ROLE OF NITROGEN IN SUBMERGED PLANT DEVELOPMENT IN MEDITERRANEAN CLIMATIC ZONE - A *MESOCOSM* EXPERIMENT

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

BY

KORHAN ÖZKAN

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
BIOLOGY

SEPTEMBER 2008

Approval of the thesis:

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

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ABSTRACT

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September 2008, 78 pages

The effects of increasing nitrogen and phosphorus loading on submerged macrophyte development was tested in a *mesocosm* experiment for three months. Experiment consisted of three NO_3-N loadings with factorial of two PO_4-P loadings in a four-fold replicated design. Twenty four enclosures placed at one meter depth were isolated from the lake but kept open to sediment and atmosphere. Each enclosure stocked with ten *Myriophyllum spicatum* shoots with underyearling fish to reduce zooplankton grazers.

Biweekly sampling and weekly nutrient additions were performed for three months. Mean total nitrogen (TN) concentrations sustained in nitrogen treatments through-

out the experiment were 0.52, 1.99, 8.07 mgl^{-1}. Both phosphorus treatments converged to a mean concentration below the targeted level, ranging between 0.05-0.1 mgl^{-1} TP. In comparison to *mesocosm* studies in temperate lakes, higher assimilation rates for nutrients were observed in Lake Pedina. Due to extraordinarily high evapotranspiration and drought in 2007, the water level decreased 0.6 m in enclosures.

Total macrophyte biomass remained indifferent to nutrient treatments with continuous growth and failed to validate any direct or indirect negative effect of increasing nutrient concentrations. Phytoplankton biomass differed significantly among factorial treatments but remained low, while periphyton biomass differed among nitrogen treatments. In comparison with other studies the phytoplankton biomass remained low and the periphyton biomass became high for reference TP concentrations, indicating a competitive advantage of periphyton over phytoplankton on nutrient utilization in the enclosures. Zooplankton:phytoplankton biomass ratio was low throughout the experiment and zooplankton community mainly consists of smaller species, reflecting high predation pressure.

Keywords: Phosphorus, Periphyton, Phytoplankton, *Myriophyllum spicatum*, Lake Pedina

ÖZ

AZOTUN AKDENİZ İKLİM BÖLGESİNDE SUİÇİ BİTKİ GELİŞİMİ ÜZERİNDEKİ ROLÜ - BİR *MEZOKOZM* DENEYİ

Özkan, Korhan

Yüksek Lisans, Biyoloji

Tez Yöneticisi : Prof. Dr. Meryem Beklioğlu Yerli

Ortak Tez Yöneticisi : Prof. Dr. Erik Jeppesen

Eylül 2008, 78 sayfa

Bu çalışmada artan azot ve fosfor miktarının suiçi bitki gelişimine olan etkisini belirlemek amacıyla üç ay süren bir *mezokozm* deneyi yapıldı. Deneyde üç NO_3-N dozu, iki PO_4-P dozu ile çaprazlanarak dört tekrarlı olarak uygulandı. Toplamda 24 *mezokozm* bir metre derinliğe, gölden yalıtılmış, sediman ve atmosfer ilişkisine açık olacak şekilde kuruldu. Her *mezokozma* on kök *Myriophyllum spicatum* bitkisi ve herbivor zooplankton popülasyonunu azaltmak için ufak boy balık yerleştirildi. Çalışma boyunca iki haftalık ve haftalık periyodlarla örnekleme ve besin eklemesi yapıldı. Uygulanan üç azot eklemesi ile deney boyunca 0.52, 1.99 ve 8.07 mgl^{-1} ortalama toplam azot derişimleri elde edilirken; uygulanan iki fosfor eklemesi

ise amaçlanan seviyeden düşük, 0.05-0.1 mgl^{-1} aralığında gerçekleşen derişimler sağladı. Sonuç olarak ılıman kuşak göllerinde yapılan diğer *mezokozm* çalışmaları ile kıyaslandığında Pedina Gölünde daha yüksek bir besin özümsemesi gözlenmiştir. 2007 yazında gözlenen yüksek buharlaşma ve kuraklık sebebiyle 0.6 m su seviyesi düşüşü kaydedildi.

Çalışma sonunda toplam bitki biyokütlesi monoton bir şekilde artmış, artan besin yoğunluğunun bitki büyümesi üzerinde tespit edilebilir bir etkisi gözlenmemiştir. Düşük seviyelerde kalan fitoplankton biyokütlesi çapraz dozlar, perifiton biyokütlesi ise azot dozları için belirgin bir farklılaşma göstermiştir. Başka çalışmalardaki benzer toplam fosfor derişimleri ile kıyaslandığında fitoplankton düşük, perifiton ise yüksek gelişim sağlamıştır. Bu durum perifitonun *mezokozm* su kolonundaki besini daha verimli kullanarak fitoplanktona baskı uyguladığına işaret etmektedir. Zooplankton:fitoplankton biyokütle oranı yüksek avlanma baskısını yansıtacak şekilde düşük gerçekleşmiş ve zooplankton topluluğu temelde ufak boyutlu türlerden oluşmuştur.

Anahtar Kelimeler: Fosfor, Perifiton, Fitoplankton, *Myriophyllum spicatum*, Pedina Gölü

ACKNOWLEDGMENTS

I would like to express my gratitude to *Meryem Beklioğlu* and *Erik Jeppesen* not only for their supervision but also for their selfless help from field to manuscript of this text. I owe much for their kind help to make sense out of confusion.

I am most thankful to my parents *Cengiz* and *Nadide* and my whole family, always offering support and love. I am gratefull that *Keziban* is always there with me to share the happiest and to endure the hardest times.

Moreover, I would like to thank;

For invaluable help in construction of the mesocosms and samplings: Mücahit Kurt and his family, Keziban Kaynar, Mukadder Arslan and Eti Levi.

For great times spent in the lab and the field: Onur Kerimoğlu, İdil Çakıroğlu, Arda Özen, Banu Akkaş, Özge Karabulut, Gizem Bezirci, Eti Levi and Nihan Yazgan.

For sympathetic cooperation and generous help in the field: Mustafa İşçioğlu, Ergün Yavaş, Ziya Babatlı, Volkan Pek and Mert Altuntaş.

For guidance on zooplankton identification: Teresa Buchaca, Liselotte Sander Johansson and Karina Jensen.

For assistance on Figure 2.1: Mert Ayaroğlu.

I would also like to thank to ananomous contributors for two marvellous open source projects, R and LaTeX. They make the process much less painful.

This project was funded by The Scientific and Technological Research Council of Turkey (project no: 105Y332), Middle East Technical University Office of Scientific Research Projects Coordination (project no: BAP-2006-07-02-00-01) and Ministry of Environment and Forestry.

To my beloved Keziban

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INTRODUCTION

1.1 Shallow Lakes and Role of Macrophytes

Only 0.009 % of earth's water is found in freshwater lakes; however, this tiny fraction is home of a rich biodiverstity and essential for terrestrial life (Wetzel, 1975). Lakes with rivers and wetlands are estimated to constitude over 25% of the total services that nature provides for human societies and survival (Costanza et al., 1997). Human civilizations has long being developed around the wetlands and lakes throughout history (Moss, 1998) and resulted in pronounced changes in ecological states of lakes, particularly after the industrial revolution. Antropogenic sources of nutrients, in particular sewage from human settlements and nutrient leakage from agricultural fields, caused severe eutrophication and catastrophic shifts in lake ecosystems world-wide.

Most of the world's lakes are shallow (Moss, 1998), their littoral communities are dominated by macrophyte and associated algae constitute the majority of primary production, especially in pristine clear water conditions (Vadeboncoeur et al., 2003; Liboriussen and Jeppesen, 2003). In contrast to shallow lakes, phytoplankton dominates the primary production in stratified deep lakes. The benthic-pelagic coupling is stronger in shallow lakes than deep lakes, resulting in a stronger sediment influence on nutrient turnover and trophic dynamics (Jeppesen, 1998). Futhermore, fish biomass and and production per unit area at a given nutrient availability is found to be independent of depth after analysis of various lakes (Hanson and Leggett,

1982; Downing et al., 1990). Thus productivity, fish biomass and most probably predation pressure on zooplankton per unit volume is higher in shallow lakes.

Shallow lakes can be classified into two different ecological states defined by the competition among primary producers along a nutrient gradient. Either macrophyte dominates the primary production with clear water conditions or phytoplankton dominates the primary production with turbid water conditions. It was initially assumed that increasing nutrient supply linearly stimulates phytoplankton and epiphyton growth that detoriates the light climate for macrophytes and gradually diminish their abundance (Phillips et al., 1978).

However, this definition of self-perturbating gradual process contradict with observations and a new hypothesis was formed as *alternative equilibria* in shallow lake research (Jeppesen et al., 1990; Moss, 1990; Scheffer, 1990). In short, alternative stable states theory accounts for both macrophyte dominated clear water and phtoplankton dominated turbid water states may occur at intermediate nutrient levels and the prevalence of one over the other is based on stochastic events mediated by some buffer mechanisms (Scheffer et al., 1993, 2001).

According to this theory, oligotropic lakes are dominated by macrophytes and piscivorous fish has a strong control over planktivorous fish in general. Increasing nutrient supply would initiate a productivity increase in the lake but grazers (zooplankton and snails for example) free from predation pressure are able to control phytoplankton and epiphyton, while preventing any substantial change in the ecological state. However, subsequent increase in nutrient supply would eventually reach a threshold, after which a catastrophic shift from clearwater to turbid state occurs. Turbid state is characterized by dominance of phytoplankton with none or a few macrophytes and abundant planktivorous fish community. High predation pressure on zooplankton due to abundant planktivorous fish and some other buffer mechanisms, prevent turbid state to shift back to clearwater state with the same threshold level at which the shift from clearwater to turbid state occured. Unless there is a major perturbation, turbid state shift back to clearwater state at a lower nutrient level, provided that there is a reduction in nutrient supply

(Figure 1.1). Observations on northern temperate lakes indicate that those shifts between macrophyte dominated clear water and phytoplankton dominated turbid conditions occur within an aproximate range of 0.025-0.100 mgl^{-1} TP (Jeppesen et al., 1990; Hosper and Jagtman, 1990).

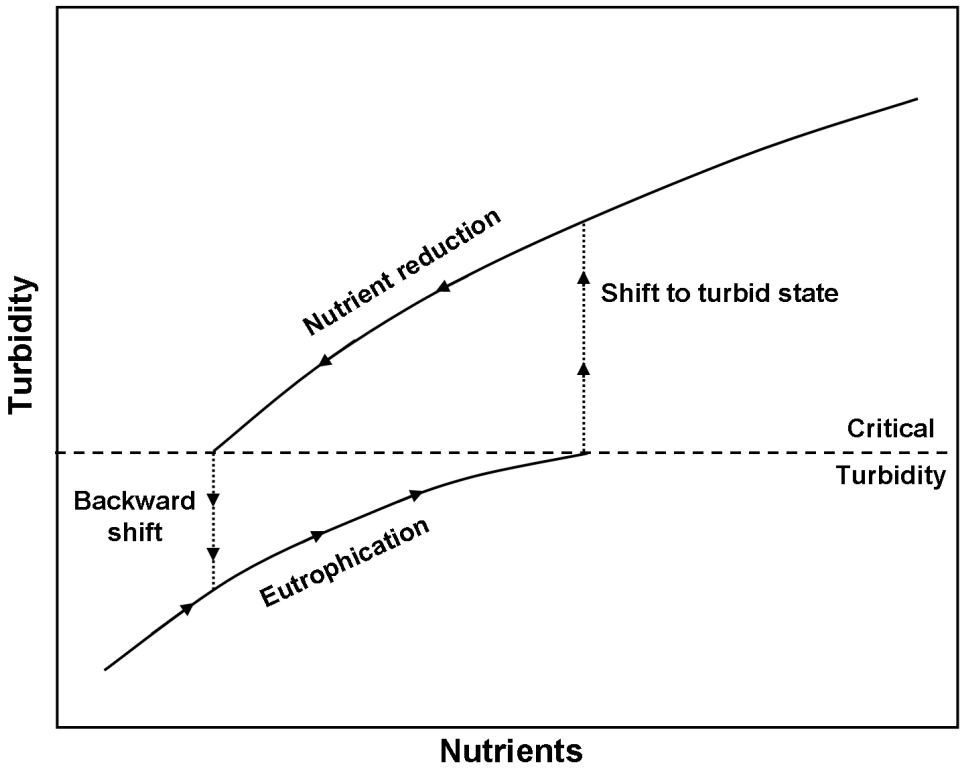

Figure 1.1: Conceptual model of the alternative stable states for shallow lakes. Critical turbidity is the treshold for the loss of macrophytes; clearwater conditions prevail below and turbid conditions prevail above that line. Arrows indicate the shifts first between clearwater to turbid state due to eutrophication and then turbid to clearwater state due to nutrient reduction. The buffer mechanisms at the turbid state prevent the lake from shifting back to clearwater condition at the same nutrient concentration that clearwater condition shifted to turbid state. A lower level of nutrient concentration should be achieved before to initiate a shift to clearwater conditions again. Figure was taken from Scheffer (1998).

Macrophytes are central to alternative stable states theory and of great importance for maintaining preferable ecological conditions by enhancing the resilience of clearwater state (Figure 1.2). Macrophyte growth responding to nutrient addition integrates some portion of the nutrients into biomass and decrease the available

nutrients for phytoplankton (Sand-Jensen and Borum, 1991). They reduce sediment resuspension (Barko and James, 1998; James and W., 1990) and thus prevent nutrient release from lake sediment (Søndergaard et al., 1992). Macrophytes may enhance nitrogen (N) loss by denitrification occured in the opiphyte layer on macrophyte surfaces (Weisner et al., 1994). Furthermore, low oxygen concentrations among macrophyte beds, especiall through night may enhance denitrification (Frodge et al., 1990). Macrophytes can shade out phytoplankton and locally diminish their population (Pokorny et al., 1984; Wetzel, 1975). Lastly, they may inhibit phytoplankton growth by secreting chemicals, called alleopathy (Van Donk and Van de Bund, 2002). All those interactions act as buffer mechanisms to enhance resilience capacity of clearwater conditions and prevent phytoplankton from proliferating.

In addition to the direct mechanisms counted above, there are several indirect effects that macrophytes contribute to the resilience capacity of clearwater state. Studies performed in temperate lakes showed that lakes containing abundant macrophytes are more transparent on average in comparison to the expected water clarity from the phosphorus (P) level (Jeppesen, 1998; Canfield et al., 1984). One of the key roles of macrophytes is that they provide refuge for grazing zooplankton (Timms and Moss, 1984; Burks et al., 2002; Lauridsen and Buenk, 1996) as well as periphyton grazers (Brönmark and Vermaat, 1998) against planktivorous fish predation, which in turn suppress phytoplankton abundance and enhance clearwater conditions. The capacity of macrophytes acting as a refuge to zooplankton grazers varies with macrophyte density and bed size (Lauridsen and Buenk, 1996; Burks et al., 2001; Schriver et al., 1995) or plant structure (Nurminen and Horppila, 2002). Trophic level of the lake (Lauridsen et al., 1999; Jeppesen et al., 1997b) and fish comunity structure (Jeppesen et al., 1997a) also effect the extend that macrophytes act as a refuge for grazers. Lastly, macrophytes favor piscivorous fish at the expense of planktivorous fish and reduce the predation pressure on zooplankton (Jeppesen, 1998).

