + \left(0.5 * Y_{Cy}\right)\right]/2.5$$

PMPL indice should be transformed to normalized EQR by using the following equation:

$$y = -0.2 * PMPL + 1$$

After the normalization PMPL values represent the EQR class boundary values (Appendix-C Table 11).

4.2.5.4 New Mediterranean Assessment System for Reservoir's Phytoplankton (NMASRP)

NMASRP was developed during the second Mediterranean region intercalibration studies and applied to the reservoirs located in Cyprus and Portugal. To be able to apply NMASRP indice, water samples for phytoplankton should be retrieved three times during the growing season. Being a multimetric index, it includes four metrics and all the metrics have equal weights;

Biomass	• Chl a (μg/L^{-1}) • Biovolume (mm^3/L^{-1})
Composition	• IGA index • BV of cyanobacteria(mm^3/L^{-1})

Intercalibration class boundary and reference site values were applied to our data set and calculation details were given in Chapter 5.

4.2.5.5 Plankton trophic ındex (PTI)

PTI index was developed during WISER EU FP7 project, by employing a large phytoplankton data set, which was derived from 1795 lakes, covering 20 countries in Europe. The objective of developing this PTI index was to create a pan-European phytoplankton index which can be used as a common metric for all geographic intercalibration groups and for countries that still does not have any national phytoplankton indices. For each phytoplankton species an optimum value (s_j) was derived from a canonical correspondence analysis along the eutrophication gradient and PTI index was calculated using the equations below (Philips *et al*., 2012);

$$PTI = \frac{\sum_{j=1}^{n} a_j s_j}{\sum_{j=1}^{n} a_j}$$

Where; a_j is proportion of j^{th} taxon in the sample and s_j is the optimum of the taxon in the sample.

$$EQR_{PTI} = \frac{PTI_{Obs} - PTI_{Max}}{PTI_{Ref} - PTI_{Max}}$$

Where; PTI_{Obs} is the mean sample PTI for each lake year, PTI_{Max} is the maximum PTI score for each type, PTI_{Ref} is the reference site PTI score.

PTI index also includes Chl-*a* as a phytoplankton biomass metric and EQR ratio was calculated as followed;

$$EQR_{Chl} = \frac{Chl_{\square ref}}{Chl}$$

Where; Chl-*a* is the observed mean Chl-*a* for the growing season (March–October) and Chl_{Ref} is the reference chlorophyll-*a* (Poikane *et al*., 2010).

4.3 Results

Lakes were classified as LCB-1 (mean depth 3-15 m) and LCB-2 (mean depth < 3 m), according to Central-Baltic typology criteria, comprising 9 and 31 lakes, respectively (Table 4.4). Only 9 of the LCB-2 type lakes were located at low altitudes (<200 m) and all LCB-1 type lakes were located at high altitudes (>200 m). Median of the alkalinity values were 10.9 for LCB-1 type and 11.9 for the LCB-2 type lakes. (Table 5.4). Trophic status of the study sites represented high variability from hypertrophic to oligotrophic (Carlson, 1977). TP concentrations were between ca. 15-61 and 18-633 μgL^{-1} and TN concentrations were between 264-1177 and 239-2476 μgL^{-1} for LCB-1 and LCB-2 types, respectively. While the highest Secchi depth was 9.0 m for the clearest lake, lowest Secchi depth was 0.2 m (Table 4.4).

Table 4.4 Minimum-Maximum and Median (in brackets) values of environmental and biological parameters measured.

Variable	Unit	LCB1 (n=9)	LCB2(n=31)
Maximum Depth	m	3.8 - 17.4 (7.3)	0.95 - 7.6 (3)
Secchi Depth	m	1.4 -9 (2.2)	0.2 - 2.5 (0.8)
Altitude	m	1328 - 785 (1216)	0-1423 (970)
Alkalinity	$meqL^{-1}$	10.9 - 0.9 (2.6)	11.9 -0.5 (3)
TP	μgL^{-1}	15.0 - 61 (37)	18.4 - 633 (116)
TN	μgL^{-1}	264 - 1177 (543)	239 - 2476 (1132)
SRP	μgL^{-1}	5.6 - 18 (4.3)	4.3 - 160.4 (25.1)
Salinity	gL^{-1}	0.1 - 0.3 (0.1)	0,1 - 6 (0.2)
Total phytoplankton biovolume	mm^3L^{-1}	0.2 - 7.7 (1.32)	0.01 - 79.2 (5.8)
Chl-*a*	μgL^{-1}	1.8 - 77.3 (6)	0.4 - 95.1 (16.3)

4.3.1 Water Quality Classifications

4.3.1.1 Denmark: Danish Lake Phytoplankton Index (DLPI)

According to DLPI index, EQR values varied between 0.7 and 0.2 for both of the lake types, respectively and represented an ecological classification of being good (n=9), moderate (n=20) and poor (n=9). Furthermore, reference sites for both typologies were classified as in moderate and good conditions, respectively. Only EQR-TN (R^2 = 0.57) correlation was negatively significant for deeper (LCB-1) lakes. For LCB-2 type lakes, however, trophic gradient-EQR relations were generally good and negatively correlated (except Secchi depth). The relationship was the strongest for TP (R^2 = 0.32; p = 0.001), followed by TN (R^2 = 0.28, p = 0.05), Secchi depth (R^2 = 0.25, p=0.01), SRP (R^2 = 22, p = 0.05) and it was the weakest for temperature (R^2 = 0.1) (Figure 4.3).

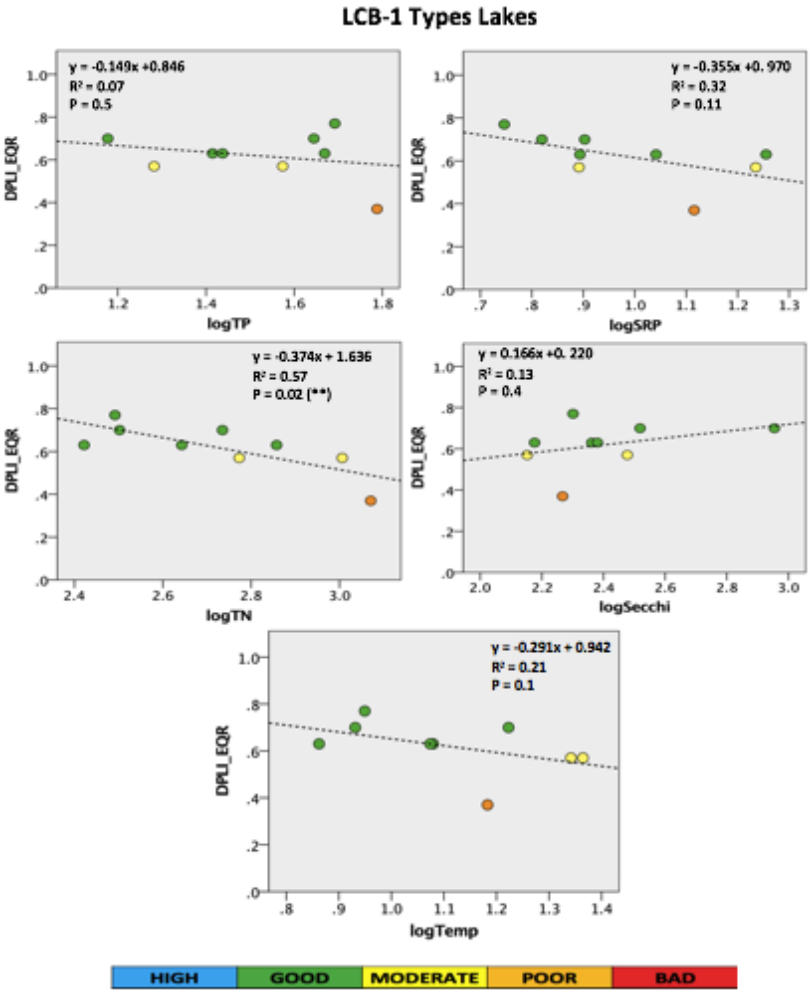

Figure 4.2 EQR based on DLPI versus TP, SRP, TN, Secchi Depth and temperature gradients for deeper LCB-1 type lakes.

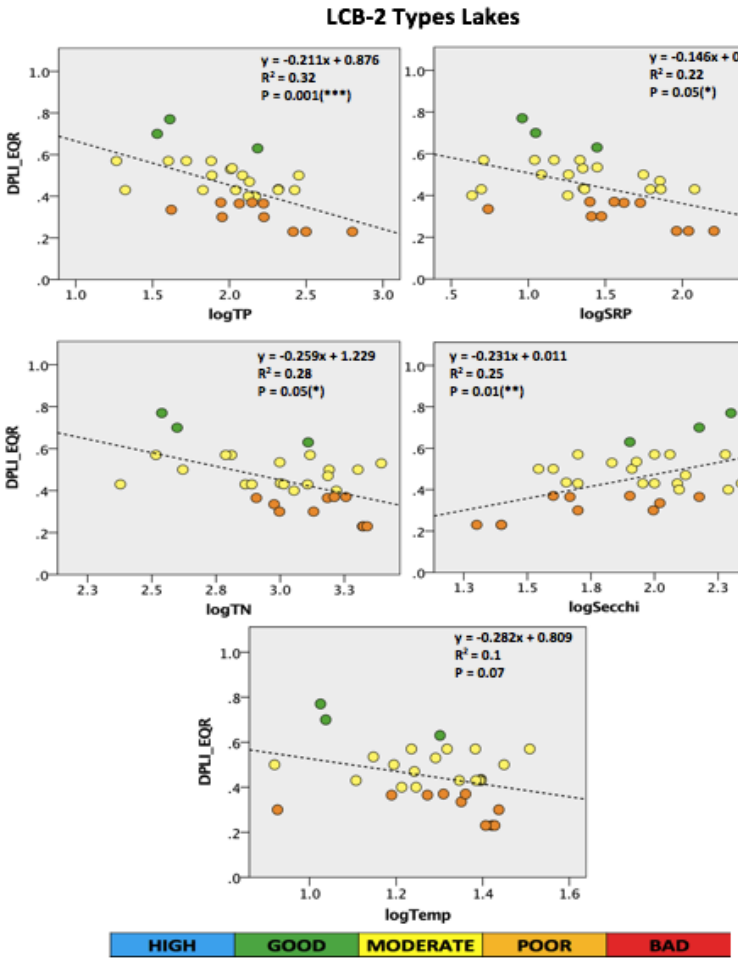

Figure 4.3 EQR base on DPLI versus TP, SRP, TN, Secchi Depth and Temperature gradients for shallower LCB-2 type lakes.

4.3.1.2 Netherlands Phytoplankton Indice

The EQR values varied between 0.20 and 0.85 for both lake types. Lakes were classified as high (n=2), good (n=13), moderate (n=14) and poor (n=11) conditions. Reference sites were grouped in high and moderate classes. Trophic gradient-EQR relation for LCB-1 lakes were not significant however, it was strongest for TN (R^2 = 0.23) and weakest for SRP (R^2 = 0.13) (Figure 4.4). For the LCB-2 type lakes TP (R^2 = 0.42), TN (R^2 = 0.46) and SRP (R^2 = 0.28) correlations were negative and significant. Moreover, the correlation of EQR values with Secchi depth was positive and significant, while with temperature no significant relation was found (Figure 4.5).

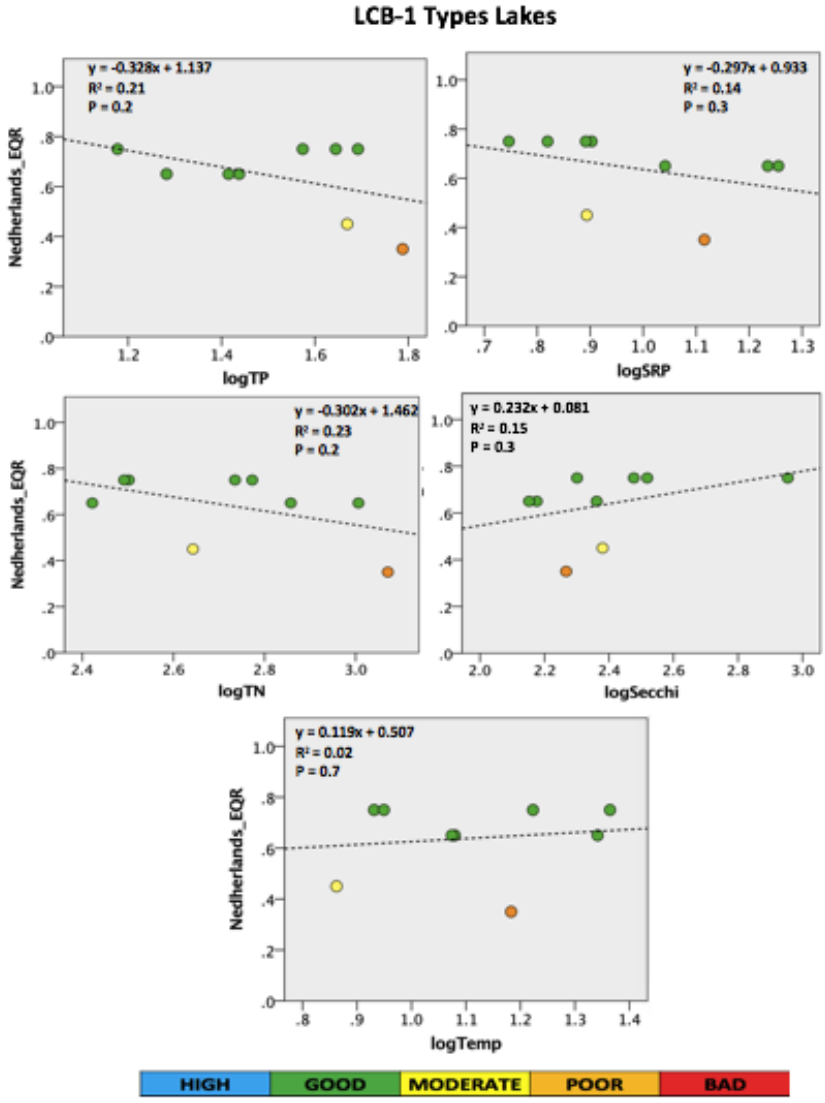

Figure 4.4 EQR based on Netherlands versus TP, SRP, TN, Secchi Depth and Temperature gradients for deeper LCB-1 type lakes.

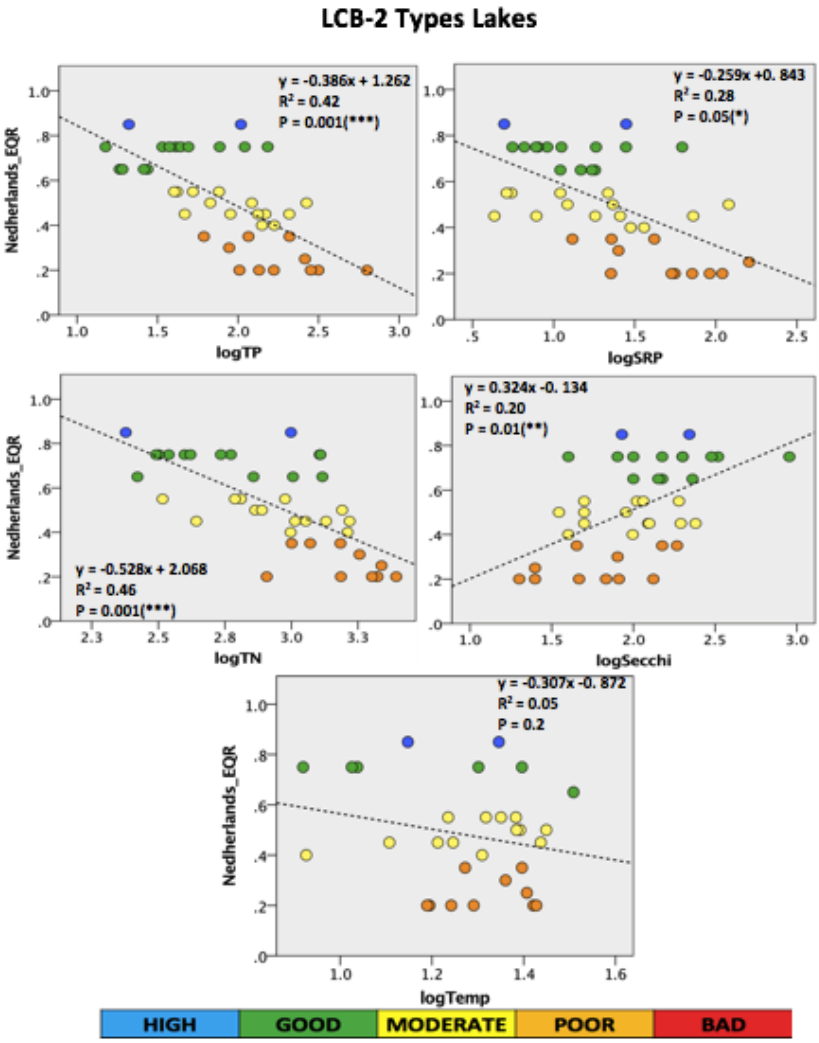

Figure 4.5 EQR based Netherlands versus TP, SRP, TN, Secchi Depth and Temperature gradients for shallower LCB-2 type lakes.

4.3.1.3 Phytoplankton Metrics For Polish Lakes (PMPL)

PMPL EQR values ranged between 0.90 and 0.06. While most of the lakes were classified as being high (n=15), rest of the lakes were classified in good (n=9), moderate (n=8) and bad (n=1) quality classes. Both of the reference sites were classified in high quality condition. The results indicated a low correlation between trophic parameters and none of them was statistically significant for LCB-1 type lakes. On the other hand, for LCB-2 type lakes, all the environmental parameters, except temperature, were significantly correlated with EQR values.

While the correlation of EQR values with TP (R^2 = 0.28), TN (R^2 = 0.56) and SRP (R^2 = 0.12) was negative, with Secchi depth there was a positively significant correlation (Figure 4.7).

