IMPACTS OF EUTROPHICATION AND WATER LEVEL CHANGE IN TURKISH SHALLOW LAKES: A PALAEOLIMNOLOGICAL APPROACH UTILIZING PLANT REMAINS AND MARKER PIGMENTS

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ABSTRACT

IMPACTS OF EUTROPHICATION AND WATER LEVEL CHANGE IN TURKISH SHALLOW LAKES: A PALAEOLIMNOLOGICAL APPROACH UTILIZING PLANT REMAINS AND MARKER PIGMENTS

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Current study provides further understanding of plant macrofossil and sedimentary pigment utilization in comparison with modern and historical environmental variables. Moreover, it contributes to investigations for determining suitable macrophyte-based indices (water framework directive-WFD) that can be employed in Turkey. In total 44 small and mostly shallow lakes, covering the whole latitudinal gradient at western half of Turkey were sampled with snap-shot sampling and from another set of three large lakes cores were retrieved.

Plant remains and pigments acquired from surface sediment samples showed significant accord to present-day plant and phytoplankton assemblages, respectively. Conductivity and trophic state were key environmental variables correlated with plants, also temperature and nutrients with phytoplanktonkton. Comparison of instrumental water-level data (40-100 years) with core samples

from three lakes showed that effect of longer-term and pronounced water-level changes reflected in sediment record. Furthermore, employing plant remains in macrophyte-indice calculations increased the reliability of the results, which suggested that for shallow lakes, metrics based on species scores (including plant remains), and for deep lakes metrics using colonization depth may be more suitable.

Being particularly useful for lakes in Mediterranean regions, that are especially vulnerable to hydrological constraints under climate change, these comparisons were conducted for the first time in Turkish shallow lakes and this study confirmed that sedimentary plant and phytoplankton remains are reliable indicators of environmental change. Furthermore, importance of conductivity, nutrients and temperature underpins the concerns on salinisation and eutrophication in Mediterranean region, supporting exacerbation of these problems as predicted by climate change projections.

Keywords: Surface sediment, Sediment core, Multi-proxy studies, Water framework Directive

ÖTROFİKASYON VE SU SEVİYESİ DEĞİŞİMİNİN TÜRKİYE'DE BULUNAN SIĞ GÖLLER ÜZERİNDEKİ ETKİLERİ: BİTKİ KALINTILARI VE PİGMENTLER İLE PALAEOLİMNOLOJİK BİR YAKLAŞIM

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Bu tez göl çökellerinde bulunan bitki kalıntıları ve fitoplankton pigmentlerinin güncel ve geçmişe yönelik çevresel değişkenler ile karşılaştırılmasına ve ayrıca Türkiye için uygun olabilecek sucul bitki indekslerinin (Su Çerçeve Direktifi-SÇD) belirlenmesine katkıda bulunmaktadır. Bu bağlamda kuzeyden güneye Türkiye'nin batı yarısında bulunan toplam 44 göl örneklenmiş ve ayrı üç büyük gölden karot örnekleri alınmıştır.

Bu çalışma sonucunda yüzey çökelinde bulunan bitki kalıntıları ve pigmentlerin günümüzde göllerde bulunan bitki ve fitoplankton toplulukları ile uyumlu olduğu gözlemlenmiş ve bitki topluluklarının iletkenlik ve trofik durum ile, fitoplanktonun ise sıcaklık ve besin tuzları ile ilişkili olduğu bulunmuştur. Üç gölden alınan karotlardaki değişkenlerin 40-100 yıllık su seviyesi ölçümleri ile

ÖZ

karşılaştırılması ise uzun dönemli ve daha belirgin olan su seviyesi değişimlerinin etkilerinin çökel kayıtlarında gözlemlenebildiğini göstermiştir. Su çerçeve direktifi kapsamında hesaplanan makrofit indeksleri, sığ göllerde yüzey çökelindeki bitki verisinin de kullanılabildiği bitki tür skorlarını temel alan indeksler ile derin göllerde bitki kolonizasyon derinliğini kullanan indekslerlerin Türkiye'deki göller için daha uygun olabileceğini önermiştir.

Iklim değişikliğine bağlı olarak hidrolojik değişimlerden özellikle etkilenebileceği öngörülen Akdeniz iklim kuşağında bulunan bölgeler için bilhassa yararlı olabilecek güncel ve çökel veri setlerinin karşılaştırılması Türkiye'deki göller için ilk defa gerçekleştirilmiştir ve bu çalışma çökelde bulunan bitki kalıntıları ile pigmentlerin göllerdeki ekolojik durumun ve değişimin belirlenmesinde güvenilir belirteçler olduklarını doğrulamıştır. Ayrıca, bu çalışmada iletkenlik, besin tuzları ve sıcaklık değişkenlerinin bitki ve fitoplankton türleri için belirleyici etmenler olarak bulunmaları, iklim değişikliği ile birlikte Akdeniz iklim kuşağında daha da artacağı öngörülen tuzlanma ve ötrofikasyon problemlerinin önemine dikkat çekmektedir.

Anahtar Kelimeler: Yüzey çökeli, Karot, Çok değişkenli çalışma, Su çerçeve direktifi

To my family and friends

*

One child, one teacher, one book & one pen can change the world. (Malala Yousafzai)

*

Do not go around saying the world owes you a living. The world owes you nothing. It was here first. (Robert Jones Burdette)

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CHAPTER 1

INTRODUCTION

1.1 Shallow Lakes

Freshwaters are essential ecosystems in the world, since they provide habitat for a wide variety of organisms, thus supporting complex ecological communities, and they are also essential in the conservation of terrestrial-based wildlife (Moss, 1998; Beklioğlu et al., 2011). Furthermore, freshwater ecosystems are very important for humans, since they can be used as energy sources, as drinking water supplies, also for recreation, like swimming and angling (Moss, 1998). Even though larger and deeper water bodies have usually been considered as more valuable, most of the freshwater sources in the world are consisted of shallow and small lakes (Moss, 1998; Wetzel, 2001). Shallow lakes can be defined with <3 m mean depth and continuous mixing of water column, causing unstable thermal stratification (i.e. polymictic) (Moss, 1998). These water bodies usually have a sufficient underwater light penetration owing to their shallowness (large littoral area), therefore allowing the dominance of littoral communities, like submerged plants (Beklioğlu *et al.*, 2011) and as a result their species richness is higher than the deep lakes (Jeppesen et al., 1997; 2003a). Unfortunately, disturbances usually have more significant impact on shallow lakes compared to deep ones, due to the probability of nutrient re-suspension being higher and to the more pronounced impact of climatic events (Dokulil, 2014).

1.2 Palaeolimnology

Information on temporal trends in ecological conditions of a lake can be preserved in sedimentary deposits, thus sediments that are accumulated in the lakes are used as stratigraphic archives, especially if they are relatively undisturbed (Smol, 2008). Palaeolimnology (or paleolimnology), which is a study based on these deposits, employs biological (Figure 1.1; Table 1.1), geochemical and physical sedimentary proxies (i.e. indicators), from both allochthonous (from outside the lake-catchment or airborne) and autochthonous (within the lake itself) sources, to gather and uncover (reconstruct) information on past environmental changes either occurred locally and/or regionally (Figure 1.1) (Frey, 1988; Smol *et al.,* 2005). Overall, palaeolimnological studies are used for determining the response of lake ecosystems to historical changes (annual to milennial scale) in e.g. trophic state, hydrology, climate, vegetation and volcanic activity, also for defining possible causes of these changes (Frey, 1988; Last & Smol, 2001).



Figure 1.1 Lake sediment composition originating from allochthonous autochtonous sources (Modified from Smol, 2008 and www.bthompson.net/lake)

Table 1.1 Animal and Plant remains that can be found in lake sediments (modified from the Quaternary Terrestrial Palaeoecology course notes prepared by Prof. Dr. Bent Vad Odgaard)

Biological Remains in Lake Sediments							
Pollen and Spores							
Algae							
Diatoms							
Chrysophytes							
Biogeochemical fossils / Pigments	Microfossils						
Crustaceae							
Cladocerans							
Foraminiferans							
Ostracods							
Aquatic Insects							
Chironomids							
Chaoborus	Macrofossils						
Remains of submerged macrophytes							
Fish Scales							

The principal of palaeolimnological studies is based on "Law of Superposition", which states that if there is no disturbance in the sedimentary sequence the deeper deposits are the older ones and the overlying sediments are younger. (Douglas, 2013). Therefore, this process results with accumulation of a depth-time profile (Smol, 2012). The use of sediment corers enables palaeolimnologists to retrieve a vertical sediment column, covering the past periods of deposition (e.g. Figure 1.2) and dating techniques, especially the ones based on radioactive decay (e.g. ²¹⁰Pb and ¹⁴C), are employed to determine the age of the gathered cores (Smol, 2012; Douglas, 2013).

Physical (e.g. grain size) and geochemical (e.g. stable isotopes) characteristics of the sediments can be used to obtain information on palaeoenvironmental conditions, like palaeoclimate and palaeoproductivity (Frey, 1964; Douglas, 2013). Physical structures of the sediments, such as fly ash particles and charcoals can further be used for reconstructing the industrialization process around the lakes and structures like Loss on ignition (LOI) are used to find out on inorganic

and organic composition of the sediments (Douglas, 2013). Various biological indicators like plant macrofossils, algal pigments, cladocerans and insects provide information on the change of biota and in biological communities (e.g. Levi, 2009; Çakıroğlu, 2013). Furthermore, an overall information on the lakes conditions, like trophic state changes (e.g. Bennion *et al.*, 2015) and water level fluctuations (Harrison & Digerfeldt, 1993) can also be acquired using all these three types of proxies (physical, geochemical and biological).



Figure 1.2 An example to the sediment coring with a Kajak Corer, which is a kind of Gravity Corer (Smol, 2008)

Since the early 1900s, palaeolimnological studies has taken part in investigating the historical changes in lake basins and currently it become a quickly progressing science (Douglas, 2013). At the beginning, these studies were mostly descriptive (Frey, 1988; Douglas, 2013), however during the subsequent decades the area of research shifted to a quantitative and applied approach (Birks, 1998; Smol, 2008). Especially around 1980s and 1990s increasing environmental problems, like eutrophication caused by anthropogenic activities and acid precipitations,

triggered a significant growth in palaeolimnological studies (Frey, 1988; Cohen, 2003; Douglas, 2013). In addition to these, lack of historical data showing conditions of the lakes during pre-impact period led scientists to turn to the sedimentary deposits (Frey, 1988). Within the last decades new palaeolimnological approaches has developed revealing the possibilities of differentiating the impact of natural and anthropogenical changes (Birks & Birks, 2006). Furthermore, while the earlier studies usually focused on only one proxy variable, multiproxy studies, which especially aim to reconstruct the past environments and climate change (Lotter, 2003), has come into prominence (Birks & Birks, 2006; Douglas, 2013)

1.2.1 Plant Macrofossils

Aquatic plants, which are called as macrophytes can be found in both lentic (standing water) and lotic (running water) systems and these plants include submerged, floating-leaved and emergent plants, also some larger algae (e.g. *Chara* spp.). Most of the submerged macrophytes have roots in the sediment, while some of them do not root and float freely in water. Some of the species may have more than one type of leafs, like both submerged and floating (e.g. *Potamogeton natans*). Due to their sensitivity to environmental changes, like water quality (e.g. nutrient concentrations) and hydrology (Canfield *et al.*, 1985; Sagrario *et al.*, 2005; Hrivnák *et al.*, 2013), they have been considered as good indicators for lakes (e.g. Penning *et al.*, 2008; Søndergaard *et al.*, 2010) and the recovery of their presence/abundance is considered as a crucial part of lake restoration projects (Gulati & Van Donk, 2002). Therefore, their sedimentary remains, called as plant macrofossils or plant remains, comprise an important part in palaeolimnological studies.

Plant remains, have a median size of 0.5-2.0 mm. In lake sediments there are two main components of plant remains, being aquatic macrofossils, which belong to various macrophyte types (e.g. submerged), and catchment derived terrestrial

plant macrofossils (Koff & Vandel, 2008). Remains of plants, such as seeds, leaves and bud scales, are used for elucidating past environmental changes (for some examples see Figure 1.3) (Birks & Birks, 2000; Birks, 2007; Koff & Vandel, 2008).

Key literature, such as Beijerinck (1947), Nilsson (1961), Berggren (1969; 1981), Birks (1980), Jacomet (1986), Haas (1994), Birks (2007), Mauquoy and Van Geel (2013), also comparison with recent reference materials that are collected during fieldworks or that are elaborated by Herbariums can be used to conduct plant remain identifications.



Figure 1.3 Examples of submerged plant macrofossils a- b- c) *Najas marina* L. seeds, d) *Potamogeton crispus* L. turion spine e) *Potamogeton* sp. seed f) *Zannichellia palustris* L. seed g) *Elodea canadensis* Michaux. leaf tip h) *Chara* sp. oospore

Brief History of Plant Macrofossil Studies

Plant macrofossil studies were the only available technique, which was employed in determining Quaternary vegetation history, prior to pollen analysis, around 19th century (Birks, 2001). Although macrofossil identification quality was high, remains were not counted and the focus was on determining the flora, instead of defining environmental conditions (Birks, 1980). Subsequent to the development of quantitative pollen analyses by Von Post (1916) studies based on plant macrofossils were faded (Birks, 1980; Birks & Birks, 2000). However, around 1960s, when the limitations in pollen analyses started to surface, the usage of plant remains has increased one more time, also because these two techniques complement one another (Birks & Birks, 2000; Birks, 2001). The first modern stratigraphic plant macrofossil analyses was conducted by West (1957) from a last interglacial sequence in England and first diagrams were presented by Baker (1965) and by Watts and Winter (1966) (Birks, 2001). Therefore, development of quantitative analyses demonstrated the importance of plant macrofossil studies for reconstructing past environmental conditions (Birks, 2001). Currently, plant remains are employed to investigate changes in past lake ecosystems, such as lake levels and temperature, also to obtain information on human impacts, like eutrophication and pollution (Hannon & Gaillard, 1997; Davidson et al., 2005; Birks, 2007; Koff & Vandel, 2008).

1.3 Marker Pigments for Phytoplankton

Phytoplankton are corporate of photosynthetic microorganisms found in seas, lakes, ponds and rivers (Findlay & Kling, 2003; Reynolds, 2006). They can either be colonial or solitary ranging in size from $<1 \mu$ m to colonies larger than 500 μ m (Findlay & Kling, 2003). They are mostly eukaryotic algae or prokaryotic blue-green algae (Cyanobacteria) (van der Valk, 2006; Moss, 2010). Phytoplankton, are mainly affected by physical factors, like light and temperature; by chemical factors, such as nutrient concentrations in euphotic zone, and also by the organism

interactions within the plankton community (e.g. grazing- edible/inedible) (Findlay & Kling, 2003; Romero-viana *et al.*, 2010; Azari *et al.*, 2010). Thus, when there is no historical phytoplankton data their sedimentary remains (pigments) may take up a substantial part of palaeolimnological research.

Pigment contributions to the lakes as organic detritus can be from all the primary producers, like phytoplankton, aquatic plants and phototrophic bacteria (Sanger, 1988; McGowan, 2013). In some cases the only indicators may be pigments, because morphological remains of many soft-bodied photosynthetic organisms are generally prone to decomposition (McGowan, 2013). Photosynthesising organisms which are represented by sedimentary pigments include chlorophylls (Chls), carotenoids, their derivatives and biliproteins (Jeffrey *et al.*, 1997; Reuss, 2005; McGowan, 2013). Chlorophyll and carotenoid pigments found in phytoplankton are the main focus for this study (Table 1.2).

<u>Chlorophylls:</u> Phytoplankton being one of the crucial primary producers in aquatic systems contain one or more types of chlorophyll pigments. (Wright, 2005; Babani, 2007). Chlorophyll molecules are cyclic tetrapyrrol compounds comprising a magnesium atom in the center of the ring and a non-polar hydrocarbon chain bonded to the ring (See Figure 1.4 for an example) (Wright, 2005; Kirk, 2011). The peaks of chlorophyll molecules mostly appears in the blue and red ends of the absorbance spectrum (Figure 1.5) (Wright, 2005). Chlorophylls might give information on the source phytoplankton. For example, even though chlorophyll-a is present in all phytoplankton groups (Leavitt & Hodgson, 2001; Watanabe *et al.*, 2012), chlorophyll-b is mainly found in green algae (e.g. Chlorophyta) (See Table 1.2 for more details) (Jeffrey & Vesk, 1997; Leavitt & Hodgson, 2001; Babani, 2007).



Figure 1.4 Chlorophyll molecules. X and Y are functional groups, that varies with chlorophyll types (commons.wikimedia.org/wiki/File:Chlorophyll_a_b_d.svg)



Figure 1.5 Absorbance spectra of free chlorophyll *a* and *b* and carotenoids in a solvent (Modified from Collin *et al.*, 2010)

<u>*Carotenoids:*</u> Carotenoids are other types of photosynthetic pigments (Wright, 2005), found in freshwaters that can be used in palaeolimnological studies (Whitmore & Riedinger, 2002). Their molecular structure is different than the chlorophylls, comprising yellow to red isoprenoids (Wright, 2005), which mostly consist of isoprene units forming circles at both ends (e.g. Figure 1.5) (Babani, 2007; Kirk, 2011). Moreover, carotenoids can absorb light at the shorter

wavelengths (blue and green) of the visible spectrum (Figure 1.5) (Wright, 2005; Kirk, 2011). There are two types of carotenoids namely carotenes (hydrocarbons) and xanthophylls (oxygenated carotenoid derivatives) (e.g. Figure 1.6) (Wright, 2005). Some carotenoids, like diatoxanthin (diatom) and alloxanthin (cryptophyta) (see **Table 1.2** for more details) (Buchaha *et al.*, 2011), are source specific, thus they are useful in defining phytoplankton communities.



Figure 1.6 Examples for Carotenoids. (a) Xanthophyll (Diatoxanthin) (b) Carotene (β -carotene) (en.wikipedia.org/; ca.wikipedia.org/)

<u>Phycobiliproteins</u>: Other types of pigments that found in phytoplankton, like cyanobacteria and rhodophyta, are phycobiliproteins (Wright, 2005). Even though they are taxonomically specific, they are not used as markers in palaeolimnological studies based on HPLC analysis, due to their solubility in water (not extractable by organic solvents) (Wright, 2005) and thus, disabling their consolidation to the sediment (Sanger, 1988).

Marker pigments are identified by comparing relative retention time and absorption spectra of the pigments to the reference spectra library extracted from known algal cultures (e.g. obtained from DHI Water and Environment (www.dhigroup.com/) (for an example see Figure 1.7).



Figure 1.7 Chromatogram of phytoplankton pigments isolated by HPLC, showing the retention times of the peaks together with an example absorption spectra of Lutein pigment which is an indicator of Chlorophyta division.

Marker Pigments	Bacillariophyta*	$Chlorobiaceae^*$	Chlorophyta*	Chromophyta*	Chrysophyta*	Cryptophyta*	Cyanobacteria*	$Diatoms^*$	Dinophyta*	Dinoflagellates*	Euglenophyta*	Eustigmatophyta*	$Haptophyta^*$	Herbivore tissues	Invertebrates	Prasinophyta*	Prochlorophyta*	Rhodophyta*	Bacteria	Zooplankton	Plantae
Alloxanthin						+															
Aphanizophyll							+														
a-phorbins							+														+
Astaxanthin			+												+					+	
Bacteriophyll a																			+		
Bacteriophyll e																			+		
Bacteriophytyn																			+		
Canthaxanthin			+				+							+						+	
Chlorophyll-a			+		+	+	+	+		+	+	+	+				+	+			+
Chlorophyll-b			+								+					+	+				+
Chlorophyll-c	+				+				+												
Chlorophyll-c1				+	+	+		+					+								
Chlorophyll-c2				+	+	+		+		+			+								
Chlorophyll-c3					+			+					+								
Chlorophyllide-a								+													
Divynil Chl-a																	+				
Divynil Chl-b																	+				
Crocoxanthin						+															
Cryptoxanthin			+				+											+			
Diadinoxanthin	+				+	+		+	+	+	+		+								
Diatoxanthin	+				+	+		+	+	+			+								
Echinenone							+													+	
Fucoxanthin	+				+	+		+	+				+								
Isorenieratene		+																	+		
Lactucaxanthin																					+
Lutein			+								+							+			+
MgDVP																	+				
Modadoxanthin						+															
Myxoxanthophyll							+														
Neoxanthin			+								+										+
Okenone																			+		
Oscillaxanthin							+														
Peridinin									+	+											
Phycobiliproteins						+	+											+			
Scytonemin							+												+		
Violaxanthin			+		+						+	+									+
Zeaxanthin			+				+				+						+	+			
β,α -carotene			+		+	+			+									+			
β,β -carotene			+		+		+	+		+	+	+	+				+	+	+		+

 Table 1.2 Examples of marker pigments used in palaeolimnological studies. *;

 indicates phytoplankton taxa.

Brief History of Pigment Research

Pigments have been used in palaeoecological studies for more than 50 years (Sanger, 1988; Leavitt & Hodgson, 2001). In the early research, spectrophotometric techniques, thin layer and open column chromatography was used in pigment studies (Hodgson et al., 1997). Due to the relatively limited possibilities, the main focus was to understand the preservation and the deposition of the pigments, thus to understand pigment biogeochemistry and problems in sampling and preservation (Sanger, 1988; Hodgson et al., 1997). Sanger and Gorham (1970) and Leavitt and Hodgson (2001) stated that pigments were also used as biochemical markers to define former source populations, especially phototrophic prokaryotes, or to infer possible changes in lake production over time. Moreover, they pointed out that the development of advanced techniques (e.g. High performance liquid chromatography-HPLC, Mass spectrometry-MS) for chemical identification enabled better taxonomic resolution and identification of source populations. Since 1980, HPLC has been the main technique used for quantitatively determining sedimentary pigments (Leavitt & Hodgson, 2001; Hobbs et al., 2010). At the present time, pigments are commonly used in reconstructing past environments (e.g. changes in eutrophication, acidification and climate) (Lami et al., 2000; Leavitt & Hodgson, 2001; McGowan, 2013) and generally studies with phytoplankton pigments are more common (Sanger, 1988).

1.4 Palaeolimnological Research in Turkey

Freshwater resources in Turkey are rich with around 200 natural lakes, 700 ponds and 75 dams (Kazancı *et al.*, 1995). Total surface area of these lakes cover approximately 10,000 km² and they are mostly shallow with highly biodiverse communities (e.g. aquatic plants) (Seçmen & Leblebici, 1982; Beklioğlu *et al.*, 2006).

In Turkey, first palaeolimnological studies were mostly based on defining the vegetation history of the area. To our knowledge the first study was conducted in 1957 using the pollen records from the cores of Lakes Abant and Yeniçağa (Beug, 1967). Furthermore, various pollen studies by Sytze Bottema, Willem van Zeist, Henk Woldring and Burhan Aytuğ were carried on in large amount of lakes to determine the late Quaternary vegetational history of the Mediterranean (e.g. van Zeist *et al.*, 1968; van Zeist & Woldring, 1978; Bottema & Woldring, 1984; Woldring *et al.*, 1986; Bottema *et al.*, 1993; Bottema, 1995). According to a recent review by Şenkul (2014) the first reconstruction of the vegetation history covering the whole Anatolia was conducted by van Zeist and Bottema (1991). Furthermore, Bottema *et al.* (1986) and Bottema and Woldring (1990) identified the Beyşehir Occupation Phase (BOP), which is the human impact period occured around mid-late Holocene (Eastwood *et al.*, 1999). Şenkul (2014) further added that vegetation in conjunction with climate was first investigated by van Zeist (1975) in Anatolia.

Subsequently, since around 1990s, there seem to be an increase in climate and environmental change related studies, also in investigating human impact (e.g. Reed *et al.*, 1999; Eastwood *et al.*, 1999; Vermeore *et al.*, 2002; Kashima, 2003; Jones *et al.*, 2005; Reed *et al.*, 2012; Dean *et al.*, 2014; Ocakoğlu *et al.*, 2015 etc.). For example, Roberts *et al.* (2001) studied a crater lake, Eski Acıgöl, with the aim of determining the impact of climate on hydrology and chemistry of the lake by employing isotopes, diatoms and pollens. In a study from Lake Nar (central Turkey) England *et al.* (2008) used pollen, stable isotopes and charcoal remains to show the changes in the lake related to climate and human impacts. More recently, Çakıroğlu *et al.* (2014) compared the surface sediment cladoceran assemblages with contemporary pelagic and littoral species from 40 lakes, with further investigation of cladoceran datasets to environmental variables. As a result, they showed that sedimentary cladoceran assemblages can be used instead of contemporary ones and they also pointed out to the importance of salinity for Turkish Lakes (Çakıroğlu *et al.* 2014). Moreover, within the context of the PALEOVAN drilling campaign (Litt *et al.*, 2009), marker pigments were also studied as indicators of primary productivity change in Lake Van (Huguet *et al.*, 2011).

1.5 Water Framework Directive (WFD)

Lakes thoughout the world are exposed to intense anthropogenic pressures, like eutrophication, invasive species, and these pressures are exacerbated by climate change (Brucet *et al.*, 2013; Poikane *et al.*, 2014). As a result of these pressures, many lakes have experienced ecological structure degradation (e.g. loss of plants) and their functioning has changed (primary production shift between littoral and pelagic systems). Hence, their ecosystem services were negatively affected by restricted use of water as a source or for recreational and touristic purposes (Poikane *et al.*, 2014). Therefore, in recent years various legislative measures has been generated and employed worldwide, in order to assess the ecological conditions of water bodies, like lakes and rivers (Poikane *et al.*, 2009; 2016). Some examples to these legislations are Clean Water Act in the USA (CWA), National Water Act in South-Africa, and Water Framework Directive in Europe (Poikane *et al.*, 2016).

The European Water Framework Directive (EC, 2000) was adopted in 2000 by the EU member states (MS) and since then the studies on ecological assessment of the water bodies has advanced significantly in Europe (Birk *et al.*, 2012; Poikane *et al.*, 2016). Unlike other legislations the processes to determine ecological status and management goals are defined in the WFD, with a further aim to harmonise the ecological assessment systems established by each MS (Poikane *et al.*, 2015). Furthermore, with the establishment of this directive general management objectives has changed from only pollution control to insuring ecosystem unity (Birk *et al.*, 2012), with the main aim of attaining at least "good ecological status with a sustainable ecosystem, which is suitable for human use", by latest 2027 (Poikane *et al.*, 2009; 2014). According to the WFD agreement, in order to establish a river basin management plan, ecological status of different water categories (rivers, lakes, transitional waters and coastal waters) in Europe are required to be assessed by employing different organism groups (phytoplankton, macrophytes and phytobenthos, benthic invertebrates and fish), which are called as "Biological Quality elements (BQE)", while physicochemical and hydromorphological data are used as supporting information (Figure 1.8) (EC, 2000; Hering *et al.*, 2010).

The main steps for defining the ecological status of lakes are (Birk *et al.*, 2012; Portielje *et al.*, 2014; Poikane *et al.*, 2016);

- a. Monitoring of the lakes
- b. Defining typologies based on variables, like altitude, mean depth, surface area, alkalinity and geology.
- c. Determining the reference sites for each typology, by using information on nutrient and oxygen concentrations of the lakes, together with the information on catchment use/stress data, such as land use, population etc. Thus, these sites are under no or very low anthropogenic pressure.
- d. Calculating the Ecological Quality Ratios (EQRs) the ratio of observed and expected/reference states – for each lake, using the biological elements.
- e. Defining the status of the lakes by comparing the EQRs of reference and other lakes. There are five status classes being: high (not different from reference conditions), good (slightly different), moderate (moderately different), poor and bad (major differences) (Figure 1.8).



Figure 1.8 'Undesirable disturbance' concept to determine 'good–moderate' boundary setting (modified from Poikane *et al.*, 2014)

The characteristics of biological elements that should be used in defining the status of the lakes (e.g. taxonomic composition, abundance) are stated in the WFD. However, each MS should develop their own metrics from the BQEs, and indices based on these metrics (Hering *et al.*, 2010). This individuality of the members reveals the need for harmonization of ecological assessment systems with a process called intercalibration, which ensures the protection and restoration of the lakes by comparing the assessment results (Nõges *et al.*, 2009). Therefore, intercalibration exercise which have started in 2003 provides the members to compare the ecological status of the lakes (Poikane *et al.*, 2009). The geographical intercalibration groups (GIG) in the WFD are determined according to the limnofaunistic ecoregions suggested by Illies (1967/1978) (Zogaris *et al.*, 2008) and to the data analysed by Nõges *et al.* (2005). In total 62 methods from

24 countries were intercalibrated and 20 methods were submitted for the lakes (EC, 2013; Poikane *et al.*, 2015).