The influence of macrophytes seems to disappear in lower lattitudes through the (sub)tropics. A survey of 319 shallow and polymictic subtropical Florida lakes revealed no direct relationship between macrophyte abundance and water trans-

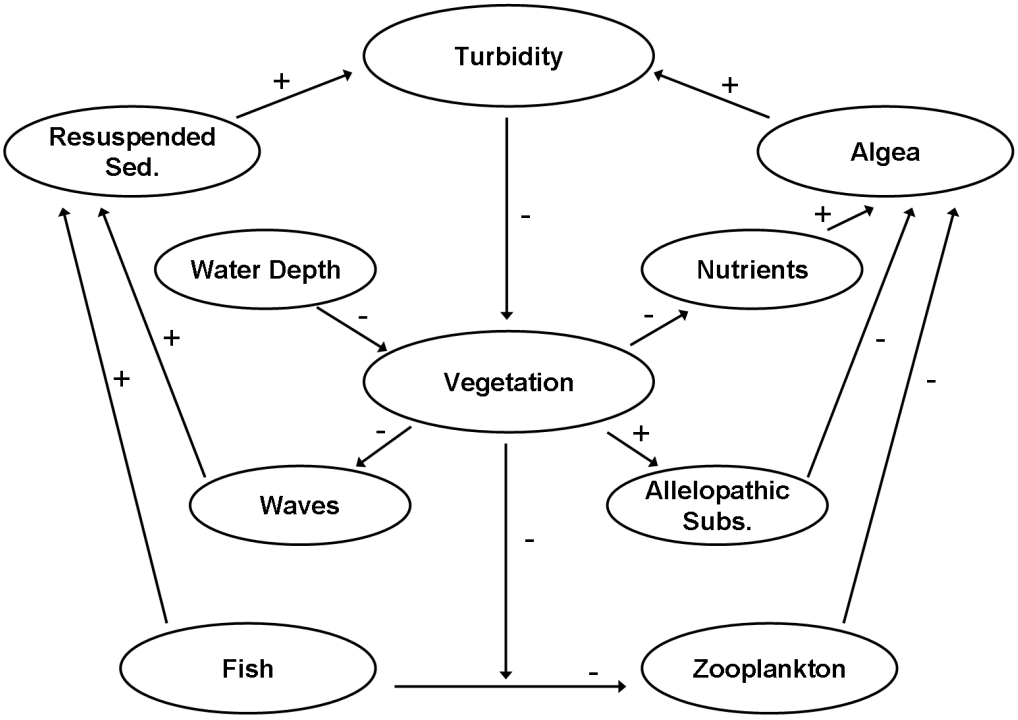

Figure 1.2: Conceptual model summarizing the effects of macrophytes in shallow lakes. Qualitative effects between components indicated by positive or negative signs on the arrows. Figure was taken from Scheffer et al. (1993).

parency (Bachmann et al., 2002). Aggregation of fish inside macrophyte beds in tropical and (sub)tropical shallow lakes impede the role of macrophytes as refuges for zooplankton and positive effect of macrophytes on water clarity (Meerhoff et al., 2003). Laboratory experiments conducted with (sub)tropical species indicate that *Daphnia* did not take refuge among the plants but rather swam away when exposed simultaneously to plants and alarm signals of fish and conspecific (Meerhoff et al., 2006b). Meerhoff et al. (2006b) concluded that macrophytes in the (sub)tropics may not initiate cascading effects via large-bodied grazers on phytoplankton as seen in temperate lakes.

Mediterranean lakes are characterized in between (sub)tropic and temperate lakes. Ecological functioning of Mediterranean lakes differs from northern temperate lakes by exhibiting extreme seasonality with rainy winters and hot, arid summers (Álvarez Cobelas et al., 2005). Water level fluctuations is an important component

of Mediterranean shallow lake hydrology (Coops et al., 2003; Beklioglu et al., 2007). The fish community is mainly dominated by omnivorous and benthivorous fish with frequent spawning (Blanco et al., 2003; Beklioglu et al., 2007). Thus the predation pressure is high on zooplanton and zooplankton community is composed of mainly small-sized members (Beklioglu et al., 2003; Romo et al., 2005).

High temperatures and water level drop in growing season are frequently observed in Mediterranean lakes and may have significant consequences. High temperatures through growing season may result in higher rate of denitrification (Talling and Lamolle, 1998) and mitigate the negative effects of nitrogen loading on macrophytes. Longer hydrologic residence time due to low water input and higher sediment interaction of water column may result in more intense internal loading in Mediterranean lakes (Romo et al., 2005). Some studies reported that nutrient concentrations in Mediterranean lakes were of greater importance than temperate lakes for submerged macrophyte development (Karapinar, 2005; Romo et al., 2004), whereas some studies reported higher resilience of macrophytes to increasing nutrient loading (Bécares et al., 2007). Lastly, low water level throuhout growing season may also result in better light conditions at lake bottom for macrophyte development (Beklioglu et al., 2006).

1.2 Factors Affecting Submerged Macrophyte Growth

Nutrient availability may increase but generally do not strictly limit macrophyte growth as rooted submerged macrophytes have access to nutrients in sediment (Moss, 1998). However, relative depletion of nitrogen in the sediment due to effective denitrification may favor nitrogen limitation on macrophyte growth (Hameed et al., 1999; Vitousek and Howarth, 1991; Moss, 1998). On the other hand, nutrient concentrations have pronounced indirect effects on macrophytes. High nutrient concentrations initiate phytoplankton and periphyton proliferation, which in turn detoriate underwater light climate and suppress macrophyte growth by shading (Jeppesen, 1998).

Water level fluctuation in conjuction with lake morphometry was found to be deterministic for macrophyte development in Mediterranean lakes (Beklioglu et al., 2006, 2007). Low water level in growing season may increase the littoral area that receive adequate light for macrophyte growth (Blindow, 1992). However, extreme low water level in winter may expose littoral zone to freezing which may destroy the regenerative capacity of macrophytes for the coming spring (Cooke et al., 1993).

Both community structure and abundance of fish can directly or indirectly affect macrophytes (Crivelli, 1983; Parkos et al., 2003; Breukelaar et al., 1994). Benthivorous fish such as carp can graze on macrophytes or cause up-rooting (Crivelli, 1983). Benthivorous fish also cause sediment resuspension through feeding activity. Thus in turn facilitate nutrient release from sediment and affect macrophytes indirectly by degraded underwater light environment due to phytoplankton growth (Breukelaar et al., 1994; Parkos et al., 2003).

Waterfowl act similarly to fish as abundance and composition of waterfowl has an important effect on macrophytes. Several waterfowl species feed partially or almost entirely on macrophyte species (Noordhuis et al., 2002) and apply a strong grazing pressure on macrophyte community.

1.3 Nitrogen Limitation in Ecosystems

Nitrogen is well known as an essential nutrient for life, producers need nitrogen in larger quatities than other nutrients and they need to invest more energy to obtain or use it (Gutschick, 1981). Atmosphere contains huge amounts of evenly distributed nitrogen that is available to a diverse community of symbiotic and non-symbiotic organisms being capable of fixing atmospheric nitrogen and found in every major ecosystem on earth. It would be trivial to assume any of such nitrogen fixing organism would gain enormous competitive advantage where there is nitrogen limitation over primary production and those organisms would in turn proliferate in great numbers and convert atmospheric nitrogen into more readily available forms. Consequently, available nitrogen produced by nitrogen fixers would alleviate nitrogen

limitation in the corresponding ecosystem. This reasoning constituded an important argument against possible nitrogen limitation, first in marine (Redfield, 1958), then in terrestrial (Walker and Syers, 1976) and freswater ecosystems (Schindler, 1977).

However, the accumulation of emprical results coming from observation on elemental ratios of various terresterial ecosystems and nitrogen fertilization experiments pointed out a wide-spread occurance of nitrogen limitation or co-limitation in terrestrial ecosystems (Vitousek and Howarth, 1991). Especially after the atmospheric nitrogen deposition became a major concern, several nitrogen enrichment studies performed in terrestrial ecosystems revealing a decline in species richness with moderately high-level nitrogen additions (Bobbink, 1991; Wedin and Tilman, 1996; Gough et al., 2000). Recent studies suggested that even chronic low-level nitrogen deposition performed over a decade may have a greater impact on diversity than previously thought, as this chronic low-level nitrogen addition reduced plant species numbers by 17% relative to controls (Clark and Tilman, 2008).

Similar to terrestrial ecosystems, evidences for wide-spread nitrogen limitation was also accumulated from marine studies constituded mainly short-term bioassays, elemental ratios of nutrients and lack of abundant cynobacteria and nitrogen fixation over the world's oceans (Vitousek and Howarth, 1991; Howarth, 1988).

The observations on terrestrial and aquatic environments indicate no certain negative feedback mechanism between nitrogen limitation and nitrogen fixation, in which nitrogen limitation results in dominance of nitrogen-fixing organisms, those in turn provide available nitrogen to the whole ecosystem and allaviate nitrogen limitation. The factors that may prevent nitrogen fixation from fully compensating for nitrogen limitation in various ecosystems were summarized in Vitousek and Howarth (1991) as:

- Energetic constraints on the activity of nitrogen-fixing organisms keep the nitrogen fixation rate low.
- Rate of nitrogen limitation is subject to limitation of other nutrients.
- Ecological and physical constraints prevent nitrogen-fixing organisms becoming

established or performing nitrogen fixation.

The nature of biogeochemical cycle of nitrogen compared to other nutrients in lakes, especially phosphorus provides more understanding on mechanisms of nitrogen limitation (Figure 1.3, Figure 1.4). The ultimate source of phosphorus is rock weathering, whereas atmosphere for nitrogen. Cycling of nitrogen and phosphorus through lake sediment differs and can favor either nitrogen or phosphorus limitation. Phosphorus, mineralized through decomposition can be readily released from the sediment and became available to the aquatic life with respect to resuspension (Søndergaard, 2007; Søndergaard et al., 1992), redox potantial (Mortimer, 1941) and pH (Andersen, 1975) observed in a lake. However, denitrification taking place in sediment or on macrophyte surface (Weisner et al., 1994) accounts for a major loss in mineralized nitrogen. Therefore, nitrogen is relatively depleted compared to phosphorus in sediment nutrient flux, which may account for a potential nitrogen limitation (Vitousek and Howarth, 1991).

Organic nitrogen is carbon-bonded and often in complex forms, while organic phosphorus is usually ester bonded and often soluble. Several organisms are able to secrete extracellular phophatases that cleve the esterphosphate bond and easily utilize resulting available phosphorus (Howarth, 1988). Contrastingly, an investment in complex multiple enzyme systems must be made to digest structural or phenolic nitrogen-containing organic compounds to convert nitrogen into available forms. This difference may make balancing requirement and nutrient supply more difficult for nitrogen than phosphorus; and nitrogen cycling can be hindered more than phosphorus cycling at lower rates of decomposition (Vitousek and Howarth, 1991; Jackson and Williams, 1985). It was also suggested that some herbivorous zooplankton regenerate phosphorus more effectively than nitrogen because relatively more phosphorus is excreted in soluble forms and relatively more nitrogen is retained in fecal pellets (Knauer et al., 1979; Lehman, 1984). Such additional mechanisms may also favor nitrogen over phosphorus limitation.

In freshwater ecosystems, different groups of phytoplankton can be limited by different nutrients. Silicon is the major limiting nutrient for diatoms in many

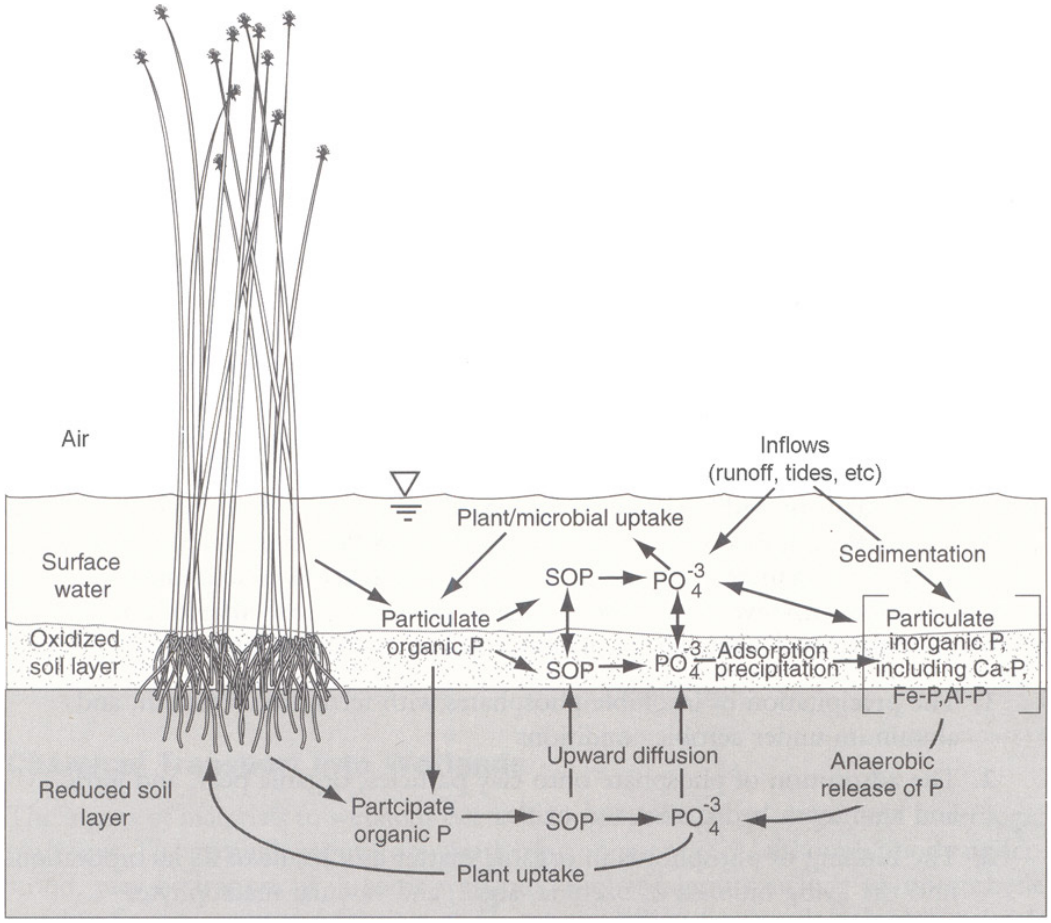

Figure 1.3: Phosphorus cycle in shallow lakes. SOP denotes for soluble organic phosphorus. Phosphorus mineralized through decomposition is readily released from the sediment. Figure is taken from Mitsch and Gosselink (2000).

freshwater lakes (Tilman et al., 1982) or nitrogen-fixing cyanobacteria blooms have been recorded if the nitrogen availability is limited (Tilman et al., 1982; Schindler, 1977). However, silicon and nitrogen proposed to have no effect on primary production as the limited group of agea in deficiency of a particular nutrient was supposed to be replaced by another group and several studies concluded that primary production in temperate-zone lakes is limited by phosphorus (Schindler, 1978, 1977; Schindler and Fee, 1974). Limitation or co-limitation of nitrogen especially on macrophyte development in lakes had been emphasized as well (Moss, 1990, 2001) but lacked the detailed quantification. However, recent studies concluded that nitrogen limitation or co-limitation with phosphorus is the norm on algal production of several lake ecosystems with varing phosphorus concentrations (Talling and