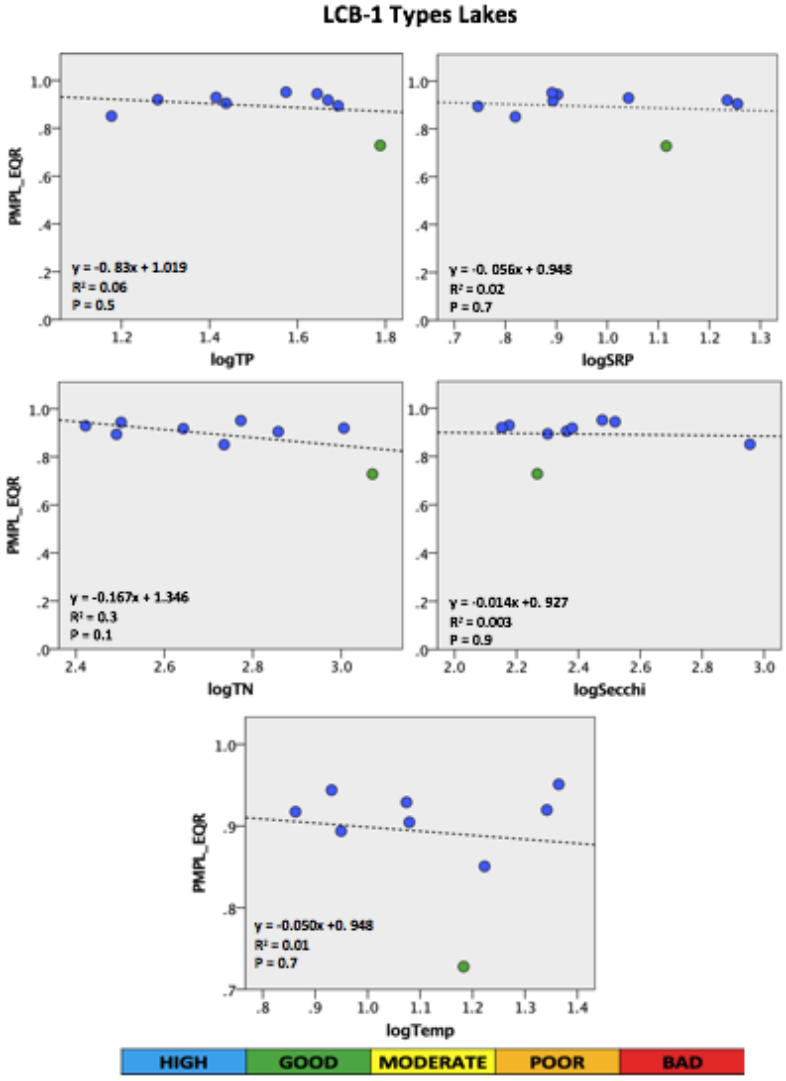

Figure 4.6 EQR based on PMPL versus TP, SRP, TN, Secchi Depth and Temperature gradients for deeper LCB-1 type lakes.

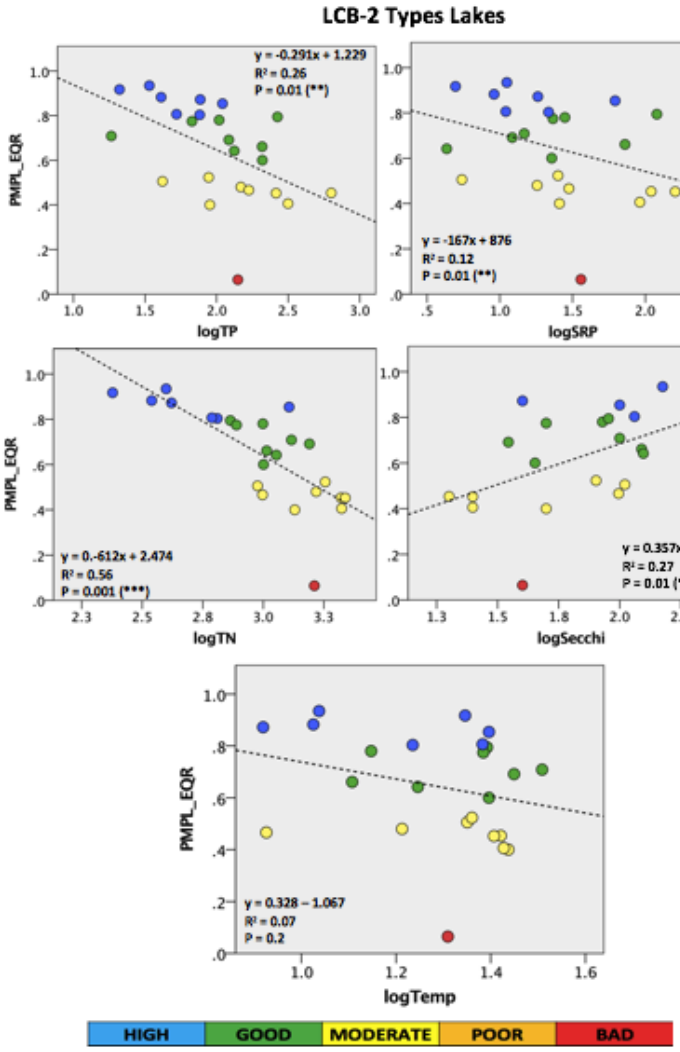

Figure 4.7 EQR based PMPL versus TP, SRP, TN, Secchi Depth and Temperature gradients for shallower LCB- 2 type lakes.

4.3.1.4 New Mediterranean Assessment System For Reservoir's Phytoplankton (NMASRP)

According to NMASRP index calculation results, EQR values changed between 0.7 and 0.1. Most of the lakes were classified as being in moderate condition (n=18), while good, poor and bad classes was represented with 6, 12 and 4 lakes, respectively. Moreover, reference sites were grouped as moderate and good conditions. For LCB-1 type lakes, only TP (R^2 = 0.45) and for LCB-2 types TN (R^2 = 0.20) and temperature (R^2 = 0.17) had significant negative correlation with EQR-values. Moreover, Secchi depth EQR correlation (R^2 = 0.18) was also positively significant for LCB-2 type lakes.

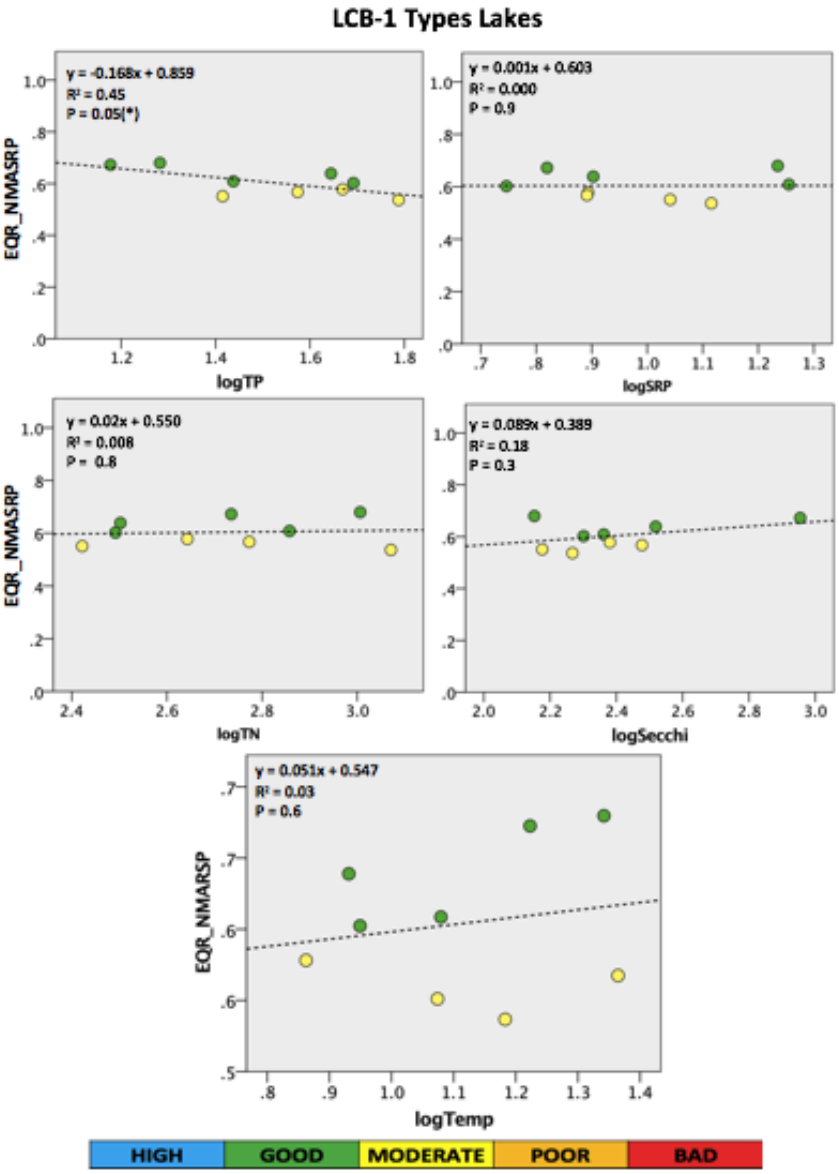

Figure 4.8 EQR based on NMASRP versus TP, SRP, TN, Secchi Depth and Temperature gradients for shallower LCB-1 type lakes.

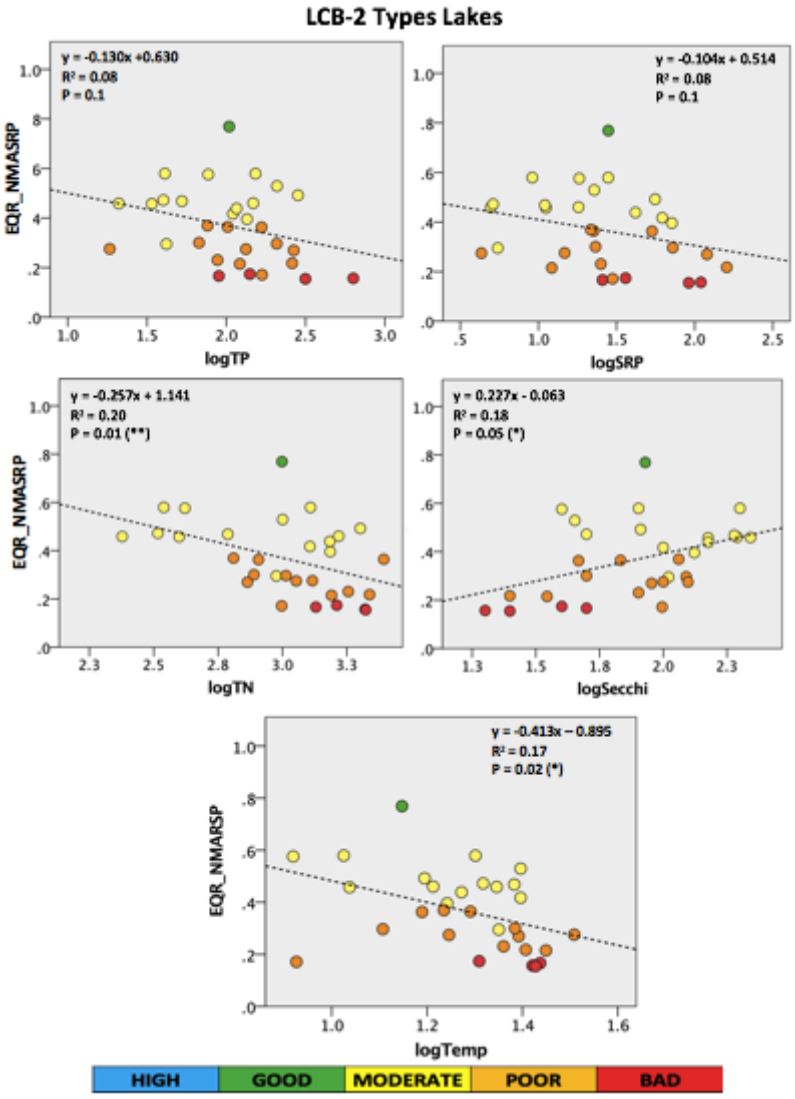

Figure 4.9 EQR based on NMASRP versus TP, SRP, TN, Secchi Depth and Temperature gradients for shallower LCB-2 type lakes.

4.3.1.5 Plankton Trophic Index (PTI)

The range of calculated EQR values were between 1 and 0. Unlike the results of other indices According to PTI results lakes were classified as in bad (n=10), poor(n=10), moderate(n=16), good (n=2) and high status (n=2) status Moreover, both of the reference lakes were classified as being in good condition. For none of the LCB-1 lakes EQR values-environmental variables correlation was significant (Figure 4.10). For LCB-2 types the correlation of EQR values with TP (R^2 = 0.16)

and temperature (R^2 = 0.19) was negatively significant, while it had a significant positive relation with Secchi depth (R^2 = 0.31) (Figure 4.11).

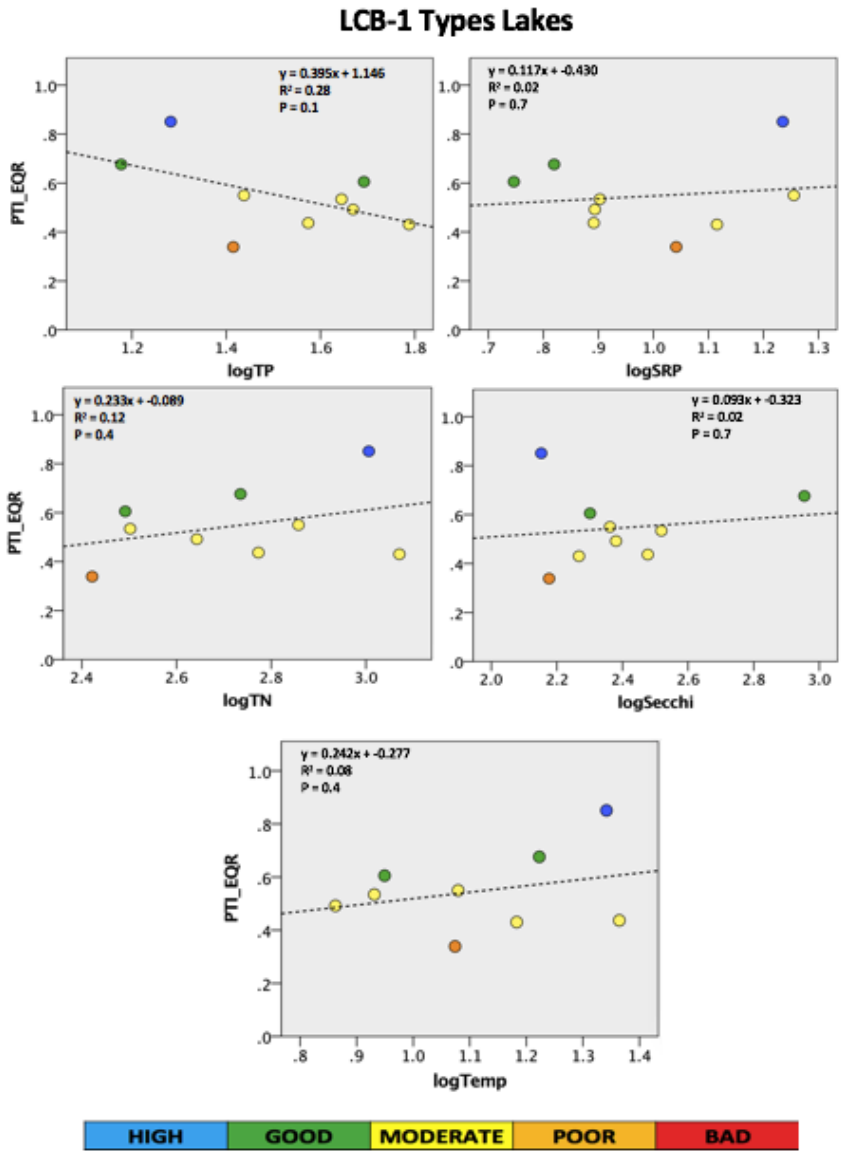

Figure 4.10 EQR based on PTI versus TP, SRP, TN, Secchi Depth and Temperature gradients for shallower LCB-1 type lakes.

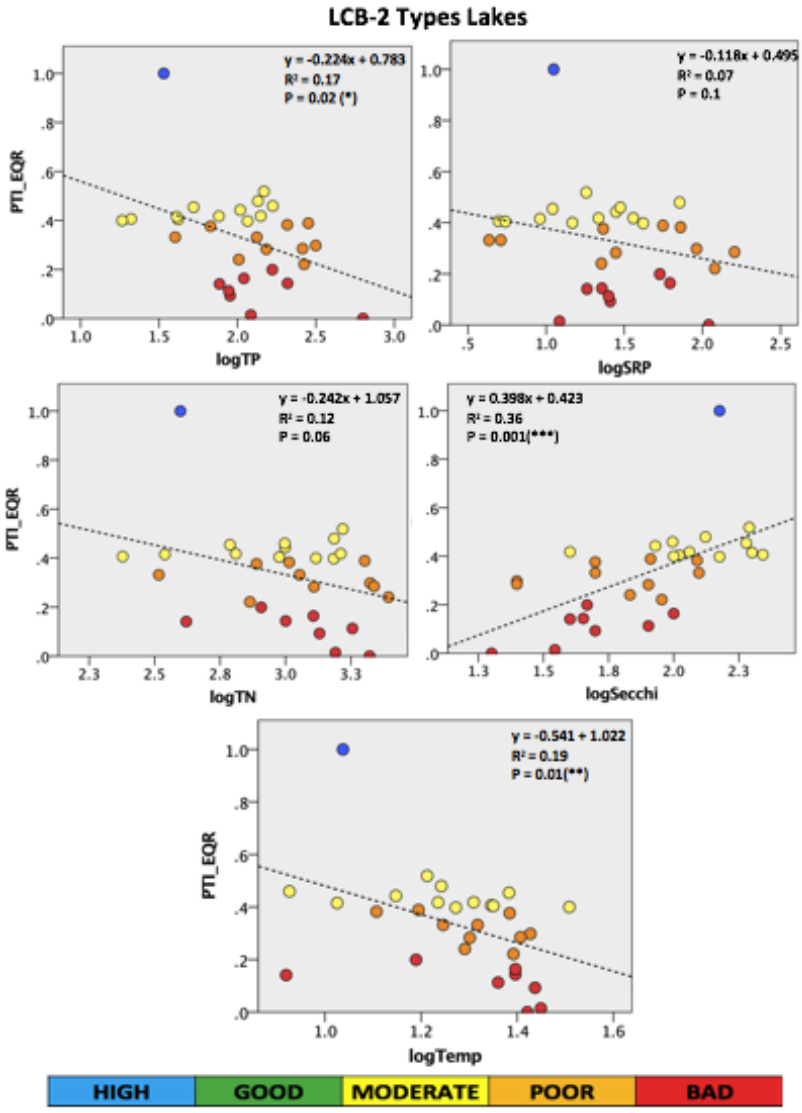

Figure 4.11 EQR based on PTI versus TP, SRP, TN, Secchi Depth and Temperature gradients for shallower LCB-2 type lakes.