The intercalibration groups were defined as follows (Figure 1.9) (Poikane *et al.*, 2009);

- Alpine GIG: Austria, France, Germany, Italy, Slovenia
- <u>Atlantic GIG</u>: Ireland, UK
- <u>Central-Baltic GIG</u>: Belgium, Czech Republic, Denmark, Estonia, France, Germany, Hungary, Latvia, Lithuania, Netherlands, Poland, Slovakia, UK
- <u>Mediterranean GIG</u>: Cyprus, France, Greece, Italy, Malta, Portugal, Romania, Spain
- Northern GIG: Finland, Ireland, Norway, Sweeden, UK



Figure 1.9 Map showing the location of Turkey and the countries joined to WFD studies (dark grey shading). The stars indicate countries that are included in more than one GIG.
Macrophytes indices developed for Lake Assessment

Macrophytes comprise an important part of WFD studies and to this day there are 17 intercalibrated and 3 submitted macrophyte-based indices (Poikane *et al.*, 2015). These metrics/indices are mostly based on taxonomic composition (sensitive vs. tolerant species) (Kolada *et al.*, 2014) and abundance (e.g. plant colonisation depth or percent coverage/plant volume inhabited) (Søndergaard *et al.*, 2010). Most of the indices and intercalibration studies indicated a significant pressure-response relation between macrophytes and eutrophication parameters, like nutrient and chlorophyll-a concentrations (Portielje *et al.*, 2014). However, the delayed and non-linear (considering alternative stable state theory) reaction of macrophytes to these parameters may reveal some difficulties (Penning *et al.*, 2008; Pall & Moser, 2009; Poikane *et al.*, 2015).

1.6 Water Framework Directive and Turkey

The water management policies in Turkey started to change after the Helsinki European Council in 1999 and since the start of 21st century, Turkey has been working on facilitating the harmonization with WFD (Sümer, 2011). For this reason, pilot projects like EU-TWINNING (2008-2009), which determined 25 River Basins in the whole country, have been conducted (Sümer & Muluk, 2011) and the legislation on water has been revised, together with further evaluation on the water management policies (Sümer, 2011).

Besides the legislative and economic point of view, a further challenge of implementing WFD in Turkey is to either develop new metrics/indices based on BQEs for ecological classification or to find and adapt metrics/indices that are suitable for lakes in Turkey. However, Turkey has a very diverse ecosystem, thus complicating the inclusion of the data collected from the lakes located here to the intercalibration groups. For example, western and southern parts of the country is located in Mediterranean region (around in Aegean and Mediterranean Seas), which can be included in Mediterranean GIG, while north-west part can be

defined as Baltic (Marmara region) (Figure 1.9). The classification of northern and mid- to eastern- parts are more complicated since there is no lakeintercalibration groups that can include the data from the lakes located in these areas.

Another challenge for Turkey comes from the Mediterranean GIG itself, since intercalibration exercise in this group encountered with difficulties in developing ecological assessment methods, because the number of lakes is relatively low and the lakes are very diverse (Poikane *et al.*, 2015). Most of the lakes are reported from Spain, however, Ruiz *et al.* (2011) showed highly diverse lake types with, 24 different typologies, which can be included in the WFD. Thus, as a consequence of lacking suitable amount of lakes with mutual types, intercalibration of the natural Mediterranean lakes has not been possible (Poikane *et al.*, 2015).

Overall, one of the important tasks that should be completed by Turkey is to define the regions and which metrics/indices to be used for different BQEs. This is a challenge that has still being argued in the Turkish Ministry of Forestry and Water Affairs (personal communication with the Expert "Hümeyra Bahçeci").

1.7 Objectives

The main aim of the current study is contributing to the studies investigating the relation of sedimentary remains (plant macrofossil and pigments) with presentday and historical environmental variables, to understand the effectiveness of using the remains in combination with present-day biological data and in longterm palaeoecological studies. Another aim is to compare the ecological status and classifications of the lakes in Turkey. For these reasons in total 44 mostly small and shallow lakes were sampled with a well calibrated snap-shot sampling protocol and another set of three large lakes were only sampled by coring. Therefore, the objectives of this thesis are;

- i. To investigate the congruence between aquatic plant remains in surface samples and present-day macrophyte assemblages in relation with environmental variables (Chapter 2),
- To determine the concordance between sedimentary pigments and phytoplankton classes observed from water column and to compare the main environmental drivers of these two phytoplankton datasets (Chapter 3),
- iii. To elucidate the effects of lake water-level changes on benthic-pelagic primary production throughout the last 50-100 years by employing biological and non-biological sedimentary proxies and to determine the factors that may lead to poor correlation between water-level changes and proxies (Chapter 4),
- To determine the possibility of employing macrophyte-based WFD indices developed by various countries for defining the ecological qualities of the lakes in Turkey (Chapter 5).

CHAPTER 2

SIMILARITY BETWEEN CONTEMPORARY VEGETATION AND PLANT REMAINS IN THE SURFACE SEDIMENT IN MEDITERRANEAN LAKES¹

2.1 Introduction

Macrophytes are important primary producers, particularly in shallow lakes. When abundant, they have a key influence on assemblage structure and ecosystem processes, including carbon and nutrient cycling (Moss, 1990; Scheffer et al., 1993) and trophic dynamics (Jeppesen et al., 1998). Water depth, clarity and chemistry affect macrophyte species composition, distribution and abundance and their temporal assemblage dynamics (Van der Valk, 1987; Gafny & Gasith, 1999). In particular, nutrients, especially nitrogen (N) and phosphorus (P), influence the abundance and species composition of aquatic plants (Van et al., 1999; James et al., 2005; Thomaz et al., 2007) by affecting phytoplankton and periphyton growth and thereby the light availability for macrophytes (Phillips et al., 1978). Contemporary and palaeolimnological studies have shown that as nutrient concentrations increase, aquatic macrophyte species shift from short - (e.g. Charophytes) to tall - growing forms (e.g. Potamogeton spp.) (Moss, 1988; Blindow, 1992; Ayres et al., 2008; Sayer et al., 2010a; Sayer et al., 2010b; Davidson et al., 2011). At high nutrient concentrations, the abundance and diversity of submerged plants (James et al., 2005) often decrease or macrophytes may even disappear (Gonzalez Sagrario et al., 2005). In addition to the impact of nutrients, salinity can influence aquatic plant composition and diversity,

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especially in salt marshes or lakes located in semi-arid and arid regions (Nielsen *et al.*, 2003; Lacoul & Freedman, 2006; Sim *et al.*, 2006; Beklioğlu & Tan, 2008).

As a consequence of their sensitivity, macrophytes are useful indicators of the ecological status of lakes (Kienast et al., 2001; Søndergaard et al., 2005, 2010; Birks, 2007). It follows therefore that historical records of plant assemblages can provide information on the past ecology of lakes. Furthermore, they may be useful when trying to identify the reference plant assemblages according to the EU Water Framework Directive (WFD, European Union, 2000) or before initiating restoration projects on degraded lakes (Bennion et al., 2011). Due to the general lack of historical data, the sediment record may be the only source of such information (Birks & Birks, 2001; Davidson et al., 2005; Ayres et al., 2008). A prerequisite, however, is that the plant remains in surface sediments have a welldefined relationship with the present-day vegetation (Davis, 1985; Zhao et al., 2006). Studies by Davis (1985) and Zhao et al. (2006) indicate that plant remains are useful for determining the dominant taxa in lakes. However, these and other studies (Birks, 1973; Dieffenbacher-Krall, 2007; Koff & Vandel, 2008) showed that the relationship between aquatic plant remains in the surface sediment and the contemporary vegetation may be complex and influenced by the variation in macrofossil dispersal, production and loss (such as decomposition), location of sampling site and basin characteristics. A number of single-site comparisons have been conducted so far (e.g. Davis, 1985; Davidson et al., 2005; Zhao et al., 2006), but no study of the relationship between contemporary macrophyte assemblages and their surface sediment remains exists for a large range of lakes.

Most lakes in Turkey located in a warm temperate to semi-arid Mediterranean climate are shallow and are threatened by the warmer and drier conditions predicted for the future (Bates *et al.*, 2008; Giorgi & Lionello, 2008). In addition, these waters are at present exploited primarily for irrigation and are subject to water level fluctuations through intense abstraction as well as through natural oscillations occurring during alternating wet and dry periods of the Mediterranean

climate (Coops *et al.*, 2003; Beklioğlu & Tan, 2008; Özen *et al.*, 2010; Jeppesen *et al.*, 2011), with potentially profound effects on the distribution, growth and species composition of macrophytes (Beklioğlu *et al.*, 2006). The importance of eutrophication for aquatic plant assemblages has been observed in mesocosm experiments conducted in lakes in the Mediterranean region, in Spain (Romo *et al.*, 2004) and Turkey, where reduced water level largely counteracted the effect of nutrient enrichment (Özkan *et al.*, 2010; Bucak *et al.*, 2012). However, the lack of historical records and the limited palaeolimnological studies in most of the shallow lakes in Turkey limit our knowledge of the macrophyte assemblage composition and dynamics in the past. Such information could provide valuable information on past ecological changes in these lakes.

Here, we compared aquatic plant remains in surface sediment samples from 35, mainly shallow, Turkish lakes with their contemporary plant assemblages. We hypothesised that (i) remains of submerged and floating plant communities retrieved from the sediment surface would provide a good indication of the contemporary living assemblage and that (ii) plant remains would elucidate the environmental conditions in these lakes.

2.2 Methods

2.2.1 Study sites, sampling and analysis

During the summers 2006–2011, 35 lakes along a latitudinal gradient from North (42°00′54″N, 34°55′20″E) to South (37°06′55″N, 29°36′03″E) Turkey, spanning around six degrees of latitude (Figure 2.1; Table 2.1), were sampled to determine physical, chemical and biological properties.

Samples for the analysis of various physical, chemical and biological parameters in each lake were taken only once during the peak of the growing season. To determine general chemical characteristics of the lakes, such as total phosphorus (TP), total nitrogen (TN) and chlorophyll-a (Chl-a), water samples were taken using a Ruttner sampler covering the entire water column from the deepest point. In addition, Secchi depth was measured together with conductivity, pH and dissolved oxygen (DO) using a YSI 556 MPS multiprobe field meter (YSI Incorporated, OH, USA) (Table 2.1). Water chemistry samples were kept frozen until analysis. The acid hydrolysis method was used to determine TP (Mackereth *et al.*, 1978). For TN, a Skalar Autoanalyzer (San++ Automated Wet Chemistry Analyzer, Skalar Analytical, B.V., Breda, The Netherlands) was employed. Chl-a was measured spectrophotometrically after ethanol extraction (Jespersen & Christoffersen, 1987).



Figure 2.1 Map of Turkey showing the main study sites together with the location of the three main cities (Ankara, Istanbul, Izmir) and seven geographical regions (http://commons.wikimedia.org).

Variable	Unit	Median	Mean	Minimum	Maximum
Area	ha	20.0	80.93	0.17	635.0
Maximum Depth	m	3.4	3.95	0.55	17.4
Secchi Depth	m	0.8	1.45	0.2	9.0
Total Phosphorus	$\mu g L^{-1}$	88	130	15	633
Total Nitrogen	$\mu g L^{-1}$	955	1034	239	2340
Conductivity	$\mu S \text{ cm}^{-1}$	506	2004	104	24392
Salinity	‰	0.24	1.11	0.05	14.5
Dissolved Oxygen	mg L ⁻¹	6.6	6.6	0.6	13.4
pН	- log[H+]	8.1	8.2	6.3	9.5
Chlorophyll-a	$\mu g \ L^{-1}$	15.4	25.1	1.8	95.1
Plant Volume Inhabited	%	14.6	25.2	0	94.2

Table 2.1 General characteristics of the 35 study lakes

The lakes ranged in size from 0.17 to 635 ha (Table 2.1) and were mostly shallow, except lakes Abant and Büyük (maximum depths of 17.4 and 15.2 m; mean depth of 4.8 and 3.8 m, respectively). Thirty-two of the lakes were fresh, while three were saline (lakes Mert, Sarıkum and Erikli) with mean conductivities (Cond) of 24.4, 13.0 and 10.3 mS cm⁻¹, respectively. The lakes were meso-hypertrophic with TP concentrations ranging from c. 15 to 633 μ g L⁻¹, Chl-a from c. 2 to 95 μ g L⁻¹ and TN from 239 to 2340 μ g L⁻¹ (Table 2.1). Thirty-three of the lakes had submerged macrophytes, and the mean percentage lake volume inhabited (PVI%) (sensu Lauridsen *et al.*, 2003) of all lakes was c. 24%.

2.2.2 Sampling aquatic macrophytes

Aquatic macrophytes, comprising floating-leaved, floating and submerged plants, were surveyed along parallel transect lines spaced out at even intervals around the lake. The number of lines depended on lake area (Table 2.2). For lakes with very low or very high coverage of submerged plants, the number of transects was reduced by c. 50%. GPS coordinates, water depth, species identity, average plant

height and surface cover of each submerged and floating-leaved species were recorded at each sampling site, located at approximately even intervals along the transect lines. Percentage cover was recorded for floating-leaved and submerged species, and percentage plant volume inhabited (PVI%) was calculated using coverage, average height and water depth (Canfield *et al.*, 1984).

Lake area (ha)	Number of transects
0 - 20	10
21 - 50	15
51 - 101	15 - 20
102 - 301	20
> 301	> 20

 Table 2.2 Minimum transect number relative to lake area

2.2.3 Coring

From each lake, surface sediment samples (0-2 cm), retrieved with a KC-Denmark Kajak Corer (internal diameter: 5.2 cm), were taken from between seven and 10 different locations in the pelagic zone, then pooled and stored frozen (18 °C) until analysis.

2.2.4 Surface plant remains sample preparation

Analysis of plant remains from the surface sediment was conducted for all the lakes. To facilitate counting, $10-300 \text{ cm}^3$ (depending on the sample size) of each sediment sample was washed through sieves with mesh sizes of 500 µm and 200 or 212 µm (Brodersen *et al.*, 2001; Odgaard & Rasmussen, 2001). Subsequently, the entire residue on the 500 µm sieve and, depending on sample size, a quantitative subsample of the residue (c. 25%) on the 200- or 212-µm sieve were identified and both counted using an OLYMPUS SZX12 stereo-micro-scope at

Aarhus University (Denmark) or a LEICA MZ 16 stereo-microscope at Middle East Technical University (Turkey) at 10–80 and 10–110 magnification, respectively. For identification, we used Beijerinck (1947), Nilsson (1961), Berggren (1969), Birks (1980), Berggren (1981), Jacomet (1986), Haas (1994), Birks (2007), Mauquoy and van Geel (2007) and recent reference material curated at the Aarhus University Herbarium.

2.2.5 Statistical analysis

The representation of each taxon in the sediment is biased towards plants leaving numerous, persistent and identifiable subfossils (Birks, 1980b; Odgaard & Rasmussen, 2001). The sedimentary remains of different plants found in lake sediments vary from small numerous leaf spines, through intermediate-sized *Chara* oospores to large seeds, and they are highly variable in abundance between taxa. To reduce the bias produced by such differential production and to prepare the data for analysis using ordination methods, the log-transformed data were scaled by centring by the mean (i.e. the mean was subtracted from each observation) and subsequently standardised by dividing by the standard deviation. Furthermore, in order to record absence correctly, which is important when using the Bray–Curtis distance used here, the taxon-specific mean to standard deviation ratio was added to each value. This procedure returned the original zero values (changed by the centring and standardising) to zero while the presences were scaled to allow a fair comparison of abundance among taxa, and ensuring that absence was correctly recorded and negative values were avoided. Taxa with more than one type of sedimentary remains (e.g. Ceratophyllum leaf fragments and spines) were represented by the fragment type with highest frequency in a given lake (Odgaard & Rasmussen, 2001). The same standardisation procedure used for plant remains was also employed for contemporary data (PVI%). Furthermore, we restricted the taxonomic resolution to the generic level due to the frequent difficulties in identifying plant fragments to species (such as *Potamogeton* spp. seeds). We tested the distribution of environmental variables

using the Kolmogorov–Smirnov tests, and if they deviated from normal, they were log-transformed.

Following transformation, non-metric multidimensional scaling (nMDS) using Bray–Curtis dissimilarity was conducted on both present-day PVI% data (taxa with >1% coverage) and plant macrofossil data (having more than five macrofossil remains per 100 cm³). The resultant ordinations were compared with procrustes rotation analysis to determine the overall degree of concordance between these ordinations (Gower, 1971; Mardia *et al.*, 1979). This analysis was followed by a PROTEST permutation test to assess the significance of the degree of correlation between the ordinal results (Jackson, 1995; Peres-Neto & Jackson, 2001).

The data sets analysed here are typical of ecological data containing many zeros. Thus, not all measures of assemblage similarity are appropriate. The Bray–Curtis distance matrix was preferred since it ignores joint absences (Legendre & Legendre, 1998; Quinn & Keogh, 2002) and is also suitable for species data (Legendre & Legendre, 1998; Legendre & Anderson, 1999). Using the Bray–Curtis dissimilarity index, distance-based redundancy analysis (db-RDA) was then carried out on the transformed data.

R version 2.15.1 (R Development Core Team, 2012) was used to perform all the transformations and analyses. NMDS, procrustes analysis, PROTEST and db-RDA were conducted using the vegan R package (version 2.0-5) (Oksanen *et al.*, 2012).

Table 2.3 Table showing the results of the comparison of present-day submerged and floating-leaved macrophyte data obtained from snap-shot contemporary sampling of plants and plant remains from surface sediment [O shows plant remains found in surface sediment; + shows present-day modern plants; grey boxes with X show representation of modern plants as remains] [KaragölK: Karagöl-Kıbrısçık, KaragölD: Karagöl-Denizli, GölcükS: Gölcük-Simav, GölcükB: Gölcük-Bolu, K.Akgöl: Küçük Akgöl, B.AkGöl: Büyük Akgöl]

	Ham	Poyra	Aban	Buyu	Nazl	Serin	Pedir	Eymi	Mog	Taşkı	K.Ak	B.Ak		Gölc	Gölh	Mert	Erikl	Saka	Emre	Gök	Kara	Azap	Gölc	Yayla	Sakh	Gici	Tatlı	Sarık	Koca	Gere	Keçi	Kara		Kaya
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Plants		-												<u> </u>							_		_									~		
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Ceratophyllum demersum L.	х	Х				Х	Х										Х	Х	X	Х				Х		х	х	$^+$	Х	Х	Х			
Ceratophyllum submersum L.																																х		
Ceratophyllum spp.				0	X			0	0	0	0	х	X		0	0					0	0	0		0								į	хх
Elodea canadensis MICHAUX																			X															
Elatine sp.																								Х										
Isoetes cf. anatolica																						0		0										
Myriophyllum spicatum L.			+											+				+	+				X	Х	х	+	x	+			+	+	ţ	хх
Myriophyllum verticillatum L.			+																											+				
Myriophyllum spp.												+			+																			
Najas marina L.								X	X		+	+			0	Х	X										х							
Najas minor ALL.																	0																	
Najas sp.								+	•												+													
Potamogeton crispus L.							0			0	0								х				0						х				-	ł
Potamogeton pectinatus L.								Х	+															Х										
Potamogeton perfoliatus L.		+										+																						
Potamogeton cf. trichoides													+																					
Potamogeton spp.										0	0									+		х				+	х	х				+ 1	x -	+ +
Ranunculus sceleratus L.																0																		
Ranunculus sect. Batrachium																				0	0			Х		0	0							
Ruppia maritima L.																х												$^+$						
Utricularia cf. australis			+																		+			+	+						+			
Vallisneria spiralis L.		+																																
Zannichellia palustris L.							0								0	0	0			0		0						Х				0	0	0
Nymphaea alba L.	+	+								Х		х												Х					+	+	+			
Nuphar lutea (L.) SM.			Х							Х		х		+																				
Nymphaceae																											0							
Trapa natans L.	х	Х					Х				+	+									0													
Potamogeton natans L.		+	+	+	+																		+	+								+		
Hydrocharis morsus-ranae L.												+																						
Polygonum amphibium L.																			ł			+		+								+		
Characeae		0	Х			X	0	0	0	0		X	0	0		0	0		+	0	0	0		х	0	0	X	Х				0 2	K	0

2.3 Results

2.3.1 Comparison of surface sediment remains with modern vegetation

Approximately 50% of the modern plant taxa were represented as sedimentary remains (Table 2.3). Of the 15 submerged (including Characeans) and floatingleaved plant families found in the vegetation survey, four taxa (*Utricularia* spp., Polygonum amphibium, Vallisneria spiralis and Hydrocharis morsus-ranae) were not represented by their subfossils, for example leaf fragments, leaf spines and seeds. Conversely, three taxa (Ranunculus sect. Batrachium, Callitriche sp. and *Isoetes* sp.) were recorded only as sedimentary remains (Table 2.3). In five of the lakes, there was no overlap between submerged or floating-leaved flora and the plant macrofossils found (e.g. lakes Gölhisar, Gölcük-Bolu). The most frequently occurring and abundant submerged plant in the vegetation survey, Ceratophyllum spp., was also the most abundant in the sediment (Table 2.3). Characean species were present in the contemporary macrophyte vegetation in eight lakes, and their oospores were recorded in the sediments in seven of those lakes. However, Chara oospores were found in the sediments of a further 16 lakes where the plants were not recorded in the contemporary survey. Potamogeton crispus was represented in the sediments of two of three lakes where it was found in the modern survey, and additionally in four other lakes, only sedimentary macrofossils were found. Remains of the floating-leaved species Trapa natans and Nymphaea alba or *Nuphar lutea* were also well represented in the sedimentary samples (Table 2.3). In general, however, floating-leaved taxa were better recorded in contemporary surveys than in the sediment (Table 2.3).

Stress values in two-dimensional nMDS ordinations were 17.4% and 21.3% for contemporary and surface sediment plant data, respectively, suggesting a reasonable fit (Kruskal & Wish, 1978; Clarke, 1993). The procrustean superimposition approach and permutation test (PRO-TEST) indicated a relatively strong correlation (reflected by the m_{12} value of 0.47, which is the equivalent of

 r^2) with a P-value of 0.001 and a root mean square error (RMSE) value of 0.50 (Figure 2.2). The residuals (arrows in Figure 2.2) indicate the degree of congruence between the ordination results of the two data sets based on the site scores. Low procrustes residuals acquired from several sites (e.g. lakes Poyrazlar, Gici and Tatli) represented lakes where in particular *Ceratophyllum* species were recorded. The poorest correspondence (procrustes residuals >0.6) between the two data sets was mainly observed for lakes where *Utricularia* spp., *Myriophyllum* spp. or *Potamogeton* spp. were found in the modern plant survey (e.g. lakes Gölcük-Bolu, Abant and Sakli).



Figure 2.2 Procrustean plot comparing the nMDS ordination results. The black circle represents the rotated matrix (contemporary samples) and the points of the arrows represent the target matrix (surface plant remains). The length of the arrows indicates the procrustean residuals (longer arrow – higher residual – lower concordance) shown also at the right-hand side of the graph.

2.3.2 Relationship between surface sediment plant remains and environmental parameters

In a distance-based redundancy analysis (db-RDA), the chosen environmental variables captured c. 28% of the variation in plant remains in the surface sediment (Figure 2.3). The selection of variables for the analysis was based on ecological requirements of the plants and also in accordance with the explanatory strength of each variable.



Figure 2.3 db-RDA result for surface sediment plant remains (dashed arrows), environmental variables (bold arrows) and the distribution of the sampled 35 lakes (species abbreviations: MyrSpp: *Myriophyllum* spp, CerSpp: *Ceratophyllum* spp, EloCan: *Elodea canadensis*, ZanPal: *Zannichellia palustris*, NajMar: *Najas marina*, RupMar: *Ruppia maritima*, PotSpp: *Potamogeton* spp, RanBat: *Ranunculus* sect. *Batrachium*, ElaSp: *Elatine* sp, Nymphac: Nymphaceae, TraNat: *Trapa natans*, Charac: Characeae).

Distance-based redundancy analysis (db-RDA) reflected a strong relationship between the composition of the plant macrofossil assemblage and conductivity (Figure 2.3). Remains of *Najas marina*, *Ruppia maritima* and *Zannichellia palustris* correlated positively with high conductivity, whereas *Myriophyllum* was negatively related to conductivity. Total nitrogen, TP and Chl-a were strongly positively related to axis 2 and correlated with taxa such as Ceratophyllum and Potamogeton. Additionally, *Ranunculus* sect. *Batrachium* was positively correlated with the Secchi/maximum depth ratio (Sec/ M.Dep).

2.3.3 Relationship between present-day macrophytes and environmental parameters

Distance-based redundancy analysis (db-RDA) with the same environmental variables as those used for the surface sediment data explained 29% of the total variance of percentage plant volume inhabited (PVI%) (Figure 2.4). Present-day macrophyte conductivity, and to a lesser degree TP and TN, was positively related to db-RDA axis 1 (Figure 2.4). *Najas marina* and *Ruppia maritima* (recorded in two lakes, one having only 0.1% PVI) and *Zannichellia palustris* were positively correlated with these three variables (Figure 2.4). In contrast, floating-leaved species (*Nuphar lutea, Nymphaea alba* and *Trapa natans*) were negatively correlated with the same variables. Characeans, together with *Ceratophyllum* species, were more related to the Sec/M.Dep ratio. Moreover, *Utricularia* species were strongly negatively related to TP and TN variables.



Figure 2.4 db-RDA result for contemporary macrophytes (dashed arrows), environmental variables (bold arrows) and the distribution of the sampled 35 lakes (species abbreviations: UtrSpp: *Utricularia* spp, PotNat: *Potamogeton natans*, PolAmp: *Polygonum amphibium*).

2.4 Discussion

We found a variety of plant macrofossils in the surface sediments, and notwithstanding the lack of sedimentary remains of some taxa and an apparent over-representation of others, there was a significant positive relationship between present-day macrophyte assemblages and their sedimentary remains.

The relatively good agreement between the two plant data sets was driven by taxa which were well represented and well preserved in the sediment, such as *Ceratophyllum* spp. and *Najas marina*. In contrast, at some of the sites (e.g. lakes Gölcük-Bolu and Saklı) the similarity between the contemporary and plant macrofossil assemblages was lower, as indicated by high procrustes residuals (Figure 2.5). This may be attributable to sites where aquatic plant abundance is

lower than average and thus fewer subfossil remains are produced or to the presence of taxa that are not well represented in the sediments (e.g. *Potamogeton*, *Myriophyllum* and *Utricularia*). The increase in procrustes residuals as PVI% decreases (Figure 2.5) supports the former explanation.



Figure 2.5 Procrustes residuals against percent plant volume inhabited (PVI%) data of 35 lakes (lakes on the right-hand side of the graph are arranged according their residuals; top lake having the highest number of residuals).

As the sediment samples were retrieved from the deepest part of the lakes, some species may be under-represented in the sedimentary assemblage, depending on the characteristics of the seeds, such as size and buoyancy. For some species, their subfossils are mainly found close to the source vegetation and, thus, usually restricted to the littoral zone of the lakes (Birks & Birks, 1980; Zhao *et al.*, 2006; Koff & Vandel, 2008). For example, water lilies were present in 10 of the lakes although their seeds were found in the sediments of only four, which may reflect low seed production (Dieffenbacher-Krall, 2007). How-ever, since trichosclereids

were also found in only two of these lakes, it may be that remains of these plants cannot be expected to be abundant in sediments from the middle of the lake away from the sheltered littoral where these plants are typically found. In agreement with this, Davidson *et al.* (2005) found that Nymphaeaceae trichosclereid cells were less abundant in a central lake core than in samples from the littoral zone of a very small shallow lake. By contrast, *Potamogeton* seeds are potentially well dispersed (Davis, 1985). However, lower seed production by *Potamogeton* species, and also rapid decomposition of the vegetative parts (e.g. *Potamogeton* spp. leaf tips), might lead to under-representation as seen in this and other studies (Davidson *et al.*, 2005; Dieffenbacher-Krall, 2007; Salgado *et al.*, 2010).

There were also a number of cases where the contemporary record did not include taxa that were found as remains in the sediments. Characean oospores were recorded in the sediments of 23 of the 35 study lakes but were only found in seven of the lakes in the contemporary plant survey. Zhao *et al.* (2006) and Koff and Vandel (2008) also found characean oospores in lakes where charophytes were apparently absent or rare in the modern vegetation, perhaps indicating between-year variability in species frequency of plant communities (Capers, 2003). Other taxa that are better represented in the sediment record include *Potamogeton crispus*, *Najas marina* and *Zannichellia palustris*.

The apparent absence of macrophytes in the contemporary survey where their remains were recorded in the surface sediments shows that the relationship between the samples of living macrophytes and their sedimentary remains is not always straightforward (Zhao *et al.*, 2006; Dieffenbacher-Krall, 2007; Koff & Vandel, 2008). Firstly, surface sediments are the reflection of a number of years of accumulation, depending on sedimentation rates and disturbance (Dieffenbacher-Krall & Halteman, 2000), and are therefore a temporal integration of the former (typically 1–5 years) plant assemblage. Thus, interannual or seasonal shifts in macrophyte assemblages may be reflected in the sediment, but not in a single contemporary sample (Capers, 2003; Sayer *et al.*, 2010b). It has

been suggested that such variations occur more often in mesotrophic–eutrophic lakes (Jeffries, 1998; Capers, 2003; Titus *et al.*, 2004), the category to which our study lakes belong. Moreover, contemporary surveys of submerged plants were conducted with a rake, which may leave rare species undetected and, where those undetected taxa produce abundant sedimentary remains, they are likely to appear in the sedimentary assemblage. This appears to be the case here for the *Chara* taxa and in other studies for *Zannichellia palustris* (Davidson *et al.*, 2005; Zhao *et al.*, 2006). It appears therefore that in some cases, contemporary surveys of submerged macrophyte vegetation may not provide a full record of the aquatic flora of a site, whereas surface sediment samples may reveal the (recent) presence of some rare taxa.