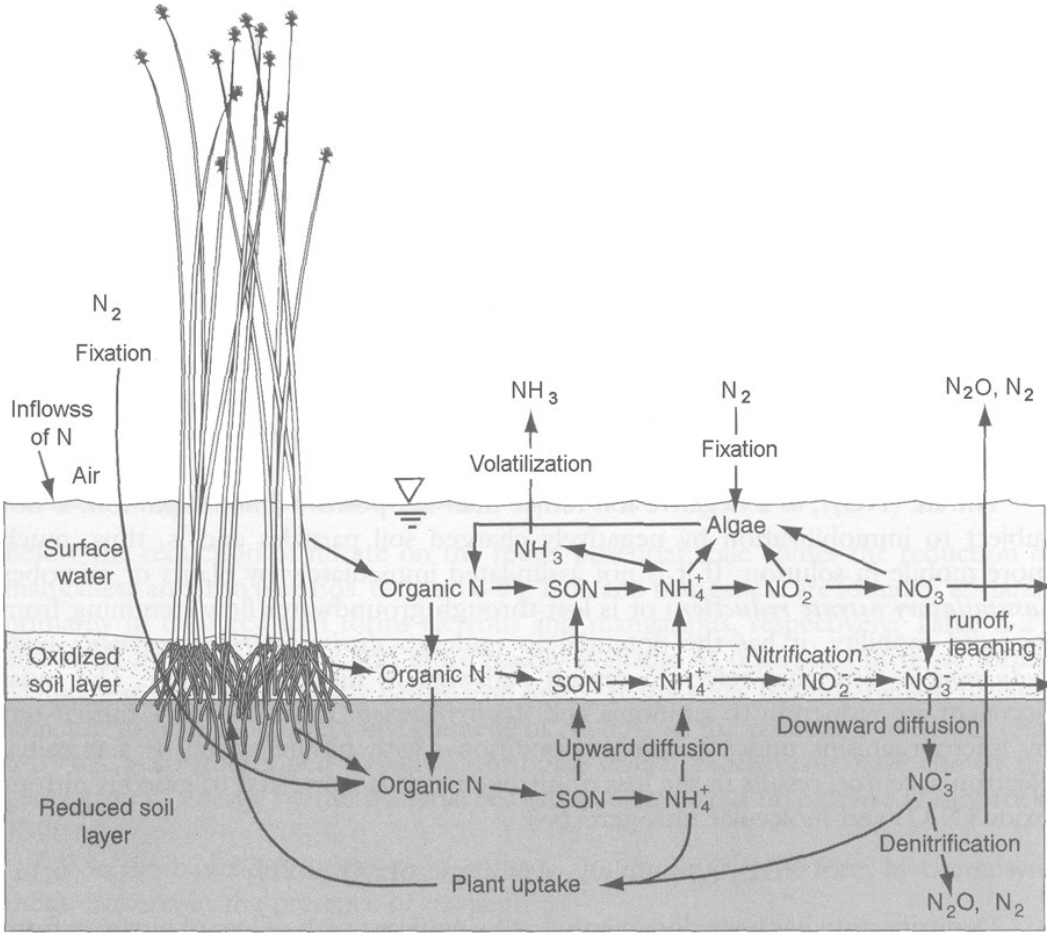

Figure 1.4: Nitrogen cycle in shallow lakes. SON denotes for soluble organic nitrogen. Denitrification is a major process occured in the sediment or on macrophyte surface removing available nitrogen mineralized through decomposition. Figure is taken from Mitsch and Gosselink (2000).

Lamolle, 1998; Hameed et al., 1999; Maberly et al., 2002; James et al., 2003; González Sagrario et al., 2005; Dzialowski et al., 2005; Lacoul and Freedman, 2005; McMaster and Schindler, 2005; Barker et al., 2008).

Barker et al. (2008) summarized the history of the ideas of nutrient limitation in limnology, starting with the fact that pioneer limnolojists regarded both nitrogen and phosphorus collectively in discussions of eutrophication problems (Hutchinson, 1969). Barker et al. (2008) also noted that the declerations of chemical industry scientists (Kuentzel, 1969) of late 1960s that claimed phosphorus was not responsible for increased algal growths in lakes, initiated a mass of research on phosphorus; which in turn proved that phosphorus, rather than carbon, was indeed frequently responsible

(Schindler, 1978, 1977; Schindler and Fee, 1974). The accumulated evidence for the phosphorus limitation resulted in underestimation of nitrogen limitation in lakes. Barker et al. (2008) concluded that recent reasearch on limitation of primary production in lakes reclaimed the neglected importance of nitrogen as a limiting nutrient.

Bergstrom and Jansson (2006) and Moss (1990) even proposed that nitrogen limitation or collective limitation of both phosphorus and nitrogen is the general pattern for lakes in pristine state and deposition of antropogenically produced nitrogen is responsible for phosphorus limitation in northern hemisphere lakes, in which algal production was naturally limited or co-limited by nitrogen.

A similar pattern of speciess richness loss in terrestrial nitrogen enrichment experiments was observed in a complied data set from England and Poland (James et al., 2005). James et al. (2005) used winter nitrate or total nitrogen concentrations to estimate nitrogen loading and they concluded that diverse communities were to be established in lakes only with concentrations lower than 1 mg N l^{-1}. *Mesocosm* experiments conducted in Denmark with changing nitrogen and phosphorus concentrations proposed a nitrogen treshold as 1.2-2 mgl^{-1} at P levels higher than 0.1-0.2 mgl^{-1}; over which there is high probability of loosing submerged macrophyte in temperate lacustrine ecosystems (González Sagrario et al., 2005). Those quantifications emphasize that nitrogen may be of a greater importance for ecological state of shallow lakes than *hitherto* anticipitated; due to the fact that it may reduce the resilience of clear water conditions by restricting the macrophyte development or decreasing macrophyte richness, leaving the resilience capacity of ecological state to the fluctuations of few macrophyte species.

Majority of the research on shallow lakes was conducted in temperate zones. Ecological functioning of lakes at lower latitudes in a sub-temperate environment differs significantly from northern temperate lakes (Beklioglu et al., 2007). Higher temperatures observed in growing season of Mediterranean lakes may result in higher rate of denitrification (Talling and Lamolle, 1998) and mitigate the negative effects of nitrogen loading on macrophytes. Longer hydrologic residence time due to high

evaporation and resulting higher sediment interaction of water column may result in more intense internal loading in contrast (Romo et al., 2005).

1.4 *Mesocosm* in hypothesis testing

Experimental studies on ecological interactions can be conducted at various scales. The essential component is a controlled environment in which the responses of organisms or ecological units to manipulated parameters can be monitored for a relatively long time scale. The size of the experimental setup can change from a 100 ml flask in laboratory to a whole natural lake in freswater ecology research.

At lower end, controlled environments are small containers enclosing limited number of entities to observe their response. These are called *microcosm*, meaning little world in latin. There are several important advantages to work with *microcosms*. They are very effective in examining specific interactions among limited number of entities. They enable researches to work with large amount of replicates with comfortable environmental conditions, mostly in laboratories. Lastly, such studies can be performed with relatively small budgets. However, this reductionist aproach have limited capacity to mimic natural conditions realistically or enable complex ecological interactions to be carried out in controlled environment.

At higher end, a natural habitat or ecological unit can be controlled and manipulated to examine the effects of changing parameters on monitored organisms or interactions within the *whole*, which is called *macrocosm*. Basic example for the *whole* is a lake ecosystem and lake manipulation experiments in freswater ecology research. These large scale experiments are very powerful in realistic results for ecological research. However, replication in those studies is not likely, site-specific conditions may interfere with the repeatability of the results, they need large budgets and they arise ethical concerns.

There is a comprimise between these two extremes; reductionist and holistic aproach. *Mesocosm* mean midle world in latin and owing its name to one of the important figures in ecology, Eugene Odum. Odum introduced mesocosm in his article "The

Mesocosm" in 1984 as:

> ... To bridge the gap between the laboratory and the real world in environmental science, more effort needs to be invested in the use of bounded and partially enclosed outdoor experimental setups, or *mesocosms*...
> ... The *mesocosm* provides an environment where parts (populations) and wholes (ecosystems) can be investigated simultaneously...

Mesocosms enable researchers to have moderate number of replicates while examining ecosystem level interactions. More realistic results for interactions under investigation can be obtained in controlled environments by utilizing *mesocosms*. Variety of forms and sizes of *mesocosms* can be used in freshwater ecology *in situ* or *ex situ*. *In situ* approach consists of studies where the *mesocosms* are constructed in natural ecosystems by enclosing some portion of it (Figure 1.5). Having a part of an actual ecosystem for manipulation comes with some handicaps as it may not be easy to have a fine control on every parameter and it often needs extensive logistics in field conditions.

Therefore, it may be preferable to have the *mesocosm* in an environment close to research facility and mimic the natural condition as much as possible inside the *ex situ mesocosms*. *Ex situ mesocosm* studies have better control on manipulation of the parameters and have less logistic needs; however, they need construction of large facilities with large budgets (Figure 1.6).

1.5 Scope of the Study

The conventional anticipation that the primary production in lacustrine ecosystems is limited by phosphorus has been changing in recent years. Several recent studies on temperate lakes concluded that nitrogen is very important in primary production and for biodiversity of lakes as a limiting nutrient (Barker et al., 2008; González Sagrario et al., 2005; James et al., 2005). There are established differences between temporal and (sub)tropic lake ecosystems which may alter the interaction among different

Figure 1.5: *In situ* experimental *mesocosm* in the present study. The *mesocosms* are constucted inside a natural lake, in contact with sediment and atmosphere, exposed to natural environmental conditions and stocked with members of authentic biotic communities.

lake communities, nutrient dynamics and the role of macrophytes (Bachmann et al., 2002; Meerhoff et al., 2006a, 2007). Studies in the Mediterranean region indicate that the lakes have characteristics in between temperate and (sub)tropic ecosystems (Beklioglu et al., 2007). Therefore, there was a need for a better understanding of the role of nitrogen on macrophyte growth and interactions among primary producers in low-lattitude lakes.

To elucidate the effect of nitrogen on macrophyte growth in low lattitudes, we performed a mesocosm experiment in Turkey to record the direct and indirect effects of increasing nitrogen availability on submerged macrophyte growth at moderate phosphorus loading. Two phosphorus and three nitrogen loadings were employed to have moderate-high and natural-high nutrient concentration ranges for phosphorus and nitrogen, respectively. The influence of nutrient enrichment on macrophyte development were monitored by stocking *Myriophyllum spicatum* L. as it is a rooted submerged macrophyte found frequently and abundantly in Turkish wetlands. Relatively higher fish density was used to mimic realistic community compositon for enclosures and prevent high zooplankton grazing pressure on phytoplankton. The

Figure 1.6: A large-scale *ex situ mesocosm* experiment setup at National Environmental Research Institude, Denmark. This fully automatized system enables automatic sampling and temperature manipulation in 24 enclosures stocked with natural lake sediment. Whole setup is reported to cost 140.000 €, excluding construction and maintenance labor. Photograph and information are taken from Liboriussen et al. (2005b).

hypothesis in this study follows the results of González Sagrario et al. (2005) that increasing nitrogen loading would negatively affect macrophyte growth, probably to a lesser extent owing to higher nitrogen removal by denitrification and higher growth rate under the warmer climate in our region.

CHAPTER 2

MATERIAL & METHODS

2.1 Experimental Setup and Sampling

The *mesocosm* experiment was conducted between June 27 to September 26, 2007 for three months, spanning the major portion of the submerged macrophyte growing season, in Lake Pedina, Turkey. Experiment was performed as a full factorial replicated block design including three nitrogen and two phosphorus treatments with four replicates.

A short survey was performed in Lake Pedina prior to the construction of *mesocosm* to identify a suitable site with flat one-meter deep lake bottom and extensive submerged macrophyte growth. After a suitable site was identified, the enclosures were constructed on littoral zone with an approximate distance of 30 meters from the shore (Figure 2.2). The replicated treatments distributed randomly in a frame including 24 enclosures in two rows. The enclosures were designed as being isolated from the lake but open to both sediment and atmosphere interaction.

The body of each enclosure was constructed by using transparent polyethelene (PE) tube with 1.2 m diameter and 0.11 mm thickness. The PE tubes attached to polyvinyl chloride (PVC) rings on both end to achieve a cylindric shape. The bottom of this PE tube was inserted into the sediment at least 0.3 m by manually pushing it. Several bricks were attached to the bottom ring to ensure that the enclosures were firmly burried into the sediment. The top ring was attached to a floating frame. The frame was made of detachable aluminum poles and sustained

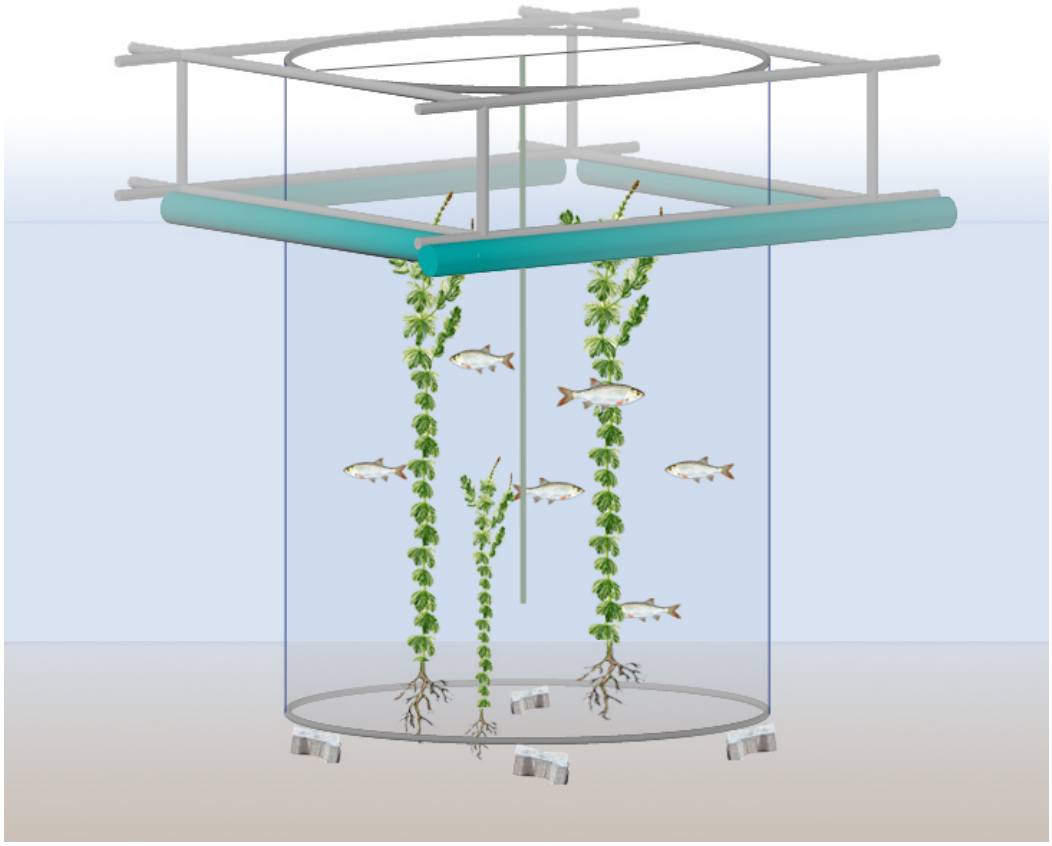

Figure 2.1: Illustration of an enclosure. Brown, blue and white regions represent sediment, water column and air respectively. Cylindric and transparent PE tube was buried into the sediment with the aid of attached bricks and sustained above the water surface by an aluminum frame, which is made buoyant with blue polyurethane foams. Healthy *Myriophyllum spicatum* shoots and underyearling fishes were stocked to each enclosure. Lastly, PE strips spanning the entire water column were installed for periphyton colonization.

top rings 0.3 m above the surface.