4.3.1.6 Indice Comparison

According to the WFD-Annex-V, specifically total phytoplankton biomass, species composition and bloom frequency-intensity parameters should be considered as main phytoplankton BQEs for the lake ecosystems (EC, 2000). However, all the applied indices do not include bloom frequency metrics (Phillips *et al*., 2014). All the indices included abundance metrics and 4 out of 5 indices (except PMPL index) comprised taxonomic metric based on indicator species (Table 4.3; Figure 4.12). Ecological classifications based on the resultant EQR values indicated that, reference lakes (Abant and Poyrazlar with a TP of 15 and 21 μgL^{-1}, respectively) was grouped between high and moderate classes, and highly eutrophic lakes (Lakes Küçük-Akgöl and Azap with a TP of 633 and 316 μgL^{-1}, respectively) were classified from moderate to bad conditions. Even though with most of the indices hypereutrophic lakes (e.g. Lakes Küçük-Akgöl, Azap) were classified as being in poor-bad condition, PMPL index, which not include indicator species metric, as it only employs total cyanobacteria biomass, classified them as having a moderate quality. Moreover, PMPL index also classified 15 lakes as being in high quality classes. The correlations between EQR values and environmental variables were not consistently high for a certain index (Table 4.5). EQR-temperature had a significant negative relation according to NMASRP and PTI index calculations, while the best correlation between EQR and TP was observed for NMASRP index.

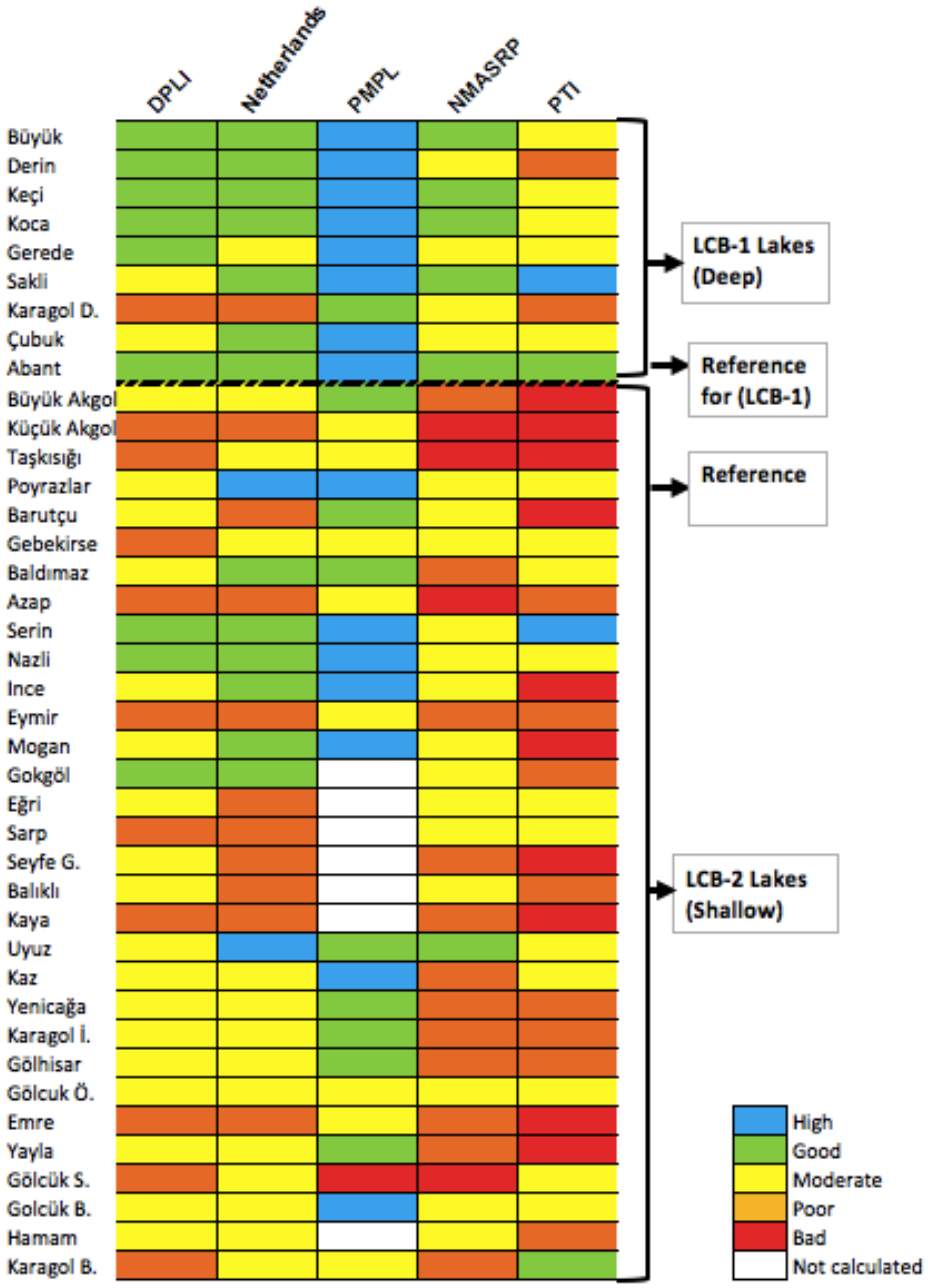

Figure 4.12 Comparison of lake ecological classifications calculated according to the indices developed by Denmark (DLPI, Netherlands, Poland (PMPL), Mediterranean (NMASRP) and PTI.

Table 4.5 Comparison table of the calculated indices, showing the number of the lakes used for each index and R^2 values indicating the relation between EQRs and trophic variables.

INDICES	Number of Lakes		TP (μgL^{-1})		SRP (μgL^{-1})		TN (μgL^{-1})		Secchi Depth (m)		Temperature °C	
	LCB-1	LCB-2	LCB-1	LCB-2	LCB-1	LCB-2	LCB-1	LCB-2	LCB-1	LCB-2	LCB-1	LCB-2
DLPI	9	31	0.07	0.32 ***	0.32	0.22*	0.57 **	0.28*	0.13	0.25**	0.21	0.1
Netherlands	9	31	0.21	0.42 ***	0.14	0.28*	0.23 **	0.46***	0.15	0.20**	0.02	0.05
PMPL	9	24	0.06	0.26**	0.02	0.12**	0.3	0.56***	0.003	0.27**	0.01	0.07
NMASRP	9	31	0.45*	0.08*	0.00	0.08	0.008	0.20	0.18	0.18***	0.03	0.17*
PTI	9	31	0.28	0.17	0.02	0.07	0.12	0.12**	0.02	0.36*	0.08	0.01**

Coefficients are donated as follows: <0.05*; <0.01**, <0.001***

4.4 Discussion

Prediction of lake ecological status based on phytoplankton data has been widely used, however as demonstrated in the present study, applying different national indices may lead to a decision that exhibits high uncertainty.

4.4.1 Biomass Metrics

All the countries applied concentration of Chl-*a* as a main biomass parameter, however NMASRP and PMPL indices also comprise total phytoplankton biovolume as biomass parameter (Table 4.1). However, EQR class boundary values for Chl-*a* concentration were different for each indice. Although Chl-*a* is generally accepted as a main proxy for nutrient increase, especially as an indicator of TP pressure, nutrient is not the only parameter that has an impact on Chl-*a* amount, since physical parameters like temperature and light intensity may also affect its concentrations (Dillon and Rigler, 1974; Kasprzak *et al.*, 2008).

According to Mazumder *et al.* (1998) for the same amount of TP, Chl-*a* production is higher in subtropical small herbivore (SH) or temperate SH lakes than temperate large herbivore lakes. Thus class boundary values, which were determined by using Chl-*a*, may not give a trustworthy result for Turkish lakes, which have warmer temperatures compared to the other countries that originally developed the indices. Moreover, Turkey was not included in the intercalibration studies, which were carried out to cope with the differences in climatic regions in each GIG. Ecological class boundary values also revealed high variability among different indices and GIG regions (Carvalho *et al.,* 2008). These differences may lead to misclassification of study sites and could be the one reason for high variability of our ecological classification results. Moreover, trophic interactions like grazing pressure on phytoplankton also show differences between temperate and warm climates, since in the former the dominant species are large size zooplankton and piscivorous fish, while in the latter small size zooplankton and omnivorous fish (Jeppesen *et al.,* 2010; Meerhoff *et al.,* 2012). Therefore, this variability in grazing pressure on phytoplankton among different climatic regions could also affect ecological status of lake ecosystems (Beklioğlu, *et al.,* 2008; Jeppesen *et al.,* 2009; Özen *et al.,* 2010).

4.4.2 Taxonomy Metrics

Taxonomic composition indices are mostly based on indicator species (except cyanobacteria % and Chrysophyta %), which are determined usually by using the information gathered from long-term monitoring data (e.g. 691 lake-years for DPLI indice). Distribution patterns of microorganisms is generally explained with the Baas-Becking (1934)’s hypothesis, stating that 'everything is everywhere: but the environment selects”. However, geographical distribution pattern of most phytoplankton species are still poorly known (Kristiansen, 1996). Since microorganisms have no dispersal limitations due to their small size and dormancy ability, environmental filtering is generally accepted as the main

determinant of phytoplankton spatial distribution (Foissner, 2006). One of the most important environmental factor shaping phytoplankton distribution is temperature (Bellinger, 2010). For example, some phytoplankton species, like warm-water species *Aulacoseira granulate*, are considered as pan-tropical since their distribution depends on temperature regimes (Foissner, 2006).

Correlation between EQR values and temperature was significantly negative for all the indices, except Netherlands, which indicated only a weak correlation with low R^2 values (Figure 4.12, Table 4.3). Countries that developed most of the applied indices calculated in the current study cover a relatively small climatic range, possibly preventing the EQR values to adequately represent the temperature effect.

Abundance of the species is also an important factor to determine the indicator species. For example, in DLPI taxonomic metric one species were determined as indicator if it was recorded at least 100 times during their long-term monitoring. One of these poor nutrient indicator species in DLPI metric is *Stichococcus* sp., nevertheless, this genus does not have a wide distribution in Turkey. Therefore, indicator species determined based on restricted environmental conditions in other countries may not reflect the same ecological classes in different climatic regions. Since PTI metric, which employs species specific optimum values for EQR-calculation, covers a large data set (1795 lakes, covering 20 European countries), we assumed that the ecological classification of our lakes based on this metric should give more reliable results. However, the classification of our reference sites indicated a high variability classifying the lakes between high and moderate conditions. Moreover, most of the lakes were grouped in bad, poor and moderate classes according to PTI index results. The data set that used to develop PTI species optimum values were mostly covering Central Baltic and Northern European countries, thus these defined optimum values may not reflect the ecological classes of the lakes located in Turkey correctly (Phillips *et al.*, 2012).

In addition to the climatic variations, sampling frequency is also an important factor for phytoplankton community structure. While CB countries employed the data gathered from 5 to 8 sampling, which were conducted during the vegetative period (usually April to October), Mediterranean countries only used data from 4 samples per year (in some cases 2-3) and samplings were usually done during the summer period (June-September) (Poikane *et al.*, 2010). Since phytoplankton generation time is short and seasonal taxonomic patterns differ among seasons, comparison of the samples retrieved during the vegetative period to the ones from summer period may lead to uncertainties (Søndergaard *et al.*, 2011; Carvalho *et al.*, 2013). The effect of these differences will be smallest for the Chl-*a* and will be highest for the composition metrics.

Furthermore, altitude range of the lakes located in CB region is low, and lake typologies do not comprise higher altitude lakes. On the other hand, most of the lakes used in the current study are located in higher altitudes (>200m), therefore this altitude difference between CB countries and Turkey may result in less reliable ecological quality classifications of the lakes.

Current study is the first comprehensive investigation of the ecological quality status from Turkish shallow lakes based on phytoplankton data. Considering the ecological classifications (based on EQR values) of the reference and eutrophic lakes, also the strength and the significance of the correlations between trophic parameters and EQR values, choosing a suitable phytoplankton index for Turkish lakes was not possible. The main reasons for the incompatible results may be related to climatic and typological differences and also to inadequate sample size. Therefore, starting to regular monitoring studies to develop a national phytoplankton index based on detailed physiological and biological data should be a priority for the governmental organizations.

CHAPTER 5

INVESTIGATION OF ECOLOGICAL STATUS OF BÜYÜK MENDERES RIVER BASIN USING PHYTOPLANKTON AS A BIOLOGICAL QUALITY ELEMENT

5.1 Introduction

Lakes and reservoirs are important aquatic habitats and water supply for human life. Moreover, they are highly sensitive ecosystems to biological, chemical, and physical stressors (Moss, 2010). While lakes are natural water bodies, reservoirs are man-made and generally constructing across a flowing river. Since they are generally deep, and water level changes are abrupt ecology of reservoirs different than shallow lake ecosystems. Moreover, generally reservoir do not have large littoral zone thus macrophyte growth is generally limited. These differences also have effect on phytoplankton, for instance increased turbidity due to clays and silts could prevent light penetration (Kimmel & Groeger: 1984). As a result of increased land use and human activities discharge of highly nutrient enriched content into water bodies is considered as the main factor leading to eutrophication symptoms (Jeppesen *et al.,* 2009; Beklioğlu *et al.,* 2007, 2008). Especially toxic cyanobacteria blooms come into prominence in recent decades and lead to deterioration of ecosystems services such as water shortage in many places around the world (Huisman, 2005; Wang *et al*., 2008; Paerl *et al*., 2011). To prevent deterioration of waters and promote sustainable water use, Water Framework Directive (WFD) came into force in 2000 (WFD, 2000/60/EC). It aims in achieving "good water status" all over European Union countries. According to Water Framework Directive (WFD), Biological Quality Elements (BQEs) which include macrophytes, phytoplankton, macroinvertebrates,

phytobenthos and fish, are used to determine the ecological status of natural water bodies or the ecological potential of highly modified water bodies (EC, 2000).

Phytoplankton was considered as a significant BQE for lake water bodies (WFD, 2000), due to their high sensitivity to environmental fluctuations (Ptacnik, *et al*., 2008). While WFD determines the general parameters of the BQEs that should be assessed (e.g. for phytoplankton; abundance, taxonomic composition and bloom), it does not specify which indices should be applied (WFD, 2000). Therefore, based on their ecoregional/bioregional properties and long term monitoring data, each member state (MS) established their own national indices or metrics, which were subsequently modified (if needed) in order to make results compatible with each other (Marchetto *et al*., 2009; Padisák *et al*., 2006; Phillips *et al*., 2012; Salmaso *et al*., 2006). According to Birk *et al*. (2013), 21% of the 297 different quality assessment methods, developed by the countries implementing the WFD, were based on phytoplankton data.

All the MS have agreed to use Chl-*a* concentration as the phytoplankton biomass, since generally Chl-*a* amount is the reliable parameter (Carvalho *et al*., 2008). However, the use of phytoplankton taxonomic composition response with respect to the environmental variables is not a simple task since they have a very high diversity and a short generation time (Dokulil & Teubner 2006). To develop a specific taxonomic metric for a specific type water body, long term monitoring data, climatic, biogeographic and hydro-morphological properties should be taken into account (Padisak *et al.*, 2006). Moreover, metrics should be cost effective, rapid and efficient, and also phytoplankton identification requires highly qualified personnel (Katsiapi *et al*., 2015). Because of these reasons developing a reliable metric remains a great challenge for Turkey.

The indices which are established by each MS, only covers the water bodies in their own national borders, enabling the comparison of the ecological classification results among different countries. Therefore, in order to assess the methods to be compatible, Geographic Intercalibration Groups (GIG) were determined and intercalibration studies were launched among these GIGs (Nöges *et al*., 2009). Twenty-eight countries finalized their intercalibration exercises for 230 different methods (EC, 2013). Mediterranean Lake Phytoplankton Geographical Intercalibration Group (MedGIG) studies were only carried out among four countries (Cyprus, Portugal, Italy, Spain) and only covered the reservoirs but not natural lakes (Hoyos *et al*., 2014). All the MS assessment methods for MedGIG were generally similar, comprising biomass and composition metrics. As a result of second intercalibration studies New Italian Method (NITMET) and New Mediterranean Assessment System for Reservoirs Phytoplankton (NMASRP) indices were developed and formally agreed and finalized for Mediterranean reservoirs (Hoyos *et al*., 2014).