The distance-based redundancy analysis (db-RDA) of both the contemporary assemblage and surface sediment data showed that conductivity was the most important environmental factor associated with the composition of the macrophyte flora, followed by the nutrients (TP and TN) and Chl-a. The apparent importance of conductivity shown in this study underpins the concerns about the impacts of salinisation in the lakes of this region (Beklioğlu *et al.*, 2011). Species tolerant of high salinity, such as *Zannichellia palustris*, *Potamogeton pectinatus*, *Ruppia maritima* and *Najas marina* (Comin & Alonso, 1988; Khedr, 1997; Heegaard *et al.*, 2001; Velasco *et al.*, 2006), were found in the lakes with the highest conductivity. Characeans generally prefer fresh waters (Winter & Kirst, 1990; Winter *et al.*, 1996), although their remains were recorded in both brackish and freshwater lakes, and some characean species are known to grow or even dominate in brackish waters (Stewart & Kantrud, 1972; Kirst *et al.*, 1988), supporting the presence of *Chara* oospores in our brackish lakes.

Water clarity, indicated by the Sec/M.Dep ratio, along with the strongly negatively correlated Chl-a, was an important variable for both plant remains and contemporary macrophytes. The genus *Ceratophyllum*, containing species which are among the most tolerant of eutrophic conditions (Beklioğlu *et al.*, 2003;

Stephen *et al.*, 2004; Pozo *et al.*, 2011), was associated with high TP and TN concentrations (e.g. Lake Gökgöl: 0.11 and 1.3 mg L⁻¹, respectively) for the sediment samples, although this pattern was less clear in the contemporary data. Conversely, *Utricularia* species appear to indicate nutrient-poor conditions and were negatively related to these variables (Adamec, 2008). Birks (2000) and Birks *et al.* (2001) observed that *Ranunculus* sect. *Batrachium* species decreased with increasing turbidity or water level changes, which is in good agreement with its strong positive correlation with the Sec/M.Dep ratio in the plant remains data.

Given the results of the procrustes analysis and the usage of same environmental variables for both ordination analyses, the similarity between the db-RDA results of the contemporary and fossil assemblages was inevitable. However, the results do carry information about both the similarities and differences in the responses of the subfossil and contemporary assemblages to environmental change. For example, conductivity was the most important variable explaining the variance in both data sets, but its impact was more pronounced for the sedimentary data. This difference in explanatory strength might be related to the presence of remains of salinity-tolerant species (e.g. Zannichellia palustris) in a higher number of lakes. Moreover, the interpretation of ecological status based on plant assemblages may also differ, depending on this difference in explanatory strength. For instance, db-RDA showed that characean assemblages were strongly related to conductivity in the sedimentary assemblage, whereas Sec/M.Dep was more important for the contemporary characean assemblage. Despite these differences, our analyses demonstrate that plant macrofossils may be useful indicators of past environments. These results increase the confidence with which past changes in macrofossil assemblages can be interpreted as having been driven by changes in conductivity or nutrient status or by a combination of the two.

Notwithstanding the bias in the data that results from differential production of sedimentary remains between taxa and from the different time periods represented by the contemporary and sedimentary samples, this study demonstrated, as have

others (e.g. Davidson *et al.*, 2005), that plant macrofossils provide a robust reflection of the contemporary macrophyte assemblage. There may be weaknesses associated with sampling sediments from the pelagic zone (e.g. Kowalewski *et al.*, 2013), resulting in the under-representation of some taxa. Under-representation was probably caused by low plant abundance, seed dispersal characteristics or vegetative parts that decompose relatively rapidly. Conversely, sedimentary remains appear to represent some taxa absent from the contemporary record, more effectively, perhaps due to the fact that they capture between-year variation within the macrophyte assemblage (Capers, 2003) or because they are difficult to sample in the contemporary environment (e.g. *Najas flexilis*). Thus, analysis of sedimentary remains may be a more useful record than contemporary data for some taxa, such as Characeae (e.g. Zhao *et al.*, 2006).

Our study also shows that the analysis of plant macrofossils from lake sediments can provide reliable information on past macrophyte assemblage composition that is rarely accessible in other ways. However, there are scenarios, for instance where plant abundance is low or the taxa present are not well represented, where the reliability of results would be increased by an integration of results from multiple cores (Davidson *et al.*, 2005; Sayer *et al.*, 2010b). Furthermore, the combination of plant macrofossil analysis with other palaeolimnological approaches analysing biological groups that are more reliably and less patchily distributed, such as those of cladocerans (Davidson *et al.*, 2011) or pigments (McGowan *et al.*, 2005), would increase the reliability of results.

CHAPTER 3

CONGRUENCE OF SURFACE SEDIMENT PIGMENTS WITH PRESENT-DAY PHYTOPLANKTON ASSEMBLAGES IN MEDITERRANEAN SHALLOW LAKES

3.1 Introduction

As a key primary producer, phytoplankton, are among the essential organisms in aquatic ecosystems (Bellinger & Sigee, 2010). Their role in matter circulation and energy flow is important and their occurrence influences the characteristics (e.g. growth and reproduction capacity) of other organisms (Azari *et al.*, 2010). Phytoplankton are affected by physical factors such as light and temperature, by chemical factors, such as nutrient concentrations, and by organism interactions within the plankton community (e.g. grazing- edible/inedible) (Findlay & Kling, 2003; Romero-viana *et al.*, 2010). The physical features of a habitat, like depth and/or area, are other important factors influencing the composition of a given phytoplankton assemblage (Findlay & Kling, 2003; Stomp *et al.*, 2011).

The response of phytoplankton to changes in ecosystems can be both qualitative and quantitative (Reynolds, 2006). They make up one of the essential and most studied groups within the context of the EU Water Framework Directive (WFD) (Poikane *et al.*, 2015), which has been implemented to assess the water quality of all water bodies (e.g. lakes, coastal waters) in Europe. However, phytoplankton identification to species level using microscopes is time consuming (Utermöhl, 1958) and demands a high level of taxonomic expertise. To lower the cost and time consumed, taxonomic resolution at phylum level can be used, depending on the research aim (Padisák *et al.*, 2006; Sarmento & Descy, 2008, Gallego *et al.*, 2012). Chemo-taxonomical classification of phytoplankton communities based on

marker pigments, allowing identification of phytoplankton classes (at phylum level) (Roy *et al.*, 2011), has been used as an alternative to microscopic identification in both contemporary (Buchaca *et al.*, 2005) and palaeolimnological studies (Bjerring *et al.*, 2013). Another advantage of using marker pigments in palaeolimnological studies is that they generally preserve long after the disapearance of the morphological structures of their sources (Leavitt & Hodgson, 2001; McGowan, 2013). Moreover, they reflect environmental changes in, for instance, nutrient loading, indicating their usefulness in reconstructing past phototrophic communities and production (Jeffrey *et al.*, 1997; Reuss, 2005; Mcgowan, 2013).

Various primary producers, e.g. phytoplankton and macrophyte assemblages, can be the principal source for sedimentary pigments (Leavitt & Hodgson, 2001). Nothwitstanding the advantages of using marker pigments, their degradation speed in the water column and sediment differs according to prevailing environmental conditions, thus affecting their preservation and complicating the interpretations (Reuss, 2005). Factors affecting the rate of pigment degradation in the water column are mainly related to lake morphometry (e.g. exposure to light, oxygen, heat) and zooplankton grazing but also to sediment resuspension and bioturbation (Steenbergen et al., 1994; Cuddington & Leavitt, 1999; Watanabe et al., 2012). Therefore, many studies have been conducted to understand variables affecting pigment composition and to determine the relationship between sedimentary pigments with monitoring environmental and/or phytoplankton data. For example, in an experimentally enriched Canadian boreal lake, Leavitt and Findlay (1994) showed a strong correlation between marker pigments from varved sediments and 20-year phytoplankton monitoring data. A study covering 82 lakes in the Pyrenean Mountains demonstrated the impact of deposition environment on surface sediment marker pigments and the most important factors defining pigment composition (Buchaca & Catalan, 2007). A combined palaeolimnological and monitoring study conducted in a large and deep lake (Lake Mjøsa, Norway),

monitored for nutrients, phytoplankton and zooplankton since 1972, showed that marker pigments captured the pattern related to eutrophication (Hobæk *et al.*, 2012). To our knowledge, these studies did not cover the relationship between present-day phytoplankton composition and surface sedimentary pigments, but recent similar studies have been conducted for cladocerans (Davidson *et al.*, 2007; Çakıroğlu *et al.*, 2014), macrophytes (Levi *et al.*, 2014-Chapter 2 of this thesis) and diatoms (Winegardner *et al.*, 2015), and these have revealed the importance of understanding the similarities and differences that can be deduced by comparing contemporary and sedimentary data. Moreover little is known about the representativeness of pigment in sediments from warm lakes potentially subjected to a higher degree of pigment degradation before algae sediment burial.

Phytoplankton-based studies in Turkey have mostly focussed on defining species composition (e.g. Naz & Türkmen, 2004; Çelekli et al., 2007), variations in algal communities, their biomass and size structure in relation to environmental changes (e.g. Kıvrak, 2006; Akçaalan et al., 2007; Yılmaz & Aykulu, 2010) and on determining factors causing cyanobacterial blooms (Albay et al., 2005). In recent years with the increase in WFD-related projects (Sümer & Muluk, 2011), studies to determine the ecological quality of lakes have been conducted (e.g. Demir et al., 2014) to fulfill the requirements of the WFD. The combination of Mediterranean climate characteristics (İyigün *et al.*, 2013) inducing significant water level fluctuations in Turkish lakes, intense anthropogenic pressures such as water extraction for irrigation (Coops et al., 2003; Beklioğlu et al., 2006) and eutrophication (e.g. Beklioğlu et al., 2003; Salioğlu & Karaer, 2004; Beklioğlu & Tan, 2008), have impacted lakes in Turkey and few monitoring programmes were initiated prior to the systems being impacted. Thus, the past condition and trajectory to current ecological conditions are unknown, with such information required to establish optimum protection/restoration measures.

Here, surface sediments and contemporary phytoplankton classes from 30, mostly shallow, lakes in Turkey were compared to reveal the degree of concordance between sedimentary pigments and contemporary phytoplankton classes and to identify the environmental conditions affecting these assemblages.

3.2 Methods

3.2.1 Study sites

The study sites (30 lakes) are located in six different climatic zones, defined according to the Köppen classification system (Peel *et al.*, 2007), with 14 lakes located in the hot/warm-summer Mediterranean zone (Csa/Csb, respectively), five in the semi-arid zone (Bsk), seven in the oceanic zone (Cfb), three in the humid temperate zone with hot summers (Cfa) and one lake in the warm humid continental (Dsb) zone. WorldClim Global climate data (30-arc-seconds resolution) obtained for the years 1950-2000 (http://www.worldclim.org/) indicated that total annual precipitation ranged between ca. 350 and 1000 mm, while mean annual temperature was approximately 8-18 °C. The altitudinal range covered was between ca. 0 and 1400m and the latitudinal gradient 36°41'-42°00'N, all situated in the western half of Turkey (Figure 3.1). The area of the lakes varied between ca. 0.1 and 700 ha, with 22 lakes being smaller than 100 ha.

The lakes were mostly shallow with maximum depths varying between ca. 1 and 17 m, and mean Secchi depth was 1.5 m (Table 1). The majority of the lakes were freshwater, but four were moderately saline, with mean salinity ranging from ca. 3 to 14‰. The trophic status of the lakes were meso-hypertrophic with total phosphorus (TP) concentrations between ca. 15 and 633 μ g L⁻¹, chlorophyll a (Chl-*a*) concentration between ca. 2 and- 90 μ g L⁻¹ and total nitrogen (TN) concentrations between 239 and 2622 μ g L⁻¹. Mean coverage of submerged and floating-leaved macrophytes was ca. 39%; however, no plants were recorded in four of the lakes. Mean total zooplankton biomass was 49.9 μ g L⁻¹.



Figure 3.1 Map of Turkey showing the study sites

Variables	Unit	Median	Mean	Minimum	Maximum
Altitude	m	910	666	0	1423
Area	ha	17.2	76	0.1	714.3
Maximum depth	m	3.3	3.9	0.7	17.4
Secchi depth	m	1.0	1.5	0.2	9.0
Mean water temperature	°C	23	22	8	32
Total phosphorus	μg L ⁻¹	78	119	15	633
Total nitrogen	$\mu g L^{-1}$	799	961	239	2622
Salinity	‰	0.3	1.3	0.1	14.5
Alkalinity	meq L-1	1.7	3.2	0.5	13.5
pН	-log[H+]	8.1	8.2	6.3	9.5
Chlorophyll a	$\mu g L^{-1}$	13.0	23.0	1.8	90.8
Phytoplankton biovolume	$mm^3 L^{-1}$	3.9	11.1	0.1	76.7
Zooplankton biomass	$\mu g DW L^{-1}$	9.7	49.9	0.1	466.8
Submerged & Floating- leaved Plant Coverage	%	40	39	0	89

Table 3.1 General characteristics of the 30 study lakes

3.2.2 Water Sampling and Analysis

Summer "snap-shot" measurements were made once to determine physical, chemical and biological properties of the study lakes. Samples including the entire water column were taken at the deepest point of the lakes and were subsequently analysed for TP and TN concentrations and alkalinity using standard analytical methods (Mackereth *et al.*, 1978; San++ Automated Wet Chemistry Analyzer, Skalar Analytical, B.V. Breda, The Netherlands; Golterman *et al.*, 1978). Secchi depth was measured with a 20 cm diameter white disc and pH, salinity and temperature with a YSI 556MPS multiprobe field meter (YSI Incorporated, OH, USA).

Zooplankton and phytoplankton samples were obtained from depth-integrated water samples and preserved in Lugol's solution (for details see Tavşanoğlu *et al.*, 2014; Erdoğan *et al.*, in prep.). Aquatic plants were sampled along parallel transects to determine the composition and coverage of submerged and floating-leaved species (for details see Levi *et al.*, 2014-Chapter 2 of this thesis).

Phytoplankton counting/identification was made according to the Utermöhl technique (1958) by settling phytoplankton samples for 24 hours prior to counting under a Leica DMI 4000B inverted microscope at 40x or 63x magnification. At least 400 individuals from the most abundant species were counted and 10 individuals from each species were measured to calculate biovolume according to the closest geometrical shapes (Hillebrand *et al.*, 1999). For the current study, phytoplankton data at class level was used and the results for each phytoplankton class were given as biovolume (mm³ L⁻¹). The countings and calculations were conducted at the Limnology Laboratory of Middle East Technical University (Erdoğan *et al.*, in prep.).

3.2.3 Coring and Pigment Analyses

A KC-Denmark Kajak Corer (internal diameter: 5.2 cm) was used to retrieve surface sediment samples (0-2 cm) from seven or ten different locations in the pelagic zone; the samples were subsequently pooled.

Water and organic matter contents of the sediments were estimated by heating the sediments for 12 hours at 105 °C and subsequently two hours at 550 °C, respectively (Nesje & Dahl, 2001; Heiri *et al.*, 2001; Smol, 2008).

For pigment analyses 2 cc sediment from each lake was transferred into 5 cc brown bottles and stored frozen (-18 °C) and then freeze-dried prior to extraction of pigments. Hereafter approximately 0.2 g freeze-dried sediment was used for extraction of pigments in 4 ml 90% acetone with a probe sonicator (2 min). The extracts were separated from the sediment by centrifugation for 4 min at 3000 rpm and then filtered through 0.2 μ m syringe filters. Finally, marker pigment composition was analysed by high performance liquid chromatography (HPLC). The HPLC system (Waters, Milford, MA, U.S.A.) was equipped with a 600E solvent delivery system, a 717 autosampler set at 4 °C, a C18 column (dimensions: 250 x 4.6 mm, particle size: 5 μ m, Spherisorb-ODS1 Waters, Milford, MA, U.S.A.) and a 996 photodiode array detector (set at 440 and 660 nm). Separation of pigments was performed at a constant flow rate (1.2 ml/min) using the system described by Buchaca and Catalan (2007).

Pigment identification was made by comparing the relative retention time and absorption spectra of the pigments with the reference spectra library elaborated by Dr. Teressa Buchaca and Lissa Skov Hansen, Department of Bioscience, Aarhus University, Denmark, using also reference material obtained from DHI Water and Environment (www.dhigroup.com/) and Jeffrey *et al.* (1997). Pigment concentrations were calculated using the LOI data and given as nmol g^{-1} OM. Marker pigments and their affinities are shown in Table 3.2. The ratio of chlorophyll-*a* to *a*-phorbins was used as an indicator of the pigment preservation

in the lakes (Figure 3.2-A). Even though not all a-phorbins were recorded, the ratio provides a good indication of pigment preservation, with 0 implying "no preservation" and 1 "full preservation" (Buchaca *et al.*, 2011).

Table 3.2 Sedimentary pigments and their taxonomic affinities defined according to Leavitt and Hodgson (2001), Buchaca and Catalan (2007), Buchaca and Catalan (2008) and Buchaca *et al.* (2011).

Pigment	Taxa affinity
β,β-Carotene	Cyanobacteria, Chlorophyta, Eukaryotic algae, Vascular plants
β,α-Carotene	Cryptophyta, Chrysophyta, Dinophyta, some Chlorophyta
Alloxanthin	Cryptophyta
Diatoxanthin	Bacillariophyta
Fucoxanthin	Chrysophyta, Bacillariophyta
Diadinoxanthin	Dinophyta, Chrysophyta, Bacillariophyta
Aphanyzophyll	Cyanobacteria (N2-fixing groups)
Canthaxanthin	Cyanobacteria, Zooplankton (Cladocera)
Echinenone	Cyanobacteria, Zooplankton (Cladocera)
Zeaxanthin	Cyanobacteria, Chlorophyta
Lutein	Chlorophyta
Chlorophyll-b	Chlorophyta, Euglenophyta, Vascular plants
Chlorophyll-a	The molar ratio Chl-a/a-phorbins is an indicator of preservation
Pheophytin-a	Chl-a degradation product (senescence)
Pheophorbide-a	Chl-a degradation product (grazing processes)

3.2.4 Numerical analysis

Detrended Correspondence Analyses (DCA) was employed for both pigment and phytoplankton data, and the length of the first axis was used as a measure of defining the species response along an ordination axis. The axis length was lower than 3 standard deviation (SD) units, suggesting that linear ordination methods should be used (ter Braak, 1995). Principal component analysis (PCA) and redundancy analysis (RDA) were therefore applied to investigate the relationship between sedimentary pigments and contemporary phytoplankton data. Prior to these analyses, Hellinger transformation, proven to have good statistical properties and being an appropriate transformation for linear ordination methods (Legendre & Gallagher, 2001), was employed for both pigment and phytoplankton data. The distribution of environmental variables was tested by Kolmogorov-Smirnov tests, and if they deviated from the normal pattern, they were either $log_{10}(x+1)$ or square root transformed.

Following transformation, PCA was conducted on sedimentary pigment (nmol g⁻¹ OM) and phytoplankton biovolume (mm³ L⁻¹) data. Subsequently, Procrustes rotation analysis followed by a PROTEST permutation test were applied to the resultant ordinations in order to explore the overall compatibility between these two ordinations and to assess the significance of the concordance between these results (Gower, 1971; Jackson, 1995; Peres-Neto & Jackson, 2001). Another PCA was carried on to investigate the relationship between Procrustes residuals (errors) and environmental parameters, which were chosen based on simple linear regressions between residuals and each environmental variable.

Redundancy analysis (RDA) was also conducted for both sediment and contemporary data allowing a comparison of the results obtained from the two data sources. Environmental variables used in the analyses were chosen according to variance inflation factors (VIF), enabling selection of the parameters explaining maximum variation in the species data, and by employing Monte Carlo permutation tests that are used to deduce significant variables. VIF values are used to determine the degree of colinearity between environmental variables, and where VIF values were larger than 20 they were excluded from further analyses. Furthermore, after the Monte Carlo permutation test with 999 random permutations, environmental parameters with low degree of significance (p > 0.05) were removed from the analyses.

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R version 3.2.3 (R Development Core Team, 2012) was used for all transformations and analyses. DCA, PCA, procrustes analysis, PROTEST and RDA were conducted using the vegan R package (version 2.3-3) (Oksanen, 2016).

3.3 Results

In total, two types of chlorophyll pigments, four types of chlorophyll degradation products and eleven carotenoid pigments were found (Figure 3.2-A and B). The affiliations of the pigments can be seen in Table 3.2. Moreover, seven phytoplankton classes – Bacillariophyta, Chrysophyta, Dianophyta, Cryptophyta, Chlorophyta, Euglenophyta and Cyanobacteria – were observed in the contemporary samples. Total phytoplankton concentrations varied between ca. 0.1 and 77 mm³ L⁻¹ (mean value of 13 mm³ L⁻¹).

3.3.1 Comparison of marker pigments from surface sediments with phytoplankton from the water column

Most of the lakes had a chlorophyll-*a*: *a*-phorbins ratio higher than 0.25, only five were <0.25 (Figure 3.2-A). In 12 of the lakes preservation was higher with a ratio >0.5 (closer to "full-preservation; 1.0" than to "no-preservation, 0.0"). It should be noted, that the change in the chlorophyll-*a*:*a*-phorbins ratio is less indicative of the preservation of carotenoid pigments since chlorophylls are less stable (Leavitt & Carpenter, 1990; Leavitt & Hodgson, 2001; Buchaca *et al.*, 2011).

Comparison of the marker pigments in each affinity group (i.e. for Cyanobacteria: echinenone vs. canthaxanthin) showed that in each lake most phytoplankton classes were represented as pigments (Figure 3.2-B). From the seven phytoplankton classes recorded in the water column, Chlorophyta, Cyanobacteria and Bacillariophyta were found in more than 25 lakes each, Dinophyta and Euglenophyta in 18 lakes each and Crysophyta in 10 lakes (Figure 3.2-B). Furthermore, Cyanobacteria were the dominant group in 14 lakes, followed by Chlorophyta, present at six of the study sites. Similarly, lutein (Chlorophyta)

marker), zeaxanthin (cyanobacteria and Chlorophyta) and diatoxanthin (Bacillariophyta) were the most recorded marker pigments in the surface sediment and were observed in 30 and 31 (the latter two) lakes, respectively. Furthermore, the cyanobacteria marker pigment concentration was higher in 11 lakes, notwithstanding their relatively lower concentrations in the dataset on contemporary phytoplankton. Fucoxanthin (Chrysophyta, Bacillariophyta marker) was the other dominant pigment for a different set of 11 lakes. Compared with the water column Cryptophyta and with Dinophyta phytoplankton data, alloxanthin (Cryptophyta marker) and diadinoxhanthin (Dinophyta, Chrysophyta, Bacillariophyta marker) pigments were recorded in a higher and lower number of lakes, respectively (Figure 3.2-B).



Figure 3.2 Study sites with A) a-phorbins (chlorophyll-*a*, chlorophyllide-*a*, pheophytin-*a*, pheophorbide-*a*) and ratio of chlorophyll-*a* and *a*-phorbins (chlorophyll-*a*, chlorophyllide-*a*, pheophytin-*a*, pheophorbide-*a*), indicating preservation of the pigments (0: no preservation; 1: full preservation of chlorophyll-*a*) B) presence-absence data of phytoplankton classes (black bars) and marker pigment data (nmol g⁻¹ OM) (grey bars). It should be noted that pigment data indicate the general trend and do not show the exact concentrations (due to a high scale difference the larger of the bars were cut). Study sites were sorted according to their pigment preservation from low (top) to high (bottom).
Variances explained by the first two PCA axes were 53% and 50% for contemporary phytoplankton data and sedimentary remains (pigments), respectively. The procrustean superimposition approach and permutation test (PROTEST) indicated a significant correlation (reflected by the m_{12} value of 0.73, which is the equivalent of r^2) with a *P*-value of 0.001 (Figure 3.3). However, root mean square error (RMSE) with a value of 0.93 and the Procrustes sum of squares of 25.95 were relatively high (Figure 3.3). The residuals (arrows in Figure 3.3) indicate the degree of concordance between the ordination results of the two datasets based on the site scores.

There was overall a good correspondence between sedimentary and water column data for most of the lakes, but 10 lakes had relatively high residuals. Fucoxanthin was dominant in four of the lakes (Lakes Poyrazlar, Koca, Serin and Mogan), where no Chrysophyta and/or Bacillariophyta were recorded. Therefore, the direction of the Procrustes plot moves towards fucoxanthin pigment (see overlying PCA plot - Figure 3.3). Moreover, diadinoxanthin was over-represented in Lake Mogan, while in the other three lakes Dinophyta was found in the contemporary samples as well. Three other lakes (Lakes Abant, Yayla and Balıklı) were clustered close to alloxanthin and diatoxanthin pigments. In Lakes Abant and Yayla, the dominant pigments were alloxanthin and diatoxanthin, while in the water column the dominant phytoplankton classes were Dinophyta and Cyanobacteria, respectively. Moreover, even though the dominant phytoplankton class was Cryptophyta in Lake Balıklı, the dominance was shared between fucoxanthin and diatoxanthin pigments in the sediment. In the last set of lakes (Tatli, Gici and Baldimaz) the dominant phytoplankton class and representative sedimentary pigments belonged to Cyanobacteria; however, the underrepresentation of alloxanthin (Cryptophyta marker) in Lake Tatlı and of lutein (Chlorophyta marker) in Lake Baldımaz likely led to a higher procrustes residuals (Figure 3.3).



Figure 3.3 Procrustean plot comparing the PCA ordination results. The black circle represents the rotated matrix (contemporary samples), and the points of the arrows represent the target matrix (surface pigment remains). The length of the arrows indicates the procrustean residuals, (longer arrow – higher residual – lower concordance). The overlaid species PCA plot was obtained from sedimentary pigment PCA.

Simple linear regression analysis revealed that suspended solid concentration and percent macrophyte (submerged and floating-leaved) coverage had marginally significant effect on procrustes residuals. The PCA, which was conducted using these variables, revealed that most lakes with high errors were positively related to these two variables (Figure 3.4), while Lakes Serin and Mogan, also Lake Baldimaz were negatively correlated with suspended solid and coverage, respectively.



Figure 3.4 PCA result, indicating the classification of the lakes according to procrustes residuals (shown with different circle sizes, small circles: low residuals, large circles: high residuals) and environmental variables (arrows) that could have an impact on the degree of similarity between surface remains and contemporary phytoplankton data (Coverage: percent submerged and floating-leaved plant coverage).

3.3.2 Relationship between sedimentary pigments and environmental parameters

In the RDA environmental variables, mean temperature, TP, alkalinity, macrophyte coverage and zooplankton biomass, captured approximately 38% of the variation in sedimentary pigments (Figure 3.5). The selection of significant (p<0.05) variables was based on the Monte Carlo permutation tests with 999 random permutations. RDA showed a strong relationship between marker pigments and mean water temperature, together with TP for the first axis, while the second axis was strongly related to total zooplankton biomass and alkalinity

(Figure 3.5). Cyanobacteria marker pigments, aphanyzophyll, echinenone, canthaxanthin and zeaxanthin were positively correlated with high temperature and TP, whereas fucoxanthin, diadinoxanthin and chlorophyll-*b* were negatively related to these variables. Axis 2 was strongly negatively correlated with alloxanthin marker pigment. Canthaxanthin was also positively correlated with cladoceran biomass. Additionally, chlorophyll-*b* was positively correlated with macrophyte coverage.



Figure 3.5 RDA result for sedimentary pigments (dashed arrows), environmental variables (bold arrows) and the distribution of the sampled 30 lakes (environmetal variable abbreviations: Mean Temp: Mean Water Temperature, TP: Total Phosphorus, Cladocera: Cladoceran biomass, Coverage: Mean submerged and floating-leaved plant coverage).

3.3.3 Relationship between contemporary phytoplankton and environmental parameters

Redundancy analysis (RDA) with the significant environmental variables, mean water temperature, TN and TP explained approximately 24% of the total variance of phytoplankton class composition (Figure 3.6).

Total phosphorus had higher positive correlation with RDA axis 1, followed by temperature. Moreover, while cyanobacteria were positively related to axis 1, all the other classes had a negative correlation. Only temperature was positively correlated with RDA axis 2, which was strongly positively correlated with Dinophyta and to a lesser degree with Chrysophyta. In contrast, other phytoplankton classes, especially Euglenophyta, were negatively correlated with the second axis.



Figure 3.6 RDA result for phytoplankton class (dashed arrows), environmental variables (bold arrows) and the distribution of the sampled 30 lakes (environmental variable abbreviations: Mean Temp: Mean Water Temperature, TP: Total Phosphorus).

3.4 Discussion

The data show a clear correspondence between sedimentary pigments and contemporary phytoplankton classes and demonstrated the similarity of the most significant environmental factors explaining both surface sediment pigments and contemporary phytoplankton datasets. Moreover, discrepancy between the two datasets was possibly caused by under- or over- representation of the contemporary data depending on the environmental variables affecting pigment degradation and sedimentation in the lakes, respectively.