The *mesocosm* is illustrated in Figure 2.1. Polyurethane foams were attached to the lower part of the frame to provide appropriate bouancy. The frame was constructed to ensemble all 24 enclosures in two rows, 12 in each row for minimun interference on natural light (Figure 2.3). The frame was fixed firmly at the location by attaching 4 concrete blocks at each corner. Each segment of the aluminum frame had a dimention of 1.2 m $\times$ 1.2 m $\times$ 0.3 m and whole frame constitudes a body with 15 m lenght and 2.5 m width. Frame was constructed on the shore and transported to the lake in two parts using a narrow corridor opened through the reed belt on the

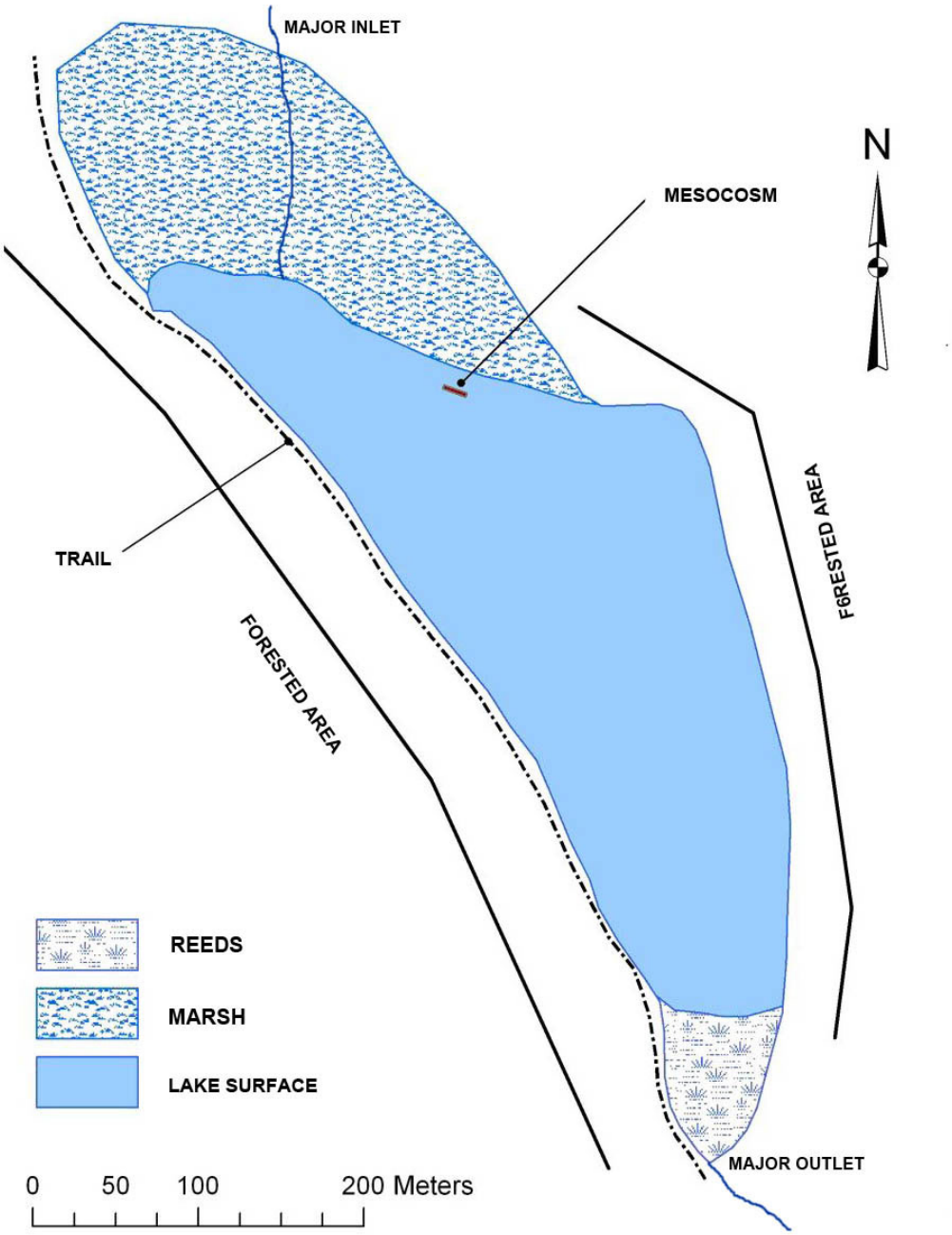

Figure 2.2: Map of Lake Pedina and mesocosm site.

Southern edge of the lake. It was transported to the *mesocosm* site on the northern edge by rowing through the lake.

The site chosed for the *mesocosm* experiment was cleaned from macrophytes with a hand rake prior to the construction of enclosures and checked again afterwards

to ensure there were no remaining macrophytes. After the establishment of the enclosures, all the lake was survayed to collect appropriate amount of *Myriophyllum spicatum* shoots, each having healthy roots, similar length and number of shoots. 10 of those collected macrophyte were stocked to each enclosure with iron weights attached to their roots to ensure they are in contact with the sediment.

While lowering the PE tubes through the water column, some amount of fish was enclosed. The approximate amount of enclosed fish were recorded after the construction and necessary amount of fish was stocked to sustain approximately 16 underyearling fish per m^{-2} (body lenght $<$ 10cm) in each enclosure. Fish that used to stock the enclosures were collected from the same lake by a sweep net or an electro-fishing equipment and they were kept in a bucket of water for a couple hours prior to stocking for observation. Approximately 10 fish were stocked into the enclosures prior to the experiment and another 10 were stocked in the first week of the experiment. Such high fish density was employed for a better replication of natural fish community structure in Lake Pedina (Figure 2.9).

Ten PE strips with 3 cm width were installed through water column; attached to a string covering the diameter of an enclosure on floating frame and stretched to the sediment by a metal weight to monitor periphyton growth on hard substrata. Lastly, all enclosures were covered with a nylon net (4×4 cm mesh size) to prevent interference of bird predation on macrophyte or fish. The fish abundance was checked visually for a month to ensure they are healthy and no dead fish was observed throughout the experiment other than a singular incidence. The whole process were summarized by a compilation of photographs in Figure 2.3.

Table 2.1: Sampling and nutrient addition dates.

Week	1	2	3	4	5	6	7	8	9	10	11	12	13
Sampling	Jun 27	Jul 06	Jul 14		Jul 29		Aug 13		Aug 27		Sep 10		Sep 26
Nutrient	Jun 28	Jul 07	Jul 15		Jul 30	Aug 07	Aug 14	Aug 21	Aug 28	Sep 04	Sep 11	Sep 18	

Figure 2.3: Sequences in the construction of the *mesocosm*.

After the setting up the enclosures, *mesocosm* was left untouched for two days to let suspended sediment settle. Three different loadings of NO_3-N including one control with factorial of two PO_4-P loadings constitute six nutrient treatments that were replicated four times in the experiment. Four and ten mgl^{-1} TN concentrations were aimed as moderate and high NO_3-N loadings (MN and HN, respectively) in nitrogen treatment. No NO_3-N addition was performed as a control (CN) to nitrogen treatment. In factorial to nitrogen treatments 100 and 250 μgl^{-1} TP concentrations were aimed as moderate and high PO_4-P loadings (MP and HP, respectively) in phosphorus treatment. Eleven nutrient additions as total were performed weekly by $Ca(NO_3)_2$*$4H_2O$ and $Na_2H_2PO_4$*$2H_2O$ as nitrogen and phosphorus sources, respectively. Whenever weekly nutrient additions were matched with bi-weekly sampling, additions were performed after the corresponding sampling. The dates of the nutrient additions and samplings are summarized in Table 2.1.

First two additions were performed intensely achive the desired starting concentrations. Rest of the additions were based upon the results of samples taken after the previous additions and targeted levels for each treatment. Whenever it was not possible to take or process an extra water sample for nutrient addition calculations, an aproximate value was estimated with respect to the previous additions and used for each treatment. The average nitrogen and phosphorus additions were summarized in Table 2.2, in Figure 2.4 and Figure 2.5.

Table 2.2: Average NO_3-N and PO_4-P additions to the treatments (mg day^{-1} m^{-2}). The first figure is the average of all additions and the second figure is the average excluding the first two start-up loadings. First row is for NO_3-N and second row is for PO_4-P additions. CN, control nitrogen; MN, moderate nitrogen; HN, high nitrogen; MP, moderate phosphorus and HP, high phosphorus.

CNMP	CNHP	MNMP	MNHP	HNMP	HNHP
0/0	0/0	122.4/60.2	137.8/75.6	313.3/117.8	329.9/134.4
4.3/2.1	11.8/6.2	4.2/2	12.6/7	4.7/2.4	12.7/7.1

Samplings were performed weekly for the first three weeks and biweekly for the rest of the experiment. First sampling was done as a control as it was performed

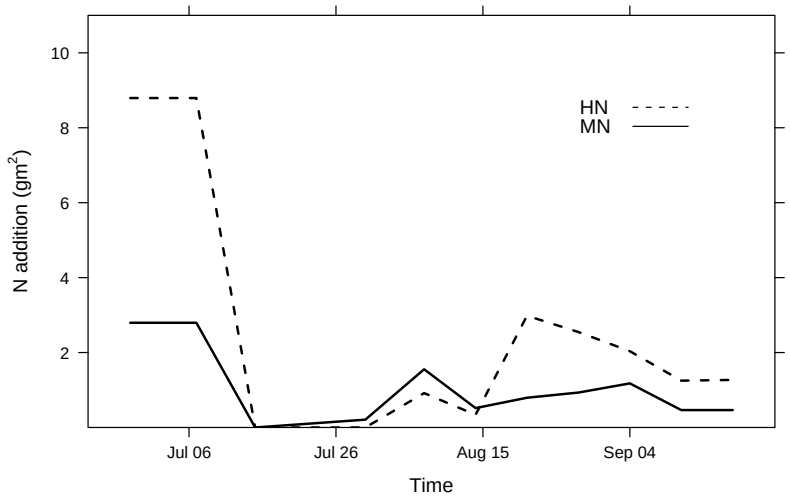

Figure 2.4: Average NO_3-N additions for MN and HN treatments through time. The values are averages for each nutrient addition date. For legend details, see Table 2.2.

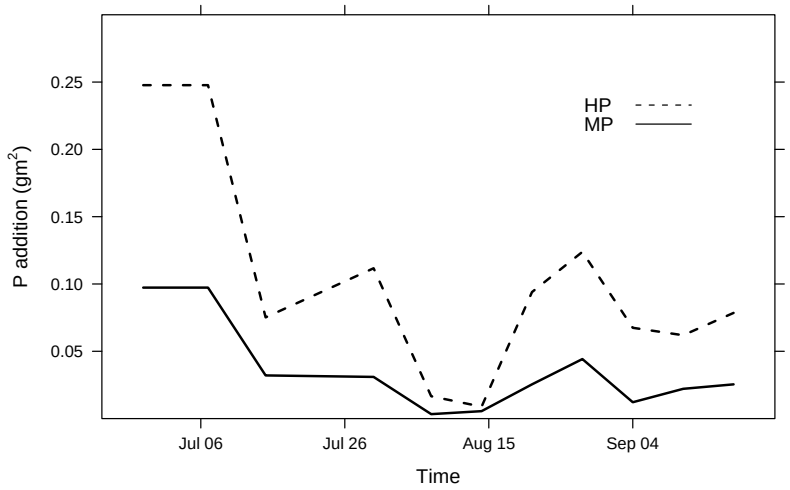

Figure 2.5: Average PO_4-P additions for MN and HN treatments through time. The values are averages for each nutrient addition date. For legend details, see Table 2.2.

prior to the first nutrient addition. Water depth, dissolved oxygen, temperature, conductivity, total dissolved solids (TDS), salinity and acidity were recorded in each sampling with a YSI 556 MPS sensor. 2 l composite water sample was taken with a 1 l syringe sampler (Figure 2.6) at 0.1 m below surface and middle-depth of enclosures with enough care not to disturb sediment or macrophytes. Subsamples were taken from the water sample for suspended matter (SS) (0.25 l), chlorophyll *a*

(chl *a*) (0.25 l), water chemistry analysis (0.25 l) and phytoplankton identification (0.05 l). SS and chl *a* samples were filtered through Whatman GF\C glassfibre filter.

Water column was spanned with a tube sampler and 3 l of water was filtered through 50 μ filter for zooplankton identification. Percent volume inhabited (PVI) for macrophyte development and percent cover for filamentous algea development were recorded in each sampling. One periphyton strip was taken out in each sampling starting from the third sampling and 0.1 m length of strip corresponding to 0.1-0.2 m water depth were sliced and enclosed in a zip-lock bag for periphyton chl *a* analysis. Whenever possible an extra water sample was taken with a 1 l-syringe sampler after the nutrient additions. Those samples was analyzed to calculate the necessary amounts of nutrient additions on the following occasion.

Figure 2.6: Syringe sampler used to take water samples in enclosures with minimum possible diturbance on periphyton and sediment. It is made of plexyglass tube with 4 cm diameter and one 1 m length and have 1 l sample capacity. It works like a piston and sucks up water through an opening with 4 mm diameter at desired depth.

Sediment cores before and after the experiment were taken and first 0.1 m were sieved through 212 μ for asessing the macroinvertebrate abundance. A sampling was performed for macrophyte associated macroinvertebrates at the end of the experiment. A Kornijóv sampler was manifactured to perform the sampling (Kornijów, 1998). However, it was broken in the field after used in the first three enclosures. Sampling was done for the other enclosures by placing 212 μ sieve with 0.3 m diameter under macrophyte on the surface and taking out of water slowly with enclosed macrophyte. The macrophyte was shaked vigorously in water and the water was filtered through 212 μ to collect associated macroinvertebrate. The macrophyte was kept to relate macroinvertebrate abundance to macrophyte dry

weight.

Three 0.1 m, healthy *Myriophyllum spicatum* shooths at least 0.05 m under surface were cut and transferred into a PE bottle with care not to disturb associated periphyton. These shoots were shaked vigorously in bottles filled with tap water to seperate periphyton on macrophyte (epiphyton) and the remaining water was filtered through Whatman GF\C glassfibre filter. Lastly, all macrophyte for each enclosure was harvested with a hand rake, their roots were excluded, cleaned from excess periphyton and macroinvertebrates and seperated for species.

Because of some technical problems with YSI 556 MPS sensor, dissolved oxygen and temperature could not be recorded for the first three samplings; while, conductivity, TDS, salinity and acidity could not be recorded for the second and the third samplings.

2.2 Sample Preparation and Analysis

All samples other than macroinvertebrate, zooplankton and phytoplankton identification were frozen in the field and kept frozen until corresponding analysis were done. Freezing is a widely used for an effective preservation in aquatic studies (Canfield et al., 2002). Zooplankton and phytoplankton samples were fixed with acid Lugol's solution (4% and 2%, respectively). All macroinvertebrate samples were preserved with 60% ethanol.

The water chemistry samples were processed with potassium persulphate digestion at 125 °C - 1.5 ATM - 1 h for total phosphorus (TP) analysis (Mackereth et al., 1978). Soluble reactive phosphorus (SRP) analysis was performed with molybdate reaction method (Mackereth et al., 1978) on filtered water. Total nitrogen (TN), ammonium (NH_4) and nitrite-nitrate (NO_2-NO_3) analysis was performed with Scalar autoanalyzer with certified methods (Houba et al., 1987; Krom, 1980; Kroon, 1993; Searle, 1984). Silicate analysis was done by molibdate reaction method (Golterman et al., 1978)and alkalinity analysis was performed by HCl titration method (Mackereth et al., 1978).

Strips for periphyton growth and filters for epiphyton and phytoplankton were submerged in ten ml ethanol for chl *a* extraction (Jespersen and Christoffersen, 1987). The remaining filter papers and strips were examined for any remaining pigment and all the extractions were checked for efficiency. Following, chl *a* concentrations was determined spectrometrically on 663 nm wavelenght with a correction on 750 nm wavelength. Phytoplankton chl *a* was converted to phytoplankton biomass by the ratio of 1:30:66 (μg chl *a*:μg C:μg DW) (Reynolds, 1984). All macrophyte samples were kept seperate for each species and for each enclosure, while the samples taken for macroinvertebrate and periphyton on macrophyte were processed seperately. Any remaining macroinvertebrate was cleaned and the samples were dried at 80-100 °C for 1-3 days for DW determination.