Since Turkey is a candidate country for the membership of European Union it has to fulfill the requirements of WFD. However, Turkey did not join the intercalibration studies and the implementation of WFD-compliant monitoring of water bodies raises a number of technical challenges in Turkey due to a lack of experienced technical staff on biological quality. In this study 12 reservoirs and 2 lakes were sampled in Büyük Menderes River Basin (BMRB) as a part of EuropeAid/131199/D/SER/TR "Technical Assistance for Capacity Building on Water Quality Monitoring" project. The main objective of the present study was to determine the ecological status of these Turkish reservoirs located in Büyük Menderes River Basin (BMRB) using phytoplankton as BQE and New Mediterranean Assessment System for Reservoir's Phytoplankton (NMASRP) as index. For this purpose, we calculated current ecological status of the study sites by applying MedGIG intercalibration reference and class boundary values, which were defined by employing the information gathered from all the reference sites in Mediterranean MS (Hoyos *et al*., 2014). Moreover, we also calculated ecological

status by using reference and class boundary values extracted from the reference sites located in BMBR, Turkey. We hypothesized that intercalibration reference and class boundary values which were established from a large data set would lead to more reliable classifications.

5.2 Material and Methods

In total 12 reservoirs and 2 lakes were sampled in Büyük Menderes River Basin (Figure 5.1, Table 5.1), (Sampling campaign was conducted by Republic of Turkey, Ministry of Forestry and Water Affairs and Enveco S.A. (Greece). The first sampling campaign was conducted during low flow conditions in September 2013, while the second sampling campaign was in March 2014 during the high flow conditions. Samples were obtained from two locations, first one being from the deepest point (if known) and the second sample was form near to the inlet or as far as possible from the lake outlet. Furthermore, additional sampling points were used if the water body had an area larger than 50 km^2, more than one main inlet and/or hydro-morphological features, which were distinctly different between the different parts of the water body. For example, 4 sampling points were selected for Lake Bafa, which is a large lake with different basin structures and having more than one inflow. Water samples were taken with a Ruttner sampler from the euphotic zone (2.5 x Secchi disc depth, 1-2 m intervals) in each sampling point. Subsequently, for further phytoplankton countings, collected water samples were pooled, mixed and approximately 400 ml sample was fixed with Lugol's iodine (1%) solution and formaldehyde (1%).

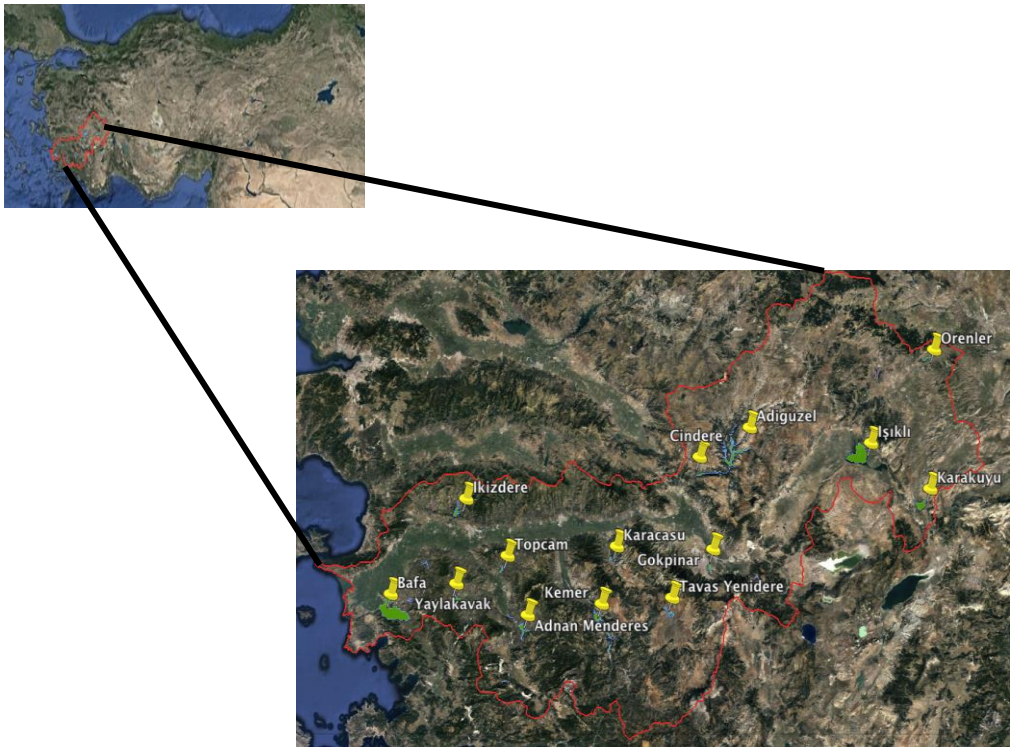

Figure 5.1 Map of Turkey and the sampling sites from BMRB included 12 reservoirs and 2 lakes

Table 5.1 Variables that are used for typology of the reservoirs and lakes, according to the Twinning project.

Province	Sampling Sites	Altitude (m)	Mean precipitation (mm)	*Mean Temp (°C)	Mean depth (m)	Area (km2)	Chachment (km2)	Alkanity (mg/l)	Alkanity (meq/l)
Denizli	Adiguzel	434	552.5	16.21	_	17.82	9723	246	4.20
	Cindere-Adıguzel 2	232	552.5	16.21	_	2.70	10052	232.5	4.64
	Gokpinar	310	552.5	16.21	_	1.05	203	Na	_
	Isikli	814	552.5	16.21	_	58.37	3840	223	4.5
	Tavas-yenidere	875	552.5	16.21	_	2.18	1150	191.5	3.82
Aydın	Bafa	0	874	16.38	10	66.67	25299	_	_
	Cine Adnan Menderes	210	621.4	17.72	_	6.90	1457	169	3.38
	Topcam	101	621.4	17.72	_	2.77	271	62.5	1.24
	Yaylakavak	176	621.4	17.72	_	0.91	183	57	1.14
	İkizdere	137	621.4	17.72	_	3.67	166	Na	_
	Karacasu Dam	275	621.4	17.72	_	0.62	548	Na	_
Afyon	Karakuyu	1006	420.8	11.29	4	12.77	90	179	3.58
	Örenler	1153	420.8	11.29	17	2.42	205	94.5	1.88
Aydın Denizli Mugla	Kemer	286	766.8	16.32	_	6.59	3435	245.5	4.9

*Mean temperature from 1954 to 2013.

Na. Not applicable

5.2.1 Phytoplankton Counting

Phytoplankton samples were counted according to Utermöhl technique (Utermöhl, 1958) and countings were continued until the number of the most abundant species reached to 400 individuals. Identification of phytoplankton species was carried out based on morphological characteristics including color, shape, motility and colony structure by using some reference books (Whitton *et al*., 2002, Prescott *et al*., 1973, Cox 1996, Komarek 1999, Krammer 1986, 1988, Popovski, 1990). In order to calculate mean biovolume ($mm^3\ L^{-1}$) of each species in each sample, dimensions of at least 30 individuals were measured (when it was

possible) and calculations were conducted according to the standard volume formulas of the geometric shapes that best fits the organisms' morphological features (Hillebrand *et al*., 1999) (Appendix-E Table 2).

5.2.2 Determining Reservoir/Lake Typologies

Given the limitations of available biological monitoring data from all water bodies in Turkey, typology of the study lakes was defined according to the Mediterranean intercalibration study results (Table 5.2) (Hoyos *et al*., 2014). All the reservoirs and lakes of the Büyük Menderes River Basin were assumed to correspond to L-M8 typology (Table 5.2). However, besides being a brackish lake (salinity = 9.6 ‰) (Altınsaçlı, 2014), Lake Bafa was a unique case, since the mean depth of the lake (5 m) did not meet the depth requirements (>15 m) of Mediterranean reservoir typology (Erdoğan, 2011). Moreover, the biological data that could allow statistically powerful assessment of class boundaries for this lake type, was inadequate for defining a new typology for Lake Bafa. Therefore, this lake was regarded as L-M8 type, as well.

Table 5.2 Description of final common inter-calibration water body types (Hoyos *et al*., 2014).

Type	Lake characterization	Altitude (m)	*Precipitation (mm), Temperature (°C)	Mean depth (m)	Area (km^2)	Catchment (km^2)	Alkalinity (meq/l)
L-M5/7	Reservoirs, deep, large, siliceous "wet" areas	< 1000	> 800 and/or<15	> 15	0.5-50	< 20000	< 1
L-M8	Reservoirs, deep, large, calcareous	< 1000	−	> 15	0.5-50	< 20000	> 1

*****Annual mean precipitation and temperature

Evaluation of Ecological Potential of Reservoirs

5.2.3 New Mediterranean Assessment System for Reservoir's Phytoplankton (NMASRP) metrics

For classifying the lake water bodies (WBs) of the Büyük Menderes River basin, the NMASRP method has been chosen since it was applied to Cyprus, which had similar number of sampling (2 samples per site) as the current study. NMASRP is a multimetric index that is composed of 2 biomass metrics, being Chl-*a* (μg L^{-1}) and phytoplankton biovolume (mm^3 L^{-1}), and 2 composition metrics, being Index Des Groups Algals (IGA) - also known as Catalan index - and Cyanobacteria biovolume (mm^3 L^{-1}). According to the NMASRP all the metrics have equal weights in the final index.

IGA metric is calculated by using biovolume of certain phytoplankton groups in the following equation:

$$CI = \frac{[1 + 0.1Cr + Cc + 2(Dc + Chc) + 3Vc + 4Cia]}{[1 + 2(D + Cnc) + Chnc + Dnc]}$$

Where:

CI: IGA Index; Cr: Cryptomonads; Cc: Colonial Chrysophyte; Dc: Colonial Diatoms; Chc: Colonial Chlorococcales; Vc: Colonial Volvocales; Cia: Cyanobacteria; D: Dinofagellates; Cnc: Chrysophyte not colonial; Chnc: Chlorococcales

5.2.4 Extracting Reference or Maximum Ecological Potential (MEP) sites

Reference or MEP sites, which should have minimum anthropogenic disturbance, were determined using a three-step procedure described in the Mediterranean intercalibration study (Hoyos *et al*., 2014), as given below;

i. Screening environmental parameters criteria.

To define the possible reference sites 'one is out all is out' principle (i.e. if one of the environmental parameters fails to pass through the rejection limit, then a site cannot be identified as reference) has been applied. Screening environmental parameters included artificial land use percentage (ALU), intensive agriculture percentage (IA), natural and semi-natural land use percentage (NASN), population density (PD), which are extracted from CORINE database, and total phosphorus (TP) concentration of the water body. If three of these five pressure parameters were lower than the threshold reference limits, the water body is regarded as reference (Table 5.3).

Table 5.3 Rejection limit and Reference thresholds of environmental variables used in screening the lake water bodies for reference conditions (Hoyos *et al*., 2014).

Environmental Variables	Lake Water Bodies	
	Rejection	Refences
Artificial Landuse % (ALU)	4%	1%
Total % Agricultural Land Uses (IA)	20%	10%
Natural Seminatural Land Use % (NASN)	70%	80%
Population density (PD) hab/km2	30	10
Average Total Phophorus (µg/L)	30	12

i. Elimination of sites with extremely high biological values.

In this step, probable reference sites were chosen according to their environmental parameters were eliminated based on their biological values. All metrics used in the NMASRP (Chl-*a*, Cyanobacteria biovolume, Total Biovolume, IGA Index) were checked against the G/M boundary values established in the first phase of the intercalibration exercise (Decision 2008/915/EC) (Table 5.4).

Table 5.4 Good / Moderate boundaries for L-M8 type of reservoirs derived from the first phase of the intercalibration exercise and published in the Decision 2008/915/EC. For Chl-*a* a range was given in the decision.

	Total Biovolume (mm^3/L^{-1})	Catalan Index (IGA)	Cyanobacteria Biov. (mm^3/L^{-1})	Chl-*a* ($\mu g/L^{-1}$)
Good / Moderate boundary for L-M8 type	2.5	6.5	0.5	5.3

i. Inclusion of sites with good biological scores though they fall out of the screening criteria based on the first step.

In this step, the sites, which did not meet the requirements for the environmental parameters (e.g. TP, ALU, PD etc.), but having good biological scores were included as possible reference sites.

5.2.5 Good / Moderate class boundary values and EQR calculations

In order to calculate the EQR values it is also necessary to establish the good/moderate (G/M) boundaries for different metrics. In the current study, two sets of class boundary values were employed, one set was obtained from the results of intercalibration exercise and the second was determined from the reference sites in BMRB. Thus, class boundaries, EQR values and finally ecological quality classifications were presented both according to the intercalibration dataset (Mediterranean reservoirs) and to the BMRB dataset, enabling the comparison of these two methods. For the latter, to define the G/M class boundaries, two parallel methodologies, based on statistical distribution of the lake dataset in different total phosphorus (TP) groups and on equidistant divisions (Hoyos *et al.*, 2014) have been employed (Calculation details were given in Appendix-D Table 1). The rest of the class boundaries were defined based on these G/M boundary values (Table 5.4).

It should be noted that both procedures (based on intercalibration reservoir dataset and on the BM reservoir dataset) were used exactly the same principles while the only difference was in the reference/MEP values, employed in the EQR calculations. Two sets of EQR values for each metric were calculated with the equations given in Appendix-D Table 2-3, by employing both the reference/MEP values determined from BMRB sites and from the intercalibration study. The last step to reach the final results prior to aggregating the outcomes of different metrics, was the normalization of the resultant EQR values of each metric (EQR

→ nEQR). This step was carried out in order to scale EQR values between zero to one, where 0.6 represented G/M boundary (Calculation details were given in Appendix-D Figure 1, Table 4).

5.2.6 New Mediterranean Assessment System for Reservoir's Phytoplankton (NMASRP) index

After normalizing the EQR estimations for all samples based on each metric, NMARSP index calculation was carried out by using following formula:

$$NMASRP = \frac{\left(\frac{nEQR(Chl) + nEQR(BV)}{2}\right) + \left(\frac{nEQR(IGA) + nEQR(CyaBV)}{2}\right)}{2}$$

5.3 Results

5.3.1 New Mediterranean Assessment System for Reservoir's Phytoplankton (NMASRP) Metric Calculation Results

For all the metric results, the highest values were observed in Örenler reservoir for the high flow sample. The lowest values for total phytoplankton biovolume and Chl-*a* were observed in Lake Işıklı) and Karakuyu Reservoir for the high flow samples, respectively. Total cyanobacteria biovolume and IGA metric results were zero for 19 and 5 samples, respectively (Table 5.5).

Table 5.5 Phytoplankton metrics results in the Büyük Menderes River Basin. The table presents the average values of the two samples in each lake

WB NAME	Season	Total BV	Catalan	Cyan BV	Chl-*a*
Adiguzel	High Flow	9.8	0.0	0.0	20.5
	Low Flow	9.6	0.2	0.2	10.7
Bafa	High Flow	2.4	0.0	0.0	14.5
	Low Flow	3.8	1.5	0.3	67.8
Cindere-Adiguzel 2	High Flow	1.4	0.0	0.0	3.1
	Low Flow	39.1	0.1	1.1	32.8
Cine Adnan Menderes	High Flow	0.7	27.5	0.0	6.6
	Low Flow	5.4	0.0	0.0	1.8
Gokpinar	High Flow	11.1	0.0	0.0	19.0
	Low Flow	12.9	1.5	0.3	63.0
Ikizdere	High Flow	0.2	1.6	0.0	3.8
	Low Flow	6.7	0.1	0.2	1.8
Isikli	High Flow	0.1	2.9	0.0	7.6
	Low Flow	8.4	21.5	7.1	4.6
Karacasu Baraji	High Flow	0.9	0.1	0.0	7.2
	Low Flow	5.3	0.4	0.0	26.8
Karakuyu	High Flow	0.0	1.9	0.0	1.5
	Low Flow	0.0	11.1	0.0	1.8
Kemer	High Flow	3.7	0.2	0.0	16.7
	Low Flow	1.8	1.5	0.0	5.3
Orenler	High Flow	13.7	0.9	1.8	93.3
	Low Flow	152.1	203.2	143.7	254.8
Tavas-Yenidere	High Flow	5.5	0.3	0.0	5.5
	Low Flow	0.5	0.4	0.0	10.7
Topcam	High Flow	1.3	0.7	0.0	6.6
	Low Flow	1.5	0.3	0.1	10.2
Yaylakavak	High Flow	3.7	33.8	0.0	18.1
	Low Flow	3.1	0.1	0.0	12.2

5.3.2 Extracting Reference or Maximum Ecological Potential (MEP) conditions

i. Screening environmental parameters criteria

Topçam Reservoir was the closest water body to a reference state as it has low total agricultural area (IA), natural and semi natural land use percentage (NASN) and population density (PD) (Table 5.6). Even though Topçam Reservoir failed to meet the TP limit, which is the criterion that makes the most reservoirs to fail, it presented a reference profile regarding to 3 criteria (IA, NASN and PD) and a good profile regarding to one criterion (ALU). Therefore, it was considered the best available choice for a reference reservoir within the Büyük Menderes River Basin (Tables 5.6).

Table 5.6 Artificial land use percentage (ALU %), intensive agriculture percentage (IA %), natural and semi-natural land use percentage (NASN %), population density (PD), total phosphorus concentration (TP). Red colored cells are for the parameters that fail the acceptance limits. Green cells are for the parameters that fail the reference limits. Blue cells are for the parameters that pass the reference limits.

Lake WB	Artificial landuse % (ALU)	Total % Agricultural Land Uses (IA)	Natural Seminatural Land use Percentage (NASN)	Population density (PD) hab/km^2	Average Total P (µg/L)
Cine Adnan Menderes	2.5	19.1	69.9	26.6	1990.0
Adiguzel	1.9	42.1	46.9	42.3	41.3
Bafa	2.1	35.2	53.7	67.3	119.3
Cindere-Adiguzel 2	1.8	42.0	47.0	41.7	200.5
Gokpinar	7.9	15.7	75.3	493.2	22.5
Ikizdere	0.3	39.7	52.3	0.0	41.5
Isikli	1.4	36.7	57.0	26.2	230.0
Karacasu	0.9	19.0	69.7	15.8	32.0
Karakuyu	0.6	22.0	71.7	4.5	48.0
Kemer	0.7	19.3	68.4	14.8	60.5
Orenler	0.8	29.5	63.2	47.8	133.0
Topcam	1.9	3.7	89.0	0.0	68.8
Yaylakavak	2.1	4.8	72.6	11.3	238.3
Tavas-Yenidere	1.5	36.7	55.0	35.8	18.5

ii. Elimination of sites with extremely high biological values.