The generally good agreement between the two datasets was probably a result of the mutual dominance of relatively stable pigments and their affinity phytoplankton classes (e.g. echinenone pigment-cyanobacteria) in sedimentary and contemporary assemblages, respectively. However, higher procrustes residuals indicated that the correspondence between the pigment and phytoplankton datasets was lower at some of the sites (e.g. Lakes Mogan and Balıklı). This higher difference might be attributed to the environmental variables, possibly increasing the rate of pigment degradation, thus resulting in disappearance of the labile pigments. The simple linear regression and the PCA revealed a positive correlation of the procrustes errors with suspended solids that possibly points to lower pigment preservation (Leavitt, 1993; McGowan, 2013). Our study sites, with larger errors, mostly had higher than average suspended solid concentrations. Shallow lakes are more prone to wind disturbance (leading to higher concentrations of suspended solids), likely preventing the settling out of the pigments, and warmer temperatures recorded at the study sites, may contribute to the degradation of pigments (McGowan, 2013). Accordingly, in large shallow Lake Võrtsärv, Estonia, Freiberg et al. (2011), attributed the low correlation between phytoplankton dynamics in the water column and sedimentary pigments to sediment resuspension. Even though it was not a significant variable, the impact of depth was likely a contributing factor to lower pigment preservation in some of our study sites. For instance, in deep Lake Abant (maximum depth 17 m), the dominant phytoplankton class in the water column was Dinophyta; nevertheless, diadinoxanthin (main pigment for Dinophytes), which is a relatively labile pigment (Leavitt & Hodgson, 2001), was not recorded in the surface sediment. This condition highlights the possible impact of high water levels causing slow settling out of pigments, resulting in their degradation and thus under-representation in the sediment (Cuddington & Leavitt, 1999).

Depending on sedimentation rates and disturbance, surface sediments may reflect the accumulation of the last several years. Therefore, while contemporary phytoplankton data represent the period when samples were retrieved (e.g. seasonal phytoplankton studies, Anneville et al., 2002), surface sediments show a temporal integration (typically 1-5 years) of the last few years (Brothers et al., 2008). This could explain the greater discrepancy between the sedimentary pigment and contemporary phytoplankton datasets from the study sites, probably causing over-representation of the contemporary assemblages. For example, one of the study sites, Lake Mogan, has been bi-weekly (approximately) monitored since 1997 (Özen et al., 2010) and thus has a long record of diverse water-column phytoplankton assemblages. However, the phytoplankton classes used in the current study only derived from one sampling event (simultaneously with the surface sediment coring), revealing that cyanobacteria species were the only ones present in the lake. Nevertheless, the marker pigment assemblage indicated a more diverse community (Figure 3.2) and was thus closer to the longer-term phytoplankton records. Correspondingly, as a part of their study, Winegardner et al. (2015) compared the diatom species composition from the surface sediments of a large set of lakes (in total 468) with diatom assemblages determined from a single visit and from temporally averaged water-column samples. They revealed a stronger correlation between the averaged water column samples and rhe surface sediment than with the results of the single sampling event (Winegardner et al., 2015).

The relation between sedimentary pigments and phytoplankton assemblages was not dependent only on under- or over- representation of the species, calling attention to a more complex relation. Higher dissimilarity between sedimentary and contemporary datasets in relation to aquatic vascular plant coverage of the study sites (indicated by simple linear regression followed by PCA) probably shows the contribution of macrophytes to pigment pool, since they can also be the principal pigment sources in the sediment (Leavitt & Hodgson, 2001). Concordantly, in a core retrieved from Lagunillo del Tejo pond, Spain, Romero-Viana *et al.* (2009) identified the shifts between the planktonic and littoral (mostly submerged plants) primary producers for the last two centuries by employing the sedimentary pigments and thus pointing out to the plant pigment contribution to sediments.

Redundancy analyses of both pigment and phytoplankton data were comparable, showing that nutrient concentrations (TP) together with mean water column temperature were the most important environmental factors explaining the variance in phytoplankton communities. Accordingly, cyanobacteria communities and their representative pigments (canhaxanthin, echinenone, aphanyzophyll and zeaxanthin) were generally found in warmer lakes, with higher nutrient concentrations (Dokulil & Teubner, 2000). The importance of nutrients along with warmer temperature underpins the concern regarding eutrophication in this region, since it is predicted that climate change will lead to further exacerbation of eutrophication in the Mediterranean region (Özen et al., 2010). Correspondingly, Cryptophyta, comprising species tolerant to relatively low water temperature (Reynolds, 2006), and its pigment (alloxanthin) were found to be negatively associated with water temperature, and chrysophytes (fucoxanthin pigment) and dinophytes (diadinoxanthin pigment), known to prefer oligo-mesotrophic conditions (Watson et al., 1997; Ptacnik et al., 2008), showed a negative relation to TP in our lakes. These results provide further evidence supporting use of marker pigment studies, proving the reliability of pigments use for investigating the eutrophication history (Leavitt & Hodgson, 2001; Waters et al., 2009; Watanabe et al., 2012) or the impact of climatic changes (Hodgson et al., 2006; Fietz et al., 2007; Reuss et al., 2010).

Notwithstanding the similarity of environmental factors forming the sedimentary and contemporary assemblages, our results also identified the differences, with a set of more versatile environmental variables explaining the variation in sedimentary pigments. Mean plant (submerged and floating-leaved) coverage and cladoceran biomass were two of the additional factors (compared with contemporary assemblages) related to pigment data and compatibly these variables were shown to be important in defining phytoplankton assemblages from the water column (e.g. Scheffer *et al.*, 1993; Meerhoff *et al.*, 2003). For example, cryptophytes (alloxanthin pigment) whose abundance can be promoted under strong grazing conditions (Leavitt *et al.*, 1989; Søndergaard *et al.*, 2008), were strongly positively associated with cladoceran biomass in the study lakes. This difference in the explanatory environmental variables may be a result of the temporal integration of surface sediments (e.g. Winegardner *et al.*, 2015), representing the last few years and likely pointing out to more comprehensive representation of lake conditions by pigments.

More versatile environmental variables explaining the variance of the pigment dataset, may also point out to the impact of other parameters, such as preservation (Leavitt, 1993). For instance, the positive association between the relatively labile pigment diadinoxanthin (Leavitt & Hodgson, 2001) with alkalinity might be a result of the better preservation of pigments in the lakes with higher alkalinity (Buchaca & Catalan, 2007). It should also be noted that most of the pigment markers have affinities with several phytoplankton classes, likely affecting the clustering of the pigments relative to environmental variables, leading to differences in the response of pigments to environmental change. For example, even though chlorophyll-b pigment can be regarded as a representative of chlorophytes one may expect that it would be clustered together with other representative pigments, like lutein. However, they may also be found in vascular plants (Jeffrey & Humphrey, 1975; McGowan, 2013), supporting the positive correlation of chlorophyll-b with submerged and floating-leaved plant coverage in the study sites. A similar case was also observed for the cyanobacteria-related pigment canthaxanthin, which was found to be positively correlated with zooplankton (Buchaca & Catalan, 2007).

Notwithstanding pigment preservation issues and temporal integration of surface sediments, the marker pigment and contemporary phytoplankton class datasets showed good correspondence. Our study demonstrates that sedimentary pigments provide a relatively good representation of the contemporary phytoplankton assemblage. Additionally, as previous studies showed (Leavitt & Findlay, 1994; Hobæk et al., 2012), it indicates that pigments could elucidate the environmental conditions in the lakes. Moreover, the use of pigments has a relatively simple and less time consuming identification process and pigment analysis may also be the only way to obtain signatures of soft-bodied photosynthetic organisms (McGowan, 2013), yielding reliable tracking of past environmental changes. Nevertheless, care should be taken when interpreting the results since low pigment preservation may have a significant impact, possibly causing underrepresentation or preventing interpretations (e.g. Lake Beysehir in Levi et al., 2016-Chapter 4 of this thesis). Therefore, the reliability of the results would increase with multiproxy approaches (e.g. Buchaca et al., 2011), enabling the combination of various biological and/or geochemical proxies. Conversely, sedimentary remains reveal absence of taxa from the contemporary record, perhaps due to the fact that they capture between-year variation within the phytoplankton community. Thus, analysis of sedimentary pigments may be a more useful and faster method for defining ecological conditions of lakes than single-year contemporary data.

CHAPTER 4

MULTI-PROXY PALAEOECOLOGICAL RESPONSES TO WATER LEVEL FLUCTUATIONS IN THREE SHALLOW TURKISH LAKES²

4.1 Introduction

The hydrology and ecosystem dynamics of shallow Mediterranean lakes respond to the natural cycle of seasonal dry and wet periods of variable annual and interannual periodicity and intensity. Along with intense seasonality and climatic change, long term anthropogenic impacts of irrigation, damming, soil erosion and groundwater drawdown have greatly affected lake water levels and their continued variability in the Mediterranean region (Coops *et al.*, 2003).

Lake ecosystems respond to water-level change, as their inter-linked habitats adjust to dynamic environmental parameters. Prediction of future ecological patterns and trajectories of change due to lake water-level change is therefore difficult, but can be assisted by assessment of recent palaeoecological and sediment evidence from lakes with documented water-level records. Individual lakes will respond to water-level changes in a unique way, depending on their basin morphometry, climate, duration and magnitude of change and resilience of ecological communities, but some general processes can be identified. Water-level fluctuations expand and contract spatial patterns of sedimentation and determine edaphic–hydrological conditions that control the zonation of littoral vegetation (Harrison and Digerfeldt, 1993). A significant rise in lake level causes

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transgressive overlap of fine-grained muds (low energy, deep water) on top of shoreline sediments (high energy, shallow water), expanding the area for aquatic and marginal plants. Conversely, a regressive fall in lake level will reduce the areal extent of littoral habitats and may generate a hiatus in deposition due to erosion of previously deposited high lake-level fine mud. This simple model is of course complicated by basin morphology; i.e. a fall in level, revealing a wide shallow margin that may increase littoral habitats. Reduced water depth may also cause a greater proportion of catchment-derived matter to be transported towards the centre of the lake basin. Equally, a fall in water depth can extend the photic zone to the bottom of shallow, turbid lakes, increasing the potential area of aquatic plant growth and organic sedimentation. Periods of water-level stability allow development of marginal wetland peats, carbonate benches and chemical precipitates depending on water chemistry (Jones and Jordan, 2013).

Indirect effects of water-level change on biological communities include changing nutrient availability, turbidity (water clarity) and fish predation (Jeppesen *et al.*, 2015). Reduced water levels in shallow lakes can cause a decrease or increase in water clarity depending on the morphometry and amplitude of water-level reduction (e.g. proportion of sediment exposed to the resuspension), thus, either negatively or positively affecting aquatic macrophyte and benthic/epiphytic diatom growth (Jones and Jordan, 2013). Furthermore, increased water levels reduce light penetration to the lake bottom, having a negative impact on the growth of light-demanding species. The direction of water-level change can have dual effect on fish, zooplankton and phytoplankton numbers. Where macrophytes expand, small planktivorous fish can take refuge in macrophyte beds, especially in warmer climates (Meerhoff *et al.*, 2007), causing an increase in small-bodied zooplankters (e.g. rotifers and small cladocerans) and in phytoplankton due to the enhanced fish predation on large-bodied zooplankton (Amsinck *et al.*, 2007), Conversely, very low water levels can induce fish kills (Nõges *et al.*, 2007),

expectedly resulting in higher abundance of large-bodied zooplankters (e.g. *Daphnia* spp.) and lower phytoplankton biomass (Iglesias *et al.*, 2011).

Lake ecosystems in Turkey are subject to annually variable water volumes, depths and salinities due to the Mediterranean climate of extreme summer heat with high evaporation and variable autumn/winter precipitation (Beklioğlu *et al.*, 2006; İyigün *et al.*, 2013). As in many other parts of the world where arid to semi-arid climates predominate, lake catchments in Turkey have been heavily modified by human activities, especially in the last century due to agricultural and population demands on water usage.

Climate change projections indicate that catchments in arid and semi-arid Mediterranean regions are likely to show an approximate decrease of 25-30% in precipitation and enhanced evaporation accompanied by an even stronger reduction in runoff by the end of the 21st century (Giorgi and Lionello, 2008). This would magnify seasonal and multiannual water-level amplitudes and enhance hydrological stresses and thus cause prolonged hydraulic drought periods.

Developing adaptive management plans in order to protect lake environments and their ecosystem services is therefore a necessity. However, to develop suitable adaptive strategies, it is essential to investigate the response of lakes and resilience of ecological communities to past water-level fluctuations as a key to understanding current and future conditions. Multi-proxy palaeolimnological techniques can contribute to such an understanding, especially where there is no long-term biological monitoring data. To elucidate this further, a range of palaeolimnological techniques was employed on dated sediment cores from three lakes (Lakes Beyşehir, Marmara and Uluabat) for which long-term instrumental water-level monitoring records (50-100 years) were available. This paper addresses three key questions:

- i. Do recent sedimentary (biological/non-biological) records accurately reflect known water level changes?
- ii. Do measured proxies respond in synchrony to known water level changes?
- iii. If relationships between proxy data and known water-level changes are complex or poorly-correlated, what factors might account for this?

4.2 Methods

4.2.1 Study sites

The three study lakes are located in the mid-western part of Turkey (Table 4.1, Figure 4.1). According to Köppen-Geiger Climate classification Lake Beyşehir is located in the Warm-summer Mediterranean climatic zone (Csb), while Lakes Marmara and Uluabat are located in Hot-Summer Mediterranean zone (Csa). The instrumental water-level data from these lakes were compiled by the General Directorate of State Hydraulic Works (DSI) and the General Directorate of Electrical Power Resources (EIE) of Turkey (here onwards DSI-EIE Database). The three lakes have been classified as important bird areas (IBA) since 2004 (BirdLife International, 2015). Moreover Lake Beyşehir is one of the most important plant areas (IPA) in Turkey (PlantLife International, 2015) and since 1991 it has been classified as a 1st degree Natural Site protection area (a site protection status defined by the Turkish Ministry of Culture) (Nas *et al.*, 2009), while Lake Uluabat has been listed as a Ramsar site since 1998 (Salihoğlu & Karaer, 2004).

Lakes	Location		Surface	Maximum	Trophic
	Ν	E	Area (km ²) ^a	Depth (m) ^b	status
Marmara	38°36'50.0"	27°60'55.0"	68	5.0	Eutrophic
Beyşehir	37°45'10.0"	31°30'50.0"	730	9.0	Oligo- mesotrophic
Uluabat	40°10'45.0"	28°35'30.0"	240	4.5	Eutrophic

Table 4.1 General characteristics of the study sites

^a Information on surface areas received from Beklioğlu *et al.* (2006)

^b Information on maximum depths received from Bulkan (2009) for Lake Marmara, Beklioğlu *et al.* (2014) for Lake Beyşehir and Karaer *et al.* (2012) for Lake Uluabat.



Figure 4.1 Location map of Turkey and the three study lakes, showing the littoral (black stars) and pelagic (white stars) core locations and the main outflows (lines with black arrows) and inflows (black lines) of the lakes. The black rectangle on the south-east cost of Lake Marmara shows the impoundment

4.2.1.1 Lake Marmara

At the beginning of the 20th century, Lake Marmara was endorheic, saline and around 50% smaller than at present (Girgin, 2000). Inflow (17 hm³ yr⁻¹) to the lake was originally derived from temporary streams and fault-generated springs (Altınayar et al., 1994). The water level and area of the lake increased in the early-mid 20th century, after converting the lake to a reservoir for irrigation purposes between 1932 and 1953 (Girgin, 2000). The construction of the first canal network south of the lake, which is used to divert the surplus lake water to the Gediz River, was completed in 1945. In the north a diversion canal and a regulator were built to divert river water to the lake (completed in 1952), and in the south another regulator was constructed to control the outflow from the first drainage canal and to ensure a lake level of 79 m.a.s.l (completed in 1953). Another canal on the eastern side carrying the water from a dam to the lake was completed in 1955. An impoundment was also made in the eastern part (finished in 1963) to prevent flooding (Girgin, 2000). Maximum water storage capacity was reached around 1960, with a level of 79.2 m.a.s.l and area of 6800 ha (Arı & Derinöz, 2011). Prior to the early-mid 20th century, the lake level was measured as ca. 73-74 m.a.s.l (Girgin, 2000). Downstream irrigation demands are probably the most significant control on water loss from the lake (Altinayar et al., 1994).

Turkish State Meteorological Service data (available at http://mgm.gov.tr/) indicated that the months with higher precipitation had been between November and March for the 1980-2012 period. Pronounced intra-annual, spring/summer low water-level periods and inter-annual water-level fluctuations have subsequently been recorded (Beklioğlu *et al.*, 2006; DSI-EIE Database).

During very shallow periods, previously flooded areas of the lake and surrounding wetlands have been cultivated (Arı & Derinöz, 2011). Agricultural data retrieved from the Turkish Statistical Institute (TÜİK) (available at http://tuik.gov.tr) shows that between 1995 and 2012 there has not been a significant change in the area of cultivated land around the lake. Conversely, the area of artificial land use, such as

continuous urban areas and industrial units, increased from 1995 to 2006 (available at http://aris.ormansu.gov. tr/csa/). In addition, increased nutrient inputs due to population growth and agricultural intensification has led to hypertrophic conditions in the lake, especially in the last 20 years (Arı & Derinöz, 2011; Gülersoy, 2013). A study conducted in Gediz River Basin showed that the mean annual total phosphorus concentration of the lake decreased from 0.6 to 0.2 mg L⁻¹ at 2001-2002 period (Gündoğdu & Kocataş, 2006).

Several fish species have also been introduced; piscivorous *Sander lucioperca* was introduced in 1955, followed by omnivorous *Carassius carassius* and herbivorous *Ctenopharyngodon idella* (Innal & Erk'akan, 2006). Massive fish kills occurred in the lake in the mid-1990s corresponding with extreme drought, thus reduced water levels and eutrophication (Arı & Derinöz, 2011).

4.2.1.2 Lake Beyşehir

Rivers, streams and groundwater from the Anamas and Sultan mountains in the western and eastern catchments feed Lake Beyşehir (Beklioğlu *et al.*, 2014; Nas *et al.*, 2009). Annual and intra-annual precipitation totals fluctuate in accordance with the North Atlantic Oscillation (NAO)/Mediterranean oscillation (Türkeş & Erlat, 2003). The main water loss from the lake occurs via evaporation, with an average of around 570 hm³ yr⁻¹ for the years 1971-2010, and through the controlled outflow (average ca. 300 hm³ yr⁻¹ between 1950 and 2010) that was built between the years 1908-1914 for downstream catchment irrigation (Altınayar *et al.*, 1998; DSI-EIE Database).

Within the lake catchment, agriculture dominates with c. 50% dry and c. 30% irrigated farming (Çiftçi *et al.*, 2010). Data from the Turkish Statistical Institute (TÜİK) (available at http://tuik.gov.tr) indicate that, at the nearby cities there has been a decrease, in the area of the cultivated lands between the years 1995-2012, while there was an increase in artificial lands during 1990-2006 (available at http://aris.ormansu.gov. tr/csa/). Despite intensive land use, monthly data gathered

between the years 2010 and 2012 show that the lake is oligo-mesotrophic, with minimum and maximum chlorophyll-*a* (Chla), total nitrogen (TN) and total phosphorus (TP) concentrations between 0.6-9.9 μ g L⁻¹, 0.0-0.7 mg L⁻¹ and 0.01-0.04 mg L⁻¹, respectively (Beklioğlu *et al.*, 2014).

The introduction of piscivorous *Sander lucioperca* in 1978 (Çubuk *et al.*, 2006) has changed the fish community in the lake and contributed to the extinction of the omnivorous endemic fish species *Alburnus akili* (Yeğen *et al.*, 2006), possibly due to predation. Omnivorous *Carassius gibelio* and benthivorous species *Tinca tinca* (from 1990s onwards) and *Atherina boyeri* (in 2002) have also been introduced to the lake (Innal & Erk'akan 2006; Yeğen *et al.*, 2006). *Carassius gibelio* has been considered as invasive in Turkey (Aydın *et al.*, 2011), and unfortunately is a widespread species recorded in many lakes (Boll *et al.*, 2016). Futhermore, Beklioğlu *et al.* (2014) found that the invasive benthivorous *Pseudorasbora parva* was the dominant fish species in Lake Beyşehir in 2010 and 2011.

4.2.1.3 Lake Uluabat

Lake Uluabat is fed by groundwater and the Mustafakemalpaşa River. The main outflow connects the lake to Susurluk (Simav) River and eventually to the Sea of Marmara. Water loss from this outflow is compensated by the main inflow. Historical lake and catchment data are fragmentary, though most authors agree that a progressive reduction in lake area has occurred over recent decades (Reed *et al.*, 2008), from c. 160 km² with a maximum depth of 7.5 m.a.s.l. to c. 120 km² with depths now ranging between c. 5.5 m.a.s.l. in winter/spring to c. 3 m in summer/autumn (Aksoy & Özsoy, 2002; Salihoğlu & Karaer, 2004; DSI-EIE Database). Water levels have been regulated since 1990 (Kazancı *et al.*, 2010) with the construction of a weir at the outflow to raise minimum water levels (Tağıl, 2007). Precipitation data for the city close to the lake indicates that for the period 1973-2012 higher precipitation occurs around November-March (available

at http://en.tutiempo.net/). The Mustafakemalpaşa River has carried a significant amount of suspended sediment to the lake during the late Holocene (Aksoy & Özsoy, 2002; Tağıl, 2007) and formed a large delta at the river-mouth on the lake, being very important for maintenance of the lake's RAMSAR status.

Diatom based reconstruction of lake water phosphorus indicated that the lake has been eutrophic (>0.1 mg L⁻¹ TP) since prior to the 19th century, showing a trend of increased anthropogenic eutrophication since the start of the 20th century (Reed *et al.*, 2008). Multiple sources of domestic and industrial effluent (including tanneries, slaughterhouses) and fertilizers from the predominantly agricultural catchment area have contributed to the current eutrophic status (Salihoğlu & Karaer, 2004). Aksoy and Özsoy (2002), further showed that between 1974 and 1998 the agricultural land in the delta had increased three times in size, as a result of transforming the reed areas to cultivated lands. This is supported by statistical data from the nearby cities, which indicates an increase in the area of cultivated land from 1995 to 2012 (available at http://tuik.gov.tr). Furthermore an increase in artificial land cover from 1990 to 2006 was recorded (available at http://aris.ormansu.gov. tr/csa/).

Fish populations in the lake have been manipulated by introductions (e.g. omnivorous *Carassius gibelio* after 2000s) (Aydın *et al.*, 2011), overfishing and variable connectivity of the lake to the sea (Çınar *et al.*, 2013). Salt-water/euryhaline fish species (piscivorous *Alosa maeotica*, *Anguilla anguilla*, zooplanktivorous *Mugil cephalus* and *Syngnathus* sp.) were recorded in the lake in 2006 (Çınar *et al.*, 2013). A crustacean (*Astacus leptodactylus*) fishery was extensive in the lake in the 1980s. However, due to plague, which was peaked around 1986, and overfishing their numbers have declined and fish have become economically more important (Baran & Soylu, 1989).

4.2.2 Field methods

Sediment cores from Lakes Beyşehir, Marmara and Uluabat were retrieved with a Livingstone piston corer from littoral and pelagic locations in October 2011 (Figure 4.1, **Hata! Başvuru kaynağı bulunamadı.**). The length of the cores were . 0.6 m and 0.7 m from Lake Marmara, c. 0.9 m and 1.6 m from Lake Beyşehir, and 0.9 m and 1.1 m from Lake Uluabat for littoral and pelagic locations, respectively. For the expediency of the current study, however, only the lengths corresponding to instrumental or known water level data are presented, and not the whole cores. It should also be noted that, due to the bad weather conditions in Lake Uluabat, it was not possible to reach the mid-lake location, therefore the core was retrieved from the nearest deep area, 1.0-1.5 km from the vegetation.

Cores were sliced into 0.5 cm thick layers for the first 20 cm (30 cm for Lake Beyşehir) and at 1 cm intervals to the base of the cores. Before chemical, biological and micro/macrofossil analyses samples were refrigerated at 4°C, while for pigment analysis, they were stored frozen.

Lake and Core Location		Water depth (m)	Core length (m) ^c	N (wgs84)	E (wgs84)
Marmara	Pelagic	4.1	0.73	38°36'31.8"	27°58'53.0"
	Littoral	2.5	0.64	38°35'59.5"	28°00'09.6"
Beyşehir	Pelagic	7.5	1.65	37°45'01.4"	31°30'48.9"
	Littoral	3.0	0.95	37°45'18.0"	31°37'34.5"
Uluabat	Pelagic	1.5	1.14	40°12'04.0"	28°29'08.5"
	Littoral	1.2	0.87	40°12'53.1"	28°29'06.2"

 Table 4.2 Summary of cores collected in October 2011

^c The results from the cores presented in this study cover only the periods with instrumental or known (e.g. Lake Marmara) water level data, not the whole core.

4.2.3 Laboratory methods*

A range of geochemical and physical proxies were analysed in radiometrically dated (using ²¹⁰Pb) littoral and pelagic cores. However, diatom valve preservation was low in the pelagic cores of all three lakes. Therefore, to obtain a comparable dataset, diatoms, sub-fossil cladocerans and plant macrofossils were analysed in the littoral cores and when possible in the pelagic ones. Conversely, pigment analysis was carried out only on the pelagic cores due to their better preservation here than in the littoral cores.

Freeze-dried sediment samples from the littoral and pelagic cores of each lake were analysed for ²¹⁰Pb, ²²⁶Ra, ¹³⁷Cs and ²⁴¹Am by direct gamma assay in the Environmental Radiometric Facility at University College London using ORTEC HPGe GWL series well-type coaxial low-background intrinsic germanium detectors. Corrections were made for the effect of self-absorption of low energy gamma rays within the sample (Appleby *et al.*, 1992). For all the lakes, ²¹⁰Pb chronologies were calculated using the constant rate of supply (CRS) dating model (Appleby & Oldfield, 1978).

Sediment water (dry weight: DW), organic matter (loss-on-ignition: LOI550) and carbonate content (LOI950) were measured at 105°C, 550°C and 950°C, respectively, following Heiri *et al.* (2001). Wet density measurements were undertaken on every fourth sample by weighing a 2 cc vial of wet sediment.

^{*} Geochemical and physical proxy analysis were conducted by Dr. Simon Turner from University College London, while cladoceran and diatom countings were done by Dr. Ayşe İdil Çakıroğlu and Gizem Bezirci from Middle East Technical University.

Sediment samples were analysed for geochemical and selected trace elements using a Spectro XLAB2000 X-ray fluorescence (XRF) spectrometer at University College London. Freeze-dried sediments were milled to a fine powder. Approximately 1 g (weighed to 4 d.p) sediment was placed in nylon cups with a base of prolene foil (4 µm thickness). Reference sediment samples (Buffalo River Sediment, National Institute of Standards and Technology (NIST) - RM8704; Canadian Certified Reference Materials Project (CCRMP) - LKSD-2) were included in each sample batch run to identify any machine drift error and assess measurement accuracy.

The diatom analysis was conducted according to the method described by Battarbee (1986). Identification and counting of the diatom slides were conducted with a Leica DMI4000B inverted microscope at Middle East Technical University at 1000x magnification. From each sample approximately 500 valves were counted. Diatom taxa were grouped into two categories, planktonic and non-planktonic, according to their habitat classes.

The method for taxonomic analysis and enumeration of cladocerans was an adaptation of the techniques described by Korhola and Rautio (2001) and the method of Jeppesen *et al.* (1996, 2001) with heating ca. 3 g sample in 10 % KOH. Identification and counting of the samples were carried out using a stereo microscope (LEICA MZ 16) and an inverted light microscope (LEICA DMI 4000) at Middle East Technical University. Identified cladoceran species were classified into four habitat groups, as planktonic, macrophyte-associated, macrophyte and sediment-associated, and sediment-associated (Flöβner, 2000; Hann, 1989).

Plant macrofossil sample preparation was carried out from sediment samples of 3 to 25 cm³ (depending on the sample size) following the method described by Brodersen *et al.* (2001). Identification and counting were conducted at Middle East Technical University using a LEICA MZ 16 stereo-microscope at 10x - 110x magnification. Haas (1994) was used for the identification of charophyte oospores.

Sedimentary pigment analysis samples were stored frozen until pigment extraction. The analysis was performed using the system described by Buchaca and Catalan (2007), except the filtration was done through 0.2 μ m syringe filters. Pigment analysis was carried out at the Department of Bioscience and the Arctic Centre (ARC) of Aarhus University, Denmark. Pigment groups were ascribed to different algal groups according to the information given in Leavitt and Hodgson (2001), and Buchaca and Catalan (2007).

4.2.4 Numerical analysis

To reveal the timing of major changes in the sediment records and to be able to compare these changes with the instrumental water level records from all three lakes, principal curves analysis (PCs) was employed for the geochemical, physical and biological variables in all the cores using the pcurve package (Hastie *et al.*, 2011) in R version 3.1.2 (R Core Team, 2015). Prior to analysis, geochemical and physical variables were log-transformed. Since there is a high variability in the numbers of plant remains (e.g. low versus high seed producing species), plant macrofossil data were square-root transformed, centered and standardised and the values which were zero before this were returned to zero (Davidson *et al.*, 2013). Moreover, diatom and cladoceran data were converted to relative abundances and subsequently square root or Hellinger transformed depending on the dissimilarity measures used (Davidson *et al.*, 2013).

For biological variables, Nonmetric Multidimensional Scaling (nMDS) axis scores using Bray-Curtis dissimilarities (Legendre & Legendre, 1998) or Principal Component Analysis using Euclidean dissimilarities (with Hellinger transformed species data) (Legendre and Gallagher, 2001) were used as the starting curves. The choice between these two matrices was made according to the species response curves and the variation explained after PC analysis. For environmental variables Euclidean distance matrix was employed (Legendre & Gallagher, 2001). The smoothing function used for all the variables was LOESS and the optimal degree of smoothing was determined by a generalised cross validation procedure.