Zooplankton samples on first, fifth and eighth samplings were processed using a streo microscope. Counting was performed until at least 100 individual of two or three dominant species were counted in a sample or subsamples with respect to the density of the original sample. *Cladocera* and *Rotifera* were identified to genus or species level. Only *Cyclopoid Copepoda* found in samples and given as males, females, copepodites and nauplii. Koste (1978); Alonso (1996); Flössner (2000) were used as main resources for identification of related taxa. Zooplankton biomass was estimated using average species dry weight values obtained over 3 years for 37 Danish lakes included in the Danish Nationwide Monitoring Programme of the Aquatic Environment (Hansen et al., 1992). All cladocerans, rotifers and nauplii were included in the calculation of zooplankton:phytoplankton biomass ratio, excluding adult cyclopoids, copepodites and *Asplanchna sp.* due to their predatory behaviour.

Zooplankton samples for other samplings were not processed as those three samples at the beginning, middle and end of the experiment were found to be appropriate to assess general trend in zooplankton community. Phytoplankton samples were not processed as there were no strong response of phytoplankton chl *a* to treatments. Macroinvertebrate samples in the sediment were excluded because half of the samples included no identifyable specimens, while the others included only some few *Chironomida*. Macroinvertebrate on macrophyte samples were also excluded as

they were not reliable because the sampling equipment was broken while sampling was performed.

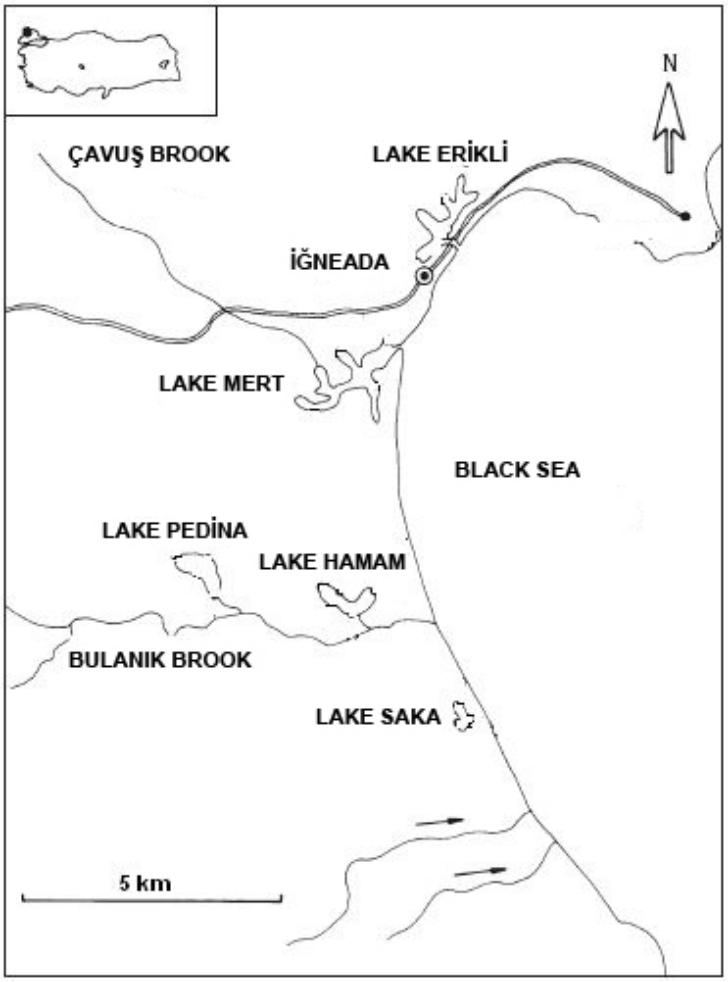

Figure 2.7: Map of Lake Pedina and surrounding area. Redrawn from Altınsaçlı (2000).

2.3 Study Site

Lake Pedina (41.83016°N 27.93531°E, datum EUR50) is a small shallow lake with seven ha surface area and 2.1 m maximum depth. Lake is located within a pristine decidious forest at 20 m altitude and three km away from Black Sea at the north-west coast of Turkey (Figure 2.8, Figure 2.7). Lake Pedina is subject to a two year

monitoring study prior to the experiment (Özkan and Beklioğlu, 2007). Lake is drained through a small channel and receives water from a small stream that dries out completely through the end of summer (Figure 2.2). Lake Pedina is mostly dependent on this input and probably on groundwater to some extend and regularly experiences a seasonal water level fluctuation of approximately 0.3 m. This shallow freswater lake is free from antropogenic effects but a small seedling nursery located along the inlet, which is in function temporarily. The morphometric characteristics, and physical properties of Lake Pedina is summarized in Table 2.3.

The lake is in mesotrophic level with a mean Secchi depth of 0.7 m through the year and having moderate chl *a* levels. Annual mean concentrations of chl *a*, Secchi disk, SS and water chemistry of this shallow freshwater lake is given in Table 2.4. The TN concentration given in the table was derived from very few samples taken from the shore and they are likely overestimates of the actual TN levels in Lake Pedina. Furthermore, Lake Pedina was sampled for pelagic water chemistry in September 2007, the sample was processed with the same methods used in present study and TN concentration was estimated as 0.25 mgl^{-1}. Extensive macrophyte cover is observed regularly throughout the summer on lake surface. The macrophyte community is dominated by floating-leaved plant *Trapa natans* L. and includes submerged macrophyte species *Myriophyllum spicatum*, *Ceratophyllum demersum* L. and *Potamogeton crispus* L. with significant abundance. The fish community is composed of three species from *Cyprinidae* family; namely: crucian carp (*Carassius carassius* L.), carp (*Cyprinus carpio* L.) and rudd (*Scardinious erythropthalmus* L.). According to a gill-net survey performed in May 2007, rudd is the dominant species in the lake while the others constutude a small minority (Figure 2.9). It is apparent from Figure 2.9 that smaller size-class fishes are extensively dominant in Lake Pedina.

Table 2.3: Morphometry and annual mean concentrations and standard deviations of pH, salinity, conductivity and TDS of Lake Pedina.

Area (ha)	Max-Mean Dep. (m)	pH	Sal. (‰)	Cond.(mS)	TDS (mgl^{-1})
7	2.1-1.2	7.6 ± 0.4	0.1 ± 0.0	0.3 ± 0.1	136.4 ± 57.6

Figure 2.8: Aerial photograph of Lake Pedina. Red mark indicates the *mesocosm* site. Photograph was taken by Aykut İnce.

Table 2.4: Annual means and standard deviations of Secchi disc, suspended solids (SS), chl *a*, total phosphorus (TP) and total nitrogen (TN) of Lake Pedina.

Secchi disk (m)	SS (mgl^{-1})	Chl *a* (mgl^{-1})	TP (mgl^{-1})	TN (mgl^{-1})
0.7 ± 0.4	7.7 ± 15.5	0.016 ± 0.011	0.032 ± 0.010	1.5 ± 0.4

2.4 Statistical Analysis

All the statistical analyses were performed by R statistical package and with relevant libraries (R Development Core Team, 2008). Initial values of all sampled parameters prior to the nutrient additions were analyzed with one-way ANOVA to test any difference among treatments. The observations on the last sampling was also tested for differences with one-way ANOVA where appropriate. Tukey HSD pairwise comparison with 0.95 confidence level was applied to parameters having significant differences among treatments in one-way ANOVA. Effect of nutrient additions and

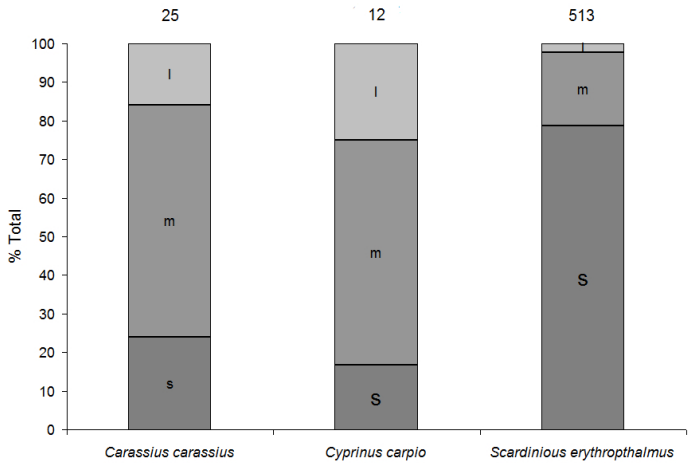

Figure 2.9: Percent contribution of each size class to the total catch of each fish species in Lake Pedina. The unit is catch per unit effort (CPUE, catch for 2 nets^{-1} night^{-1} on littoral). s, m and l denotes for small, medium and large size classes for each species. Total number of cought speciemens were mentioned at the top of the corresponding bars. The size classes correspond to mm fork lengths: s<90<m<140<l for *Carrasius carrasius*; s<100<m<200<l for *Cyprinus carpio* and s<100<m<180<l for *Scardinious erythropthalmus*.

other relevant parameters was tested with linear mixed-effects model (Pinheiro and Bates, 2000). It is common in biological sciences to use repeated measures of ANOVA for longitidunal data. However, mixed effect models is better in handling the pseudoreplicated repeated measures data (Pinheiro and Bates, 2000; Crawley, 2008). The mixed effect model (MEM) was constructed as the treatments are factorial fixed effects and repated measures through time are random effect as pseudoreplication.

The behaviour of the data both for one-way ANOVA or mixed effect model was examined with relevant diagnostic plots (boxplots of residuals, standardized residuals versus fitted values for each group, observed versus fitted values, normal plot of residuals for each group, etc.). If there is a violation of the related assumptions, log transformation was applied. If there is apparent heteroscedasticity in mixed effects model data, a modified model for heterescedastic fit was employed. If mixed effects model cannot fit the data because of the extremely low variation in the first sampling, the first sampling was excluded from the analysis.

CHAPTER 3

RESULTS

3.1 Water Level, Temperature and Physical Properties of Water

The mesocosm was placed on a site having a depth range between 0.9-1 m. A water level drop of 0.3 m was observed while setting up the enclosures. Therefore the experiment had to be started with an average depth of 0.72 ± 0.07 m (mean ± standard deviation (SD)). A continious decrease in water level was observed through the three-month duration of the experiment and resulted in an average depth of 0.43 ± 0.06 m at the end of the experiment. This trend is shown in Figure 3.1. It is usual to experience two-week of rainy period in İğneada at the end of August. Although a very short and less intensive rainy period was observed during the experiment, Lake Pedina experienced a water level increase of a couple of cm. This quick response was a basic indication of the dependence of hydrology of Lake Pedina on surface water sources. Water temperatures higher than 25°C was recorded 0.25 m below water surface through July and August (Figure 3.2).

There were no significant differences among treatments for depth, salinity, conductivity, TDS, alkalinity and pH at the first sampling prior to the nutrient additions (one-way ANOVA, $P > 0.10$; for all of the variables). There was no difference among treatments for salinity (MEM, $P > 0.10$) through the course of experiment with a mean and SD of 0.097 ± 0.017 ‰, being similar to lake salinity level (Table 2.3). Both TDS and conductivity only differed significantly for N treatment, while an

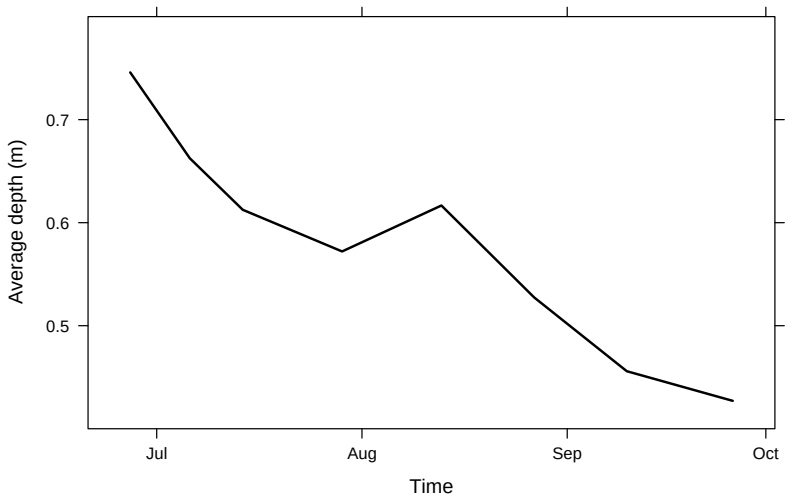

Figure 3.1: Water level in the *mesocosms* through the experiment

increasing trend observed for all tretments through time (MEM, $P < 0.01$ for N and $P >$ 0,10 for P and NP treatments, Figure 3.3, Figure 3.4). Both TDS and conductivity had water levels around 100 - 160 mgl^{-1} and 0.16 - 0.24 mS, which are complementary to the values observed in Lake Pedina in previous years (Table 2.3). There were no significant differences among treatments for alkalinity through the experiment (MEM, $P > 0.10$). There was also no apparent change through time in alkalinity with a mean and SD of 1.3 ± 0.3 meq^{-1} (Figure 3.5). Total alkalinity is largely dominated by bicarbonate alkalinity, while carbonate alkalinity is around 0 - 10%. pH measurements differed only for N treatments (MEM, $P < 0.004$ for N and $P > 0.10$ for P, NP treatments), having a decreasing trend with the highest values at the HN treatment (Figure 3.6). Lastly, dissolved oxygen had a decreasing trend over time (Figure Figure 3.7) with no significant differences among treatments (MEM, $P > 0.10$).

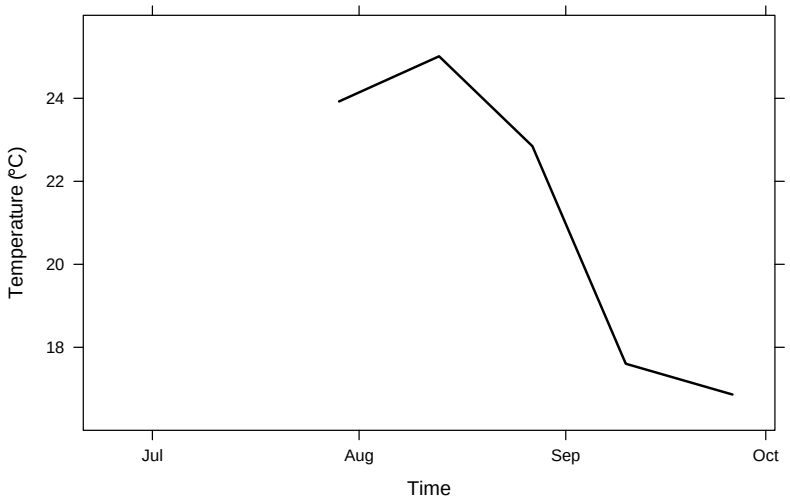

Figure 3.2: Water temperature in the enclosures at 0.25 m depth through the experiment, the data for the first month lacked due to a technical problem.

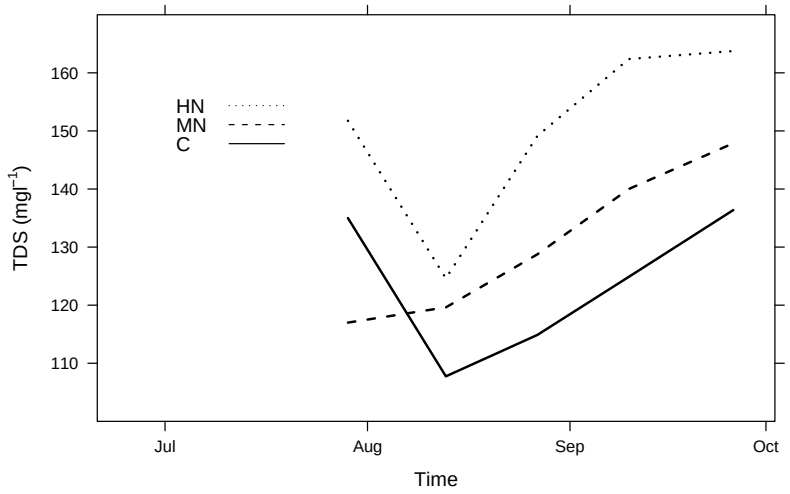

Figure 3.3: Changes in total dissolved solids in water column for nitrogen treatments, the data for the first month lacked due to a technical problem. For legend details, see Table 2.2.