The results of NMASRP metric calculations showed that Topçam reservoir have met the requirements for Total BV, IGA and Cyan BV, but failed only for Chl-*a* value, though it was close to acceptable range (Table 5.5 and 5.6). Due to lack of reference reservoirs it was decided that Topçam reservoir should still be included as a MEP lake water body.

iii. Inclusion of sites with good biological scores though they fall out of the screening criteria based on the first step

İkizdere and Tavas Yenidere reservoirs passed the same number of biological quality criteria as Topçam Reservoir (Table 5.5-5.6). Therefore, due to their high quality biological values both of these sites were defined to be the MEP sites. This approach was needed for determining class boundaries and calculating EQR values that would fit specifically to the Büyük Menderes River Basin.

5.3.3 Good/Moderate class boundary values and EQR calculations

Calculated BM-G/M boundary values and intercalibration-G/M boundary values are given in Table 5.7. For all the metrics, calculated G/M boundary values were higher than intercalibration values (Table 5.7).

Table 5.7 Good / Moderate boundaries for L-M8 type of reservoirs derived from the first phase of the inter-calibration exercise and published in the Decision 2008/915/EC.

Method for G/M boundary	Total BV ($mm^3 L^{-1}$)	IGA	Cyan BV ($mm^3 L^{-1}$)	Chl-a ($\mu g L^{-1}$)
Average G/M Boundary according to the Büyük Menderes dataset	7.8	6.5	1.1	9.7
G/M Boundary according to the Med. Indercalibration dataset for L-M8 Type	***2.5***	***6.5***	***0.5***	***5.3***

EQR values indicated that except IGA metric all the calculated reference/MEP values were higher for Büyük Menderes MEP sites (Appendix-D Table 2). EQR values for all seasons in the BMRB reservoirs were given in Appendix-D Table 1-3.

Final classification results were given in Table 5.8. Moreover, average of low flow and high flow results were also given in Table 5.9. As can be seen in the Table 5.8, the ecological status derived from taking into account the Büyük Menderes dataset was very similar to the one that was obtained through the intercalibration exercise derived threshold values. However, on the high flow samples of Cine Adnan Menderes reservoir, and on the low flows of the Lake Bafa and Topçam reservoir, the intercalibration classification differed from the one derived from Büyük Menderes dataset (Table 5.8). In the case of Lake Bafa the quality according to intercalibration exercise was better by one class, while in the Cine Adnan Menderes and Topçam reservoirs they were one class worse (Table 5.8).

Table 5.8 Overview of the NMASRP index results for the samples of each season

Büyük Menderes lake water bodies	Season	nEQR according to the Büyük				NMASRP	Buyuk	Intercalibration
		Total BV	IGA	Cyan BV	Chl -*a*		Ecological Potential	Ecological Potential
Adiguzel	HF	0.5	1.0	1.0	0.3	0.7	Good	*Good*
	LF	0.5	1.0	0.7	0.5	0.7	Good	*Good*
Bafa	HF	0.8	1.0	1.0	0.4	0.8	High	*High*
	LF	0.7	0.9	0.6	0.1	0.6	Moderate	*Good*
Cindere-Adiguzel 2	HF	1.0	1.0	1.0	1.0	1.0	High	*High*
	LF	0.1	1.0	0.6	0.2	0.5	Moderate	*Moderate*
Cine Adnan Menderes	HF	1.0	0.6	1.0	1.0	0.9	High	*Good*
	LF	0.6	1.0	1.0	1.0	0.9	High	*High*
Gokpinar	HF	0.4	1.0	1.0	0.3	0.7	Good	*Good*
	LF	0.4	0.9	0.6	0.1	0.5	Moderate	*Moderate*
Ikizdere	HF	1.0	0.9	1.0	1.0	1.0	High	*High*
	LF	0.6	1.0	0.7	1.0	0.8	High	*High*
Isikli	HF	1.0	0.8	1.0	0.8	0.9	High	*High*
	LF	0.6	0.6	0.1	1.0	0.6	Moderate	*Moderate*
Karacasu Baraji	HF	1.0	1.0	1.0	0.9	1.0	High	*High*
	LF	0.6	1.0	1.0	0.2	0.7	Good	*Good*
Karakuyu	HF	1.0	0.9	1.0	1.0	1.0	High	*High*
	LF	1.0	0.6	1.0	1.0	0.9	High	*High*
Kemer	HF	0.7	1.0	1.0	0.3	0.8	Good	*Good*
	LF	0.9	0.9	1.0	1.0	1.0	High	*High*
Orenler	HF	0.3	1.0	0.4	0.1	0.4	Moderate	*Moderate*
	LF	0.0	0.3	0.0	0.0	0.1	Bad	*Bad*
Tavas-Yenidere	HF	0.6	1.0	1.0	1.0	0.9	High	*High*
	LF	1.0	1.0	1.0	0.5	0.9	High	*High*
Topcam	HF	1.0	1.0	1.0	1.0	1.0	High	*High*
	LF	1.0	1.0	0.8	0.6	0.8	High	*Good*
Yaylakavak	HF	0.7	0.6	1.0	0.3	0.6	Good	*Good*
	LF	0.7	1.0	1.0	0.5	0.8	High	*High*

The following Table 5.9 summarizes the results of the classification for all water bodies in the Büyük Menderes River Basin by applying the 'one out all out' principle for the different seasons' samples in each lake. All the classification results were same both for BMRB and intercalibration classifications except Lake Bafa and Cine-Adnan Menderes, which were classified as being in moderate and high classes according to BMRB calculations, while both of them were classified in good classes according to intercalibration calculations (Table 5.9).

Table 5.9 Final classification of all reservoirs of the Büyük Menderes River Basin, by applying the 'one out all out' principle (i.e. the classification of the water body is the worst classification of each season)

Büyük Menderes lake water bodies	**Buyuk Menderes**	**Intercalibration**
Adıguzel	Good	*Good*
Bafa	Moderate	*Good*
Cindere-Adıguzel 2	Moderate	*Moderate*
Cine Adnan Menderes	High	*Good*
Gökpınar	Moderate	*Moderate*
Ikizdere	High	*High*
Işıklı	Moderate	*Moderate*
Karacasu Barajı	Good	*Good*
Karakuyu	High	*High*
Kemer	Good	*Good*
Örenler	Bad	*Bad*
Tavas-Yenidere	High	*High*
Topçam	Good	*Good*
Yaylakavak	Good	*Good*

5.4 Discussion

All the water bodies were classified in the same ecological quality class both for intercalibration- and for Büyük Menderes- reference/MEP site based class boundary values, with the exception of Lake Bafa and Cine Adnan Menderes Reservoir (Table 5.9). However, it should also be noted that none of our study sites met the reference site requirements, thus leading to the conclusion that intercalibration results were more reliable than ours as we hypothesized. Moreover, we expected our reference/MEP sites should be in high or good classes due to their relatively high biological and chemical status. Accordingly, our results showed that these sites were classified in high (İkizdere, Tavas Yenidere Reservoirs) and good (Topçam Reservoir) ecological conditions. Moreover, dense cyanobacteria bloom was observed in Örenler Reservoir, which was classified in bad ecological status. These findings also support the applicability of NMASRP index to Turkish reservoirs.

Since WFD requires harmonisation of national classification results, to make them comparable among all European countries, intercalibration studies were carried out in all GIGs (Hoyos *et al*., 2014; Poikane *et al*., 2015) though Turkey was not part of it. Even though Turkey covers various climatic regions, Büyük Menderes River Basin mainly covers Mediterranean climate. NMASRP indice was used successfully for reservoirs in Cyprus and Portugal (Hoyos *et al*., 2014) thus it was the most appropriate one for our study sites though there were some variations between member states and our sampling frequencies. While Cyprus used two summer samples, Portugal used 9 samples per year (3 growing season, 3 autumn and 3 winter), our data set comprised only two samples (spring and summer). This difference could lead to misclassification of the status.

We also observed quality class differences between low flow and high flow seasons for our study sites. The water quality classification in Büyük Menderes water bodies showed that the worst quality occurred at the low flow period. An exception to this was noted for Kemer Reservoir where the low flow was the high water quality period whereas the high flow period was the good quality. During the high flow, surface runoff water may bring high amount of nutrients into the lake that may have lowered the water quality (Jeppesen *et al*., 2009). On the contrary, higher pressure with bad water quality may occur in the water bodies during the low flow season when water levels are lower, thus enhancing the effect of eutrophication through up-concentration of nutrients as the lake volume decrease, also due to higher internal P loadings during warmer periods and hindered denitrification (Özen *et al*., 2010, Naselli-Flores & Barone, 2005; Beklioğlu *et al*., 2007; Coppens *et al*., 2016). It should also be noted that different water management practices in the reservoirs could also cause high pressure even at the high flow season (Sechi & Lugliè, 1992; 1996).

Lake Bafa was classified as being in moderate ecological status, contradicting with the findings of cyanobacteria blooms starting as early as in May and persisting throughout the summer for several years in the lake (Erdoğan, 2011, Altınsaçlı, 2014). In this study, Lake Bafa was sampled during September from two sampling stations, during which lake had abundant species of diatoms (e.g. *Thalassionema nitzhioides*) and dinophyta (e.g *Prorocentrum micans*), but cyanobacteria species were not abundant. Wind induced mixing might have been critical during the sampling period as the lake is shallow with large open surface area to catch larger fetch. Thus timing and frequency of the sampling are very critical to reveal the true nature of the water bodies as well as expanding the monitoring into a national level (Moss *et al*., 2003). On the other hand, the classification scheme that we used does not comply with Lake Bafa as it is neither a reservoir, nor a freshwater lake (Demir, 2007). Therefore, it should belong to a different type, with different reference conditions and reference values, which were not possible to do with the current study, since no such reference data exist.

Hence, the results of the current study should not be taken as a case study in a process of reaching the goal of better monitoring, in a broader geographic area such as the national level.

It is evident from our results that long term monitoring data and by using more and better reference sites are needed to better classify the status of Turkish freshwaters. Furthermore, among the study lakes some of them were shallow (Lakes Işıklı, Karakuyu and Bafa) whose governing mechanisms for the ecosystem status are completely different than deep lakes and reservoirs such as morphology, water level regimes, or physico-chemical differences, macrophytes (Wetzel, 1990). That's why our reference sites being reservoirs are very unlikely to be good reference sites for these shallow lakes. Furthermore, for determining ecological class boundaries, assuming that the factors determining class boundaries (metrics of the multimetric NMSRP Index) have equal weight would be grossly error bounded. This also needs to be understood better with in-depth research through long term monitoring.

CHAPTER 6

CONCLUSION

This study had four main aims. The first aim was determining the effect of climate change on phytoplankton size structure by using major abiotic and biotic drivers in 46 Turkish shallow lakes. The thesis also aimed to explore nutrient, depth and temperature effects on phytoplankton community structure along a latitudinal climate gradient from Sweden to Greece by mesocosm experiments. The last two aims were calculating phytoplankton-based Mediterranean and Central Baltic WFD indices to investigate their search applicability to Turkish reservoirs and lakes, also to evaluate the ecological status of the water bodies by employing WFD approaches.

The results revealed that phytoplankton size structure response to changing environmental conditions and that top-down regulation was more pronounced in eutrophic and hypereutrophic lake ecosystems. Indirect nutrient effect was also observed via zooplanktivorous fish increase. Moreover, due to rotifer abundance increase, grazing pressure on small sized phytoplankton also increased with increasing eutrophication. Additionally, mean variance in phytoplankton unit size also increased with eutrophication due to higher colonial and filamentous species. The results of our study highlight the sensitivity of cell size structure to both biotic and abiotic variables like eutrophication and grazing. Furthermore, our results imply that phytoplankton size traits can be used as a tool to understand ecological responses to climate change in lake ecosystems (Chapter 2).

The mesocosm experiment that was performed in six European countries showed the response of phytoplankton community structure to nutrient, temperature and water level changes. While cyanobacteria biovolume was high in high nutrient treatments of warm countries (Turkey and Greece), clear state indicator group (e.g. Chrysophyta) abundances were low. Moreover, rather than singular nutrient temperature and depth effects, interactions between these variables were more pronounced for taxonomic and functional classifications, also for diversity, and richness parameters. Besides the importance of nutrient and temperature the results also suggested that high macrophyte coverage also suppressed phytoplankton growth probably due to nutrient competition and allelopathic interactions. On the other hand, severe water level decrease caused macrophytes to die back and lead to phytoplankton increase in Greece. We also found that phytoplankton diversity and richness were mainly negatively affected by temperature, nutrient and depth interactions in all treatments probably due to long term growing season in warm countries (competitive exclusion principle) (Chapter 3).

MedGIG reservoir intercalibration class boundary values and Büyük Menderes River Basin class boundary values that were extracted from the reference sites located in Büyük Menderes River Basin were generally similar. Moreover, none of our reference sites met the reference site requirements thus we can say that intercalibration results were more reliable than ours. However, there is not much difference between two calculation results. On the other hand, since there is no intercalibration study for Mediterranean lake ecosystems, Central Baltic, MedGIG reservoir and common PTI indices were applied to our lake data, however we did not find a consistent correlation for any of the trophic parameters or calculated indice values. Climatic and typological difference and inadequate sample size could be the main reason of inconsistent results (Chapters 4 and 5).

This study confirmed the reliability of using phytoplankton size and community composition as an indicator of climate change and a parameter for determining the lake water quality. However due to small sample size and lack of long term monitoring data water quality calculations need further improvements. To meet requirements of WFD, Turkey should start regular monitoring studies, and afterwards national metrics might be developed based on long-term detailed data, national typologies and reference sites.

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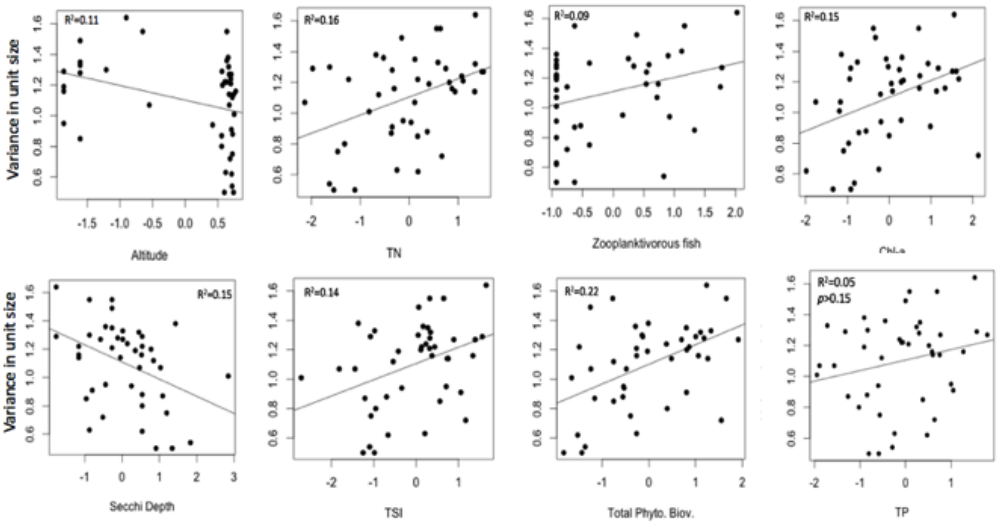
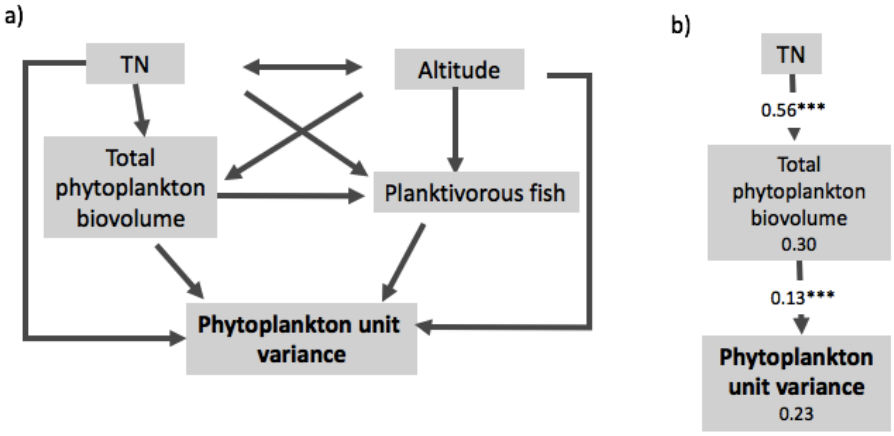
APPENDIX A

Appendix-A Table 1 Bivariate regression results.

	Coefficient	R^2	*p* value	*F* value
Variance 3 lakes excluded				
Altitude	-0.096	0.11	<0.05	$F_{1,41}$=5.121
TN	0.1185	0.17	<0.01	$F_{1,41}$=8.18
Zooplanktivorus fish	0.0943	0.09	<0.05	$F_{1,41}$=4.11
Chl-*a*	0.11	0.15	<0.05	$F_{1,41}$=7.01
Sechii depth	-0.1215	0.16	<0.01	$F_{1,41}$=7.80
TSI	0.1107	0.14	<0.05	$F_{1,41}$=6.70
Total biovolume	0.1346	0.23	0.001	$F_{1,41}$=11.98



Appendix-A Figure 1 Bivariate regression plots for variance in size and environmental variables.