4.3 Results

4.3.1 Lake Marmara

4.3.1.1 Water-level change

Instrumental water-level data from Lake Marmara covered the period from 1970 to 2011. The mean water level for this period was 75.7 m.a.s.l and varied between 73.2 and 77.7 m.a.s.l. A period of higher water levels was observed around the 1970s to the 1980s, followed by a very significant decline from the late 1980s until the mid-1990s. The lowest level occurred in 1992-3 (ca. 2.0 m decrease from the mean) when most of the lake dried out. Another large, but short-term, water-level drop was in 2007-2008. After 2008 with the construction of a dam (1998-2012), the water level in the lake has increased.

Calculated local water depth at the pelagic core site ranged between 0.7 and 5.0 m, while at the littoral core site it was between -0.9 and 3.4 m, indicating that the littoral core site has been regularly exposed.

4.3.1.2 Chronology

In the littoral core of Lake Marmara ²¹⁰Pb activities are low (Appendix-B Figure 1-A, Appendix-B Table 1). The equilibrium depth of total ²¹⁰Pb activity with the supported ²¹⁰Pb occurs deeper than 20 cm. Unsupported ²¹⁰Pb activities decline irregularly with depth , and the maximum activity appears at c. 8.3 cm while the surface sediment activity is very low (Appendix-B Figure 1-B). Sedimentation rates in the core have varied in the last 70 years and disturbance to sedimentation has occurred in recent years. As unsupported ²¹⁰Pb activities are low and counting errors are relatively high, there is some uncertainty with interpretation of the chronology for this core. However, the 1963 depth derived from the ²¹⁰Pb CRS model was placed at c. 14.3 cm, which was in reasonable agreement with the depth suggested by the ¹³⁷Cs record (Appendix-B Figures. 1-C and 4-A). The sedimentation rate showed some regular increases and decreases, ranging between 0.05-0.50 g cm⁻² yr⁻¹, with a marked increase towards the surface of the core.

²¹⁰Pb activities in the pelagic core are also low. The equilibrium depth of total ²¹⁰Pb activity with the supported ²¹⁰Pb occurred at a depth of c. 38 cm (Appendix-B Figure 1-D, Appendix-B Table 2). Unsupported ²¹⁰Pb activities decline irregularly with depth with the maximum activity at c. 6.3 cm (Appendix-B Figure 1-E), suggesting changes in sedimentation rates and an increase in recent years. The ¹³⁷Cs activity versus depth shows a peak at 28.5 cm, which is almost certainly derived from the 1963 fallout maximum from the atmospheric testing of nuclear weapons (Appendix-B Figure 1-F). However, the CRS dating model placed the 1963 depth at 18.5 cm (Appendix-B Figure 4-B), which was not in agreement with the depth suggested by the ¹³⁷Cs record. Corrected chronologies and sedimentation rates were calculated by the CRS model using the ¹³⁷Cs peak at 28.5 cm for 1963 as a reference level. Corrected chronologies showed that 15.3 cm was dated to 1986. Also, a distinct peak in the sedimentation rate, with an increase of 0.27 g cm⁻² yr⁻¹ at 20.5 cm (dated 1976±5) in the pelagic core agreed with the peak rate at 11.3 cm (dated to 1978±11) in the littoral core.

4.3.1.3 Sediment composition (LOI) and bulk geochemistry

The DW and LOI profiles of the littoral core were more variable than the pelagic core (Figure 4.2-A and B). Prior to the constructions, during lower water-level periods (40-23 cm, pre 20th century), LOI950 and LOI550 were low with less than 10% and 5%, respectively, while DW was relatively high, being c. 80% (Figure 4.2-A). The distinct low LOI950 in this period (ca. 34-24 cm) is visible in the Ca element profile but was more evident by the concomitant increase of Ti and erosion resistant Zr (more sand silt). DW showed a significant shift, decreasing to c. 40-50% above 24 cm, indicating a change in the hydraulic properties of the sediment (less compact and higher silt content). Around 20-22 cm, coinciding with the construction period, a fall in mineral (Rb:Sr, Ti, Zr) indicators and a rise in LOI950 from c. 5% to c. 12% was observed. There was a change in sediment composition around 12 cm (1973 \pm 13), with a 1% decline in Fe, 20 μ g g⁻¹ drop in Rb and a noticeable c. 0.5% decrease in Mg, Al and K. Between 8-9 cm (c. 1990-1995), LOI550 values show a marked c. 3.5% decrease. PC-XRF scores also indicated that the main changes in the core were above 24 cm coinciding with the construction period, also around the long-term drought in 1990s with a significant and sudden change.

Before water-level regulation in Lake Marmara, XRF and LOI measurements of the pelagic core suggest a stable carbonate-/lithogenic-mixed mud system (Figure 4.2-B). LOI550 values were low (<10%) throughout the core, indicating low organic matter content and/or preservation. The principal geochemical change in the pelagic core followed the compositional changes measured by LOI950 with a significant decrease of Ca by c. 5% and Sr by c. 500 μ g g⁻¹ above 40 cm during the last half century (after c. 1950 AD). Also, more non-carbonate/soil mineral-associated elements (Rb:Sr, Ti) gradually increase during this time. This soil input phase appeared to finish around 23 cm (c. 1975) (Figure 4.2-B). LOI950 showed some variability, with a peak of c. 3% increase, in carbonate deposition at 14 cm (late 1980s), coinciding with low water levels. From c. 1995, especially after

1997, water levels increased and the lake refilled with soil/mineral matter continuing to be deposited in the pelagic area. The short-term water level decrease in 2007-2008, however, was not registered in the core, with the exception of a slight increase in LOI950.

4.3.1.4 Biological variables

Before constructions began in 1932 (likely below c. 23 cm), notwithstanding the possible low water levels, dominant cladocerans were planktonic *Bosmina longirostris*, with c. 25-90% and *Daphnia* spp. with 2-4%, but the abundance of benthic species such as *Leydigia* spp., with c. 5-10% was also relatively high (Figure 4.2-A). Moreover, short-growing, low turbidity-tolerant macrophyte species, Characeae and *Ranunculus*, comprised the dominant macrophyte remains. At around 26 cm cladocerans shifted to benthic dominance, and both the abundance and diversity of macrophyte remains was sparse. Diatom preservation was poor in the lower core (below 18 cm), preventing species identification.

The change in PC at c. 23 cm indicates a significant alteration in both cladoceran and plant macrofossil records during the construction period (1932-1953), which culminated with maximum water levels in c. 1960 (around 15 cm depth) (Figure 4.2-A). *Daphnia* spp. and short-growing plant remains disappeared and remains of tall-growing plants, tolerating lower light availability, such as *Potamogeton* spp., appeared, along with macrophyte-associated cladoceran *Graptoloberis testudinaria*.

Following the construction period (14-0 cm; c. 1970-present), changes in the pelagic-benthic diatom ratio associated with water level were muted, though a slight increase in pelagic species abundance (e.g. *Aulacoseira granulata*) can be distinguished during high levels. In contrast, the variation in the relative abundance of pelagic and benthic cladocerans (excluding cosmopolitan *B. longirostris* and *Chydorus sphaericus*) was more coherent with water-level fluctuations, with an increase in pelagic-benthic species ratio of 0.3 (note that the

excluded *B. longirostris* have the highest abundance) (Figure 4.2-A). Despite higher water levels, remains of short-growing plants (Characeae) were abundant. The long-term low water-level period from c. late 1980s to mid-1990s likely led to the notable increase in macrophyte remains at this time, and the coincident very slight decrease in pelagic-benthic ratio of diatoms and cladocerans, by c. 0.10 and 0.04, respectively.

In the pelagic core, through the construction period (c. 40 to 30 cm), there is a decrease in all the pigment concentrations, with c. 0.5-1.5 nmol g⁻¹ organic matter (OM), except Cryptophyta markers, and an increase in the alloxanthin/diatoxanthin (Cryptophyta/diatom) ratio (Figure 4.2-B). A significant change was also observed in the PC scores. After the 1960s pigment concentrations increased. However, in the upper part of the core PC scores did not show any significant change until around long-term drought period in the 1990s (ca. 15-10 cm), where a significant increase (twice the previous concentration) in cyanobacteria and Chlorophyta markers was also observed.



physical variables, showing low (light grey shading) and high (dark grey shading) water level periods, also the mean Figure 4.2 Summary diagram of Lake Marmara (A) littoral and (B) pelagic cores with biological, geochemical and water level (dashed line in the water level graph) calculated from the instrumental data. Species names in bold indicate the pelagic environment. Only abundant sub-fossil cladoceran and diatom species are shown in the graph. It should be noted that, since there is no dating, the pre-construction period is an approximation according to changes in the cores.

4.3.2 Lake Beyşehir

4.3.2.1 Water-level change

The instrumental water-level data between 1905 and 2012 showed that mean water level was c. 1123.3 m.a.s.l (Figure 4.3-A and B), and included four low and four high water-level periods. High levels were around 1905-1927, 1940-1956, 1966-1973 and 1977-1988, being 1.0- 2.2 m higher than the mean level. There were two relatively long- and one short-term water level drops in c. 1928-1939, 1957-1965 and 1974-1976, by approximately 2.0 m (for the first) and 1.0 m (for the latter two) from the mean level. A longer-lasting low water-level period occurred in 1989-2011, reaching its lowest level in the mid-90s with a 2.0 m drawdown.

Water-level calculations based on the water depth at the core sites in October 2011 compared to the instrumental lake-level value, indicated that lowest local water depth at the pelagic site was 5.7 m and the highest was 9.5 m, while at the littoral core site the water depth ranged from 1.5 to 5.0 m.

4.3.2.2 Chronology

In the littoral core from Lake Beyşehir total ²¹⁰Pb activity reaches equilibrium with the supported ²¹⁰Pb at around 20 cm (Appendix-B Figure 2-A, Appendix-B Table 3). Unsupported ²¹⁰Pb activities, calculated by subtracting ²²⁶Ra activity from total ²¹⁰Pb activity, declines irregularly with depth suggesting changes in sedimentation rates (Appendix-B Figure 2-B). Maximum unsupported ²¹⁰Pb activity is at c. 5 cm, indicating a recent increase in sediment accumulation rates. The ¹³⁷Cs activity versus depth shows a broad peak between 17 and 10 cm, which is likely to be derived from the 1963 fallout maximum from the atmospheric testing of nuclear weapons (Appendix-B Figure 2-C). The relatively high ¹³⁷Cs activities in the surface might be due to soil in-wash from the catchment and resuspension of ¹³⁷Cs-labile sediment in the littoral zone. The 1963 depth derived

from the ²¹⁰Pb constant rate of supply (CRS) model was placed between 11.8 and 13.3 cm (Appendix-B Figure 4-C), which was in agreement with the depth suggested by the ¹³⁷Cs record. Because of low unsupported ²¹⁰Pb activities and relatively high counting errors in the sediments deeper than 17.0 cm, ²¹⁰Pb dates were not ascribed to depths below 16.8 cm (pre-1900s). The data showed that sedimentation rates increased greatly since the 1900s from 0.03 to 0.30 g cm⁻² yr⁻¹ at present.

In the pelagic core, the equilibrium depth of total ²¹⁰Pb activity with the supported ²¹⁰Pb is at around 6 cm (Appendix-B Figure 2-D, Appendix-B Table 4). Like the littoral core from the same lake, unsupported ²¹⁰Pb activities also decline irregularly with depth indicating recent change in sedimentation rates (Appendix-B Figure 2-E). The ¹³⁷Cs activity versus depth curve has a shoulder between 3 and 5 cm that may be derived from the 1963 fallout (Appendix-B Figure 2-F). ¹³⁷Cs activities increase in the upper core sediments, due to soil in-wash from the catchment in recent years as also observed in the littoral core. Because of the low accumulation rate in the pelagic zone, this surface increase may also indicate some bioturbation - an intact bivalve was found in the surface mud of the core and diffusion in the slowly accumulating sediment. The CRS dating model placed the 1963 depth between 3.8 and 4.3 cm, which was also in agreement with the depth suggested by the ¹³⁷Cs record (Appendix-B Figure 1-D). Sedimentation rates of the core showed a gradual increase from the 1930s to the 1970s, from 0.02 to 0.05 g cm⁻² yr⁻¹, followed by fluctuations in recent years (ranging between 0.03 and 0.09 g cm⁻² yr⁻¹).

4.3.2.3 Sediment composition (LOI) and bulk sediment geochemistry

In the littoral core, LOI950 and DW decreased gradually, by c. 10 % and 15%, from 15 cm (1932 \pm 12 AD, drought period) to present (Figure 4.3-A). LOI550 mirrors this change. The shift in sediment composition recorded post 14-15cm (mid 1930s – early 1940s) emerged with increased values of terrigenous/mineral indicators (i.e. Ti, Zr) and a fall in autochthonous Ca with 4% and Sr with 140 µg g⁻¹.

The pelagic core is formed of homogenous low organic (<6% LOI550, mean 4% sd 0.48) silt-clay/carbonate mud (Figure 4.3-B). DW remained at approximately 46-50% throughout the core. The small surface increase in LOI550, by c. 1.5%, coincided with a c. 2% decrease in Ca and a small increase in organic matter-associated elements (P, S, As and Br). The small but fluctuating nature of Al, Si, Ti, K, Zr, Rb and Sn (mineral in-wash) suggested a conservative sub-decadal record of catchment sediment inputs contrasting with chemical precipitation in the lake (Ca, Mg, Sr).

4.3.2.4 Biological variables

During the 1905-1988 period (c. 20- 7 cm), water level was fluctuating between high and low, though was dominated by high levels. Benthic diatoms, (e.g. *Navicula subrotundata* and *Amphora pediculus*), and littoral cladoceran taxa were dominant. Short growing plants, such as *Chara* spp. (mostly *C. contraria* type oospores) and *Ranunculus* sect. *Batrachium*, were also present (Figure 4.3-A). Above 15 cm, after the 1930s drought (the most pronounced in this period), the abundance of sediment-associated cladocerans decreased from c. 35% to 1%, while the abundance of the small-bodied pelagic taxon *Bosmina longirostris* increased from c. 3% till 70% to the sediment surface, becoming the dominant species around early-1990s. This change was also reflected in the PCs of cladocerans which increased above 15 cm (Figure 4.3-A). Conditions following this drought led to the first appearance of the planktonic diatom *Aulacoseira*

granulata, which is a typical species of eutrophic and turbid environments (Wolin & Stone, 2010).

Above 7 cm, during the lower water-level period c. 1989-2011, planktonic diatom taxa, such as *A. granulata* and *Cyclotella ocellata*, increased and reached together a maximum abundance of around 50% at the surface sediment (Figure 4.3-A). Moreover, during this period an increase in plant remains, especially of tall-growing, low-light tolerant species such as *Ceratophyllum* sp., was observed, and oospores of Characeae mostly belonging to *C. hispida-* and *C. globularis-* types appeared. Furthermore, the PC scores of macrophytes, cladocerans and diatoms either increased or decreased above 7 cm indicating synchronous compositional shifts in all three groups (Figure 4.3-A).

Pigment preservation indicated by the chlorophyll-*a*:phaeophytin-*a* ratio in the pelagic core was not stable and relatively low, causing the interpretation to be unclear or misleading (Figure 4.3-B). Therefore, pigment data are not interpreted further in the study. Cladoceran remains, on the other hand, were in agreement with the change in the littoral core, with a shift from benthic species dominance towards more pelagic associated species (Figure 4.3-B).



physical variables, showing low (light grey shading) and high (dark grey shading) water level periods, also the mean water level (dashed line in the water level graph) calculated from the instrumental data. Species names in bold indicate the pelagic environment. Only abundant sub-fossil cladoceran and diatom species are shown in the graph. Figure 4.3 Summary diagram of Lake Beyşehir (A) littoral and (B) pelagic cores with biological, geochemical and
4.3.3 Lake Uluabat Cores

4.3.3.1 Water-level change

Repeated annual water-level changes of approx. 1 m, have been a regular feature of Lake Uluabat since c. 1960. A long-term high water-level period occurred around 1960-1984 and a long-term low water-level period has lasted from around 1985 until the present. In the former, a few short-term decreases were observed, while short-term (one to two years) increases have occurred in the latter. The largest water-level drop was recorded in 2001, with a 1 m drop from the mean (3.96 m.a.s.l).

Local water depth indicate that water levels at the pelagic core site fluctuated between 1.0 m and 2.7 m, while at the littoral core site it varied between 0.8 m and 2.4 m.

4.3.3.2 Chronology

²¹⁰Pb activities in the Lake Uluabat littoral core are low and most counting errors are higher than the unsupported ²¹⁰Pb activities in the sediments greater than 20 cm, hence the depth where total ²¹⁰Pb activity reaches equilibrium with supported ²¹⁰Pb is uncertain (Appendix-B Figure 3-B, Appendix-B Table 5). Low unsupported ²¹⁰Pb activities that decline irregularly with depth indicate significant changes in the magnitude and regularity of sedimentation (Appendix-B Figure 3-B). The ¹³⁷Cs activity versus depth shows a broad peak between 26 and 35 cm, which is almost certainly derived from the 1963 fallout maximum from the atmospheric testing of nuclear weapons (Appendix-B Figure 3-C). The 1963 depth derived from the ²¹⁰Pb CRS model is placed at 18.5 cm, which is not in agreement with the depth suggested by the ¹³⁷Cs record (Appendix-B Figures. 3-C and 4-E). Corrected chronologies and sedimentation rates were calculated by the CRS model using the ¹³⁷Cs peak at 34.5 cm for 1963 as reference level. Sedimentation rates showed two peaks in the 1960s and the 1970s, respectively, and since then sedimentation rates were relatively uniform with a mean of 0.28 g cm^{-2} yr⁻¹. A similar break around 1960s in the sedimetation rate of a core retrieved from the North-Eastern part of the lake, was also observed by Reed *et al.* (2008), but they did not observe a second peak around 1970s, as in the current study. This difference might be a result of a higher accumulation rate found in the core we collected from a diffrent location (western part of the lake near outflow).

In the pelagic core of Lake Uluabat, the equilibrium depth of total ²¹⁰Pb activity with supported ²¹⁰Pb occurs at an approximate depth of 41 cm (Appendix-B Figure 3-D, Appendix-B Table 6). Unsupported ²¹⁰Pb activities in the sediments are low, and they decline irregularly with depth (Appendix-B Figure 3-E), indicating significant changes in sedimentation rates. Sedimentation rates in this core are relatively high. The ¹³⁷Cs activity versus depth shows a peak between 22 and 25 cm, which is certainly derived from the 1963 fallout maximum (Appendix-B Figure 3-F). In the pelagic core of Lake Uluabat, the CRS dating model places the 1963 depth at 32 cm (Appendix-B Figure 1-F), which was considerably deeper than the depth suggested by the ¹³⁷Cs record. Corrected chronologies and sedimentation rates were calculated by the CRS model using the ¹³⁷Cs peak at 23 cm for 1963 as reference level. Sedimentation rates varied with a mean of 0.22 g cm⁻² yr⁻¹.

4.3.3.3 Sediment composition (LOI) and bulk sediment geochemistry

LOI550 was higher in the littoral core (up to 18%) than the pelagic core, most likely due to the proximity of littoral vegetation, and showed an almost linear increase from 20 cm to the surface (Figure 4.4-A). In parallel, LOI950, Ca and Sr increased from c. 10 to 12%, c. 6 to 8% and c. 200 to 260 μ g g⁻¹, respectively, while concentrations of the mineral indicators (i.e. K, Al) decreased slightly from 20 cm to the present (1975-2011). At around 4 cm, both LOI550 and LOI950 profiles indicate broader changes interspersed with a marked c. 1% increase and decline, respectively. DW also showed a steady decline from 45 to 35%, then to

18% between 20 cm (c. 1974) and present reflecting less compacted mud in the upper core (Figure 4.4-A). LOI950 displayed two peaks at 10 cm with c. 10% (c. 1992) and 1 cm with c. 15% (c. 2009). The former peak appeared to coincide with a short-lived fall in the lake level (and deposition of carbonate/biogenic sediments). The latter peak coincided with a drop in Rb/Sr that indicates a recent decline in soil/mineral sediment and an increase in authigenic-organic mud (Figure 4.4-A).

The homogeneous nature of the pelagic sediment core, consisting of silty mud, was apparent in the stable LOI and element profiles, also from the PC scores (Figure 4.4-B). Less compact and slightly more organic mud occurs in the top 10 cm. The LOI550 profile showed little variation (mean 7.1 sd 1.1) except a very steady increase, between c. 7% and 11%, from the bottom to the top of the core. Br, which is often associated with organic matter (along with P), increased steadily up core, from c. 3 μ g g⁻¹ to 7 μ g g⁻¹, supporting an increase in organic carbon in the upper 20 cm. However, there was no evidence of significant organic matter accumulation, suggesting that the site was unlikely to have been colonised by aquatic/emergent plants during low-water phases. Additionally, the low LOI550 indicated that organic matter was quickly degraded and recycled rather than buried (Figure 4.4-B). At around 7-8 cm a small shell-rich (*Hydrobia* sp.) peak in LOI950 (21.7%), Ca, Sr and As (arsenic) was observed and Rb reduced, suggesting an authigenic sediment depositional phase.

4.3.3.4 Biological variables

Between 20 cm to 16 cm of the littoral core (ca. mid-1970s until the mid-1980s), when the water level was high, remains of both short-growing (e.g. *Chara* spp.) and tall-growing (e.g. *Ceratophyllum* sp.) plant taxa were found (Figure 4.4-A). Accordingly, macrophyte- and sediment-associated cladoceran taxa, such as *Graptoloberis testudinaria* were observed, along with the pelagic *Bosmina longirostris*. The diatom assemblage also contained a mixture of planktonic (e.g.

Aulacoseira granulata, Cyclostephanos dubius) and benthic (Cocconeis spp., Epithemia spp.) taxa.

Coinciding with the long-term low water-level period from 1985 to 1996 (ca. 16-9 cm), an increase in submerged and floating leaved macrophyte taxa, namely *Najas minor, Chara* sp. and Nymphaceae was seen (Figure 4.4-A). Accordingly, the abundance of *G. testudinaria* increased by c. 20% and planktonic diatom species *A. granulata* decreased by c. 26% from the 1990s. In the two consecutive short-term high water-level (ca. 8.5-7 cm and 6-5 cm; late 1990s and early 2000s respectively) periods a decline in macrophyte species richness was seen, and *Ceratophyllum* sp. remains were dominant (Figure 4.4-A). This change can also be seen in the PC scores obtained from plant remains. From 5 cm to 1 cm (ca. 2007-2010), during the low water-level period, *B. longirostris* increased by c. 20% and *G. testudinaria* decreased by c. 10%. Plant remains indicated a possible increase in *Ceratophyllum* sp. abundance especially above 5 cm. Together with these changes, the rapid increase, from c. 2% to 20%, in planktonic and eutrophictolerant diatom species *C. dubius* suggested a possible increase in nutrient concentrations in recent years.

Marker pigment concentrations showed an increasing trend from 30 cm depth to the surface (approx. 80 years) in the pelagic core (Figure 4.4-B). However, pigment preservation exhibited a parallel increase that lowers the confidence in their use for interpreting ecological changes. During the higher water-level period from 1960 to1984 (ca. 22-12 cm), cyanobacteria pigment concentration was lower (0.5-1.0 nmol g⁻¹ OM) than in the later low-water (1.0-5.0 nmol g⁻¹ OM) periods. Accordingly, macrophyte- and sediment- associated cladoceran abundances were also higher by 50%, but benthic and planktonic diatoms were equally abundant (Figure 4.4-B). In the upper part of the core, coinciding with low water levels (ca. 5-2 cm), cyanobacteria indicator pigments increased. Correspondingly, despite low water levels, a significant increase in the abundance of planktonic cladocerans occurred, along with remains of tall-growing plant species (Figure 4.4-B).



Figure 4.4 Summary diagram of Lake Uluabat (A) littoral and (B) pelagic cores with biological, geochemical and physical variables, showing low (light grey shading) and high (dark grey shading) water level periods, also the mean water level (dashed line in the water level graph) calculated from the instrumental data. Species names in bold indicate the pelagic environment. Only abundant sub-fossil cladoceran and diatom species are shown in the graph

4.4 Discussion

4.4.1 **Responses in the cores to water-level changes**

Our results from the three lakes showed that longer term and more pronounced water-level changes were reflected in the sediment record, while short-term but distinct lake-level changes left little impression (Figure 4.5). Noisiness of short-term cyclical water-level changes, low temporal resolution, and additional anthropogenic impacts on these lakes have prevented close relationships between actual changes and those recorded in the sediment, highlighting the care is required in using recent sediment records from large shallow lakes for forecasting future patterns of water-level changes.

Contemporary water-level data showed that all the lakes had both longer lasting (more than 5 years) and short-term high- and low-level periods, with some being more intense (c. 2 m shift from the mean levels). Overall an increase in total submerged macrophyte remains was observed during more protracted water-level reduction periods, coinciding with higher macrophyte coverage as judged from the contemporary records (Altinayar 1998; Altinayar et al., 1994, 1998; Beklioğlu et al., 2006). However, the extent of changes (indistinct at times) in the relative abundance of benthic-pelagic cladocerans and diatoms and also in the composition of submerged macrophytes (tall- vs. short- growing) varied among the lakes and even within the same core. Moreover, the record of sub-decadal trends in the cores was somehow blurred by the interaction between timing and magnitude of lake response and mixing. Thus, slow rates of accumulation and resuspension occurring in the study lakes were not conducive to detecting subdecadal trends. We further found that the geochemical and physical proxies in these lakes indicated better agreement of littoral cores with water-level alterations compared to pelagic cores, especially during periods of marked water-level changes as also suggested by Furey et al. (2004).

Before the water-level regulation in Lake Marmara, when the water level was low, the lake was endorheic (Altınayar et al., 1994). Geochemical and physical records, with reduced carbonate and higher mineral mater (Ti, Zr) suggested that the littoral core site consisted of more sand-silt material and was water covered but likely affected by mixing and sorting of the sediment as a result of profound seasonal water-level changes. The presence of Ranunculus sect. Batrachium remains (Birks et al., 2001) and desiccation-resistant Charophyte oospores (Soulié-Märsche & García, 2015) in the littoral core also confirmed fluctuating water levels. The conditions in the pelagic core were more stable with carbonatelithogenic mixed sediment content, representing deposition in the pre-modified endorheic lake. The long-term low water-level period in the 1990s appears to have left its mark on the pelagic and littoral sediments, suggesting a return to depositional conditions not seen since before the water level regulation. Furthermore, biological proxies in the sediment with predominance of macrophytes, and slight increase in benthic/macrophyte associated taxa of cladocerans and diatoms concur with the high Secchi depth measurements of the 1990s (Beklioğlu et al., 2006), by showing relatively low phytoplankton indicator pigment concentrations and higher contribution of low-light intolerant species (e.g. diatom Cocconeis placentula, cladoceran Graptoloberis testudinaria).

Contrary to expectations, however, the pronounced water-level drops in Lakes Beyşehir (c. 1930s and 1990s) and Uluabat (1990s and mid-2000s), did not result in an increase in remains indicative of a benthic system, but rather a decrease in littoral species abundance (e.g. cladoceran *G. testudinaria*) and an increase in tallgrowing plant remains (e.g. *Ceratophyllum* sp.). As other cores from these lakes also have suggested (Fethi *et al.*, 2014; Kazancı *et al.*, 2010; Reed *et al.*, 2008), our geochemical and physical sedimentary records indicate sediment resuspension, especially during low water-level periods as the lake's wide open large surface area makes it susceptible to wind induced resuspension. Correspondingly, results of Oğuzkurt (2001), Dalkıran *et al.* (2006) and Beklioğlu *et al.* (2014) also revealed relatively low Secchi depths for these lakes. Therefore, this change towards a system with more pelagic species may reflect that the water-level decrease enhanced resuspension and thus turbidity in these two lakes, where coverage of submerged macrophytes and thus protection against resuspension typically have been lower than in Lake Marmara, which was completely covered with submerged plants during the 1990s low level period (Altınayar *et al.*, 1994). A study conducted in large Lake Marsh (Minnesota) also showed significantly lower impact of wind, and thus lower resuspension, during the year with dense macrophyte beds, compared to a low plant covered period (James & Barko, 1994).

The effect of the deliberate water-level increase (c. 1930-1953/1960), of around 6 m (from c. 73 to 79 m.a.s.l.), in Lake Marmara shifted the lake from an endorheic to an open and deeper lake system used for irrigation (Gülersoy, 2013), occurring concomitantly with the input of water and soil materials as a result of river channelization. Corresponding with this water level increase, the littoral core sediment was compact with higher silt content while the pelagic core indicated lower light conditions at the pelagic water-sediment interface by the loss of carbonate and increased mineral input (Harrison & Digerfeldt, 1993). Accordingly, a change towards dominance of tall-growing macrophyte remains, such as Potamogeton sp, Zannichellia. palustris, and a decrease in all the pigment concentrations (except Cryptophyta) was also observed, likely indicating higher light attenuation (Bjerring et al., 2013; Nõges & Nõges, 1998) but relatively lower nutrient concentration. Despite water-level increase, however, planktonic cladoceran Daphnia spp. disappeared, indicating higher fish predation (Jeppesen et al., 2003b). Even though there are no measurements from the pre-construction period the lake was defined as saline (Altınayar et al., 1994; Girgin, 2000) and it has been claimed that with increasing water levels the salinity of the lake has decreased (Gülersoy, 2013). However, the changes in the core could not be reliably interpreted as a change in salinity, since the possible biological indicators,

such as Zannichellia palustris, have a wide salinity tolerance range (Greenwood & Dubowy, 2005; Heegard et al., 2001). Furthermore, the better preservation of the diatoms with higher water level may also be related to relatively lower alkalinity (Flower, 1993). The geochemical records indicated a new, but less distinct water level increase in Lake Marmara in the late 1990s, which may be a result of less significant increase in water level. However, unlike the previous water level increase the reaction of cladocerans was clearer, while the change in submerged macrophytes was more tentative. The observed higher pelagic/littoral cladoceran ratio, possibly draw attention to the lower fish predation pressure as a result of fish kills (Arı & Derinöz, 2011), allowing cladocerans to react as expected. Submerged macrophyte remains indicated a community with abundant short-growing plant remains (e.g. Chara sp.), which suggests a relatively good light climate (Blindow, 1992), notwithstanding the higher water levels. Furthermore, a community with higher macrophyte species richness may indicate relatively higher nutrient concentrations (Rørslett, 1991) compared to previous high water level period.