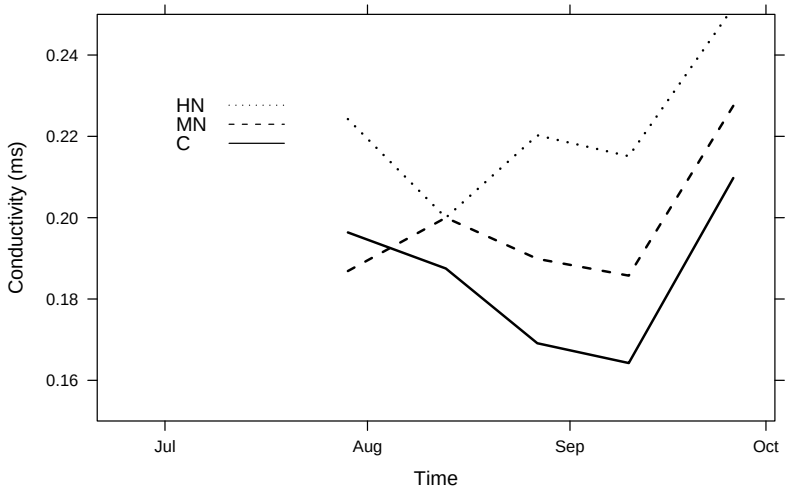

Figure 3.4: Changes in conductivity of water column for nitrogen treatments, the data for the first month lacked to a technical problem. For legend details, see Table 2.2.

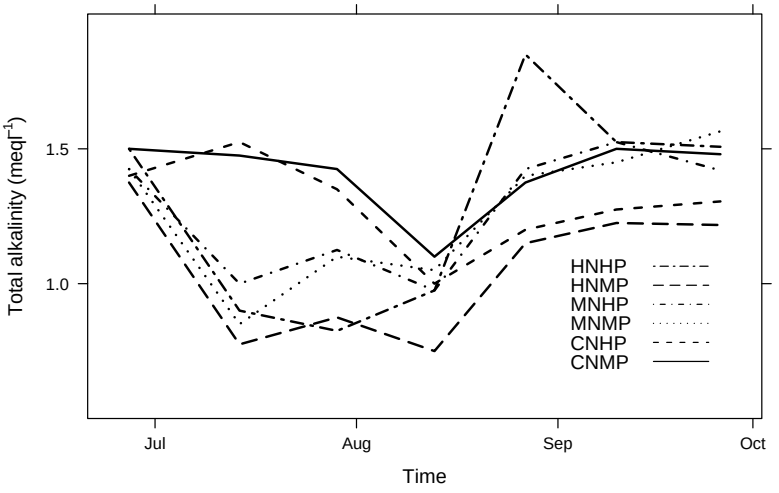

Figure 3.5: Changes in alkalinity in treatments. For legend details, see Table 2.2.

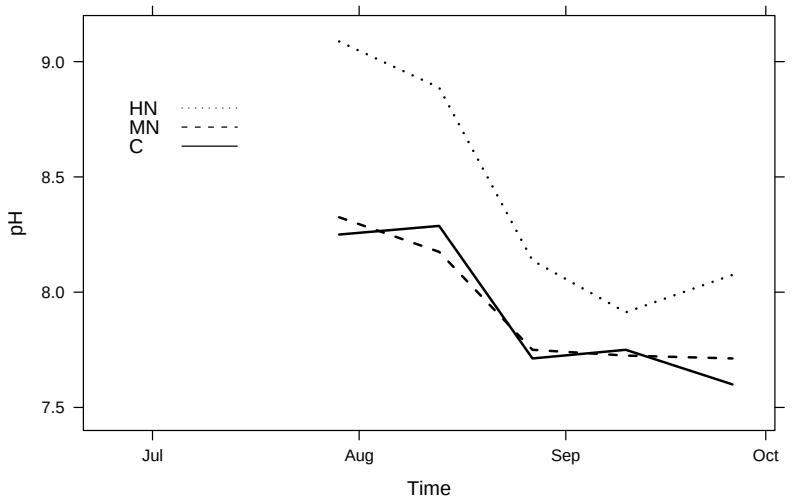

Figure 3.6: Changes in pH for nitrogen treatments, the data for the first month lacked due to a technical problem. For legend details, see Table 2.2.

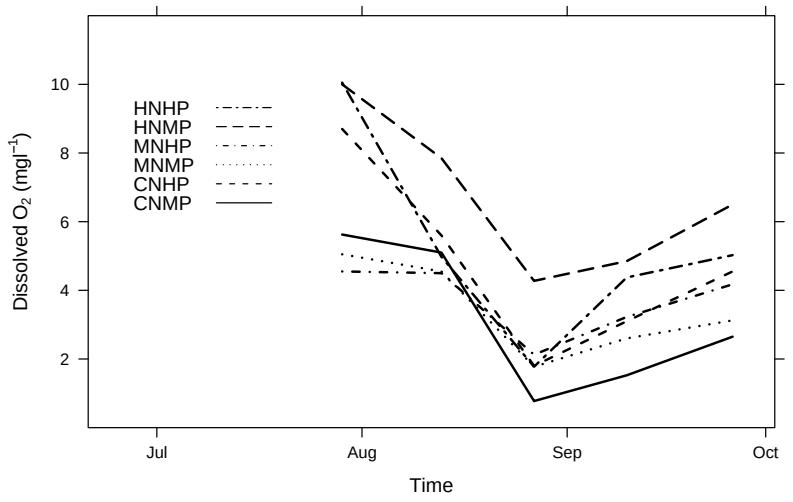

Figure 3.7: Changes in dissolved oxygen in water column for treatments, the data for the first month lacked due to a technical problem. For legend details, see Table 2.2.

3.2 Nutrients

There were no significant differences among treatments for TN, NH_4, TP, SRP and SiO_2 concentrations at the first sampling prior to nutrient additions (one-way ANOVA, $P > 0.10$ for all variables). NO_2+NO_3 concentrations were undetectable in the enclosures prior to nutrient additions. TN concentrations differed significantly for N treatment through the course of experiment as expected (MEM, $P < 0.0001$ for N treatment, $P > 0.10$ for P and NP treatments). TN concentrations prior to the nutrient additions had a mean and SD of 0.37 ± 0.07 mgl^{-1} and CN treatment had a mean and SD of 0.52 ± 0.17 mgl^{-1} through the experiment. MN and HN treatments resulted in two different levels of TN in related enclosures with similar patterns with no apparent differentiation for P treatments (Figure 3.8). N additions in MN treatment resulted in average TN concentrations of 1.99 ± 1.09 mgl^{-1}, whereas N additions in HN treatment resulted in average TN concentrations of 8.07 ± 5.88 mgl^{-1} through the experiment. NO_2+NO_3 concentrations had a similar result with TN (MEM, $P < 0.0001$ for N treatment, $P > 0.10$ for P and NP treatments, Figure 3.9). NO_2+NO_3 concentrations in the enclosures throughout the experiment were slightly less than TN levels and have a mean of 1.00 ± 1.05 and 6.16 ± 5.55 mgl^{-1} for MN and HN treatments, respectively. NO_2+NO_3 level for CN treatment was very low with a mean and SD of 0.033 ± 0.044 mgl^{-1}. NH_4 concentrations also differed only for N treatment through the experiment (MEM, $P < 0.0001$ for N treatment, $P > 0.10$ for P and NP treatments). NH_4 concentrations for CN treatment remained low and constant through the experiment with a mean and SD of 0.038 ± 0.029 mgl^{-1}. However, all the other treatments had increasing trend through the end of the experiment (Figure 3.10), while MN treatments reached a level of 0.2 - 0.3 mgl^{-1} and HNHP treatment had the highest concentration of 0.8 mgl^{-1} in late September.

TP concentrations differed for both N and P treatments (MEM, $P < 0.01$ for N, $P < 0.0001$ for P and $P > 0.10$ for NP treatments); whereas, SRP concentrations differented only among P treatment (MEM, $P < 0.01$ for P and $P > 0.10$ for N and NP treatments). Although TP concentrations differed for P treatment, MP

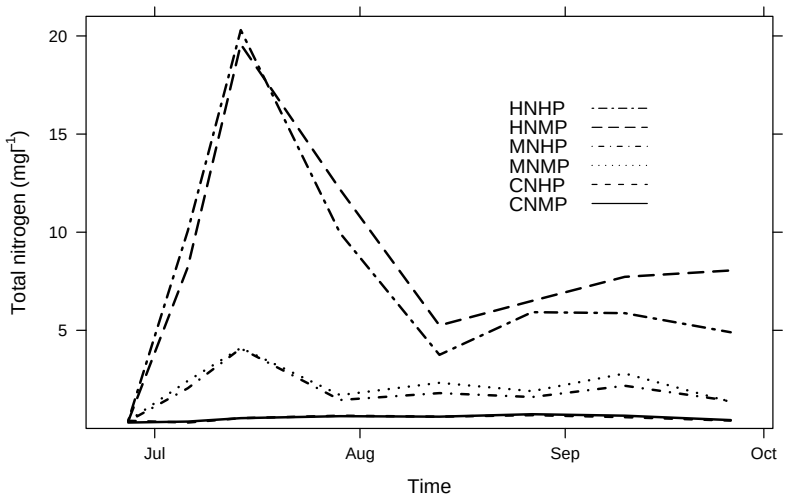

Figure 3.8: Changes in the total nitrogen concentrations in treatments. For legend details, see Table 2.2.

and HP treatments had similar concentration with means and SD‘s of 0.055 $\pm$ 0.018 and 0.072 $\pm$ 0.021 mgl^{-1}, respectively. TP concentrations for all treatments fluctuated between 50 - 100 mgl^{-1} through the experiment (Figure 3.11). SRP concentrations had a similar pattern as TP with a lower range of 0.005 - 0.015 mgl^{-1} (Figure 3.12). Lastly, silicate concentrations differed in N treatment through the experiment (MEM, $P < 0.01$ for N and $P > 0.10$ for P and NP treatments). The high concentrations at the beginning of the experiment were probably resulted from sediment resuspension during the *mesocosm* construction. After leveling off, enclosures sustained a stable level around 2 mgl^{-1} SiO_2 (Figure 3.13).

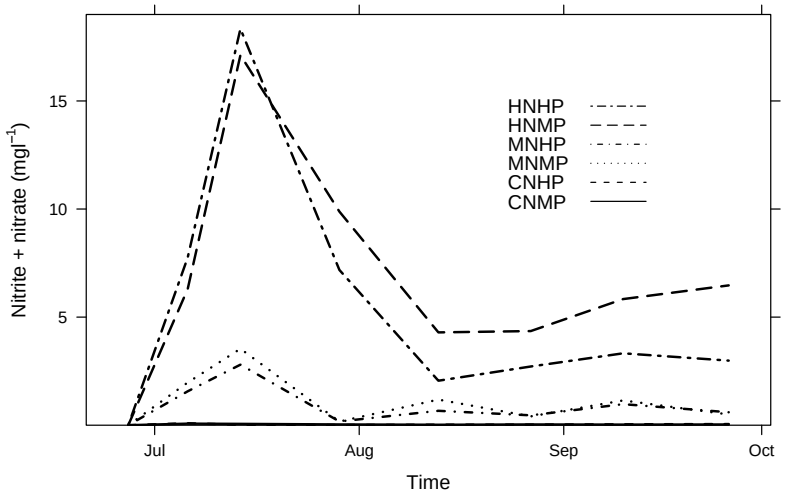

Figure 3.9: Changes in nitrite + nitrate concentrations in treatments. For legend details, see Table 2.2.

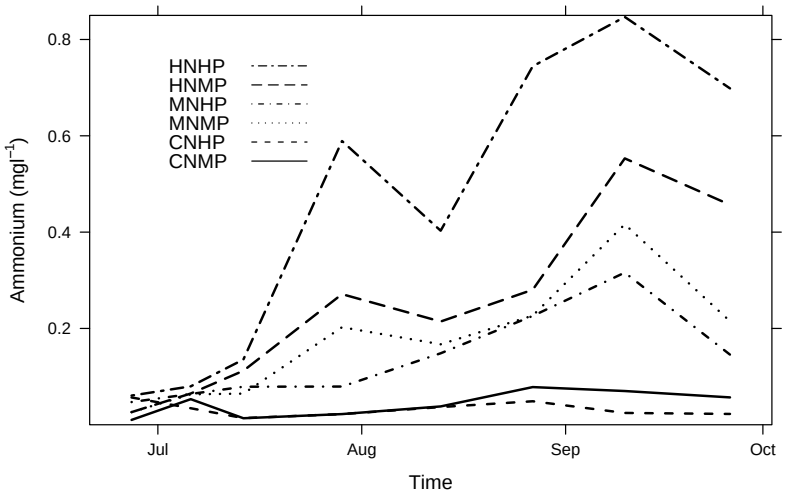

Figure 3.10: Changes in ammonium concentrations in treatments. For legend details, see Table 2.2.

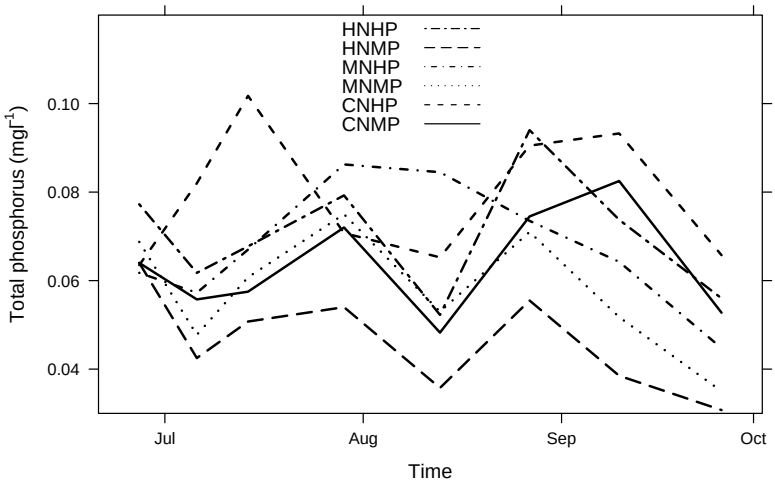

Figure 3.11: Changes in total phosphorus concentrations in treatments. For legend details, see Table 2.2.

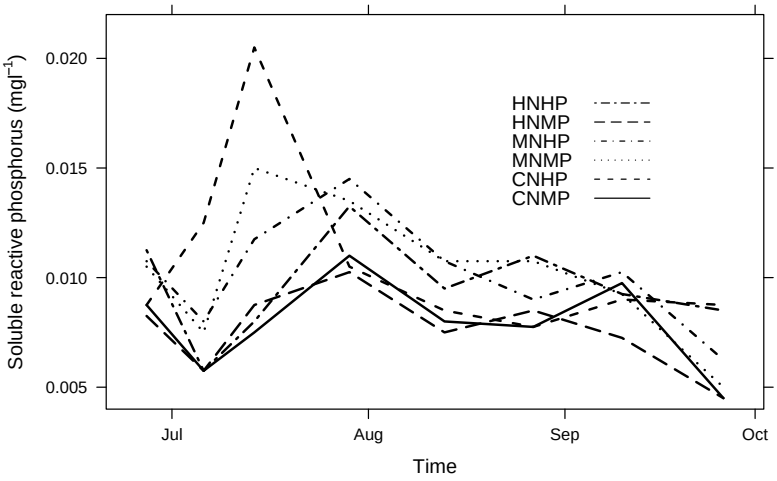

Figure 3.12: Changes in soluble reactive phosphate concentrations in treatments. For legend details, see Table 2.2.