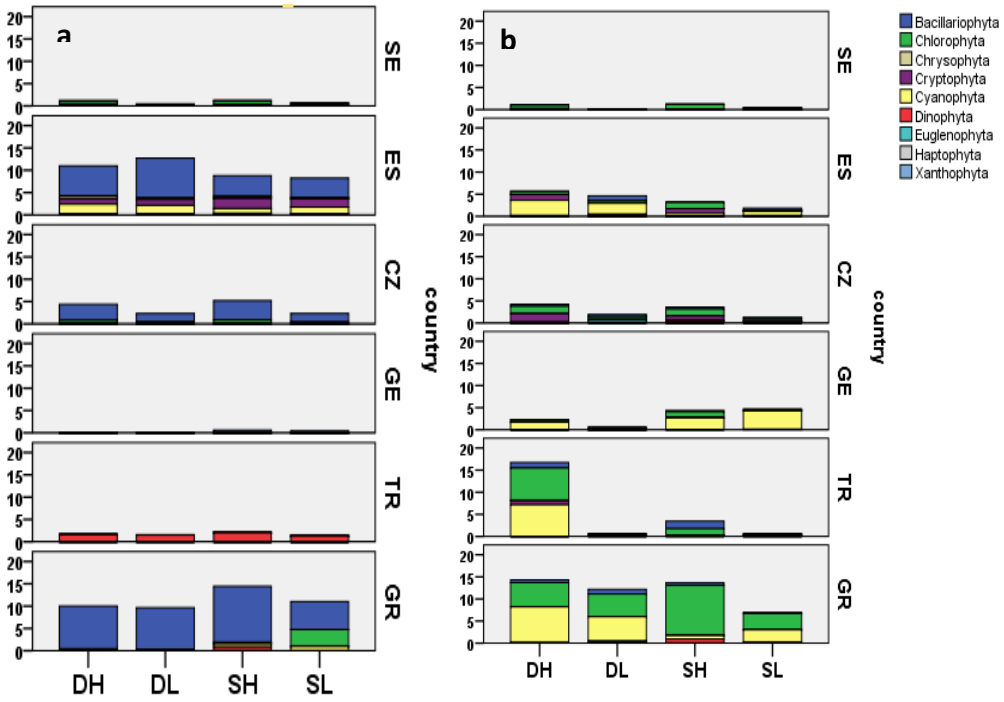


Appendix-A Figure 2 Initial variance SEM diagram (left) and final SEM results (right). Arrows represent casual positive relationship, coefficients and significance values were presented on arrow lines. R^2 values were given under variable names. p<0.05*; 0.01**; 0.001***

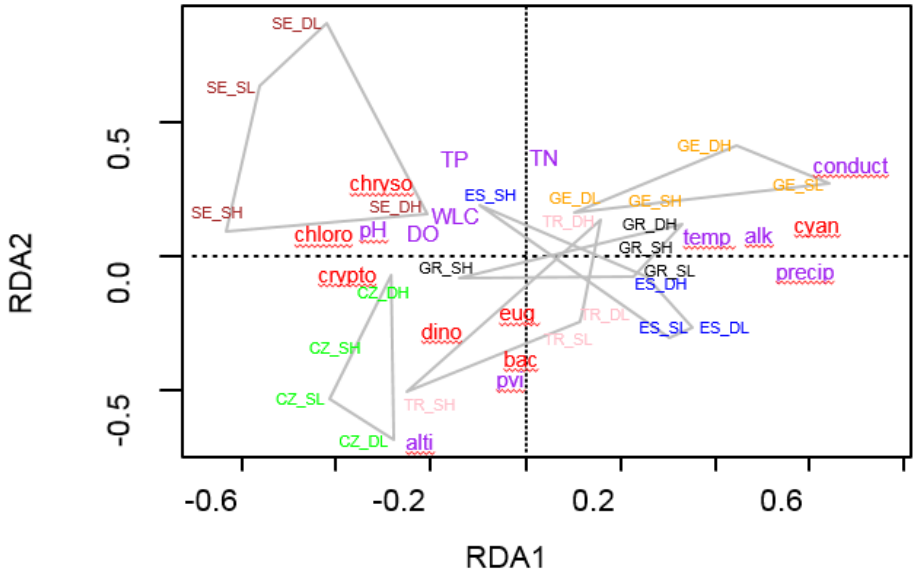
APPENDIX B

Appendix-B Table 1 Initial and bulk total phytoplankton biovolume for each treatment and country.

	Total phytoplankton biovolume ($mm^3 L^{-1}$)							
	Initial				**Pooled**			
	DH	**DL**	**SH**	**SL**	**DH**	**DL**	**SH**	**SL**
SE	1.2 ± 0.5	0.4 ± 0.2	1.3 ± 0.4	0.7 ± 0.4	1 ± 0.5	0.2 ± 0.2	1.3 ± 0.5	0.4 ± 0.4
ES	11 ± 2.3	12.7 ± 2.3	8.8 ± 6.6	8.2 ± 2.8	5.7 ± 2.1	4.6 ± 1.2	3.2 ± 2.1	1.9 ± 0.4
CZ	4.4 ± 0.6	2.3 ± 0.8	5.2 ± 1.9	3.2 ± 0.9	4.3 ± 1.5	2 ± 0.8	3.6 ± 1.4	1.3 ± 0.2
GE	0.1 ± 0.03	0.1 ± 0.09	0.6 ± 0.2	0.4 ± 0.2	2.3 ± 2.1	0.6 ± 0.3	4.4 ± 1.9	4.7 ± 8.1
TR	1.7 ± 0.9	1.6 ± 0.4	2.1 ± 0.7	1.4 ± 0.5	16.8 ± 10.5	0.6 ± 0.4	3.5 ± 3.2	0.7 ± 0.4
GR	10 ± 8.1	9.7 ± 2.9	14.4 ± 0.7	11 ± 4.8	14.3 ± 7.4	12.2 ± 5.7	13.7 ± 9.1	7 ± 2.9



Appendix-B Figure 1 a) Initial phytoplankton biovolume, b) Pooled phytoplankton biovolume



Appendix-B Figure 2 Non-Metric Multidimensional scaling results

APPENDIX C

This appendix is prepared from Hoyos *et al.*, (2014) and Phillips *et al.*, (2014) for Chapter 5

1. Danish Lake Phytoplankton Index (DLPI)

Appendix-C Table 1 Phytoplankton taxa indicative of number poor or nutrient rich conditions.

Algal class	Nutrient poor conditions	Nutrient rich conditions
Cyanophytes	*Gomphosphaeria lacustris*	*Woronichinia sp.*
	G. littoralis	*Merismopedia tenuissima*
	Synechococcus elongatus	*M. warmingiana*
		Microcystis incerta
		M. viridis
		Cyanonephron styloides
		Anabaenopsis sp.
		A. elenkinii
		Lyngbya contorta
		Oscilatoria limnetica v. acicularis
		O. plantonica
Cryptophytes	*Radiocystis geminata*	
Crysophytes	*Dinobryon divergens*	
	D. bavaricum	
	D. cylindricum	
	D. sociale	
Dinophytes	*Gymnodinium sp.*	
	G. uberrimum	
	Peridinium cinctum	
	P. inconspicuum	
	P. volzii	
	P. willei	
	P. umbonatum group	
	Mallomonas akrokomos	
	Ochromonas sp.	
	Uroglena sp.	
	Chromulina sp.	
	Apedinella/Pseudopedinella sp	
Diatoms	*Synedra acus v. angustissima*	*Synedra berolinensis*
Euglenophytes		*Phacus sp.*

Algal class	Nutrient poor conditions	Nutrient rich conditions
Chlorophytes	*Pseudosphaerocystic lacustris*	*Actinastrum hantzchii*
	Ankyra lanceolata	*Coelastrum astroideum*
	Botryococcus sp.	*Crucigenia tetrapedia*
	Botryococcus braunii	*Monoraphidium sp.*
	Eutetramorus fottii	*Pediastrum sp.*
	Spaerocystis schroeterii	*Scenedesmus spp, desmodesmus*
	Stichococcus sp.	*S. spp, acutodesmus group*
	Mougeotia sp.	*S. acuminatus*
	Oodogonium sp.	*S. acuminatus/acutus*
		S. opoliensis
		S. quadriqauda
		S. dimorphus
		Tetrastrum staurogeniaeforme
		Planktonema lauterbornii
		Closterium limneticum

Appendix-C Table 2 Calculation of phytoplankton score (mean summer values). * Total biomass, **Sum of taxa from nutrient poor lakes minus sum of taxa from nutrient rich lakes.

Lake type/indicator	3 points		2 points		1 points	
Alkalinity (meq/l)	TA*>1	TA>1	TA>1	TA>1	TA>1	TA>1
Mean depth (m)	Z **< 3	Z > 3	Z < 3	Z > 3	Z < 3	Z > 3
EU-lake type	LCB2	LCB1	LCB2	LCB1	LCB2	LCB1
Chlorophyll a (µg/l)	< 11.7	<6.5	[11.7-	[6.5-	[25,56]	[12-
% Cyanobacteria *	< 5	< 10	[5-10]	[10-	[10,20]	[20-
% Chrysophytes*	> 1	> 10	[0.5-1]	[5-10]	[0,0.5[	[0.5-5]
Indicator species**	> 4	> 4	[2-4]	[2-4]	[-1,1]	[-1-1]

**TA: Total Alkalinity*

*** Z: Mean*

Appendix-C Table 3 Calculation of phytoplankton-EQR and ecological class based on total score (0-12 points).

Total score	Phytoplankton-	Phytoplankton EQR
0	0.1	Bad (0-0.2)
1	0.23	Poor (0.2-0.4)
2	0.30	Poor (0.2-0.4)
3	0.37	Poor (0.2-0.4)
4	0.43	Moderate (0.4-0.6)
5	0.50	Moderate (0.4-0.6)
6	0.57	Moderate (0.4-0.6)
7	0.63	Good (0.6-0.8)
8	0.70	Good (0.6-0.8)
9	0.77	Good (0.6-0.8)
10	0.83	High (0.8-1)
11	0.90	High (0.8-1)
12	0.97	High (0.8-1)

2. Netherlands Phytoplankton Indice

Appendix-C Table 4 Chlorophyll-a class (Chl-*a*, μg/L^{-1}). H:High, G:Good, M:Moderate, P:Poor, B:Bad.

Typology	Reference	H/G	G/M	M/P	P/B
LCB1	3.2	5.8	10	20	40
LCB2	6.8	10.8	23	46	96

3. Phytoplankton Metrics for Polish Lakes (PMPL)

Chl-a

Appendix-C Table 5 Boundaries for ecological status classes of chlorophyll a concentration (μg L^{-1}). H:High, G:Good, M:Moderate, P:Poor, B:Bad, VQ (Schindler's ratio).

Type of lake	VQ	H/G	G/M	M/P	P/B
Stratified	<2	5.2	7.7	11.1	16.3
	>2	7.1	12.8	21.4	32.8
Polymictic	<2	10.0	19.1	30.0	42.1
	>2	10.1	22.7	40.5	67.9

Appendix-C Table 6 The values of coefficients k, z and m for equation 1 and 2.

Type of lake	VQ	*k*	*z*	*m*
Stratified	<2	-32.698	0	26.081
	>2	-18.555	0.0369	13.293
Polymictic	<2	-11.252	0.0649	0.6414
	>2	-0.3334	0.2147	0.0357

a. Chl-a Total Biomass

Appendix-C Table 7 Boundaries for ecological status classes of total biomass (mg l-L^{-1}).

Type of lake	VQ	H/G	G/M	M/P	P/B
Stratified	<2	1.1	2.4	5.2	11.3
	>2	1.2	3.2	8.3	21.9
Polymictic	<2	1.8	4.6	11.6	29.3
	>2	1.9	5.3	14.5	29.1

Appendix-C Table 8 The values of coefficients k, z and m for total biomass metric.

Type of lake	VQ	k	m	z	o
Stratified	<2	0.8727	12.900	0	0
	>2	0.8135	10.325	0	0
Polimictic	<2	0.3778	10.720	0	0
	>2	29.511	0	0.0541	-28.344

b. Cyanobacteria

Appendix-C Table 9 Boundaries for ecological status classes of biovolume of Cyanobacteria.

Type of lake	VQ	H/G	G/M	M/P	P/B
Stratified	<2	0.6	1.1	2.3	4.7
	>2	0.8	1.9	4.8	12.1
Polymictic		0.93	2.3	5.7	13.9

Appendix-C Table 10 Boundaries of PMPL and EQR for ecological status classes.

Type of lake mixing	VQ	*k*	*m*
Stratified	<2	18.112	14.113
	>2	12.835	10.898
polymictic		10.803	11.072

Appendix-C Table 11 Boundaries of PMPL and EQR for ecological status classes.

PMPL	EQR	Ecological Status Class
0-1	0.8-1.0	high
1 - 2	0.6-0.8	good
2 - 3	0.4-0.6	moderate
3 - 4	0.2-0.4	poor
4 - 5	0-0.2	bad

APPENDIX D

Good / Moderate class boundary calculation

According to Hoyos *et al*., (2014) two parallel methodologies have been employed, each of them giving weight to different aspects of the data:

1. The first one use data statistical distribution of the lake dataset in different total phosphorus (TP) groups, specifically the 75th percentile of each metric in the TP group with values 20-50 μg L^{-1}.

2. The second approach is based on equidistant division using both ends (upper and lower of the data. The median of the MEP and reference values are used as the upper and the 95th percentile of all values per metric as the lower value. The equation provided by Hoyos *et al*. (2014) is:

$$G/M = 0.6 * (E1 - E0) + E0$$

E1: MEP median
E0: Maximum (worst quality) value of 95% all of cases

Compared to the G/M Boundary obtained with the intercalibration dataset, boundary values obtained from the BM reservoirs are in general higher. This shows that the quality of the BM water bodies are in general lower compared with the average Mediterranean ones (Table 1-A).

Appendix-D Table 1 Establishing the Good/Moderate boundary in the NMASRP metrics based on the Büyük Menderes River Basin lake WBs dataset and according to the Intercalibration L-M8 type reservoirs dataset.

Method for G/M boundary		Total BV (mm^3/L^{-1})	IGA	Cyan BV (mm^3/L^{-1})	Chl-a ($\mu g/L^{-1}$)
1st Method	Best 75th percentile where TP=20-50 µg/L	3.1	0.3	0.002	2.8
2nd Method	E1=MEP Median	0.5	0.3	0.04	6.1
	E0= Maximum (worst quality) value of 95% of samples	30.2	31.6	5.2	32.5
	G/M Boundary = 0.6 * (E1 - E0) + E0	12.4	12.8	2.1	16.6
Average G/M Boundary according to the Büyük Menderes dataset		7.8	6.5	1.1	9.7
G/M Boundary according to the Med. Indercalibration dataset for L-M8 Type		***2.5***	***6.5***	***0.5***	***5.3***

EQR calculation results

EQR values was calculated with the equations given in Table 1-B. The values were greater than 1 were truncated to 1. The following table provides the EQR values for all seasons in the BMRB reservoirs. Potential values (Average of MEP WBs values) were substituted accordingly.

Appendix-D Table 2 Equations by Hoyos *et al*., (2014) for the calculations of the EQR values of the WBs. The MEP values of the current study for each parameter are used.

Metric	Reservoir IC type	MEP value (Buyuk Menderes)	*MEP value (Intercalibration)*	EQR calculation
Total BV (mm^3 L^{-1})	L-M8	1.5	*0.9*	(1/x)/(1/MEP value)
IGA	L-M8	0.6	*2.1*	(1/x)/(1/ MEP value)
Cyan BV (mm^3 L^{-1})	L-M8	0.04	*0.005*	(400-x)/(400- MEP value)
Chl-a (μg L^{-1})	L-M8	6.4	*1.9*	(1/x)/(1/ MEP value)

Appendix-D Table 3 EQR values of the Büyük Menderes water bodies per season for each metric by taking into account the reference values and EQR derived from the Büyük Menderes reservoirs and the Intercalibration reservoirs dataset