Various studies on the water level-biological proxy relationship have indicated a change towards pelagic species abundance with higher water depths (Moos *et al.*, 2005; Riis & Hawes, 2003; Sarmaja-Koronen & Alhonen, 1999). In contrast, the high water level period (c. 1900s to mid-1980) of Lake Beyşehir coincided with increased abundance of benthic species, such as the diatom *Navicula subrotundata*, cladoceran *Monospilus dispar*, and more remains of short-growing plants (e.g. *Ranunculus* sect. *Batrachium*). Since this lake is defined as oligomesotrophic, this shift may indicate improved light conditions, owing to the possible lower turbidity, compared to low water-level periods (ca. 1990s-2011).



Figure 4.5 Synthesis diagram of the changes observed at the study lakes, showing principal curve (PC) axis-1 scores and water level data. Dotted line in the water level graphs shows mean water levels calculated from the instrumental data. Dark and light grey shading indicates high and low water-level periods, respectively. Horizontal dashed lines show the start and end of the main construction periods. Black diamond shapes in Lake Marmara indicate the period without dating. It should also be noted that, since there is no dating, the start of the construction period in Lake Marmara is an approximation according to changes in the core.

4.4.2 **Possible Factors Leading to Poor Agreement**

Lake water-level changes are clearly recorded in Quaternary-Holocene lake sediment sequences (e.g. Abbott *et al.*, 2010; Shuman *et al.*, 2009; Stone & Fritz, 2004). In our lakes, however, it would be difficult to attribute the complex shifts in the palaeo-records to water-level changes were it not for the presence of the instrumental water-level data. Even though water-level data used in this study cover extensive periods as instrumental records, compared with many palaeolimnological studies (Bjerring *et al.*, 2013; Finkenbinder *et al.*, 2014), these periods are still relatively short (longest being 100 years for Lake Beyşehir). A study, covering the Holocene period, from shallow Lake Juusa, Estonia, showed that diatom assemblage change in both littoral and pelagic cores were compatible

with each other, also with c. 2-4 m water level fluctuations (Punning & Puusepp, 2007), though the much smaller size of this lake compared to our study lakes should be noted. It is also pointed out that the variation in the lake level or surface area of open basin lakes is less sensitive than closed ones, since water level in overflowing lakes depends not only on climate, but also on the outflow of the lake (Cheddadi *et al.*, 1997). Nevertheless, the study conducted in large, shallow and open-basin Lake Võrtsjärv, Estonia, pointed to the good correlation between instrumental water level data and sedimentary pelagic diatom abundance (Heinsalu *et al.*, 2008).

A stronger agreement of all the proxies with the water-level records in Lake Marmara compared to the other study lakes most likely reflects a higher amplitude of water-level change, with the difference between yearly maximum and minimum levels being highest, with an average of 2.1 m for this lake. Similarly, using at least 30 years of water level data, Evtimova and Donohue (2016) compared two sets of lakes in Ireland, which are exposed to high (mean annual >1m) and low (mean annual ≤ 0.65 m) amplitude water level changes on a monthly and yearly basis. This study showed more significant impact of high amplitude fluctuations than low level ones on the littoral area structure (Evtimova & Donohue, 2016). The flatter bottom profile of Lake Marmara than in the other lakes (Beklioğlu et al., 2006; Bulkan, 2009; Karaer et al., 2012; Mercan, 2006), may have contributed making the littoral zone more extensive with higher chances of shifting between benthic and pelagic dominated system (Vadeboncoeur et al., 2008). It is worth noting, however, that if the fluctuations are not around a "critical depth" (Sverdrup, 1953), the flatter bottom profile may also result in lower sensitivity depending on the proportion of lake area in or out of the photic zone.

In the other two lakes, the relationship between the sediment record and waterlevel fluctuations was more equivocal. Geochemical and physical proxies from Lake Beyşehir show that the littoral and pelagic cores have evolved in contrasting depositional environments. It is apparent that these variables in the pelagic core, at the resolution of sampling, did not reveal a palaeolimnological record of waterlevel change during the last century. Moreover, even though an overall trend towards more pelagic conditions can be seen in the littoral core, due to the rather coarse temporal resolution (3-33 years and 6-42 years per sample, for littoral and pelagic cores, respectively) it was not possible to identify the effect of each waterlevel change period separately. A recent review (Anderson, 2014) also points out the problems with temporal variability, drawing attention to the loss of ecological information when the lifespan of aquatic organisms and coarser temporal resolution is taken into account. Indeed, Korhola *et al.* (2005) when estimating a 4-6 m Holocene lake level change in three small and shallow lakes, highlighted the high sample-specific error (\pm 2.4-2.6 m) and low temporal resolution, which complicated the noise-signal separation.

Similarly, geochemical and physical proxies analysed from Lake Uluabat cores were poorly related to changes in water level, although with high temporal resolution. The reason for this might be the cyclical pattern of seasonal water-level fluctuations (low in September/October and high in January-March) and the positioning of the cores near the outflow (transporting fine-grained particles out of the lake). While the former can cause high variation in ecological response of biological proxies (Osborne *et al.*, 1987), leading to ambiguous interpretation, the latter can yield records reflecting suspended and re-worked sediment, thus smoothing geochemical and physical changes, resulting with homogenous records (Kazancı *et al.*, 2010).

Studies have furthermore shown that Lake Beyşehir and Lake Uluabat are largely affected by wind turbulence (Fethi *et al.*, 2014; Kazancı et al, 2010). Moreover, Karaer *et al.* (2012) pointed out the faster flow rates caused by higher wind speed at the western part of the lake, near the outflow, thus close to our coring location. Correspondingly, Nõges *et al.* (1999) demonstrated the spatial variability of sediment composition in a large shallow lake, caused by wind impact.

Woodbridge and Roberts (2010) also showed that even in varved sediments, analysed with high temporal resolution, core signals can be smoothed and aggregated through mixing at the surface sediment. Therefore, the impact of wind resuspension may also be a reason for poor agreement in these two Turkish lakes.

4.4.3 Multiple drivers of alterations

Interpretation of the palaeolimnological records with respect to changes in water level is also complicated by the occurrence of other anthropogenic pressures. Besides canal and regulator constructions, the study lakes have been, and are today, exposed to various human impacts, such as fish introductions, agriculture around the lakes and excessive destruction of reedbeds. Nevertheless the effect of such factors on the lakes differed.

In Lake Marmara, even though the biomass of bottom-feeding Cyprinus carpio rose following Sander lucioperca introduction in 1955, the drought period in the 1990s led to extensive fish kills and reduced carp abundance (Arı & Derinöz, 2011). This reduction might have resulted in lower sediment resuspension and higher zooplankton grazing on phytoplankton (Jeppesen et al., 2012), thereby maintaining a relatively high light penetration to the lake bottom even after the increase in water level. In turn, this, together with lower seasonal variation in water level towards present may have led to the higher abundance of low-light intolerant species. Moreover, contrary to communities seen prior to construction, endurance of high nutrient-tolerant species, such as macrophyte Ceratophyllum sp., during 1990s low water-level period can be related to the intensification of agricultural activities and thus enhanced nutrient loading to the lake (Arı & Derinöz, 2011). Similarly, higher abundance of diatom Cocconeis placentula and lower Epithemia sorex and Fragilaria capucina might point to higher nitrogen concentrations (Marks & Power, 2001). A study, covering around 160 years, from large-shallow Lake Võrtsjärv, Estonia, demonstrated that prior to human impact, pelagic diatom abundance was strongly related to water-level fluctuations, but this

relation was masked following eutrophication (Heinsalu *et al.*, 2008). Recent studies also draw attention to the difficulties of interpreting palaeolimnological proxies in sites with multiple drivers (Battarbee *et al.*, 2012; Bennion *et al.*, 2012).

Even though Lake Beyşehir currently is of low trophic status (oligo- mesotrophic) (Beklioğlu *et al.*, 2014), the change in littoral and pelagic cores suggested a trend towards a decrease in littoral species during lower water levels. The significant increase in bottom-dwelling fish species such as *Tinca tinca* and *C. carpio* recorded following fish introduction to this lake (Çubuk *et al.*, 2006), may have promoted greater resuspension of the sediments (Breukelaar *et al.*, 1994) and reduced zooplankton grazing on phytoplankton (Jeppesen *et al.*, 2012), possibly contributing to the indirect effect of reduced water levels. Furthermore, the study by Fethi *et al.* (2014) demonstrated that the sediment of Lake Beyşehir is dominated by silty clay, as also suggested by the geochemical records from this study. Therefore, the colloidal nature of clayey sediments, leading to slow settling out, may be another reason for the deterioration of the light climate in Lake Beyşehir.

Unlike Lake Beyşehir, studies suggest a major shift in the trophic state of Lake Uluabat from being first-second class (e.g. 0.2-1 mgNH₄ L⁻¹, 0.002-0.01 mgNO₂ L⁻¹, 5-10 mgNO₃ L⁻¹, 0.02-0.16 mgTP L⁻¹, 8-4 mgDO L⁻¹) (1970s) to second-fourth/worst class (e.g. fourth class; >2 mgNH₄ L⁻¹, >0.05 mgNO₂ L⁻¹, >20 mgNO₃ L⁻¹, >0.65 mgTP L⁻¹, <3 mgDO L⁻¹) (2000s) (Salihoğlu & Karaer, 2004; WPCR, 1988), possibly contributing to the disappearance of low-light intolerant Characeae species, also to the change towards higher amount of tall-growing plant remains (e.g. *Potamogeton* spp.) and lower abundance of benthic cladocerans (e.g. *Pleuroxus* spp.) during the lower water-level periods. Moreover, as for Lake Beyşehir suspended sediments in the water column might have been another reason for a higher light attenuation as the inflow of Lake Uluabat carries high amounts of suspended solids to the lake (Tağıl, 2007).

4.4.4 Conclusions

The major advantage of this study was to be able to use multi-proxy core data in comparison with the recorded water-level changes of the lakes. Since each proxy (biological and sedimentary) has a unique response to ecosystem changes, using multiple proxies facilitated the interpretations of changes in our cores. However, weaknesses of the proxies should not be overlooked, because the response of each proxy may vary depending on the nature and strength of the main stressors, leading to disagreement of the proxies with each other or with water level changes. Therefore, our results also showed that for this study none of the proxies could be used on their own. Correspondingly, our results highlight the lakespecific changes associated with water-level fluctuations and, once again the complexity of palaeolimnological studies covering recent periods where multiple drivers are in force (Battarbee et al., 2012; Bennion et al., 2012). The results also suggest muted responses in the proxies, especially when the lake-level change is small (as in Lake Uluabat) and/or when there is an effect from other drivers. Other plausible reasons for the muted response of the palaeo-records to water-level change are the mismatches between sampling resolution and sedimentation rates, and different depositional environments of pelagic and littoral cores (as seen in Lake Beyşehir). The inherent homogenised nature of lake sediments may result in low sensitivity of high frequency sediment data to record changes. Nonetheless, where instrumental records of lake changes are limited or do not exist, multiproxy palaeolimnological data provides an essential long-term assessment of environmental change that can be incorporated into lake management.

CHAPTER 5

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

5.1 Introduction

Water, being an essential resource for humanity and the core of natural ecosystems, is used in a diverse range of anthropogenic activities (e.g. for agricultural and recreational activities) (Costanza et al., 1997). In addition to the risk of unsustainable anthropogenic water use, studies point out to increasing risk of droughts and/or floods in the coming decades through global climate change (Giorgi & Lionello, 2008; Erol & Randyr, 2012; IPCC, 2013). Therefore, European Union Water Framework Directive (WFD) was developed and came into force in year 2000 (WFD 2000/60/EC) in order to investigate the factors affecting water sources, to ensure their sustainable use and for making right decisions on water management (EC, 2000). The main aim of the directive is to achieve "good water status" all over EU waters (EC, 2000). To accomplish this objective, member states (MS) from various geographical regions developed different indices based on Biological Quality Elements (BQEs) (Birk et al., 2012; Lyche-Solheim et al., 2013), which can be used to calculate ecological quality ratios (EQR), thus to determine the ecological status of natural water bodies or the ecological potential of highly modified water bodies (EC, 2000).

Aquatic plants (macrophytes) are considered as one of the BQEs for lakes, together with phytoplankton, phytobenthos, macroinvertebrate fauna and fish (EC, 2000; Lyche-Solheim *et al.*, 2013). Macrophytes have a significant effect on the biological structure and water quality of lakes, especially of shallow ones, by

dominating the primary production and thus promoting and helping to maintain high water clarity (Scheffer et al., 1993; Jeppesen et al., 1997). Their species composition, richness and abundance are mainly affected by water level fluctuations, light availability (Van der Valk, 1987; Gafny & Gasith, 1999) and nutrient concentrations, particularly phosphorus (P) and nitrogen (N) (Van et al., 1999; James et al., 2005; Thomaz et al., 2007). High nutrient concentrations promote the growth of phytoplankton and periphyton causing a decrease in light penetration and thus negatively affecting macrophyte communities, inducing a decrease in their richness, diversity and abundance as well as a shift towards more tolerant species (Scheffer et al., 2001). Moreover, water-level fluctuations, altering the light climate of the lakes affect the sensitivity of macrophytes to turbidity increase (Gafny & Gasith, 1999; Havens et al., 2004; Beklioğlu et al., 2006). Özkan et al. (2010) and Bucak et al. (2012) showed that even at high nutrient levels, with high turbidity and periphyton biomass, water level decrease in warm shallow lakes can help promote macrophyte growth, pointing out to higher stability of macrophytes in Mediterranean countries. Besides water level changes, faster macrophyte growth rate caused by warmer temperatures would lead macrophytes to reach the water surface in shorter time, thus increasing their tolerance to shading (Barko & Smart, 1981; Kosten et al., 2011). Therefore, owing to their sensitivity for environmental changes and to their non-mobile nature, macrophytes make up an important indicator for ecological classification of the lakes (Kienast et al., 2001; Birks, 2007; Søndergaard et al., 2005; 2010). Information on macrophyte abundance and composition from data gathered around the end of summer also has the advantages of being indicative of the whole growing season, obtained from relatively easy sampling and without the need of laboratory analysis (Poikane et al., 2011). Consequently, various lake assessment indices based on macrophyte richness, abundance and/or composition are currently in use. Poikane et al. (2011) pointed out to three classification strategies used in European countries, which are all related to eutrophication

gradient. These strategies are established using the relative abundance of different taxa based on their sensitivity, the trophic indices calculated by trophic scores assigned to species and the multimetric indices using various characteristics of macrophyte species (Poikane *et al.*, 2011).

In Turkey, as in many parts of the world, lake ecosystems are under pressure from intense anthropogenic activities, like agriculture around the lakes and irrigation (e.g. Girgin, 2000; Celik, 2006) also significant natural and/or artificial water level fluctuations caused by Mediterranean climate characteristics (İyigün et al., 2013) and by water extraction for irrigation (Coops et al., 2003; Beklioğlu et al., 2006). Moreover, climate change projections draw attention to the 25-30% decrease in precipitation and enhanced evaporation in the arid and semi-arid Mediterranean regions (Giorgi & Lionello, 2008). Hence, protecting lake ecosystems is an essential task for Turkey. Within this scope, after the Helsinki Summit, starting from early-2000s, Turkey started to renovate its water management policies in order to ease the harmonization process with the WFD (Sümer, 2011). Thereafter, projects such as MATRA (2002-2004), which focused on Büyük Menderes River Basin, and EU-TWINNING (2008-2009), which determined 25 River Basins in the whole country came into force (Sümer & Muluk, 2011). Moreover, subsequent to the start of the negotiations on Environmental Chapter, the number of WFD-related projects have been increasing (Sümer & Muluk, 2011). For example, an attempt to define the ecological status of Turkish lakes was carried out at Büyük Menderes River Basin within the scope of "Technical Assistance for Capacity Building on Water Quality Monitoring (TR2009/0327.02-02/001)" project and macrophyte-based index employed to define the quality of this basin was LEAFPACS, which is developed by United Kingdom (WFD-UKTAG, 2014). Another index, namely Macrophyte Index, was also calculated for Lake Mogan, revealing the ecological status of the lake as in moderate condition at 2003 and in bad condition at 2013 (Şanal et al., 2015).

Since recovery of macrophyte presence/abundance is considered as a crucial part of lake restoration projects (Gulati & Van Donk, 2002), it is important to define the ecological status of the lakes using macrophytes. Moreover, to our knowledge among the intercalibrated indices only calculation of the LEAFPACS index (WFD-UKTAG, 2014) has been suggested for Turkish lakes (e.g. Erkan, 2014; Bakır, 2015). Therefore, for the current study in order to determine the suitable metrics, macrophyte-based indices developed for Central-Baltic Region were applied to 35 lakes sampled along a latitudinal gradient in the western part of Turkey. Within this context, we hypothesized that in Mediterranean lakes due to the lower sensitivity of macrophytes to turbidity and faster plant growth, coverage based-metrics may result in misleading classifications, and in shallower lakes compositional metrics supported by plant richness data and in deeper lakes colonization depth metrics would show a stronger relation with trophic gradients.

5.2 Methods

5.2.1 Study Sites and Field Sampling

The study sites are located at the hot/warm-summer Mediterranean (Csa/Csb, respectively), semi-arid (Bsk) and oceanic (Cfb) climatic zones, defined by Köppen classification system (Peel *et al.*, 2007). The altitudinal position of the lakes ranged between ca. 10 - 1400 m, covering almost the whole latitudinal gradient at the western half of Turkey between 41°50'N-27°56'E and 37°07'N-29°36'E coordinates.

In total 35 lakes were sampled (Figure 5.1). Two of these lakes, have been monitoring since ca. mid-1990s (Burnak & Beklioğlu, 2000; Beklioğlu & Tan, 2008; Özen *et al.*, 2010; Coppens *et al.*, submitted) and three of them were sampled twice. Therefore, the plant survey and environmental variable data of monitored lakes from 2007 till 2014 and also data of the lakes sampled two times

were also added to the dataset as individual lakes, increasing the number of study sites up to 48 lake-years.

Physical, chemical and biological properties of these lakes, were evaluated with single, one-season measurements, which were conducted within the summer months of the 2006-2012 period. At the field, bathymetric data (depth measurements and coordinates) were collected from various points along transect lines located parallel and perpendicular to lake shores and were subsequently exported to ARCGIS 10.1 software (ESRI, 2012) for mapping. Samples, covering the whole water column, were retrieved from lakes' deepest points for total phosphorus (TP), soluble reactive phosphate (SRP), alkalinity and total nitrogen (TN) analysis. Secchi depth was also measured (for details see Çakıroğlu *et al.*, 2014 and Levi *et al.*, 2014-Chapter 2 of this thesis).



Figure 5.1 Location of the study sites

5.2.1.1 Macrophyte survey

The coverage of free floating, floating-leaved and submerged macrophytes were determined once during the summer period, by surveying the whole lake area along the parallel transect lines at equidistant intervals. The number of lines was chosen to reflect the lake areas, nevertheless the amount of transects reduced or increased according to the plant coverage. At each sampling point, situated at about equal intervals on the transect lines, GPS coordinates, water and Secchi depths were registered, also presence and coverage of each plant species were recorded using a rake with a rope. Identification of the species was carried out in the field when it was possible, and samples from macrophytes with doubtful identifications were collected in zip-lock bags for later more detailed determination. Fassett (1969), Davis (1984), Seçmen and Leblebici (1997) and Turkish Plants Data Service (TÜBİVES- http://www.tubives.com/) were used as the main sources for species identification. When necessary, experts from Gazi University, Prof .Dr. Mecit Vural and Assist. Prof. Erkan Uzunhisarcıklı, were also consulted.

5.2.1.2 Lake – Ecological Potential Evaluation Method

An ecoregional map adapted from Illies' (1967/1978), which was generated based on the distribution range and endemism of freshwater organisms (especially aquatic invertebrates), divided the continent into 25 regions and was used as a base for defining the regions of WFD (Zogaris *et al.*, 2008). Nevertheless, Turkey was not included among these regions. Considering the climatic characteristics (Köppen classification) and topographic features (e.g. elevation), Turkey can be partly considered within the Mediterranean region, for example with Spain. However, due to natural lakes being very diverse, it has not been possible to complete intercalibration works (including the macrophyte indices) for Mediterranean countries (Ruiz *et al.*, 2011; Poikane *et al.*, 2015). Therefore, since some of the Central-Baltic Countries were also included in other intercalibration groups (GIG) (e.g. United Kingdom in Northern GIG and Germany in Alpine GIG), for the current study the indices developed for Central-Baltic Countries were used.

5.2.1.2.1 Typology and Reference Sites

Typology of the lakes was defined, as LCB-1 and LCB-2, according to the Central/Baltic GIG Lake common intercalibration types, using the data on mean depth (< or > 3m) and alkalinity (< or > 1 meqL⁻¹) (Portielje *et al.*, 2014). However, most of our study lakes (29 out of 35 lakes) were found at altitudes higher than 200 m (Table 5.2-A), unlike the lakes included in the Central-Baltic intercalibration, having a maximum altitude of 200 m (Portielje *et al.*, 2014).

Each country that joined to WFD defined their reference sites based on, for example expert knowledge, historical data and modelling (Portielje et al., 2014). Therefore, reference sites among the current study lakes were determined according to the criteria defined by the Turkish Ministry of Forestry and Water Affairs based on lake environmental variables (e.g. TP <40 μ gL⁻¹, TN <1000 μ gL⁻ ¹) and catchment characteristics (e.g. >80% forest and semi-natural areas, <15%agricultural area and <0.8% artificial surface area) (Gök, 2014; Tuzkaya, 2015), also considering that reference sites should comprise at least 30% submerged plant coverage (van Wijk et al., 2003). However, the resultant reference lake number was very low, with only two sites representing LCB-1 lakes and only one site LCB-2 lakes. Thus, these lakes were only used as a means of control sites, since they are expected to be in high condition. Therefore, due to the low number of reference lakes in the current study, EQR values determined by Central-Baltic countries were used (Portielje et al., 2014) and when needed these values were normalized based on linear interpolation (Appendix-C Normalization) (Pall et al., bad: 0.0-0.2", "poor: 0.2-0.4"; 2014) to obtain an EQR classification as "moderate: 0.4-0.6"; "good: 0.6-0.8" and "high: 0.8-1.0".

5.2.1.2.2 Calculation of the Indices

A total of 10 macrophyte-indices, which are mainly based on the macrophyte taxonomic composition (floating, floating-leaved, submerged, emergent) and abundance data (percent or 5-7 scale coverage), are developed and used in the Central-Baltic Region (Portielje *et al.*, 2014). The indices which were chosen for this study represent various aspects of macrophytes, as composition-based metrics constituting "taxon-specific scores relative to TP, scores based on indicator taxa, comparison of sensitive-tolerant species and evenness index", also abundance-based metrics constituting "richness, coverage and colonization depth/index". However, some of the indices were calculated with some minor adjustments, in order to employ suitable measures for our study sites.

For determining the indices to be used, sampling methods are also important. In accordance with our study, the basic method employed by Central Baltic Countries was similar with sampling macrophytes during summer period and employing a rake-transect line method, but some of the countries also used Scubadiving and/or a water-viewer as supplements.

In the case when taxa could not be identified to species level and when its coverage was less than 5%, it was excluded from the index calculations. Moreover, for each calculated index, the study sites, thus their numbers, differed, because lakes were excluded/included in order to meet the requirements of each index. For example very high alkalinity lakes were removed from some calculations and kept in the others.

UKTAG Lake Assessment Method, Macrophytes (LEAFPACS) - United Kingdom

Suggested sampling procedure to calculate this index differed from the method used in the current study, by extra samplings at marginal and submerged shore transects and along the shoreline transects (ca. 0-1 m water depth). Since they also used boat transect lines to the depth of colonization, notwithstanding the differences, this index was also calculated for our study sites (WFD-UKTAG, 2014).

LEAFPACS index consists three main sections, as calculations of (Portielje *et al.*, 2014; WFD-UKTAG, 2014);

- Observed values,
- Site specific reference conditions by using abiotic factors (i.e. lake altitude, mean depth, area, alkalinity and weighted freshwater sensitivity class),
- EQR values for each site (see Appendix-C Index-1 for the whole method).

Each of these sections comprise metrics based on (Portielje *et al.*, 2014; WFD-UKTAG, 2014);

- <u>Composition</u>: Lake Macrophyte Nutrient Index (LMNI- calculated by using taxon-specific scores, established related to TP concentrations)
- <u>Richness</u>: Numbers of macrophyte taxa (NTAXA) and macrophyte functional groups (NFG)
- <u>Abundance</u>: Mean macrophyte cover (COV) and relative filamentous algae cover (ALG). The coverage of filamentous algae was not recorded in our macrophyte survey, thus abundance metrics were not included in our calculations.

Calculation of these metrics are sensitive to various factors, therefore some minor adjustments were needed in employing it for our study sites;

- LMNI metric is highly sensitive to the number of taxa recorded in the lakes and if less than four, the strength of the relation with TP decreases (Willby *et al.*, 2009). Therefore, surface plant remain data (Levi *et al.*, 2014-Chapter 2 of this thesis) was also included to the calculations as presence/absence of species and lakes with low number of species, were excluded.
- Species scores, used in calculating LMNI metric may get saturated when applied to high alkalinity lakes (Willby *et al.*, 2009), thus, lakes with very high alkalinities (>10 meqL⁻¹) were excluded.
- Richness metrics (NTAXA and NFG) are highly sensitive to the survey method.

Eventually, for low-moderate alkalinity lakes only LMNI metric, which is based on the taxon specific scores (established related to nutrient concentrations), and for high alkalinity (>2.5 meqL-1) lakes only richness metrics (NTAXA and NFG) were employed. The calculation of observed values are as follows;

Lake Macrophyte Nutrient Index (LMNI): Compositional metric based on species specific scores, which represents the sensitivity of the taxa to nutrients. It is calculated as,

$$LMNI = \frac{\sum_{J=1}^{n} LMNI_{J}}{N}$$

Where, "**LMNI**_j" is Lake Macrophyte Nutrient Index score for taxon "j", and "**N**" is the total number of macrophyte taxa.

<u>Number of functional groups (NFG)</u>: Lake macrophyte taxa recorded in the survey are assigned to one functional group out of 18.

Number of macrophyte taxa (NTAXA): Number of taxa recorded in the lake

Reference Index (RI) and Module Macrophyte Assessment (M_{MP})-Germany

The reference index (RI) calculation requires each recorded species to be assigned to a type-specific group, namely A (reference taxa), B (indifferent taxa) or C (disturbance indicator taxa). Therefore, this calculation is based on the relative quantities of reference, indifferent and disturbance indicator species, defined according to lake typologies (Stelzer *et al.*, 2005; Portielje *et al.*, 2014). Furthermore, as correction factors of RI values, depth of vegetation limit and dominant stands of specific taxa are employed (see Appendix-C Index-2 for the whole method). The resultant values were then converted to 0-1 scale (Module Macrophyte Assessment - M_{MP}).

Originally, RI calculation comprise dividing the lakes into 4 depth classes (0-1, 1-2, 2-4, >4 m), thus macrophytes are recorded and assigned to type-specific groups accordingly (Stelzer *et al.*, 2005; Portielje *et al.*, 2014). However, for the current study the calculation of this index is conducted according to the German-intercalibration method, which did not employ different depth classes and which proved to show good correlation with trophic state variables (Portielje *et al.*, 2014).

Calculation of reference index (RI) and transformation of this index to 0-1 scale (M_{MP}) are as below;

Reference Index (RI) =
$$\frac{\sum_{i=1}^{n_a} (Q_{Ai}) - \sum_{i=1}^{n_c} (Q_{Ci})}{\sum_{i=1}^{n_g} (Q_{gi})} \times 100$$

Q : Quantity = $(5 \text{ level-scale abundance})^3$

 \mathbf{Q}_{Ai} : Quantity of the i-th taxon of species group A

Qci: Quantity of the i-th taxon of species group C

 Q_{gi} : Quantity of the i-th taxon of all groups

n_A : Total number of taxa in group A

n_C : Total number of taxa in group C

n_g : Total number of taxa in all groups

$M_{MP} = \frac{(RI_{seen} + 100) \times 0.5}{100}$

MMP : Module Macrophyte Assessment RI_{Seen/Lakes}: type specifically calculated Reference Index Seen/Lakes

In this method helophytes were also used as indifferent taxa, but since our macrophyte data did not include the coverage of helophytes, they were excluded from our calculations. Moreover, for RI calculations *Najas marina* species was defined as disturbance indicators, but not *N. marina subsp. intermedia*. Because in our macrophyte data the subspecies was not defined, the lakes with high *N. marina* coverage were excluded from the calculations. Furthermore, the results of the German-macrophyte reference index are combined with the results from diatom index, nevertheless for our study sites only water column and surface sediment diatom data were present, thus diatom index could not be used.