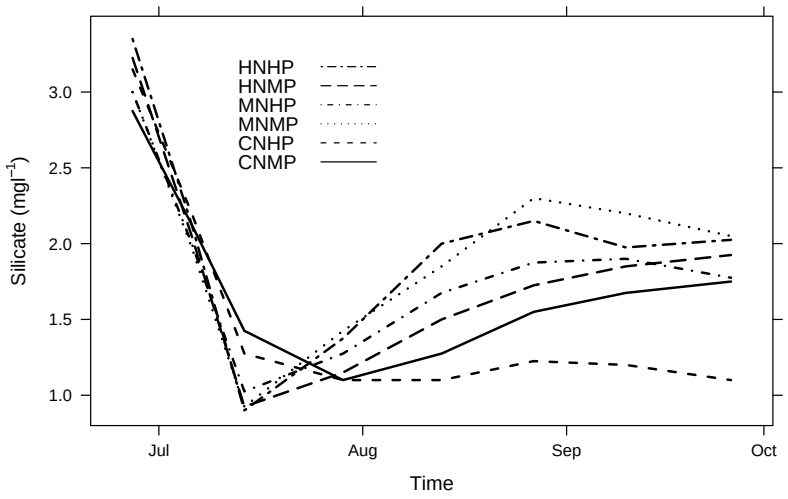

Figure 3.13: Changes in silicate concentrations in treatments. For legend details, see Table 2.2.

3.3 Phytoplankton, Periphyton and Epiphyton

Prior to the experiment, chl *a* concentrations did not differ among treatments (one-way ANOVA, $P > 0.10$) and had a level of (mean $\pm$ SD) 0.007 ± 0.003 mgl^{-1}. Through time, chl *a* results differed among factorial treatments (MEM, $P < 0.01$ for NP, $P > 0.10$ for N and P treatments). However, all of treatments scattered within a narrow range between 0.01 - 0.04 mgl^{-1} (Figure 3.14). There was a seasonal pattern in all treatments with an increase at the beginning of the experiment and a decrease at the end. Similar to chl *a* results, SS results had no difference among treatments (one way ANOVA, $P > 0.10$) prior to the nutrient additions and had slightly higher values probably owing to the sediment resuspension during the construction of the *mesocosm*. Through time, SS differed among factorial treatments (MEM, $P < 0.05$ for NP, $P > 0.10$ for N and P treatments). SS results centered around 6 mgl^{-1} and had a slightly decreasing trend over time (Figure 3.15).

The epiphyton chl *a* concentrations differed significantly among treatments (one-way ANOVA, $P < 0.05$) . Mean epiphyton abundance was highest in HNHP treatment with a mean concentration of 0.92 μg chl *a* mg^{-1} DW^{-1}, while all others had concentrations below 0.52 μg chl *a* mg^{-1} DW^{-1} (Figure 3.17). Pairwise comparisons between HNHP - CNHP (Tukey HSD, $P < 0.05$) and HNHP - CNMP (Tukey HSD, $P = 0.053$) revealed significant differences. Periphyton chl *a* concentrations on strips differed for N and factorial treatments (MEM, $P < 0.05$ for N, $P = 0.05$ for NP and $P > 0.10$ for P treatments). Periphyton abundance did not change much over time, while HNHP treatment had always the highest values (Figure 3.16). A stronger effect similar to epiphyton abundance was apparent for periphyton abundance in the third sampling (one way ANOVA, $P < 0.01$). In the third sampling as being the first sampling for periphyton after three weeks, mean periphyton abundance was highest in HNHP treatment with a concentration of 116.7 mg chl *a* m^2, while all others scattered between values of 32 - 63 mg chl *a* m^2. Pairwise comparison between HNHP and CNHP treatment on third sampling revealed significant difference (Tukey HSD, $P < 0.05$) for periphyton abundance.

Sporadic occurances of filamentous algea on the water surface in enclosures were

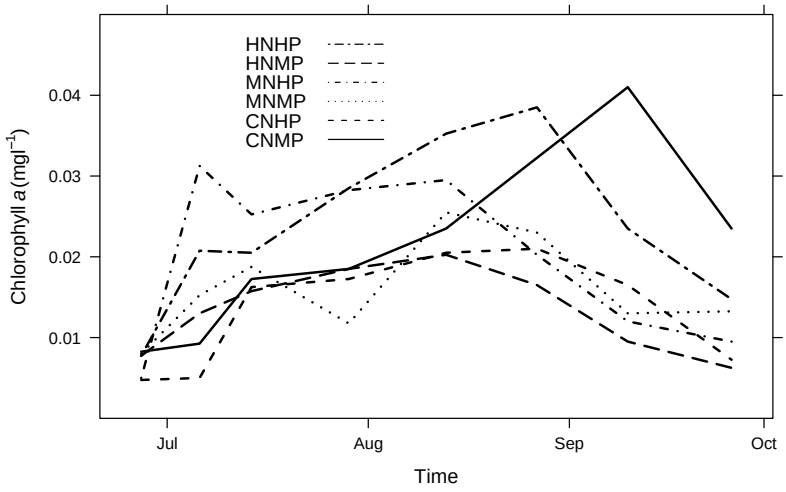

Figure 3.14: Changes in water column chlorophyll *a* concentrations in treatments. For legend details, see Table 2.2.

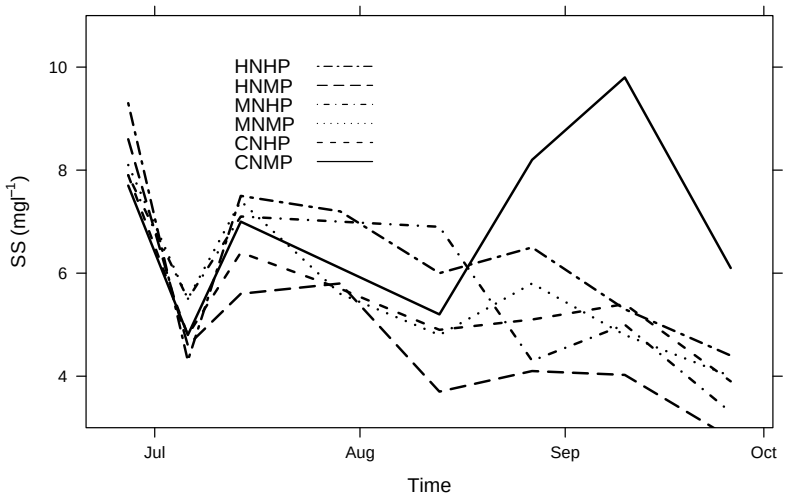

Figure 3.15: Changes in suspended solids levels in treatments. For legend details, see Table 2.2.

recorded for all treatments. There is not a significant difference among treatments for filamentous algea development (MEM, $P > 0.05$ for all treatments). Moderate coverage of filamentous algea was observed through the course of the experiment in all treatments (Figure 3.18). Occasional full coverage was also observed in enclosures independent of the treatments but not sustained more than one week in general.

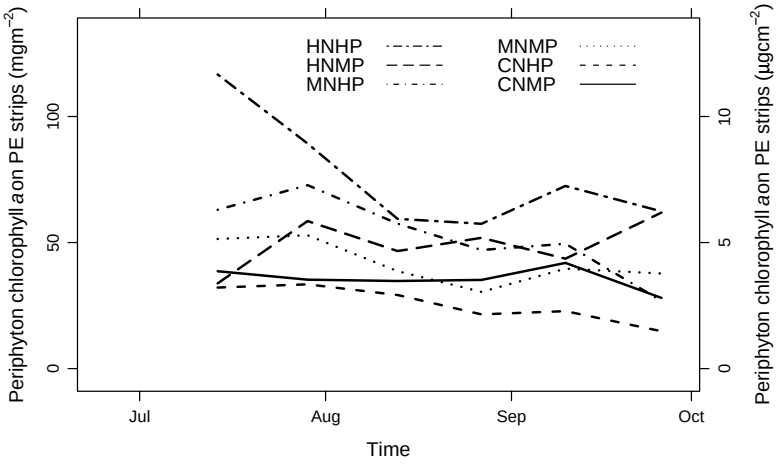

Figure 3.16: Changes in periphyton chlorophyll *a* concentrations on strips in treatments. For legend details, see Table 2.2.

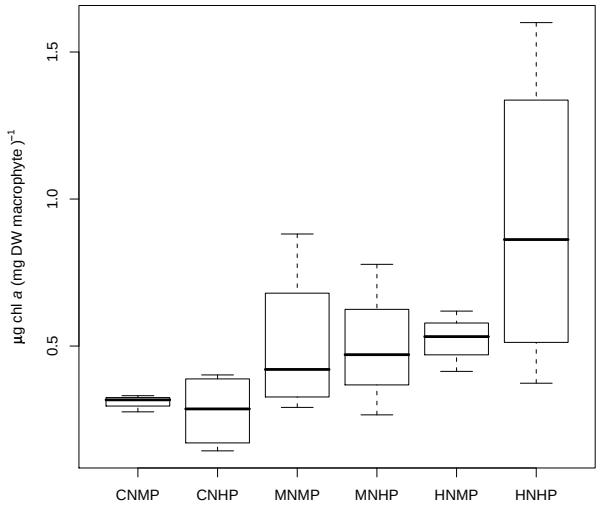

Figure 3.17: Epiphyton chlorophyll *a* concentrations on *Myriophyllum spicatum* shoots at the end of the experiment for each treatment. For legend details, see Table 2.2.

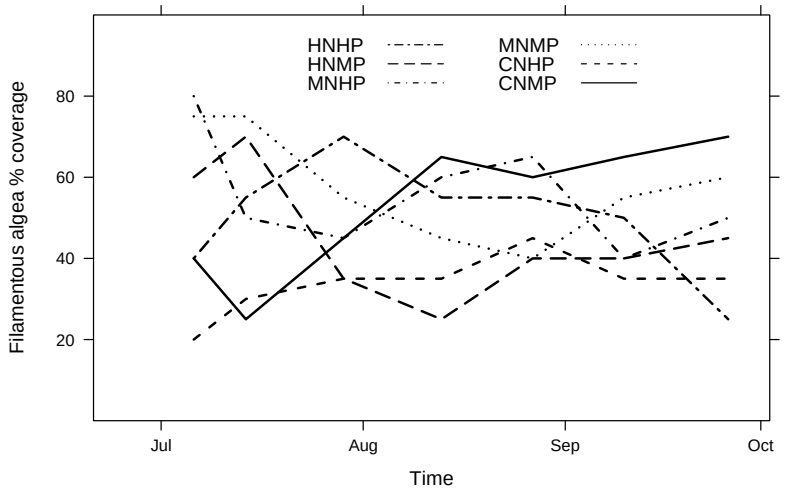

Figure 3.18: Filamentous algea percent coverage on water surface in treatments. For legend details, see Table 2.2.

3.4 Macrophyte

PVI results showed continuous growth of macrophytes in all treatments from a mean of %7 stocking density to the means of all treatmens scattering around 50% at the end of the experiment (Figure 3.19, Figure 3.21). No significant difference among treatments was observed throughout the experiment(MEM, $P = 0.086$ for N and $P > 0.10$ for P and NP treatments), while CN treatment having highest PVI means.

Although all macrophyte biomass were removed from the enclosures prior to the experiment, *C. demersum* growth was observed in all enclosures but one for CNMP treatment. *P. crispus* growth was also observed in half of the enclosures (3 out of 4 replicates in CNMP, CNHP, HNMP and 1 out of 4 replicates in MNMP, MNHP, HNHP treatments). *C. demersum* growth was extensive for most of the enclosures and comparable to *M. spicatum* biomasses (Figure 3.20). *P. crispus* growth was limited in amount and always below 3 g DW m^{-2}, were occurred. Lowest biomasss were observed for total macrophyte and *M. spicatum* at highest dual treatment (Figure 3.20) but none of the total macrophyte, *M. spicatum* or *C. demersum* or *P. crispus* DW biomass results were differed significantly with respect to treatments

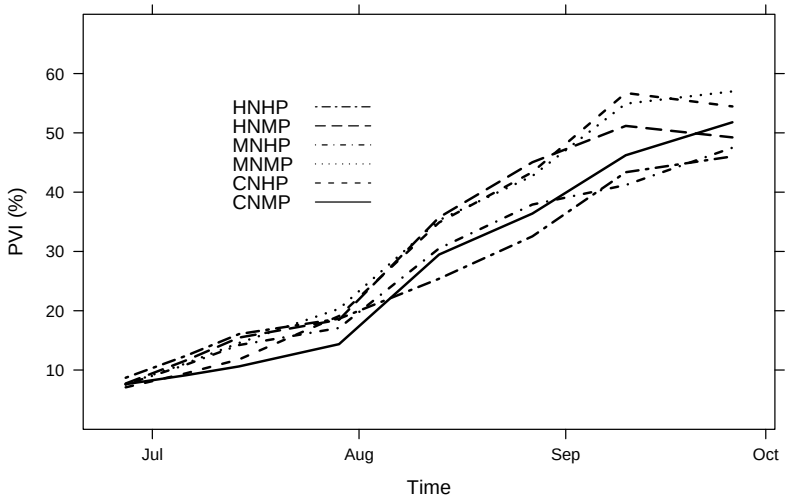

Figure 3.19: Changes in percent volume inhabited (PVI) in treatments. For legend details, see Table 2.2.

(one way ANOVA, $P > 0.05$ for all).

3.5 Zooplankton

The zooplankton biomass in all enclosures throughout the study were largely dominated by cyclopoids (Figure 3.23). A slight increase up to the level of 20% mean biomass contribution for cladocerans were observed at the end of the experiment. Neither the biomass of cladocerans, rotifers or cyclopoids nor the total biomass differed significantly with respect to the treatments at the beginning or end of the experiment (one way ANOVA, $P > 0.10$ for all; Figure 3.22). Cladocerans mainly dominated by *Simocephalus vetulus* (O.F. Müller, 1776), *Scaphaloberis rammneri* (Dumont & Pensaert, 1983), *Acroperus harpae* (Baird, 1834) and *Chydorus sphaericus* (O .F . Müller, 1776) in all treatments throughout the study; whereas only a few *Daphnia sp.* individuals observed 5 of the enclosures prior to the experiment. None of the dominant members cladocerans differed significantly at the beginning of the experiment (one way ANOVA, $P > 0.10$ for all). *A. harpae* biomass was the only responding dominant member of cladocerans to treatments significantly at the end

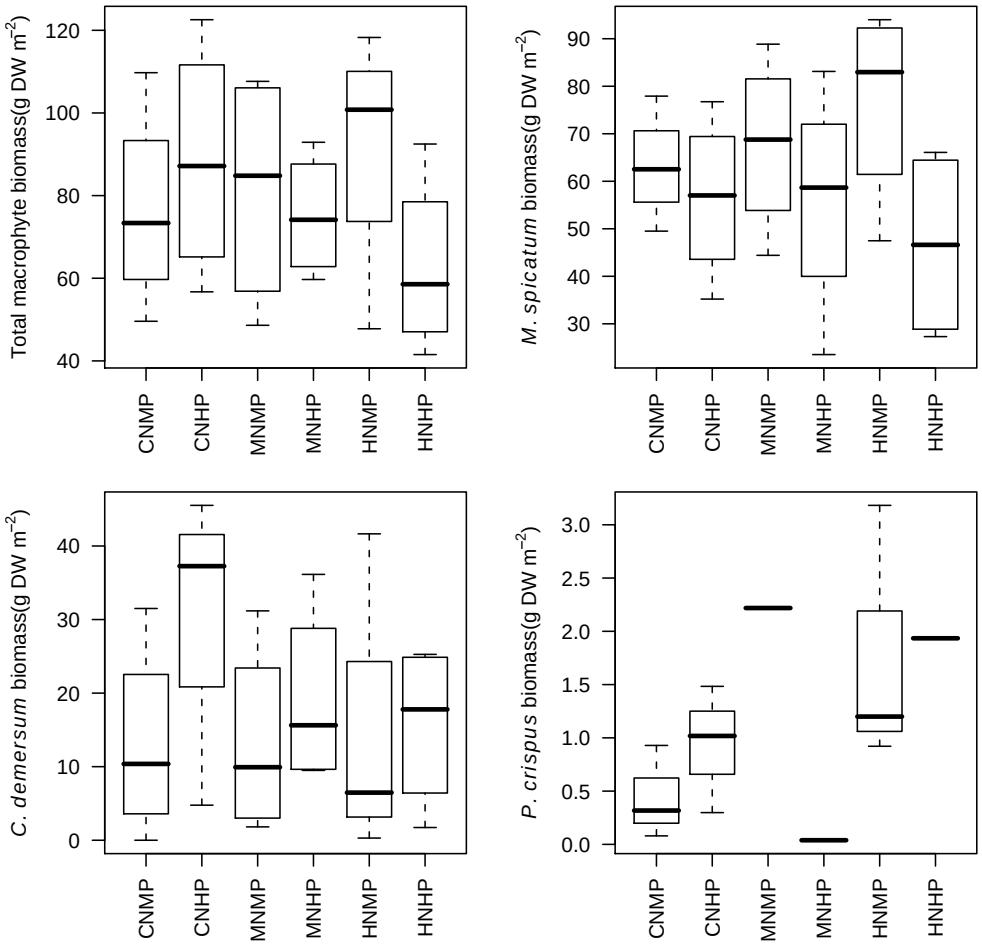

Figure 3.20: Dry weight (DW) biomasses of total macrophyte, *Myriophyllum spicatum*, *Ceratophyllum demersum* and *Potamogeton crispus* at the end of the experiment for each treatment. For legend details, see Table 2.2.

of the experiment (one way ANOVA, $P < 0.05$ for *A. harpae* and $P > 0.10$ for all the other dominant cladocerans; Figure 3.25). Only cyclopoid copepods observed in the samples and they were mainly dominated by nauplii or copepotides and neither of them had treatment effect at the beginning or the end of the experiment (one way ANOVA, $P > 0.10$ for both).