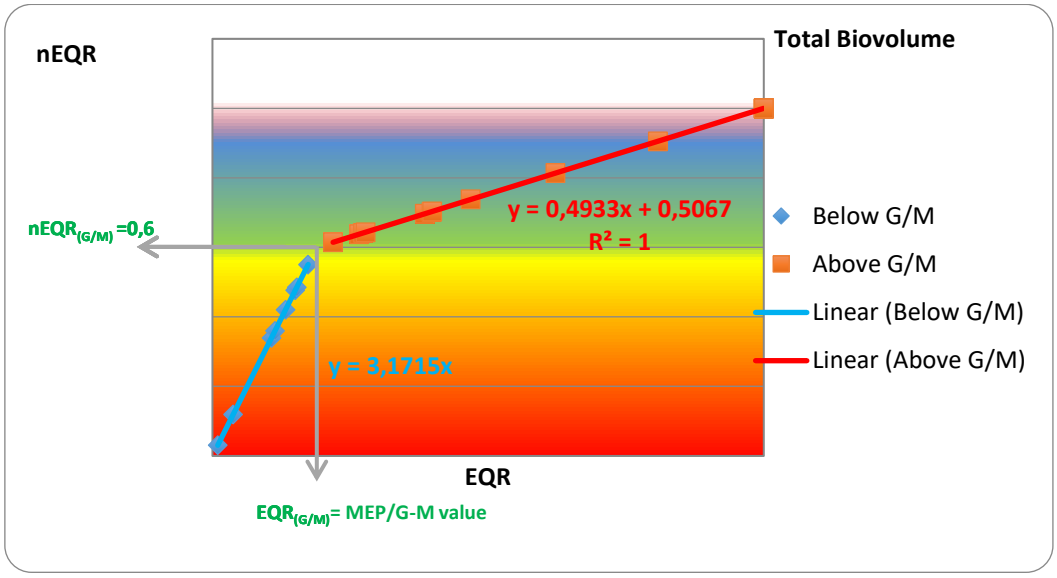
WB NAME	Season*	EQR based on the BM reservoirs				*EQR based on the*			
		Total BV	IGA	Cyan BV	Chl-*a*	*Total BV*	*IGA*	*Cyan BV*	*Chl-a*
Adiguzel	HF	0.2	1.0	1.0	0.3	*0.4*	*1.0*	*1.0*	*0.1*
	LF	0.2	1.0	0.2	0.6	*0.4*	*1.0*	*0.1*	*0.2*
Bafa	HF	0.6	1.0	1.0	0.4	*1.0*	*1.0*	*1.0*	*0.1*
	LF	0.4	1.0	0.2	0.1	*0.9*	*1.0*	*0.2*	*0.0*
Cindere-Adiguzel 2	HF	1.0	1.0	1.0	1.0	*1.0*	*1.0*	*1.0*	*0.6*
	LF	0.0	1.0	0.0	0.2	*0.1*	*1.0*	*0.0*	*0.1*
Cine Adnan Menderes	HF	1.0	0.9	1.0	1.0	*1.0*	*0.9*	*1.0*	*0.3*
	LF	0.3	1.0	1.0	1.0	*0.6*	*1.0*	*1.0*	*1.0*
Gokpinar	HF	0.1	1.0	1.0	0.3	*0.3*	*1.0*	*1.0*	*0.1*
	LF	0.1	1.0	0.2	0.1	*0.3*	*1.0*	*0.1*	*0.0*
Ikizdere	HF	1.0	1.0	1.0	1.0	*1.0*	*1.0*	*1.0*	*0.5*
	LF	0.2	1.0	0.2	1.0	*0.5*	*1.0*	*0.1*	*1.0*
Isikli	HF	1.0	1.0	1.0	0.9	*1.0*	*1.0*	*1.0*	*0.3*
	LF	0.2	1.0	0.0	1.0	*0.4*	*1.0*	*0.0*	*0.4*
Karacasu Baraji	HF	1.0	1.0	1.0	0.9	*1.0*	*1.0*	*1.0*	*0.3*
	LF	0.3	1.0	1.0	0.2	*0.7*	*1.0*	*1.0*	*0.1*
Karakuyu	HF	1.0	1.0	1.0	1.0	*1.0*	*1.0*	*1.0*	*1.0*
	LF	1.0	1.0	1.0	1.0	*1.0*	*1.0*	*1.0*	*1.0*
Kemer	HF	0.4	1.0	1.0	0.4	*1.0*	*1.0*	*1.0*	*0.1*
	LF	0.8	1.0	1.0	1.0	*1.0*	*1.0*	*0.9*	*0.4*
Orenler	HF	0.1	1.0	0.0	0.1	*0.3*	*1.0*	*0.0*	*0.0*
	LF	0.0	0.5	0.0	0.0	*0.0*	*0.5*	*0.0*	*0.0*
Tavas-Yenidere	HF	0.3	1.0	1.0	1.0	*0.6*	*1.0*	*1.0*	*0.4*
	LF	1.0	1.0	1.0	0.6	*1.0*	*1.0*	*1.0*	*0.2*
Topcam	HF	1.0	1.0	1.0	1.0	*1.0*	*1.0*	*1.0*	*0.3*
	LF	1.0	1.0	0.5	0.6	*1.0*	*1.0*	*0.2*	*0.2*
Ikizdere	HF	0.4	0.9	1.0	0.4	*0.9*	*0.9*	*1.0*	*0.1*
	LF	0.5	1.0	1.0	0.5	*1.0*	*1.0*	*1.0*	*0.2*

HF: High Flow (March 2014)

LF: Low Flow (September 2014)

Normalization equations

Normalization of the EQR values to nEQR were done with linear transform of the EQR values. This step was carried out in order to scale EQR values between zero to one scale where 0.6 represented G/M boundary. Normalization equations derived from linear transformation were given in Figure 1-A and Table 1-D and.



Appendix-D Figure 1 Example of deriving normalization equations for values above (red) and below (blue) G/M class boundary EQR. nEQR ranges from 0 to 1 and EQR for G/M boundary corresponds to the nEQR value of 0.6.

Appendix-D Table 4 Normalization equations for EQR values for all metrics based on the G/M boundaries found in the current study.

Metric	**Büyük Menderes**		***Intercalibration***	
	G/M	**Normalising equation**	***G/M***	***Normalising equation***
Total biovolume ($mm^3 L^{-1}$)	> 7.750	nEQR = 3.1715*EQR	*> 2,5*	*nEQR = 1.6667*EQR*
	≤ 7.750	nEQR = 0.4933*EQR +0.5067	*≤ 2,5*	*nEQR = 0.6250*EQR + 0.3750*
IGA index	> 6.536	nEQR = 0.6090*EQR	*> 6,5*	*nEQR = 0.6067*EQR*
	≤ 6.536	nEQR = 26.9349*EQR -25.9349	*≤ 6,5*	*nEQR = 36.1727*EQR - 35.1727*
Cyanobacteria. Biovolume ($mm^3 L^{-1}$)	> 1.054	nEQR = 15.9615*EQR	*> 0,5*	*nEQR = 60.0*EQR*
	≤ 1.054	nEQR = 0.4156*EQR + 0.5844	*≤ 0,5*	*nEQR = 0.4040*EQR + 0.5960*
Chl-a ($\mu g L^{-1}$)	> 9.715	nEQR = 0.9061*EQR	*> 5,3*	*nEQR = 1.6737*EQR*
	≤ 9.715	nEQR = 1.1842*EQR – 0.1842	*≤ 5,3*	*nEQR = 0.6235*EQR + 0.3765*

APPENDIX E

Appendix-E Table 1: List of species found in the studied shallow lakes.

1: Hamam, 2: Poyrazlar, 3: Abant, 4: Büyük, 5: Derin, 6: Nazlı, 7: İnce, 8: Serin, 9: Pedina, 10: Eymir, 11:Mogan, 12: Taşkısığı, 13: Küçük Akgöl, 14: Büyük Akgöl, 15: Çubuk, 16: Gölcük Bolu. 17: Yeniçağa, 18: Gölhisar, 19: Mert, 20: Erikli, 21: Saka, 22: Gebekirse, 23: Barutçu, 24: Karagöl İzmir, 25: Gölcük Ödemiş, 26: Emre, 27: Gökgöl, 28: Karagöl Denizli, 29: Azap, 30: Gölcük Sakarya, 31: Yayla, 32: Saklı, 33: Baldımaz, 34: Gıcı, 35: Tatlı, 36: Sarıkum, 37: Kocagöl, 38: Gerede, 39: Keçi, 40: Karagöl Bolu, 41: Uyuz, 42: Balıklı, 43: Kaya, 44: Eğri, 45: Sarp, 46: Kaz, 47: Seyfe Göleti

Bacillariophyta	
Achnantes spp.	39,46,47
Achnanthes minutissima Kützing	4,15,42,47
Amphora sp.	42
Asterionella formosa Hassall	3,4,6,7,8,16,24,37,38,39,40,47
Attheya zachariasii Brun	25
Aulacoseira granulata (Ehrenberg) Simonsen	2,4,7,12,14,15,16,17,21,25,26,29,32,39,40,37, 47
Boreozonacola sp.	6
Caloneis sp.	6,26,27
Cocconeis placentula Ehrenberg	2,3,6,7,8,14,15,16,19,20,26,27,30,31,32,34,33, 35,36,37,38,42,43,44,45,46,47,54,50
Craticula sp.	42
Cyclotella menenghiniana Kützing	8,34,36,38,39,42,37,43,44,47
Cyclotella ocellata Pantocsek	3,8,15,16,38,39,40
Cyclotella sp.	2,3,6,10,16,18,19,20,22,25,26,27,29,35,34,42, 44,46
Cymbella sp.	2,6,15,16,19,21,24,26,27,32,37,46
Cymbella turgida W Greg.	37,43
Cymbella neocistula Krammer	41,42,43
Cymbella helvetica Kützing	46
Cymbella cistula (Hemprich & Ehrenberg) O.Kirchner	47
Diatoma sp.	3,14

Bacillariophyta	
Epithemia sp.	16,31,34,36,40,46
Epithemia adnata (Kützing) Brébisson	34,46
Epithemia sorex Kützing	19,20,27,30,31,32,35,41,37,46
Fragilaria sp.	2,8,38,43,34,40
Fragilaria crotonensis Kitton.	38
Frustulia sp.	46
Frustulia rhomboides (Ehrenberg) De Toni	43
Gomphonema sp.	3,14,29,32,44,46
Gomphonema augur Ehrenberg	21
Gomphonema olivaceum (Hornemann) Ehrenberg	6,7,16,26,27,41,42,43,44,45,46,34,37
Gomphonema parvulum Kützing	42
Gomphonema truncatum Ehrenberg	15,31,41,42,43,46,37
Gyrosigma acuminatum Rabenhorst	2,3,7,16,34,39, 37
Gyrosigma attenuatum (Kützing) Rabenhorst	4,2
Melosira sp.	26
Melosira varians C.Agardh	42,43,46
Navicula viridula (Kützing) Ehrenberg	19
Navicula radiosa Kützing	34,35,45
Navicula cuspidata (Kütz.) Kütz.	34,39,40
Navicula sp.	1,3,4,6,7,8,16,19,20,21,26,32,33,34,37,39,41,44,45,46,36,50
Nitzschia amphibia Grunow	36
Nitzschia acicularis (Kützing) W.Smit	19,22,28,29,31,36,40 ,42,43,46,47
Nitzschia palea (Kützing) W.Smith	41,42,34
Nitzschia linearis West	42
Nitzschia sigmoidea W.Smith	42
Nitzschia recta Hantzsch ex Rabenh.	39
Nitzschia sp.	14,19,20,27,41,34,46,47
Planktonema sp.	2
Rhopalodia gibba O. Müller	35,40,46
Rhopalodia gibberula (Ehrenberg) Otto Müller	22,46
Rhoicosphenia curvata (Kütz.) Grunow	35
Stephanodiscus parvus Stoermer & Håkansson	39,42,43
Surirella peisonis Pantocsek	41
Surirella striatula Turpin	20
Synedra sp.	2,4,8,10,11,14,15,16,19,20,21,27,29,36

Bacillariophyta	
Synedra capitata Ehrenberg	46
Synedra acus Kützing	38,46
Synedra ulna (Nitzsch) Ehrenberg	3,6,22,24,28,31,38,39,40,41,42,43,34,35,37,45 ,46,47,50
Tabelleria sp.	46
Tabellaria flocculosa (Roth) Kützing	47
Mougeotia sp.	38
Chlorophyta	
Actinastrum hantzschii Lagerheim	24,25,27,29,34,35,39,46,47
Ankistrodesmus gracilis (Reinsch) Korshikov	28
Ankistrodesmus densus Korshikov	31
Botryococcus braunii Kützing	40
Carteria sp.	46,5,6,20,27
Carteria multifilis (Fresenius) O.Dill	47
Chlamydomonas sp.	2,31,39,42,38,43,45,
Chlorella minutissima Fott & Novakova	43
Chlorella vulgaris Beyerinck	23
Closteriopsis acicularis (G. M. Smith) J. H. Belcher et Swale	34
Closterium acerosum Ehrenberg ex Ralfs	17
Closterium acutum Brébisson in Ralfs	2,10,16,18
Closterium aciculare T.West	45
Closterium dianae Ehrenberg ex Ralfs	24,34
Closterium gracile Brébisson ex Ralfs	24,4
Closterium littorale F.Gay	34
Closterium cornu Ehrenberg ex Ralfs	24
Closterium parvulum Nägeli	31
Closterium venus Kützing ex Ralfs	46
Closterium sp.	1,11,26
Coelastrum sp.	14,15,30
Coelastrum astroideum De Notaris	15,17,18,24,26,27,29,30,31,34,38,40,43,46,47
Coelastrum microporum Nägeli in A. Braun	25,28,31,35,37,38,43,40,46,47
Coelastrum morus West & G.S.West	38
Coelastrum sphaericum Nägeli	24
Cosmarium sp.	1,3,28,46
Cosmarium bioculatum Brébisson ex Ralfs	24,31,4
Cosmarium bireme Nordstedt	40

Chlorophyta	
Cosmarium blyttii Wille	34
Cosmarium botrytis Meneghini ex Ralfs	34
Cosmarium granatum Brébisson ex Ralfs	36
Cosmarium laeve Rabenhorst	15,31,35,36
Crucigenia tetrapedia (Kirchner) West & G.S.West	10,11,18,21,23,24,28,31,32,37,38,39
Crucigenia quadrata Morren	28
Crucigeniella crucifera (Wolle) Komárek	2,16,18,23,28,31,35
Crucigeniella apiculata Komarek	40
Crucigeniella irregularis (Wille) P.M.Tsarenko & D.M.John.	27
Dictyosphaerium sp.	1,10
Dictyosphaerium pulchellum H.C.Wood	2,18,33
Euastrum binale (Turpin) Ehrenberg ex Ralfs	35
Euastrum insulare (Wittrock) J.Roy	34
Franceia sp.	24
Golenkinia radiata Chodat	21,25,26,27,29,30
Gonathozygon sp.	46
Kirchneriella sp.	1,2,3,10,11,12,15,16,18,21,29
Kirchneriella cornuta (Schmidle) Bohl	37
Kirchneriella obesa (West) Schmidle	31,37
Lagerheimia genevensis (Chodat) Chodat	10,31,37,38
Lagerheimia subsalsa Lemmermann	14,16
Monoraphidium sp.	1,2,4,10,11,14,16,21,26,27
Monoraphidium circinale Nygaard	36,37,38
Monoraphidium arcuatum (Korshikov) Hindák	24,29,40,44,46,50
Monoraphidium minutum (Nägeli) Komàrková-Legnerová	23,28,37,38,39,40,43
Monoraphidium contortum (Thuret) Komàrková-Legnerová	1,2,4,7,10,15,16,18,20,21,23,24,27,28,30,31,32,34,35,37,40,42,44,45
Monoraphidium komarkovae Nygaard	25,28,31,34,35,38,39,40,42,43,44,37
Monoraphidium dybowskii (Wolosz.) Hindak & Komark-Legn.	37
Monoraphidium griffithii (Berk.) Komark-Legn.	23,24,28,29,31,40,43,44,45,46,47
Monoraphidium tortile (West & G.S.West) Komárková-Legnerová	28,31,50
Micractinium pusillum Fresenius	2,27
Mougeotia sp.	8,44,50,46

Chlorophyta	
Nephrocytium agardhianum Nägeli	31
Oedogonium sp.	41
Oocystis sp.	4,10,11,16,26
Oocystis apiculata West	34
Oocystis lacustris Chodat	43,37
Oocystis parva West & G.S.West	34,40,43
Oocystis borgei J.Snow	3,17,18,19,25,37
Planktonema sp.	18
Pandorina sp.	2,25,31
Pandorina morum (O.F.Müller) Bory	31,34
Pediastrum boryanum (Turpin) Meneghini	12,16,30,38,42,43,47
Pediastrum duplex Meyen	12,24,25,43,37,47
Pediastrum tetras (Ehrenberg) Ralfs	13,16,25,27,28,29,30,31,40,43,46
Pediastrum simplex Meyen	25
Phacotus sp.	39
Planktosphaeria sp.	8
Pyramimonas sp.	23
Scenedesmus sp.	2,4,5,6,8,10,13,14,15,18,26,30,31
Scenedesmus apoliensis Richter	27
Scenedesmus acutus Meyen	30,42
Scenedesmus arcuatus (Lemmermann) Lemmermann	15,24,25,27,28,31,35,44
Scenedesmus armatus (R.Chodat) R.Chodat	46
Scenedesmus apiculatus (West - G.S.West) Chod.	40
Scenedesmus bicaudatus Dedusenko	8,31
Scenedesmus communis E.Hegewald	12,18,20,24,29,25,30,31,35,40,42,43,44,46,47, 50,37
Scenedesmus dimorphus (Turpin) Kützing	23,31,38,40,42,43,46,47
Scenedesmus dispar Brébisson	34,47
Scenedesmus ecornis (Ehrenberg) Chodat	24,25,34
Scenedesmus falcatus Chodat	25,31,43,45
Scenedesmus insignis (W.& G.S.West) Chodat	37,40
Scenedesmus obliquus (Turpin) Kützing	50
Scenedesmus quadricauda Chodat	25,34,37,38,47
Scenedesmus longispina Chod.	36
Scenedesmus sempervirens Chod.	39,43

Chlorophyta	
Scenedesmus obtusus Meyen	28
Scenedesmus intermedius Chodat	25
Scenedesmus regularis Svirenko	2
Scenedesmus linearis Komárek	12,16
Scenedesmus acuminatus (Lagerheim) Chodat	27,29,30
Schroederia sp.	10
Selenastrum westii G.M.Smith	28
Sphaerocystis sp.	38
Sphaerocystis planctonica (Korshikov) Bourrelly	31
Spirogyra sp.	8,42,44,46
Staurastrum apiculatum Brébisson	31,38
Staurastrum gracile Ralfs	35
Staurastrum punctulatum (Brébisson) Ralfs	38
Staurastrum pingue Teiling	40
Staurastrum sp.	1,2,12,13,15,16,18,24,25,27,40, 46
Tetraedron caudatum (Corda) Hansgirg	15,16,30,40,43
Tetraedron minimum (A.Braun) Hansgirg	2,3,10,11,12,15,16,18,20,23,24,25,26,28,30,31 ,32,34,35,36,38,39,40,43,44,37
Tetraedron triangulare Korshikov	2,3,15,23,24,25,34,35,38,43,36
Tetrastrum sp.	14,15,16,29,30,41,34,46,47
Tetrastrum staurogeniiforme (Schröder)	24
Tetrastrum elegans Playfair	24,25
Treubaria sp.	2, 14
Treubaria schmidlei (Schröder) Fott & Kovácik	39
Zygnema sp.	41
Cryptophyta	
Cryptomonas sp.	1,2,3,4,5,6,7,8,10,11,12,14,15,16,18,19,20,21, 23,25,26,27,29,30,31,36,47
Cryptomonas erosa Ehrenberg	38, 39,40,42,50,34,35, 37
Cryptomonas ovata Ehrenberg	2,3,7,10,24,25,27,28,34,35,36,38,39,40,42,43, 44,45,46,50
Cryptomonas marssonii Skuja	34
Cryptomonas rostratiformis Skuja ex Willen	28,38
Rhodomonas sp.	3,5,6,8,15,16,17,18,27,35
Rhodomonas lacustris Pascher *et* Ruttner in Pascher & Lemmermann	11,23,25,28,34,36,37,38,42,43,45,46,50