Danish Lake Macrophyte Index (DLMI)-Denmark

Danish Lake Macrophyte Index (DLMI) calculation was carried out by dividing the species into two groups as indicator and not-indicator taxa, which then used in a point scoring system combined with lake area values (Portielje *et al.*, 2014). Subsequently, the resultant scores were summed up with the outcome of other point scoring methods, which uses percent macrophyte coverage (for shallow lakes) and maximum depth of colonization (for deep lakes). These scores were then assigned to EQRs, resulting in discrete EQR values (see Table 5.1 for an example) (Portielje *et al.*, 2014). The calculation of this index can be found in Appendix-C (Index-3).

Besides the indicator species list established by Denmark, another list has also been prepared according to the information gathered from the whole Central-Baltic countries (Portielje *et al.*, 2014). Thus, indicator taxa in current study were chosen using both DLMI and Central-Baltic species lists (Portielje *et al.*, 2014).

Lake Type						Total Point Score	EQR
LCB-1 & LCB-2	Number of indicator species: ≥ 4	&	Lake Area (ha): > 100	=>	<u>Points (p)</u> (1-4 scale): 4p	ſ	
LCB-1	Maximum depth of colonization (m): 5-7			=>	<u>Points (p)</u> (1-9 scale): 8p	4p+8p = 12p	0.90
LCB-2	Coverage of lake area (%): 1-2			=>	<u>Points (p)</u> (1-9 scale): 3p	4p+3p = 7p	0.57

Table 5.1 An example for calculation of points in deep (LCB-1) and shallow (LCB-2) lakes

Ecological State Macrophyte Index (ESMI) - Poland

The calculation of this index requires the minimum potential phytolittoral area (defined by the 2.5 m isobar from the bathymetric maps), total phytolittoral area (calculated using the maximum depth of colonization), total lake area, number of identified plant communities and percent coverage of each plant community in the phytolittoral (Ciecierska & Kolada, 2014; Portielje *et al.*, 2014). The main metrics used in this method are based on evenness and macrophyte colonization depth. Furthermore, since in this method only the plant communities, defined as 25% coverage per $1m^2$ area, were taken into account, our transect points which had less than 25% coverage, was excluded from our calculations (Ciecierska & Kolada, 2014; Portielje *et al.*, 2014). Helophytes are also included in the Polish method, but for the calculations conducted on the current study lakes, these species could not be included.

• Taxonomic composition metric:

Pielou index of evenness (J) = $\frac{H}{H_{max}}$ Phytocenotic diversity index (H) = $\sum \frac{n_i}{N} \times \ln \frac{n_i}{N}$

Maximum phytopcenotic diversity index $(H_{max}) = lnS$

• <u>Abundance metric:</u>

Colonization index (Z) = $\frac{N}{izob. 2.5}$

Ecological State Macrophyte Index (ESMI) = $1 - exp\left[-J \times Z \times exp\left(\frac{N}{P}\right)\right]$

N : total area of phytolittoral in ha in ha or km²

 \mathbf{n}_{i} : % share of proporcion of the area occupied by particular plant communities in % of N

S : number of plant communities identified in phytolittoral

izob.2.5 : area where water depth is <2.5m

P : total lake area in ha or km²

5.3 Results

According to the Central-Baltic intercalibration typology criteria, the study sites were assigned to two typologies as LCB-1 (mean depth 3-15 m) and LCB-2 (mean depth < 3 m), comprising 10 and 38 lake-years, respectively. All of the LCB-1 types was located at high altitudes (over 1000 m), while only six of the LCB-2 types were below 500 m (Table 5.2-A). The area of the lakes differed between ca. 0.1 - 700 ha and 26 of which was very small. Alkalinity of the lakes was between 0.6 and 28.0 meqL⁻¹. The trophic status of the lakes represented a variability from oligo-mesotrophic with a TP concentration of 15 μ gL⁻¹ to hypereutrophic with a TP of 633 μ gL⁻¹ and the TN concentrations were between 264 and 3230 μ gL⁻¹. Secchi depth of the clearest lake was 9.0 m for LCB-1 lakes and 2.2 m for LCB-2 sites (Table 5.2-B).

Table 5.2 Characteristics of the study sites according to the LCB-1 and LCB-2 typologies A) number of lakes found in each value class (modified from Kolada *et al.*, 2014) B) Minimum-Maximum and Median (in brackets) values of environmental and biological parameters measured

	Unit	Value Class		Number of Lake-years			
Determinant			Boundary values	LCB-1	LCB-2 (Low altitude)	LCB-2 (High altitude)	
Altitude	m	Low	0 - 500	-	б		
		High	500 - 1400	10		32	
	ha	Very Small	< 50	8	2	16	
Size (area)		Small	50 - 100	-	3	3	
		Medium	100 - 800	2	1	13	
	meqL ⁻¹	Medium	0.2 - 1.0	1	-	2	
A 11 - 11 - 14		High	1.0 - 2.5	4	4	8	
Alkalinity		Very high	2.5 - 10.0	4	2	14	
		Too high	> 10.0	1	-	8	

B)

Variable	Unit	LCB-1 (High altitude)	LCB-2 (Low altitude)	LCB-2 (High altitude)		
Maximum Depth	m	3.2 - 17.4 (6.5)	1.0 - 5.0 (2.7)	1.0 - 6.5 (3.5)		
Secchi Depth	m	1.4 - 9.0 (2.4)	0.2 - 2.2 (0.4)	0.3 - 2.0 (1.0)		
TP	µgL⁻¹	15 - 216 (44)	21 - 633 (106)	34 - 402 (67)		
TN	µgL⁻¹	264 - 2622 (568)	239 - 2107 (1450)	309 - 3230 (1234)		
SRP	µgL⁻¹	3.4 - 150 (11)	5.0 - 110 (19)	3.8 - 190 (30)		
Salinity	gL ⁻¹	0.1 - 1.6 (545)	0.1 - 0.3 (0.3)	0.1 - 2.9 (0.3)		
Coverage (Submerged -Floating)	%	1.0 - 79.0 (0.3)	2 - 86 (40)	0.5 - 89 (29)		

A)

In total 27 submerged, floating-leaved and floating species were recorded. *Ceratophyllum demersum* L., *Potamogeton pectinatus* L., *Myriophyllum spicatum* L. and *Najas marina* L. were the most common submerged species recorded among the lakes and the prevalent floating-leaved species were *Nymphaea alba* L., *Polygonum amphibium* L. and *Potamgeton natans* L. (Table 5.3).

		Percent Coverage			
Macrophyte Species	Number of Lakes	Median (for >0% coverage)	Minimum (for >0% coverage)	Maximum	
Ceratophyllum demersum L.	17	5.6	0.02	87.1	
Ceratophyllum submersum L.	3	63.2	36.2	84.1	
Elatine alsinastrum L.	1	1.5	1.5	1.5	
Elodea canadensis MICHAUX	1	17.6	17.6	17.6	
Myriophyllum spicatum L.	14	4.3	0.2	40.0	
Myriophyllum verticillatum L.	2	1.3	1.0	1.7	
Najas marina L.	14	4.1	0.3	69.5	
Najas minor Allioni	1	0.3	0.3	0.3	
Potamogeton crispus L.	4	0.1	0.03	1.2	
Potamogeton lucens L.	1	2.4	2.4	2.4	
Potamogeton pectinatus L.	14	3.8	0.5	42.2	
Potamogeton perfoliatus L.	3	0.7	0.7	0.9	
Potamogeton sp.	6	2.6	0.4	50.0	
Ranunculus rionii Lagger	1	0.2	0.2	0.2	
Vallisneria spiralis L.	1	2.3	2.3	2.3	
Zannichellia palustris L.	2	1.0	0.7	1.4	
Utricularia australis R. Br.	9	4.9	0.1	20.0	
Utricularia vulgaris L.	2	1.0	0.1	2.0	
Chara vulgaris L.	2	0.1	0.1	0.2	
Chara spp.	5	16.3	0.2	47.6	
Nitella sp.	2	5.1	0.2	10.0	
Hydrocharis morsus-ranae L.	1	0.1	0.1	0.1	
Nuphar lutea (L.) SM.	4	15.0	7.1	22.0	
Nymphaea alba L.	8	4.9	0.3	19.0	
Polygonum amphibium L.	8	3.6	0.4	29.3	
Potamogeton natans L.	8	4.4	0.04	61.1	
Trapa natans L.	4	9.5	3.8	62.3	

Table 5.3 Submerged, floating-leaved and floating macrophyte species recorded in the study sites, number of lakes they were found and their percent coverage

5.3.1 Index Calculation Results

UKTAG Lake Assessment Method, Macrophytes (LEAFPACS)

This index is normally based on three types of metrics, being compositional, species richness, and abundance. For this study only compositional and richness metrics were used. The resultant EQR values were between 0.1-0.8 for both lake types and the number of lakes classified as bad, poor, moderate and good were 4, 4, 8 and 4, respectively. Moreover, for both lake types reference sites were grouped in good condition. The results indicated a weaker trophic gradient-EQR relation with deeper LCB-1 lakes (Figure 5.2), which were associated with TP ($R^2 = 0.38$) and Secchi depth ($R^2 = 0.23$). LCB-2 lakes, on the other hand were found to be related with the strongest relationship being with SRP ($R^2 = 0.52$) followed by TN, TP and Secchi depth (Figure 5.3).



Figure 5.2 EQR-LEAFPACS versus TP and Secchi Depth gradients for deeper LCB-1 type lakes.



Figure 5.3 EQR-LEAFPACS versus TP, SRP, TN and Secchi Depth gradients for shallower LCB-2 type lakes.

Reference Index (RI) and Module Macrophyte Assessment (MMP)

The reference index for the study sites changed between -7 and 100 for LCB-1 types and between -95 and 100 for LCB-2 lakes, with EQRs ranging between 0.5-0.6 and 0.25-0.95, respectively (Figure 5.4 and Figure 5.5). Therefore, LCB-1 lakes were grouped as being in moderate condition, while LCB-2 lakes had a wider range from poor to high conditions, nonetheless as many other lakes the reference site grouped as moderate. The EQR interval for deeper LCB-1 lakes was very low, even though the Trophic gradient-EQR relation indicated a strong linear relation ($R^2 = 0.58$ and 0.53 for TP and TN, respectively) and nutrient

concentrations varied between around 15-200 μ gTP L⁻¹, 3-150 μ gSRP L⁻¹ and 300-2500 μ gTN L⁻¹. Similar situation was also observed for some of the shallower lakes, where only indifferent taxa were recorded. For these lakes the EQR range was higher, nevertheless, many of them, with TP concentrations between ca. 50 and 250 μ gL⁻¹, was classified as in moderate conditions.



Figure 5.4 EQR-MMP versus TP, SRP and TN gradients for deeper LCB-1 type lakes.



Figure 5.5 EQR-MMP versus TP and SRP gradients for shallower LCB-2 type lakes.

Danish Lake Macrophyte Index (DLMI)

The DLMI-EQR values ranged between 0.30 and 0.77 for LCB-1 lakes and between 0.37 and 0.90 for LCB-2 types, grouping the lakes between poor and high conditions (Figure 5.6 and Figure 5.7). Moreover, the reference sites were classified as good for both lake types, while some of the lakes in LCB-2 were classified as being in high condition, even though the nutrient concentrations were high with a TN concentration of c. 2100 μ gL⁻¹ and TP of c. 160 μ gL⁻¹. The results indicated a relatively strong relationship of deeper lakes with trophic gradients TP (R² = 0.70), SRP (R² = 0.55), TN (R² = 0.27) and Secchi depth (R² = 0.40). For shallower LCB-2 type lakes, however, the relationship was weaker with TP (R² = 0.13) and SRP (R² = 0.18), while no relationship was observed with Secchi depth and TN.


Figure 5.6 EQR-DLMI versus TP, SRP, TN and Secchi depth gradients for deeper LCB-1 type lakes.



Figure 5.7 EQR-DLMI versus TP and SRP gradients for shallower LCB-2 type lakes.

Ecological State Macrophyte Index (ESMI)

ESMI-EQR values, which were calculated based on the total/phytolittoral areas of the lakes and diversity/evenness of the aquatic plants, were mostly between 0.0 and 0.6 assigning the lakes mostly to bad-poor and some to moderate conditions, while only two lakes were classified as in good status. Moreover, EQR values showed a strong relationship with Secchi depth ($R^2 = 0.80$) and TP ($R^2 = 0.87$), but weaker relations with SRP and TN ($R^2 = 0.35$, $R^2 = 0.14$, respectively) for LCB-1 lakes (Figure 5.8). For shallower LCB-2 lakes the strongest, relation was with SRP ($R^2 = 0.17$) and with the other trophic variables (TP, SRP and Secchi depth) a relation was not found (Figure 5.9). Nonetheless, for both typologies the results suggested that almost all the lakes were classified as between poor and moderate conditions, even the ones chosen as reference lakes, with TP, SRP and TN values of 5, 21 and 239 µgL⁻¹, respectively.



Figure 5.8 EQR-ESMI versus TP, SRP and Secchi depth gradients for deeper LCB-1 type lakes.



Figure 5.9 EQR-ESMI versus SRP gradient for shallower LCB-2 type lakes.

5.4 Discussion

The implementation of Central-Baltic region macrophyte indices to lakes located in western Turkey pointed out to differences in the strength of the relationship between the trophic variables and ecological classification of the study lakes and in some cases misclassification of the lakes (Table 5.4, Figure 5.10).

The results of the indices indicated that the reference sites determined according to trophic lake variables and catchment characteristics were classified as "good" for deep lakes, while for shallow lakes the site was grouped as "moderate" when evenness (ESMI) and type-specific group (M_{MP}) based indices were used.

Table 5.4 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.

INDICE	Number of Lakes		ΤΡ (μgL ⁻¹)		SRP (μgL ⁻¹)		TN (μgL ⁻¹)		Secchi Depth (m)	
	LCB-1	LCB-2	LCB-1	LCB-2	LCB-1	LCB-2	LCB-1	LCB-2	LCB-1	LCB-2
LEAFPACS	6	14	0.38	0.28	-	0.52	-	0.37	0.23	0.21
Ммр	6	22	0.58	0.23	0.53	0.22	0.41	-	-	-
DLMI	7	38	0.70	0.13	0.55	0.18	0.27	-	0.40	-
ESMI	5	30	0.87	-	0.35	0.17	0.14	-	0.80	-



Figure 5.10 Comparison of lake ecological classifications calculated according to the indices developed by United Kingdom (LEAFPACS), Germany (MM_P), Denmark (DLMI) and Poland (ESMI)

The adjusted UKTAG-Macrophyte Lake Assessment Index which is based on the metric calculated from species specific scores (LMNI), but also combining this with another metric based on species richness, indicated a relatively strong relation and a wide EQR range. This index also delivered the strongest TP/SRP-EQR relationship for the shallow LCB-2 lakes. Therefore, the suggestion is that combining compositional and richness metrics may be reliable for Turkish lakes. However, care should be taken while using the compositional metric, since it is suggested to be sensitive to alkalinity, and species scores seem to get saturated when applied to only high alkalinity shallow lakes (Willby et al., 2009; Dudley et al., 2013). Furthermore, Manolaki and Papastergiadou (2012) pointed out that the scores may not be in accordance with the trophic gradient in a different country, thus some species may need to be re-scored. Even though the application of species richness metrics to our high alkalinity sites improved the relationship, for some lakes the results appeared to be very subdued. Notwithstanding the usage of plant remains as supplementary data, the lower EQR values may be related with our less elaborate sampling procedure, since species richness metrics are very sensitive to survey method (Willby et al., 2009). Furthermore, the UK method produces site specific reference conditions based on abiotic indicators, like area and altitude (WFD-UKTAG, 2014). Since, Turkish lakes are different than the ones from Central-Baltic region, for example with karstic, fluctuating water level and high altitude characteristics, produced reference values may be less reliable for Turkey. Accordingly, Lauridsen et al. (2015) covered a large latitudinal gradient investigating the impact of environmental variables on submerged macrophyte assemblages in Denmark, Belgium/The Netherlands and Spain, drawing attention to the lower species richness in south. In contrast, a review study by Meerhoff et al. (2012) showed no obvious relation between macrophyte species richness and latitude.

Reference Index (Germany), is based on macrophyte community composition (type specific groups) and abundance. Nonetheless instead of trophic scores it is calculated using the relative abundance/quantity of sensitive-disturbance indicator species, supported by vegetation limit and abundance of dominant stands. Despite the strength of the EQR-TP relation for deeper, LCB-1, lakes was high ($R^2 = 0.58$), the range of EQR values was very low, categorizing all the lakes in moderate condition, which was also the case for many of the LCB-2 lakes. This was not unexpected considering the species grouped as indifferent taxa for German Lakes, generally composed the dominant stands in our lakes and also reference or disturbance indicator taxa were not present. Similarly, several studies also pointed out (Schaumburg *et al.*, 2004; Stelzer *et al.*, 2005; Penning *et al.*, 2008) to the potentially unreliable classifications in lakes lacking indicator species.

Danish Lake Macrophyte Index (DLMI) calculation is based on the maximum colonization depth (deep lakes), submerged macrophyte coverage (shallow lakes) and richness of low-nutrient indicator species (both types of lakes). The results indicated a more steep relation between the EQR values of deeper lakes and trophic variables. The correlation for shallower lakes was less strong, possibly reflecting the categorization of the lakes, having relatively high TP (>100 μ g L⁻¹) and TN (>2000 μ g L⁻¹) concentrations and low species richness, in high class. This likely misclassification was due to the higher submerged macrophyte coverage of these lakes leading to higher DLMI-point scores, likely suggesting the coverage-point scores defined using the information obtained from Danish lakes may be too low for some lakes located in warmer countries. Studies also confirmed the high resilience (Özkan et al., 2010), even enhanced growth (Bucak et al., 2012) of macrophytes when nutrient levels and turbidity are high in Mediterranean lakes that are subject to water level reductions (e.g. Beklioğlu et al., 2006). Despite the higher correlation of maximum growing depth and trophic state variables, it should also be noted that higher temperatures may lead to deeper colonization depths of submerged macrophytes (Middelboe & Markager, 1997; Rooney & Kalff, 2000) due to enhanced growth rates (Barko & Smart, 1981), leading to higher shade tolerance (Kosten *et al.*, 2011). Correspondingly, Søndergaard *et al.* (2013) pointed out to the possibility of misclassification of lakes when fixed boundaries for colonization depths are used and thus, to the importance of several samplings to determine a lakes' natural variability.

The index developed for Poland, applying diversity/evenness and colonization index, showed a relatively weak relation with trophic variables in general, but its relation with TP and Secchi depth for deeper lakes was strong. On the other hand, EQR values of the shallower lakes were mostly low and many of the lakes were classified as in bad-poor condition. This may be related to the lack of helophyte data from our lakes, since Kolada (2014) showed the significance of including helophytes. Other studies (e.g. Dudley *et al.*, 2013), however, could not justify the improvement of ecological classification by including emergent vegetation, thus their relevance remains dubious (Kolada *et al.*, 2014). Another reason for this possible misclassification may be a result of diversity/evenness metric, since the number of species recorded in our lakes were relatively low and there was one dominant plant species in most of our lakes. Despite the fact that Ciecierska and Kolada (2014) draw attention to the high strength of this metric, the lower diversity in our lakes may be a result of the warmer conditions as it was also pointed out in Lauridsen *et al.* (2015).

Overall, the results suggested that when evenness/diversity or only coverage based metrics used the lakes may be misclassified, while the usage of compositional metrics together with abundance or richness information (LEAFPACS index) increased the strength of the relation for shallower lakes. Moreover, for our deep lakes, compatible with other studies (Canfield *et al.*, 1985; Sondergaard *et al.*, 2013) depth of colonization (DLMI and ESMI indices) seems to be a suitable abundance measure, though low number of deep lakes in this study should also be noted. The higher sensitivity of shallow lakes to environmental changes might be the cause for the difference in the strength of the metrics between shallow and deep lakes. It is likely that compared to Central-Baltic countries deep lakes are less affected from the warmer temperatures in Turkey, which possibly leads to higher tolerance of macrophytes to nutrient concentrations and shading in shallow lakes (Özkan et al., 2010; Kosten et al., 2011; Bucak et al., 2012). However, the basic weakness/difficulty of applying these indices to Turkey or Mediterranean countries is probably due to the more diverse typologies (e.g. Ruiz et al., 2011; Çakıroğlu et al., 2014), possibly leading to some incompatibilities in reference systems compared to the ones in European countries. Another important point that care should be taken when using or adjusting different metrics is the significant inter-annual and seasonal water level fluctuations in Mediterranean regions (Coops et al., 2003; Beklioğlu et al., 2006). The impact of these fluctuations can be complicated, since reduced water levels may either enhance plant growth due to lower light attenuation (Özkan et al., 2010; Bucak et al., 2012) or may cause an increase in salinity concentration leading to lower species diversity.

CHAPTER 6

CONCLUSION

This study had four main aims. The first two aims were determining the congruence between surface sediment plant macrofossils and pigments with modern macrophyte and phytoplankton communities, respectively, in a large range of mostly shallow lakes. The thesis also aimed to determine the impact of long-term (c. 40-110 years) water level changes on the sediment cores from three large lakes with a multi-proxy approach. Moreover, the last aim was calculating four macrophyte-based WFD indices to investigate their suitability for Turkish lakes and to evaluate the ecological status of shallow small lakes by employing WFD approach.

The results suggested that plant remains and pigments found in the surface sediments showed a good agreement with present-day macrophyte and phytoplankton assemblages, respectively. However, another inference was that this relation is not always straightforward and in some lakes the discrepancy between sedimentary and modern data were relatively high, highlighting one more time the need for multi-proxy studies. Over- or under- representation of sedimentary remains was the probable cause for this difference, which was dependent on the seed characteristics (e.g. size, buoyancy) of macrophytes, and preservation quality of the sedimentary pigments. This discrepancy is further related to the surface sediments reflecting a temporal integration of the last 1-5 years of modern communities. Moreover, similarity of sedimentary and modern assemblages in representing corresponding environmental conditions of the lakes

demonstrated one more time that these proxies provide reliable indications of past conditions.

Comparison of instrumental water-level data with multi-proxy records from littoral and pelagic cores of three large lakes, namely Marmara, Beyşehir and Uluabat, showed that sediment records reflected long-term and more pronounced water-level changes. The main advantage of this study was to be able to employ a multi-proxy approach, which facilitated the interpretation of proxy response in relation to water-level change. Nevertheless, response of the proxies differed between lakes, through time and among pelagic-littoral areas, complicating the interpretations. Moreover, the impact of anthropogenic activities, such as fish introduction and eutrophication, on the ecological response of biological variables likely prevented a clear response of these proxies to water-level changes. Furthermore, the comparison of the changes in pigments and plant remains through the cores did not reveal a clear shift between clear (macrophyte dominated) and turbid (phytoplankton dominated) water states with changing water levels and nutrient concentrations, as expected in "alternative stable states hypothesis" (Scheffer *et al.*, 1993).

The good agreement between plant remains of surface sediment and modern macrophyte assemblages enabled the utilization of these remains in WFD index calculations, when only presence-absence data is needed. The results of the indices suggested that the combination of metrics based on species scores, allowing the use of surface sediment plant remains, with the one based on species richness increased the strength of EQR-Trophic variable relation in shallow lakes, while for deep lakes this relation was stronger with the metrics employing maximum depth of colonization. Nevertheless, the resultant EQR values of the possible reference sites indicated that these lakes are classified as in "good" status, yet the expectation was they should be classified as "high". This difference in the classifications may point out to the importance of adapting the calculations to Turkey, since the geological features, thus lake typologies, differ from Central-

Baltic countries. However, it should also be noted that this difference may also be a result of using the same EQR-classification boundaries defined by these countries.

This study confirmed the reliability of using plant remains and sedimentary pigments in order to define past ecology of the lakes, by employing a large set of lakes for the first time in literature, by comparing these proxies with long-term instrumental water-level data and by using plant remains in WFD index calculations. Overall results suggested that notwithstanding the difficulties in palaeolimnological research, these studies are crucial in long-term assessment of environmental change that can be incorporated into lake ecological assessment and management, where long-term instrumental records and monitoring programmes of lakes are limited or do not exist.

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APPENDIX A

This appendix is prepared for CHAPTERS 1, 2 and 5.



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

Appendix-A Figure 1. List and location of the lakes that were sampled with snap-shot sampling methodology

Variables	Unit	Minimum	Maximum	Mean	Median
Altitude	m	0	1423	758	977
Area	ha	0.08	714.28	61.55	15.07
Average Water Temperature	°C	8.30	32.25	21.96	23.15
Maximum Depth	m	0.55	17.40	3.76	3.20
Secchi Depth	m	0.20	9.00	1.30	0.90
Total Phosphorus	μg L ⁻¹	15.04	632.57	125.28	88.00
Soluble Reactive Phosphorus	μg L ⁻¹	3.38	160.44	32.93	18.24
Total Nitrogen	μg L ⁻¹	238.8	2340.0	1106.4	971.8
Nitrite-Nitrate-N	μg L ⁻¹	0.00	1721.98	66.11	12.58
Ammonium-N	μg L ⁻¹	0.00	714.26	105.26	44.90
Silicate	mg L ⁻¹	1.10	15.65	4.91	4.21
Alkalinity	meq L-1	0.50	27.70	4.98	2.00
pH	-log[H+]	6.29	9.64	8.18	8.02
Dissolved Oxygen	mg L ⁻¹	0.56	15.32	6.82	6.60
Conductivity	mS cm ⁻¹	0.10	24.39	1.78	0.44
Salinity	‰	0.05	14.50	0.98	0.21
Chlorophyll-a	μg L ⁻¹	1.82	181.09	28.57	13.43
Zooplankton	μg L ⁻¹	0.23	976.6	98.5	39.54
Cladocera	μg L ⁻¹	0.00	160.3	21.5	4.40
Copepoda	μg L ⁻¹	0.00	918.4	58.6	5.81
Rotifera	μg L ⁻¹	0.002	182.0	15.5	4.43
Nauplii	μg L ⁻¹	0.00	52.00	4.10	0.68
Phytoplankton	$mm^3 L^{-1}$	0.08	76.70	14.13	5.84
Bacillariophyta	$mm^3 L^{-1}$	0.00	24.99	2.02	0.20
Chlorophyta	$mm^3 L^{-1}$	0.00	47.14	2.41	0.42
Chrysophyta	$mm^3 L^{-1}$	0.00	0.66	0.03	0.00
Cryptophyta	$mm^3 L^{-1}$	0.00	49.66	1.77	0.14
Cyanobacteria	$mm^3 L^{-1}$	0.00	50.47	6.90	0.29
Dinophyta	$mm^3 L^{-1}$	0.00	7.97	0.65	0.10
Euglenophyta	$mm^3 L^{-1}$	0.00	5.64	0.36	0.04
Total Fish	NPUE	0.0	1425.0	173.8	53.5
Benthivorous Fish	NPUE	0.0	79.0	12.5	3.8
Omnivorous Fish	NPUE	0.0	412.0	31.6	7.0
Piscivorous Fish	NPUE	0.0	43.0	4.6	0.0
Zooplanktivorous Fish	NPUE	0.0	1190.8	125.7	12.4
Submerged Plant volume inhabited	%	0.00	79.93	22.53	11.59
Submerged Plant Coverage	%	0.00	89.07	32.19	21.80
Floating-leaved plant Coverage	%	0.00	62.31	8.99	0.00

Appendix-A Table 1. Physical, chemical and biological characteristics of the snap-shot sampled lakes

APPENDIX B

Information on the Water Level Data of the Study Sites (CHAPTER 4)

Water level data for the lakes were obtained from the reports published by General Directorate of Electrical Power, Resource Survey & Development Administration (EIE) and by General Directorate of State Hydraulic Works (DSI) (Appendix-B Figure 5).

The data for Lake Marmara retrieved from DSI, but from two different stations based on monthly average measurements. The first one comprises the years between 1970-2002 and the second one 1982-2012. The comparison of the coinciding years mostly indicated slight differences, with some exceptions. Therefore, the two datasets combined and 1970-1981 period from one dataset and 1982-2012 period from the other dataset were used.

Lake Beyşehir's dataset was obtained from DSI. There were two sets of data covering yearly minimum and maximum measurements for the period 1905-2001 and monthly average data from 1950 till 2012. The comparison of the water level data of the coinciding years showed very little differences, hence water level data from 1905-1949 period from the first dataset and 1950-2012 period from the second one were combined.

Water level measurements of Lake Uluabat were obtained from three different sources, namely EIE, DSI located in Ankara and DSI located in Bursa, covering the periods 1961-2000; 2001-2005 and 1999-2012, respectively. While the first two datasets composed of the "first day of the month and monthly 'minimum, maximum and mean' measurements", the last one only includes data from the "first day of the month". Therefore, for 1961-2005 period mean monthly data

from two sources (EIE and DSI-Ankara), and for 2006-2012 period first day of the month data was used.