Cyanophyta	
Anabaena affinis Lemmermann	37
Anabaena catenula Kützing ex Bornet & Flahault	40,41
Anabaena planctonica Brunnthaler	39
Anabaena verrucosa J. B. Petersen	38,40
Anabaena delicatula Lemmermann	31
Anabaena sp.	1,2,6,8,10,12,13,14,15,16,19,20,21,24,29,35,43
Anabaena flos-aquae Brébisson ex Bornet & Flauhault	24
Anabaenaopsis sp.	10,11,23,24,29,34,35,40
Aphanizomenon sp.	11,12,14,17,20
Aphanizomenon gracile Lemmermann	40,43
Aphanizomenon ovalisporum Forti	36
Aphanocapsa sp.	12,14,16,17,18,29,33
Aphanocapsa elachista West & G.S.West	3,36
Aphanocapsa incerta (Lemmermann) Cronberg & Komárek	15,28,35,43,40
Arthrospira sp.	29
Chroococcus limneticus Lemmermann	34,35
Chroococcus minimus (Keissler) Lemmermann	22,36
Chroococcus sp.	38,44,47
Chroococcus turgidus (Kützing) Nägeli	2,14,34,36,42
Coelosphaerium kuetzingianum Nägeli	35
Dolichospermum affine (Lemmermann) P.Wacklin, L.Hoffmann & J.Komárek	35,5
Gomphosphaeria sp.	31,4
Geitlerinema splendidum (Greville ex Gomont) Anagnostidis	35
Glaucospira sp.	10,11,20,29,42,50
Hydrococcus sp.	42,44
Jaaginema metaphyticum Komárek in Anagnostidis et Komárek	42,43
Jaaginema sp.	2,7,12,19,20,22,29,30,41
Jaaginema subtilissimum (Kützing ex De Toni) Anagnostidis & Komárek	36
Komvophoron sp.	27,35,34,39
Leptolyngbya lignicola (Frémy) Anagnostidis & Komárek	40

Cyanophyta	
Leptolyngbya lurida (Gomont) Anagnostidis & Komárek	38
Limnothrix sp.	10
Limnothrix redekei (Goor) M.E.Meffert	46
Lyngbya sp.	19,44
Lyngbya martensiana (Meneghini) Gomont	35,44
Merismopedia sp.	4,8,11,12,16,21,24,26,27,29,31,46
Merismopedia glauca (Ehrenberg) Nägeli	24,35,36,38,42
Merismopedia punctata Meyen	3,30,34,35
Merismopedia teunissima Lemmermann	15,17,18,20,22,24,25,31,35,37,40,42,43
Merismopedia minutissima Joosten	22,23
Microcystis sp.	3,12,13,14,17,26,29,30,46
Microcystis flos-aquae (Wittrock) Kirchner	31,34,35
Microcystis wesenbergii (Komárek) Komárek	12,13,14,17,35
Microcystis aeruginosa (Kütz) Kütz.	14,40,44
Nodularia spumigena (Mertens)Bornet *et* Flahault	41
Oscillatoria sp.	10,45,46
Oscillatoria limosa (C.Agardh) Gomont	35
Oscillatoria tenuis (C.Agardh) Gomont	37,43
Oscillatoria sancta Kützing ex Gomont	46
Phormidium sp.	6,8,15,16,26,29,35,47
Planktotrix sp.	31,44
Planktothrix agardhii (Gomont) Anagnostidis & Komárek	25,34,35,46
Planktolyngbya sp.	10,12,23,44,45
Planktolyngbya contorta (Lemmermann) Anagnostidis & Komárek	22
Planktolyngbya limnetica (Lemmermann) J.Komárková-Legnerová & G.Cronberg	2,46
Pseudoanabaena sp.	6,7,10,11,12,15,16,19,20,21,26,41,44,47
Spirulina sp.	15,26,27,29
Spirulina major Kützing ex Gomont	28,41,50
Snowella atomus Komárek & Hindák	24
Synechococcus sp.	10
Trichodesmium lacustre Klebahn	23
Woronichinia sp.	46

Cyanophyta	
Woronichinia elorantae J.Komárek & J.Komárková-Legnerová	22
Woronichinia compacta (Lemmermann) Komárek & Hindák	24
Woronichinia naegeliana (Unger) Elenkin	40
Euglenophyta	
Euglena sp.	1,2,3,5,6,7,10,11,12,14,16,20,21,26,27,29,30,47
Euglena viridis Ehrenberg	1,2,8,17,21,23,25,31,34,36,39,40,42,44,45
Euglena acus Ehrenberg	2,4,11,21,27,28,31,46,47,50
Euglena tripteris (Dujardin) G. A. Klebs	47
Phacus caudatus K. Hübner	43,44,47
Phacus curvicauda Svirenko	43,46
Phacus triqueter (Ehrenberg) Dujardin	28,31
Phacus tortus (Lemmermann) Skvortzov	31
Phacus longicauda (Ehrenberg) Dujardin	31
Phacus monilatus Stokes in Lemmerman	31
Phacus orbicularis K.Hübner	31
Phacus pleuronectes (O.F.Müller) Nitzsch ex Dujardin	46
Phacus helikoides Pochmann	44
Phacus sp.	1,2,6,7,21,27
Strombomonas deflandrei (Y.V.Roll) Deflandre	31
Trachalemonas sp.	38,39
Trachelomonas caudata (Ehrenberg) Stein	31
Trachelomonas hispida (Perty) F. Stein	23,25,28,31,35,38,40,42,43
Trachelomonas lacustris Drezepolski	31
Trachelomonas volvocina Ehrenberg	23,24,25,38,40,46,47
Dinophyta	
Ceratium hirundinella (O.F.Müller) Dujardin	3,24,26
Ceratium furcoides (Levander) Langhans	2,12,15,26
Glenodinium sp.	35,37,43
Gymnodinium sp.	3,4,10,11,16,19,20,24,26,29,32,33,44,46
Gymnodinium limneticum Woloszynska	5,36
Peridiniopsis cunningtonii (Lemmermann) Popovsky et Pfiester	25,37,38,39
Peridinium lomnickii Woloszynska	34,37,38
Peridinium umbonatum F.Stein	24,28,45,46

Dinophyta	
Peridiniopsis sp.	21
Peridinium sp.	1,2,6,8,12,15,27,28,30,32,33,35,44,47,50
Chrysophyta	
*Bitrichia longispina (*J.W.G.Lund) Bourrelly	50
Dinobryon divergens O.E.Imhof	2,4,8,15,31,37,38,39,40,42,43,46
Dinobryon sp.	6
Mallomonas elliptica (Kisselev) W.Conrad	28
Mallomonas sp.	24,29,38,39,44,47

Appendix-E Table 2 List of species found in the Büyük Menderes River Basin.

1: Gölpınar, 2: Karacasu, 3: İkizdere, 4: Cindere, 5: Adıgüzel, 6: Adnan Menderes, 7: Örenler, 8: Topçam, 9: Kemer, 10: Karakuyu, 11: Yenidere, 12: Yaylakavak, 13: Bafa, 14: Işıklı

Bacillariophyta	
Achnantes sp.	14
Achnanthes minutissima Kützing	2,10,14
Amphora sp.	14
Asterionella formosa Hassall	3,4,5,6,8,13
Aulacoseira granulata (Ehrenberg) Simonsen	1,2,5,6,7,8,9,10,12,13
Campylodiscus sp.	13
Chaetoceros sp.	13
Caloneis sp.	4,12
Cocconeis placentula Ehrenberg	10,13,14
Craticula sp.	10
Cyclotella menenghiniana Kützing	5,7,8,14
Cyclotella ocellata Pantocsek	2,3,4,5,6,7,8,9,10,11,12,13,14
Cyclotella sp.	1,8,9,12,13,14
Cymatopleura solea (Brébisson) W.Smith	7,13
Cymbella sp.	2,3,4,5,10,12,13,14
Cymbella amphicephala Näegeli in Kützing	2

Bacillariophyta	
Cymbella cistula (Hemprich & Ehrenberg) O.Kirchner	14
Diatoma sp.	2
Diatoma vulgaris Bory	2,13
Epithemia sp.	14
Epithemia sorex Kützing	3,10,12,14
Fragilaria sp.	1,2,4,5,6,8,13,14
Fragilaria brevistriata Grunow in van Heurck	10
Fragilaria ulna (Nitzsch) Lange-Bertalot	2,6,11,13
Gomphonema sp.	10
Gomphonema augur Ehrenberg	4
Gomphonema olivaceum (Hornemann) Ehrenberg	10,11,13
Gomphonema parvulum Kützing	2,13
Gomphonema truncatum Ehrenberg	12,14
Gyrosigma acuminatum Rabenhorst	7,8,10,11,12,13
Melosira sp.	8,12,13
Mastoglia sp.	6,7,9,13
Mastogloia smithii Thwaites ex W.Smith	1
Melosira varians C.Agardh	8,12,13
Navicula clementis Grunow	8
Navicula pupula Kützing	8
Navicula viridula (Kützing) Ehrenberg	1,3,
Navicula radiosa Kützing	14
Navicula rhychocephalia Kützing	2
Navicula sp.	2,7,8,10,11,12,13,14
Nitzschia acicularis (Kützing) W.Smit	2,3,8,11,13
Nitzschia circmsuta	13
Nitzschia sigmoidea W.Smith	13
Nitzschia tryblionella Hantzsch in Rabenhorst	13
Nitzschia sp.	1,2,7,8,11,13,14
Pinnularia sp.	4
Rhopalodia gibba O. Müller	13
Stephanodiscus sp.	4
Surirella sp.	5
Surirella striatula Turpin	13
Synedra sp.	2,9,11

Bacillariophyta	
Synedra acus Kützing	8,11,12,13
Synedra ulna (Nitzsch) Ehrenberg	1,3,4,5,7,8,9,10,11,13,14
Thalassionema nitzschioides (Grunow) Mereschkowsky	13
Chlorophyta	
Actinastrum hantzschii Lagerheim	7,13
Ankistrodesmus fusiformis Corda	5,7
Carteria sp.	5,11
Chlamydomonas sp.	4
Closterium acerosum Ehrenberg ex Ralfs	7,13
Closterium acutum Brébisson in Ralfs	1,3,4,5,6,9,11,12,13
Closterium aciculare T.West	8,9,13,14
Closterium dianae Ehrenberg ex Ralfs	7,13,14
Closterium gracile Brébisson ex Ralfs	7,8
Closterium littorale F.Gay	8
Closterium sp.	11,12
Coelastrum sp.	4,9
Coelastrum astroideum De Notaris	1,2,3,5,6,7,8,9,11,13,14
Coelastrum microporum Nägeli in A. Braun	9,13
Coelastrum reticulatum (P.A.Dangeard) Senn	9
Cosmarium sp.	10,11
Cosmarium bioculatum Brébisson ex Ralfs	14
Cosmarium bireme Nordstedt	6,14
Cosmarium laeve Rabenhorst	1,4,6,14
Cosmarium ornatulum Coesel	6
Cosmarium pseudopyramidatum P.Lundell	2,14
Cosmarium punctulatum Brébisson	3,6,12
Cosmarium retusum (Perty) Rabenhorst	6
Cosmarium moniliforme Ralfs	14
Cosmarium tenue W.Archer	14
Crucigenia tetrapedia (Kirchner) West & G.S.West	2,11
Crucigenia quadrata Morren	13
Crucigeniella irregularis (Wille) P.M.Tsarenko & D.M.John.	6,14
Dictyosphaerium pulchellum H.C.Wood	4,5,7
Elakatothrix sp.	14
Eudorina elegans Ehrenberg	12,14

Chlorophyta	
Euastrum binale (Turpin) Ehrenberg ex Ralfs	14
Golenkinia radiata Chodat	5,6,7,8,11,12
Gonatozygon brebissonii De Bary	7,8
Gleocystis sp.	6
Kirchneriella sp.	4,5,11,13
Kirchneriella obesa (West) Schmidle	1,7,8,11,13,14
Lagerheimia genevensis (Chodat) Chodat	1,7,11
Lagerheimia subsalsa Lemmermann	6,12,14
Lagerheimia citriformis (J.W.Snow) Collins	2
Monoraphidium sp.	2,3,5,6,7,8,11,12,13
Monoraphidium arcuatum (Korshikov) Hindák	12
Monoraphidium contortum (Thuret) Komàrková-Legnerová	5,7,8,11,12,13,14
Monoraphidium griffithii (Berk.) Komark-Legn.	3,8,11
Micractinium pusillum Fresenius	7,13
Micractinium micans	7
Mougeotia sp.	6,8
Nephrocytium agardhianum Nägeli	6,14
Oocystis sp.	1,2,3,4,6,7,12,14
Oocystis parva West & G.S.West	6,7,14
Oocystis borgei J.Snow	3,4,6
Planktonema sp.	2,5,9,13
Pandorina sp.	6,11,14
Pandorina morum (O.F.Müller) Bory	5,6
Pediastrum boryanum (Turpin) Meneghini	2,3,5,6,7,11,13,14
Pediastrum duplex Meyen	2,5,7,8,11,13,14
Pediastrum tetras (Ehrenberg) Ralfs	12,13,14
Pediastrum simplex Meyen	1,2,5,9,11,13
Phacotus lenticularis (Ehrenberg) Deising	4
Pseudosphaerocystis sp.	*2*
Pseudoschroederia robusta (Korshikov) E.Hegewald & E.Schnepf	*2*
Scenedesmus sp.	2,4,6,7,8,11,12,13,14
Scenedesmus apoliensis Richter	14
Scenedesmus acutus Meyen	1,2,4,7,9,11,12
Scenedesmus bicaudatus Dedusenko	6,7,8,11,12
Scenedesmus communis E.Hegewald	1,7,9,13,14

Chlorophyta	
Scenedesmus denticulatus Lagerheim	7
Scenedesmus disciformis (Chodat) Fott & Komárek	2,3,5,6,14
Scenedesmus dispar Brébisson	6
Scenedesmus sempervirens Chodat	1
Scenedesmus linearis Komárek	2,6,7,9,11,14
Scenedesmus acuminatus (Lagerheim) Chodat	1,8
Scenedesmus semipulcher Hortobágy	12
Sphaerocystis sp.	3,4,5,8,9,11,14
Spirogyra sp.	13
Staurastrum tetracerum Ralfs ex Ralfs	14
Staurastrum furcatum Brébisson	14
Staurastrum lunatum Ralfs	3,14
Staurastrum sp.	1,3,4,5,6,7,9,11,12,13,14
Staurodesmus cuspidatus (Brébisson) Teiling	14
Tetraedron caudatum (Corda) Hansgirg	7,8
Tetraedron minimum (A.Braun) Hansgirg	1,3,4,5,6,7,8,9,10,11,12,13,14
Tetraedron triangulare Korshikov	10,14
Tetrastrum sp.	8,11,13
Tetrastrum staurogeniiforme (Schröder) Lemmermann	7
Tetrastrum elegans Playfair	7
Cryptophyta	
Cryptomonas sp.	1,4,5,6,8,9,10,11,13,14
Cryptomonas erosa Ehrenberg	2
Cryptomonas ovata Ehrenberg	1,2,7,14
Rhodomonas sp.	2,4,5,6,8,9,11,13,14
Rhodomonas lacustris Pascher *et* Ruttner in Pascher & Lemmermann	4
Cyanophyta	
Aphanocapsa delicatissima West & G.S.West	2
Anabaena sp.	3,4,5,7,8,12,14
Anabaena flos-aquae Brébisson ex Bornet & Flauhault	7
Anabaenaopsis sp.	12,13,14
Aphanothece sp.	11
Aphanizomenon sp.	5,7
Aphanocapsa sp.	7,9
Aphanocapsa elachista West & G.S.West	6,8,9,14

Cyanophyta	
Chroococcus limneticus Lemmermann	6
Chroococcus turgidus (Kützing) Nägeli	6,14
Cyanodictyon sp.	9
Gomphosphaeria sp.	10
Glaucospira sp.	2,3
Jaaginema sp.	4
Komvophoron minutum (Skuja) Anagnostidis & Komárek	14
Limnothrix sp.	3
Limnothrix redekei (Goor) M.E.Meffert	5,7,8
Lyngbya sp.	13
Merismopedia punctata Meyen	10,14
Merismopedia teunissima Lemmermann	3,7,14
Microcystis aeruginosa (Kütz) Kützing	5,14
Oscillatoria sp.	3,10,13
Phormidium sp.	6,7,10,13
Planktolyngbya sp.	12
Planktolyngbya limnetica (Lemmermann) J.Komárková-Legnerová & G.Cronberg	1,3,4,5,8,12
Pseudoanabaena sp.	1,6,8,9
Pseudosphaerocystis sp.	1,5
Raphidiopsis sp.	4
Snowella sp.	14
Spirulina sp.	2,9
Spirulina subsalsa Oersted ex Gomont	6,13
Euglenophyta	
Euglena sp.	3,7,8,11,13
Euglena viridis Ehrenberg	1,7,14
Euglena ehrenbergii G.A.Klebs	13
Phacus curvicauda Svirenko	1,11,13
Phacus longicauda (Ehrenberg) Dujardin	11
Phacotus lenticularis (Ehrenberg) Deising	11
Prorocentrum micans Ehrenberg	13
Strombomonas sp.	11
Trachalemonas sp.	3,7,8,11,12,13
Trachelomonas hispida (Perty) F. Stein	3
Trachelomonas volvocina Ehrenberg	8

Dinophyta	
Ceratium hirundinella (O.F.Müller) Dujardin	2,3,4,5,6,7,8,11,12,14
Ceratium furcoides (Levander) Langhans	4,5,8,9,12
Gymnodinium sp.	3,4,5,6,8,11,13,14
Peridiniopsis sp.	5,8,9,11,12,13
Peridinium sp.	1,2,3,4,5,6,14
Chrysophyta	
Dinobryon divergens O.E.Imhof	2,9,11,14
Dinobryon sp.	1
Xantophyta	
Chlorocloster sp.	2
Chlorocloster terrestris Pascher	4,7

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