Appendix-B Table 1. Radiometric analysis (²¹⁰ Pb and ¹³⁷ Cs) results o	f Lake
Marmara littoral core	

Dopth	Dry	y 210Pb Supported		²¹⁰ Pt	²¹⁰ Pb			Chr	Chronology			
Dehiii	Mass	Subt	Unsupported				Date	Ag	je			
(cm)	(g cm-2)	(Bq Kg ⁻¹)	±	(Bq Kg ⁻¹)	±	(Bq Kg ⁻¹)	±	(AD)	(yr)	±		
								2011	0			
0.0-0.5	0.12	56.26	6.14	3.97	26.02	0.00	0.00	2011	0	2		
1.0-1.5	0.60	39.80	2.50	18.42	12.37	2.41	1.40	2010	1	2		
2.0-2.5	1.08	39.46	3.03	21.83	14.88	0.00	0.00	2009	2	2		
3.0-3.5	1.57	41.64	2.53	7.04	11.32	4.65	1.28	2008	3	3		
4.0-4.5	2.07	41.56	1.64	24.92	7.74	3.44	0.85	2007	4	3		
5.0-5.5	2.57	35.58	1.95	19.81	9.20	3.83	1.08	2005	6	3		
6.0-6.5	3.08	38.75	1.52	21.02	7.19	0.00	0.00	2003	8	4		
7.0-7.5	3.70	34.62	1.25	27.12	6.07	1.85	0.67	2000	11	4		
8.0-8.5	4.32	32.69	2.10	43.57	9.87	3.44	1.21	1995	16	5		
9.0-9.5	5.09	35.98	1.41	33.06	7.24	2.88	0.74	1988	23	7		
10.0-10.5	5.86	37.17	1.14	15.98	4.92	2.63	0.58	1982	29	9		
11.0-11.5	6.60	34.89	1.14	11.78	5.35	5.21	0.65	1978	33	11		
12.0-12.5	7.35	33.80	1.02	14.15	4.98	2.47	0.56	1973	38	13		
14.0-14.5	8.73	38.87	2.24	16.04	10.45	6.76	1.28	1962	49	18		
16.0-16.5	10.12	39.93	2.17	15.16	10.07	4.98	1.25	1943	68	23		
18.0-18.5	11.60	34.82	2.34	12.39	10.64	5.89	1.34					
20.0-21.0	13.57	38.11	1.40	-3.14	6.25	6.10	0.83					
22.0-23.0	15.80	34.03	1.53	-0.84	6.55	3.42	0.80					
26.0-27.0	20.78	33.59	1.01	1.77	4.58	0.00	0.00					
28.0-29.0	23.49	34.01	1.65	5.76	7.84	1.53	0.80					
30.0-31.0	26.28	27.45	1.05	0.19	4.71	0.69	0.48					
34.0-35.0	31.70	31.42	1.25	-3.96	5.69	3.04	0.63					

Donth	Dry Mass	210Dh Supr	²¹⁰ Pb		b	13700	Chronology			
Deptil	Diy Mass	D Subb	oneu	Unsuppo	Unsupported					e
(cm)	(g cm-2)	(Bq Kg⁻¹)	±	(Bq Kg⁻¹)	±	(Bq Kg⁻¹)	±	(AD)	(yr)	±
								2011	0	
0.0-0.5	0.08	57.23	4.33	35.93	13.01	13.69	2.58	2011	0	2
3.0-3.5	1.06	46.01	2.37	26.81	7.10	8.61	1.32	2008	3	2
6.0-6.5	2.16	42.57	2.58	63.92	9.22	9.19	1.62	2004	7	2
9.0-9.5	3.28	39.92	2.61	51.30	9.03	10.09	1.49	1998	13	2
12.0-12.5	4.47	40.15	2.01	29.67	6.82	17.32	1.37	1992	19	3
15.0-15.5	5.69	41.89	2.69	42.42	10.16	15.83	1.76	1986	25	4
18.0-18.5	6.87	44.06	3.09	33.12	10.62	17.74	2.14	1978	33	5
20.0-21.0	7.72	47.13	2.53	4.11	8.04	14.62	1.62	1976	35	5
24.0-25.0	9.29	41.69	2.71	28.71	9.60	21.99	2.03	1970	41	5
28.0-29.0	10.80	41.39	2.41	8.54	8.19	30.84	2.05	1963	48	7
32.0-33.0	12.20	38.33	1.76	10.84	6.06	29.66	1.49	1958	53	8
36.0-37.0	13.55	41.78	2.25	4.22	7.50	15.14	1.48			
40.0-41.0	14.92	41.12	2.26	-3.58	7.18	3.35	1.16			
44.0-45.0	16.30	42.51	1.66	7.36	5.47	0.00	0.00			
48.0-49.0	17.71	40.29	2.07	-3.53	6.52	0.00	0.00			

Appendix-B Table 2. Radiometric analysis (²¹⁰Pb and ¹³⁷Cs) results of Lake Marmara pelagic core

Appendix-B Table 3. Radiometric analysis (²¹⁰ Pb and ¹³⁷ Cs) results of La	ake
Beyşehir littoral core	

Donth	Dry Mass			²¹⁰ P	b	1370-		Chr	onolog	у
Depth	Dry Mass 210Pb Supported		Unsupp	orted		•	Date	Age		
(cm)	(g cm-2)	(Bq Kg⁻¹)	±	(Bq Kg⁻¹)	±	(Bq Kg⁻¹)	±	(AD)	(yr)	±
								2011	0	
0.0-0.5	0.13	51.31	4.06	58.03	15.13	43.92	2.99	2011	0	2
3.0-3.5	1.82	28.84	2.56	65.89	10.95	36.71	2.29	2004	7	2
5.0-5.5	3.00	30.12	2.10	80.06	9.78	36.40	1.82	1997	14	2
8.0-8.5	4.90	35.40	2.27	44.80	9.28	29.08	1.75	1985	26	3
10.0-10.5	6.22	31.00	1.97	47.85	8.49	34.79	1.65	1976	35	4
11.0-11.5	7.26	32.13	2.26	57.24	10.09	35.13	1.90	1965	46	5
13.0-13.5	8.35	33.00	2.22	29.43	8.91	36.56	1.84	1951	60	7
15.0-15.5	9.95	30.61	1.18	20.22	4.68	31.39	0.95	1932	79	12
16.5-17.0	11.26	27.86	1.26	23.15	4.83	33.06	1.05	1903	108	26
18.0-18.5	12.59	28.63	1.44	3.12	5.39	30.00	1.12			
20.0-20.5	14.36	30.96	1.61	3.07	5.94	11.82	0.99			
23.0-23.5	17.10	27.18	1.22	-4.74	4.27	0.87	0.52			

Denth Dry Mass		210 Dis Commonte d		²¹⁰ Pl	²¹⁰ Pb		1270-		Chronology			
Depth	Dry Mass	210Pb Supp	onea	Unsuppo	orted	13/08		Date	Ag	e		
(cm)	(g cm-2)	(Bq Kg⁻¹)	±	(Bq Kg⁻¹)	±	(Bq Kg⁻¹)	±	(AD)	(yr)	±		
								2011	0			
0.0-0.5	0.11	39.44	3.14	84.46	11.51	45.71	2.23	2009	2	2		
0.5-1.0	0.35	29.16	2.56	85.06	10.80	43.80	2.26	2003	8	2		
1.0-1.5	0.59	26.34	1.24	37.42	5.45	45.90	1.14	1998	13	2		
1.5-2.0	0.85	32.43	3.07	88.84	12.43	46.32	2.42	1992	19	2		
2.0-2.5	1.12	30.57	1.85	22.56	6.83	28.14	1.43	1986	25	3		
2.5-3.0	1.42	23.77	1.88	47.21	8.15	19.32	1.38	1981	30	3		
3.0-3.5	1.72	26.44	1.12	26.07	4.37	16.76	0.75	1974	37	4		
3.5-4.0	2.03	23.60	1.93	30.24	7.93	16.09	1.24	1966	45	5		
4.0-4.5	2.34	26.17	1.02	19.53	4.21	14.24	0.65	1958	53	7		
4.5-5.0	2.65	21.23	1.81	25.40	7.46	17.45	1.30	1948	63	9		
5.0-5.5	2.97	24.82	0.91	23.65	3.66	13.20	0.59	1931	80	14		
5.5-6.0	3.28	25.10	1.70	25.17	6.43	13.26	1.04					
6.0-6.5	3.60	25.00	3.18	-9.14	10.25	8.11	1.66					
6.5-7.0	3.91	22.85	1.37	18.13	5.27	5.72	0.74					
8.0-8.5	4.87	25.42	1.02	-0.86	3.88	1.97	0.48					
12.0-12.5	7.63	24.38	1.14	0.81	4.60	0.00	0.00					

Appendix-B Table 4. Radiometric analysis (²¹⁰Pb and ¹³⁷Cs) results of Lake Beyşehir pelagic core

Death	DryMass	210Dh Cumr	orted	²¹⁰ P	b	13700		Chronology		
Depth	Dry Wass	200Pb Supp	ontea	Unsupp	orted	10105	i	Date	Age	9
(cm)	(g cm ⁻²)	(Bq Kg⁻¹)	±	(Bq Kg⁻¹)	±	(Bq Kg ⁻¹)	±	(AD)	(yr)	±
								2011	0	
0.0-0.5	0.05	62.86	8.36	28.57	30.73	0.00	0.00	2011	0	2
2.5-3.0	1.06	51.94	4.33	47.50	15.62	13.08	2.50	2008	3	2
7.0-7.5	3.26	50.58	3.27	42.31	13.32	8.65	1.76	2000	11	3
12.0-12.5	5.77	48.74	2.55	28.71	10.14	11.15	1.36	1991	20	4
14.5-15.0	7.14	50.50	1.33	19.67	5.28	12.82	0.75	1987	24	4
16.5-17.0	8.31	44.96	2.06	21.90	8.26	10.96	1.16	1984	27	5
18.0-18.5	9.22	44.05	2.53	37.98	10.27	16.94	1.60	1979	32	5
19.5-20.0	10.15	47.44	2.23	11.03	8.11	13.31	1.19	1975	36	6
22.0-23.0	11.92	48.27	2.13	2.76	7.00	17.02	1.31	1974	37	6
24.0-25.0	13.20	50.09	1.40	0.60	5.39	19.13	0.93	1973	38	6
26.0-25.0	14.49	48.57	2.27	11.49	8.79	21.13	1.57	1972	39	5
28.0-29.0	15.79	47.93	2.12	25.64	8.90	20.72	1.39	1966	45	6
30.0-31.0	17.14	54.41	2.00	-6.06	7.12	19.59	1.24	1965	46	6
32.0-33.0	18.49	50.17	1.53	-2.74	5.35	21.66	1.00	1964	47	5
34.0-35.0	19.92	48.86	1.41	5.09	5.56	21.74	0.95	1963	48	5
38.0-39.0	22.74	48.94	2.22	13.83	8.84	17.92	1.48	1956	55	8
40.0-41.0	24.14	48.68	1.75	2.63	6.69	10.69	1.01			
44.0-45.0	26.84	46.22	1.41	-1.56	5.15	3.22	0.61			
56.0-57.0	35.21	45.80	1.25	1.95	4.81	0.00	0.00			

Appendix-B Table 5. Radiometric analysis (²¹⁰Pb and ¹³⁷Cs) results of Lake Uluabat littoral core

Donth	Dry Mass	210Ph Supported		²¹⁰ PI	²¹⁰ Pb		137		Chronology			
Depth	Dry Mass	210Pb Supp	ortea	Unsuppo	Unsupported					je		
(cm)	(g cm-2)	(Bq Kg⁻¹)	±	(Bq Kg⁻¹)	±	(Bq Kg⁻¹)	±	(AD)	(yr)	±		
								2011	0			
0.0-0.5	0.05	49.42	3.93	27.74	14.00	8.14	2.40	2011	0	2		
4.5-5.0	1.17	48.41	3.69	40.97	11.78	9.44	2.15	2006	5	2		
7.0-7.5	2.36	47.55	2.52	43.84	9.01	7.85	1.45	1997	14	3		
11.5-12.0	4.33	48.06	2.35	12.28	7.89	12.21	1.44	1986	25	5		
15.0-15.5	6.20	51.12	1.44	10.98	4.71	18.50	1.02	1980	31	7		
19.5-20.0	8.69	48.77	2.30	8.95	7.60	14.65	1.50	1971	40	8		
22.0-23.0	10.22	45.97	1.55	10.10	5.18	22.07	1.15	1964	47	10		
24.0-25.0	11.35	44.59	1.36	3.99	4.85	22.20	1.01	1961	50	12		
28.0-29.0	13.75	47.14	1.39	6.68	4.67	14.19	0.86	1951	60	18		
30.0-31.0	15.03	44.89	1.17	9.37	4.00	15.29	0.78	1942	69	26		
34.0-35.0	17.53	43.24	1.27	9.18	4.58	2.38	0.66					
36.0-37.0	18.75	48.12	2.33	13.09	8.11	0.00	0.00					
40.0-41.0	21.12	41.34	2.32	17.62	8.53	0.00	0.00					
44.0-45.0	23.48	41.25	1.50	-3.10	5.06	0.00	0.00					

Appendix-B Table 6. Radiometric analysis (²¹⁰Pb and ¹³⁷Cs) results of Lake Uluabat pelagic core



Appendix-B Figure 1 Lake Marmara; fallout radionuclide concentrations, showing, A) and D) total ²¹⁰Pb activity, B) and E) unsupported ²¹⁰Pb, and C) and F) ¹³⁷Cs concentrations versus depth. The upper graphs indicate the littoral core, and the lower graphs pelagic core.



Appendix-B Figure 2 Lake Beyşehir; fallout radionuclide concentrations, showing, A) and D) total ²¹⁰Pb activity, B) and E) unsupported ²¹⁰Pb, and C) and F) ¹³⁷Cs concentrations versus depth. The upper graphs indicate the littoral core, and the lower graphs pelagic core.



Appendix-B Figure 3 Lake Uluabat; fallout radionuclide concentrations, showing, A) and D) total ²¹⁰Pb activity, B) and E) unsupported ²¹⁰Pb, and C) and F) ¹³⁷Cs concentrations versus depth. The upper graphs indicate the littoral core, and the lower graphs pelagic core.



Appendix-B Figure 4 Radiometric chronology of cores A) and B) Lake Marmara, C) and D) Lake Beyşehir, E) and F) Lake Uluabat; showing the CRS model ²¹⁰Pb dates and sedimentation rates. Dating results for littoral cores are given on the left panel and for the pelagic ones on the right panel. The solid line shows age (with error bars), while the dashed line indicates sedimentation rate. Age (yr) '0' corresponds to the year 2011.



Appendix-B Figure 5 Comparison of the Water Level Data of the Study Sites. Vertical dotted lines indicate the mean of the instrumental water level measurements. Please note the same scales.

APPENDIX C

This appendix is prepared from Stelzer *et al.* (2005); Portielje *et al.* (2014); WFD-UKTAG (2014); Ciecierska & Kolada (2014) for *CHAPTER 5*.

INDEX-1: Uktag Lake Assessment Method, Macrophytes (LEAFPACS) -United Kingdom

1. Calculation of Observed Values

a. Lake Macrophyte Nutrient Index (LMNI)

Observed value of LMNI =
$$\frac{\sum_{J=1}^{n} LMNI_{J}}{N}$$

LMNI_j : Lake Macrophyte Nutrient Index score for taxon "j" N : total number of macrophyte taxa

b. Observed number of functional groups (NFG)

NFG = Functional group number

- Lake macrophyte taxa recorded in the survey are assigned to a functional group (numbered 1-18).
- c. Observed number of macrophyte taxa (NTAXA)

NTAXA = Number of species recorded

d. Observed mean percent cover of hydrophytes (COV)

Observed value of COV =
$$\frac{\sum_{j=1}^{n} \% \text{COV}_{j}}{N}$$

%COV_j: percentage cover of hydrophyte taxon "j" in the lake;

N : total number of macrophyte taxa

e. Observed relative percent cover of filamentous algae (ALG)

Observed value of ALG =
$$\frac{\sum_{k=1}^{n} \% F_k}{\sum_{j=1}^{n} \% COV_j}$$

%COV_j: percentage cover of hydrophyte taxon "j" in the lake;
%F_k : percentage cover of filamentous algal taxon "k" in the lake

2. Expected (reference) metric value calculation

For this study the calculations for "England, Scotland and Wales" were used.

a. Expected Lake Macrophyte Nutrient Index (LMNI)

• Morpho-edaphic index (MEI):

It is a relationship between lake alkalinity and mean depth, which helps providing an indication of the natural nutrient availability in a lake

$$MEI = \log_{10}\left(\frac{alk + 40}{1000}\right) \div D$$

alk: annual mean alkalinity, µeqL⁻¹

D : mean depth, m

40: fixed number added to the alkalinity value to ensure negative alkalinity values are never used

• Weighted Freshwater Sensitivity Class (wFWSC):

Freshwater Sensitivity Class (FWSC) is employed to determine the capability of geology and soils for neutralizing the acidity entrance to the lakes. For the current study in order to define weighted freshwater sensitivity class (well or poorly buffered, soft of hard calcareous geology), major soil and dominant parent material (Aksoy *et al.* 2010) maps were used.

If FWSC data is unavailable;

- FWSC = 4.0, for lakes on predominantly soft, calcareous geology
- FWSC < 4.0, for lakes on hard geologies
- Calculation of expected LMNI:

wFSC	Expected LMNI
wFSC ≥ 4.0	$= 4.969 + 1.272 \times MEI + 0.193 \times MEI^2$
wFSC < 4.0	$= 4.969 + 1.272 \times MEI + 0.193 \times MEI^2 - 0.55$

b. Expected number of functional groups (NFG)

wFSC	Expected NFG
≥ 4.0	$= \text{EXP}(0.703 - (0.049\log_{10}H) + (0.133\log_{10}S) + (0.287\log_{10}(Alk + 40)) + 0.132)$
< 4.0	$= \text{EXP}(0.703 - (0.049\log_{10}H) + (0.133\log_{10}S) + (0.287\log_{10}(Alk + 40)) + 0.132 + 0.356)$

Alk: annual mean alkalinity, µeqL⁻¹

40: fixed number added to the alkalinity value to ensure negative alkalinity values are never used

H : Lake altitude, m

S : Lake surface area, hectares

c. Expected number of macrophyte taxa (NTAXA)

wFSC	Expected NTAXA
≥ 4.0	$= \text{EXP}(1.488 - (0.098\log_{10}H) + (0.185\log_{10}S) + (0.194\log_{10}(Alk + 40)) + 0.149)$
< 4.0	$= \text{EXP}(1.488 - (0.098\log_{10}H) + (0.185\log_{10}S) + (0.194\log_{10}(Alk + 40)) + 0.149 + 0.287)$

Alk: annual mean alkalinity, $\mu eq L^{-1}$

 $40\;$: fixed number added to the alkalinity value to ensure negative alkalinity values are never used

- **H** : Lake altitude, m
- **S** : Lake surface area, hectares
- d. Expected mean percent cover of hydrophytes (COV)

An expected COV value of 8.2% should be used.

e. Expected relative percent cover of filamentous algae (ALG)

An expected ALG value of 0.05 should be used.

3. Calculating EQR for each metric

a. Lake Macrophyte Nutrient Index (LMNI) EQR

Expected LMNI	EQRLMNI
≥ 5	$=\frac{observed \ LMNI - 10}{expected \ LMNI - 10}$
< 5	$= \frac{observed \ LMNI - (expected \ LMNI + 5)}{expected \ LMNI - (expected \ LMNI + 5)}$

b. Number of functional groups (NFG) EQR

 EQR_{NFG} = observed NFG ÷ expected NFG

c. Number of macrophyte taxa (NTAXA) EQR

 EQR_{NTAXA} = observed NTAXA ÷ expected NTAXA

d. Mean percent cover of hydrophytes (COV) EQR

 $EQR_{COV} = \sqrt{\text{observed COV}} \div \sqrt{\text{expected COV}}$

e. <u>Relative percent cover of filamentous algae (ALG)</u>

observed ALG > 0.05	$EQR_{ALG} = \frac{Observed \ ALG - 1}{0.05 - 1}$
observed ALG ≤ 0.05	$EQR_{ALG} = 1$

4. Combining the ecological quality ratios to determine the overall EQR (EQR_{LEAFPACS})

<u>Step1:</u>

	Diversity adjusted EQR _{LMNI} (^A EQR _{LMNI})
If, the smaller of EQR_{NFG} and EQR_{NTAXA} is $< EQR_{\text{LMNI}}$	$= EQR_{LMNI} + (a \times 0.5)) \div 1.5$
Otherwise	$= EQR_{LMNI}$

Step2:

This step measures ${}^{A}EQR_{LMNI}$, against the EQRs relating to plant cover, EQR_{COV} and EQR_{ALG}, enabling the derivation of the final overall LEAFPACS EQR (EQR_{LEAFPACS}).

	LEAFPACs EQR (EQR _{LEAFPACS})
If, the smaller of EQR _{COV} and EQR _{ALG} is $< ^{A}EQR_{LMNI}$	$= ({}^{A}EQR_{LMNI} + (b \times 0.25)) \div 1.25$
Otherwise	$= AEQR_{LMNI}$

<u>Step3:</u>

The standardized (to 0-1 scale) ecological quality ratio (SEQRLEAFPACS) should be calculated for as follows;

EQRLEAFPACS	SEQRLEAFPACS
> 1.05	= 1
$\leq 1.05 and \geq 0.80$	$=\frac{EQR_{LEAFPACS} - 0.80}{1.05 - 0.80} \times 0.20 + 0.80$
$< 0.80 \ and \ge 0.66$	$=\frac{EQR_{LEAFPACS} - 0.66}{0.80 - 0.66} \times 0.20 + 0.60$
$< 0.66 and \ge 0.51$	$=\frac{EQR_{LEAFPACS} - 0.51}{0.66 - 0.51} \times 0.20 + 0.40$
$< 0.51 and \ge 0.35$	$=\frac{EQR_{LEAFPACS} - 0.35}{0.51 - 0.35} \times 0.20 + 0.20$
$< 0.35 and \ge 0.20$	$=\frac{EQR_{LEAFPACS} - 0.20}{0.35 - 0.20} \times 0.20$
< 0.20	= 0

<u>INDEX-2</u>: Reference Index (RI) and Module Macrophyte Assessment (M_{MP})-Germany

Each macrophyte species found in the lakes is assigned to a type specific group, A (reference taxa), B (indifferent taxa) or C (disturbance indicator taxa). Originally, RI calculation comprise dividing the lakes into 4 depth classes (0-1, 1-2, 2-4, >4 m), thus macrophytes are recorded and assigned to type-specific groups accordingly (Stelzer *et al.* 2005; Portielje *et al.* 2014). However, for the current study the calculation of this index is conducted according to the German-intercalibration method, which did not employ different depth classes and which proved to show good correlation with trophic state variables (Portielje *et al.* 2014). Therefore, for intercalibration method different groups for each depth class were reduced to only one group per taxon, by chosing the most common group or if their numbers were even by chosing group B (see Appendix-B Table 1 for an example).

Appendix-C Table 1. An example of assigning macrophytes to type-specific groups for *Ceratophyllum demersum* L. (Portielje *et al.* 2014). This species is assigned to group "B" for both lake types (LCB-1 and LCB-2).

Depth Classes	Species Group			
(m)	LCB-1	LCB-2		
0-1	С	С		
1-2	С	В		
2-4	В	В		
> 4	В	В		

The abundance of each species were determined in 5-level scale and their quantities (Q) are calculated as " \mathbf{Q} = abundance³".

$$RI = \frac{\sum_{i=1}^{n_a} (Q_{Ai}) - \sum_{i=1}^{n_c} (Q_{Ci})}{\sum_{i=1}^{n_g} (Q_{gi})} \times 100$$

RI : Reference Index

 Q_{Ai} : Quantity of the i-th taxon of species group A

 \mathbf{Q}_{Ci} : Quantity of the i-th taxon of species group C

 Q_{gi} : Quantity of the i-th taxon of all groups

 $\mathbf{n}_{\mathbf{A}}$: Total number of taxa in group A

 $\boldsymbol{n}_C~$: Total number of taxa in group C

 \mathbf{n}_{g} : Total number of taxa in all groups

After the calculation of Reference Index, the resultant values were corrected according to vegetation limit and stands of dominant taxa (Appendix-B Table 2).

Lake Type	Correcting factors
	• if RI > 0 and vegetation limit > 5 m and < 8 m → RI is reduced by 10
	• if RI > 0 and vegetation limit > 2,5 m and < 5 m → RI is reduced by 20
(German Lake Type	• if vegetation limit is < 2,5 m \rightarrow RI is reduced by 50
TKg13)	• if RI > -50 and dominant stands of one of the following taxa occur, RI is reduced by 50:
	Ceratophyllum demersum, C. submersum, Elodea canadensis/nuttallii, Myriophyllum spicatum, Najas marina subsp. intermedia or Potamogeton pectinatus
LCB-2	• if RI > 0 and vegetation limit between 2,5 m and 4 m → RI is reduced by 10, in case of a maximum depth >= 4 m
(German Lake Type TKp)	• if vegetation limit ist < 2,5 m → RI is reduced by 50, in case of a maximum depth >= 2,5 m

Appendix-C Table 2 Correcting factors defined according to lake typologies

• if $RI > -50$ and dominant stands of one of the following taxa
occur, RI is reduced by 50:
C. demersum, C. submersum, E. canadensis/nuttallii, M.
spicatum, N. marina subsp. intermedia or P. pectinatus

• Transformation of the module Reference index to a scale from 0 to 1.

$$M_{MP} = \frac{(RI_{seen} + 100) \times 0.5}{100}$$

 M_{MP} : Module Macrophyte Assessment

 $RI_{Seen/Lakes}{\hfill}$ type specifically calculated Reference Index Seen/Lakes

INDEX-3: Danish Lake Macrophyte Index (DLMI)-Denmark

Appendix-C Table 3 Calculation of points in deep lakes (LCB-1) used for calculating the total score and macrophyte-EQR

For Deep Lakes (LCB-1) (mean depth > 3 m)									
	Number of indicator species required to obtain 1-4 points (p)								
4 p			3 p		2 p		1 p		
> 100 ha: ≥ 4 10 - 100 ha: ≥ 3 < 10 ha: ≥ 3		> 100 ha: 3 10 - 100 ha: 2 < 10 ha: 2		> 100 ha: 2 10 – 100 ha: 1 < 10 ha: 1		> 100 ha: 1 10 – 100 ha: 1 < 10 ha: 0			
Max depth colonization (m) required to obtain 1-9 points									
9 p	8 p	7 p	6 p	5 p	4 p	3 p	2 p	1 p	0 p
> 7 m	5–7 m	4-5 m	3-4 m	2.5-3 m	2-2.5 m	1.5 - 2 m	1-1.5 m	0-1 m	0*

* no submerged macrophytes found

Appendix-C Table 4 Calculation of points in deep lakes (LCB-2) used for calculating the total score and macrophyte-EQR
For Shallow Lakes (LCB-2) (mean depth \leq 3 m)									
Number of indicator species required to obtain 1-4 points (p)									
4 p		3 p		2 p		1 p			
> 100 ha: ≥ 4 10 - 100 ha: ≥ 3 < 10 ha: ≥ 3		> 100 ha: 3 10 - 100 ha: 2 < 10 ha: 2		> 100 ha: 2 10 – 100 ha: 1 < 10 ha: 1		> 100 ha: 1 10 – 100 ha: 1 < 10 ha: 0			
Coverage (% of total lake area) required to obtain 1-9 points									
9 p	8 p	7 p	6 p	5 p	4 p	3 p	2 p	1 p	0 p
> 40	30-40	15-30	7.5-15	3.5-7.5	2-3.5	1-2	0.5-1	0-0.5	0*

* no submerged macrophytes found

Appendix-C Table 5 Calculation of final EQR (based on macrophytes)

Score (points)	Ecological class	Macrophyte-EQR
0	Bad	0.07
1	Bad	0.14
2	Poor	0.23
3	Poor	0.30
4	Poor	0.37
5	Moderate	0.44
6	Moderate	0.50
7	Moderate	0.57
8	Good	0.64
9	Good	0.70
10	Good	0.77
11	High	0.84
12	High	0.90
13	High	0.97

INDEX-4: Ecological State Macrophyte Index (Esmi) - Poland

Pielou index of evenness (J) = $\frac{H}{H_{max}}$

Phytocenotic diversity index (H) = $\sum \frac{n_i}{N} \times \ln \frac{n_i}{N}$

Maximum phytopcenotic diversity index $(H_{max}) = lnS$

Colonization index (Z) = $\frac{N}{izob. 2.5}$

Ecological State Macrophyte Index (ESMI) =
$$1 - exp\left[-J \times Z \times exp\left(\frac{N}{P}\right)\right]$$

N : total area of phytolittoral in ha in ha or km²
n_i : % share of proporcion of the area occupied by particular plant communities in % of N
S : number of plant communities identified in phytolittoral
izob.2.5 : area where water depth is <2.5m
P : total lake area in ha or km²

CALCULATIONS FOR EQR NORMALIZATION

These calculations are conducted to be able to standardise class boundaries (obtain equal class widths), thus enabling the comparison of resultant classifications of different indices. These normalization equations (different for each class) is based on linear interpolation and calculated as below (Pall *et al.* 2014);

Class	EQR _{norm}
High	$= 1.0 - 0.2 \times (1 - EQR) \div (1 - EQR_{HG})$
Good	$= 0.8 - 0.2 \times (EQR_{HG} - EQR) \div (EQR_{HG} - EQR_{GM})$
Moderate	$= 0.6 - 0.2 \times (EQR_{GM} - EQR) \div (EQR_{GM} - EQR_{MP})$
Poor	$= 0.4 - 0.2 \times (EQR_{MP} - EQR) \div (EQR_{MP} - EQR_{PB})$
Bad	$= 0.2 - 0.2 \times (EQR_{PB} - EQR) \div (EQR_{PB} - EQR_{min})$

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PUBLICATIONS

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HOBBIES

Scuba Diving, Latin and Tango Dancing, Photography