Zooplankton:phytoplankton ratio had no significant difference among treatments at the beginning or the end of the experiment (one-way ANOVA, $P > 0.10$ for both) and was low (Figure 3.24). Zooplankton:phytoplankton ratios for all treatments

Figure 3.21: Photograph of macrophyte growth in enclosures at the end of the experiment. Full surface coverage and 50% PVI observed in enclosures.

converged a very low level between 0.15 - 0.33 at the middle of the experiment and reached a level between 0.5 - 1.5 at the end, similar to the initial ratios. Mean total grazing zooplankton biomass increased for all treatments through the experiment from a range between 190 - 350 μg DW l^{-1} at the beginning of the experiment to a range between 350 - 700 μg DW l^{-1} at the end of the experiment with no significant difference for treatments (one-way ANOVA, $P > 0.10$ both at the beginning and the end of the experiment; Figure 3.24).

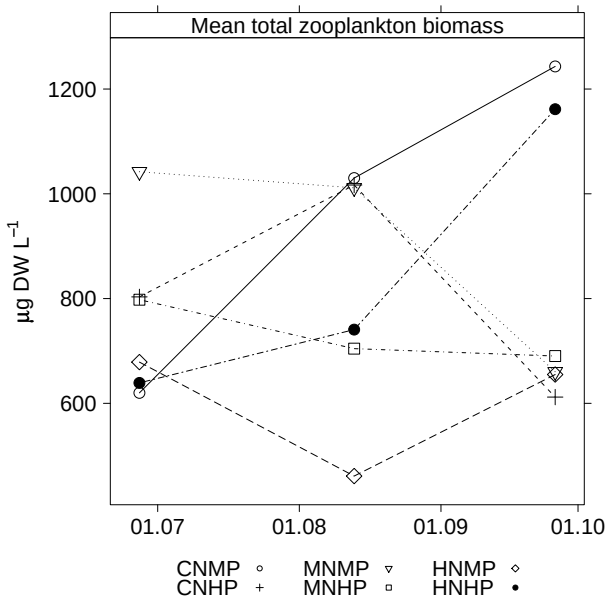

Figure 3.22: Total zooplankton biomass prior to the experiment, at the middle and end of the experiment. For legend details, see Table 2.2.

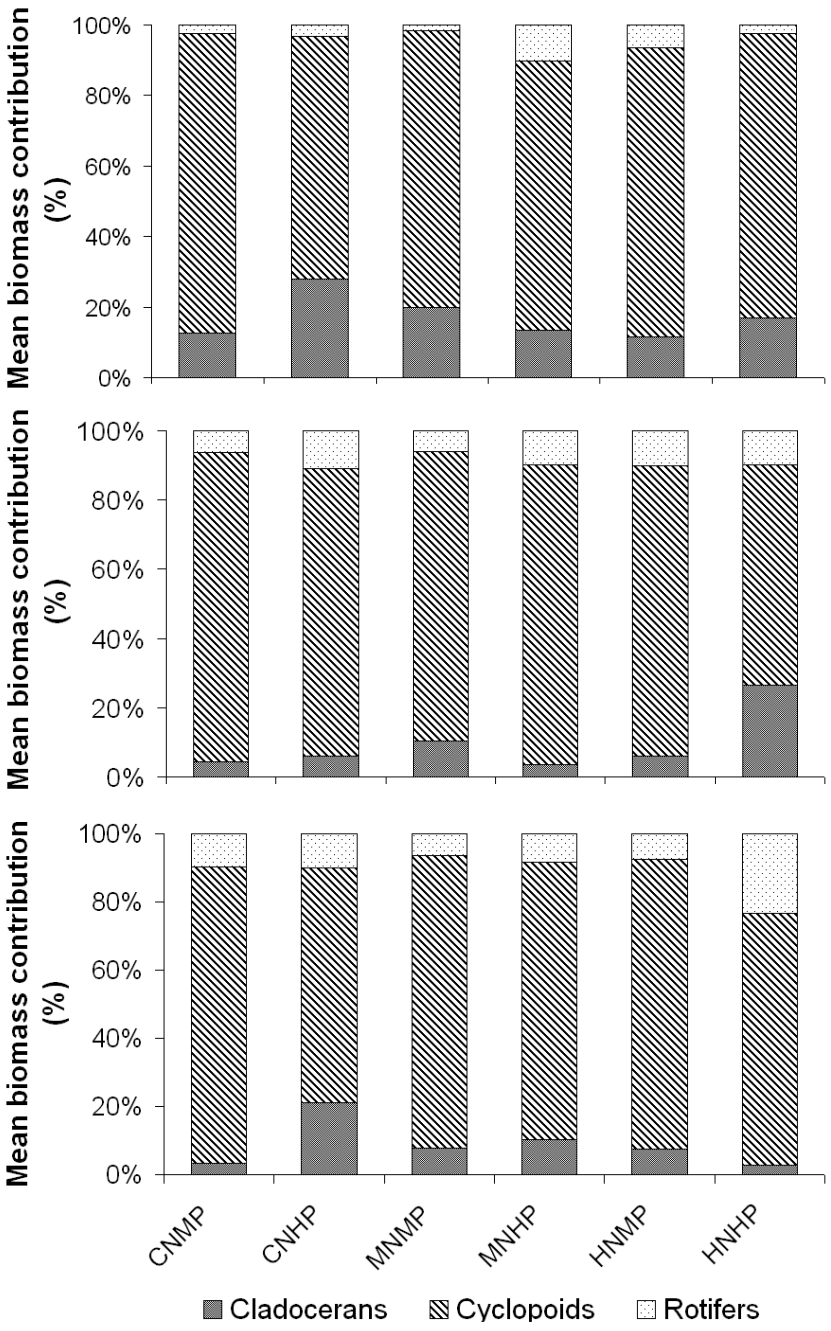

Figure 3.23: Percentage contribution of cyclopoid copepods, rotifers and cladocerans to total zooplankton biomass prior to the experiment (bottom), at the middle (middle) and end (top) of the experiment. For legend details, see Table 2.2.

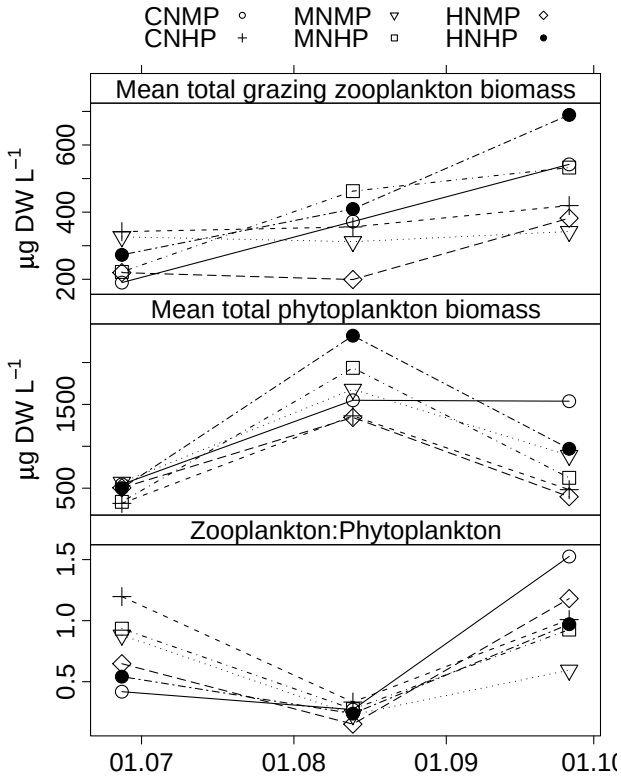

Figure 3.24: Mean total grazing zooplankton biomass (top), mean total phytoplankton biomass (middle) and zooplankton:phytoplankton biomass ratio for the beginning, middle and the end of the experiment. For legend details, see Table 2.2.

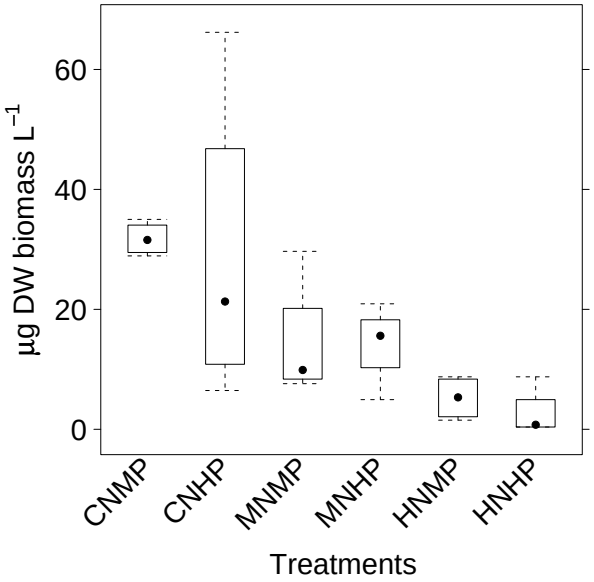

Figure 3.25: Mean *Acroperus harpae* biomass at the end of the experiment. For legend details, see Table 2.2.

Table 3.1: Summary of the results. The means and standard deviations for all samplings (mean ± SD) are given for each treatment . Only the treatment means and SD's at the last sampling are given for variables with * sign. P values for mixed effect models are given for N, P and N×P treatments. P values for one-way ANOVA are given only for N×P treatments. "ns" donates for not significant and "na" donates for not applicable. For legend details, see Table 2.2.

	CNMP	CNHP	MNMP	MNHP	HNMP	HNHP	N	P	N×P
TN	0.53 ± 0.17	0.51 ± 0.18	2.12 ± 1.08	1.86 ± 1.11	8.49 ± 5.96	7.65 ± 5.85	<0.0001	ns	ns
NO_3	0.04 ± 0.04	0.03 ± 0.04	1.11 ± 1.15	0.90 ± 0.96	6.78 ± 5.48	5.54 ± 5.65	<0.0001	ns	ns
NH_4	0.03 ± 0.03	0.03 ± 0.02	0.14 ± 0.13	0.12 ± 0.13	0.21 ± 0.28	0.41 ± 0.42	<0.0001	ns	ns
TP	0.08 ± 0.04	0.13 ± 0.13	0.07 ± 0.04	0.11 ± 0.07	0.06 ± 0.03	0.11 ± 0.08	0.006	<0.0001	ns
SRP	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	ns	0.01	ns
SiO_2	1.66 ± 0.67	1.45 ± 0.74	1.97 ± 0.78	1.80 ± 0.72	1.75 ± 0.90	1.98 ± 0.92	0.01	ns	ns
PVI*	51.79 ± 17.30	54.46 ± 15.62	56.99 ± 23.80	47.50 ± 10.67	49.27 ± 17.48	46.03 ± 8.64	0.09	ns	ns
Total MP*	76.51 ± 24.99	88.40 ± 29.11	81.47 ± 29.23	75.23 ± 15.18	91.90 ± 30.56	62.78 ± 21.92			ns
*M. spicatum**	63.12 ± 11.64	56.50 ± 17.47	67.70 ± 18.73	56.00 ± 24.59	76.86 ± 21.18	46.65 ± 20.62			ns
*C. demersum**	13.06 ± 13.49	31.20 ± 18.05	13.21 ± 13.41	19.22 ± 12.58	13.72 ± 18.85	15.64 ± 11.33			ns
*P. crispus**	0.44 ± 0.44	0.93 ± 0.60	2.22 ± na	0.04 ± na	1.77 ± 1.23	1.93 ± na			ns
Phytoplankton	0.02 ± 0.02	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.02	0.01 ± 0.01	0.02 ± 0.01	ns	ns	0.004
SS	6.86 ± 2.66	5.51 ± 1.94	5.75 ± 2.28	5.88 ± 2.75	4.90 ± 2.26	6.31 ± 2.26	ns	ns	0.03
Periphyton	35.63 ± 19.89	25.65 ± 22.48	41.79 ± 20.11	52.78 ± 28.23	49.38 ± 45.62	76.26 ± 47.03	0.02	ns	0.05
Epiphyton*	0.31 ± 0.02	0.28 ± 0.13	0.50 ± 0.26	0.50 ± 0.21	0.52 ± 0.08	0.92 ± 0.53			0.02
Fil. algea	52.86 ± 30.41	33.57 ± 34.02	57.86 ± 38.23	55.71 ± 43.67	45.00 ± 41.68	50.00 ± 41.28	0.06	ns	ns
Zoo:Phyto*	1.53 ± 1.53	1.01 ± 0.49	0.59 ± 0.41	0.93 ± 0.52	1.18 ± 1.02	0.97 ± 0.91			ns
Salinity	0.10 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.09 ± 0.02	0.11 ± 0.02	0.11 ± 0.02	ns	ns	ns
Conductivity	0.21 ± 0.05	0.19 ± 0.05	0.21 ± 0.05	0.21 ± 0.05	0.23 ± 0.05	0.24 ± 0.04	0.003	ns	0.09
TDS	132.38 ± 14.93	121.21 ± 16.67	132.00 ± 17.29	133.13 ± 22.67	146.75 ± 21.62	151.08 ± 19.56	0.002	ns	ns
Alkalinity	1.41 ± 0.19	1.29 ± 0.21	1.26 ± 0.30	1.27 ± 0.29	1.05 ± 0.30	1.30 ± 0.56	ns	ns	ns
pH	7.77 ± 0.28	8.08 ± 0.49	7.90 ± 0.41	7.98 ± 0.50	8.49 ± 0.60	8.21 ± 0.69	0.004	ns	0.09
Dissolved O2	3.13 ± 2.18	4.75 ± 2.93	3.42 ± 1.90	3.71 ± 2.84	6.68 ± 2.97	5.23 ± 3.87	ns	ns	ns

CHAPTER 4

DISCUSSION & CONCLUSION

4.1 Discussion

Turkey is located between subtropical and temperate zones and subject to Mediterranean climate pattern with hot, dry summers. As a result of this fact, majority of Turkish shallow lakes with exceptions on alpine zone, experience water level fluctuations upto a couple of meters. It is not uncommon for the lakes found in middle Anatolian plateau to disappear totally through the end of some summers. This natural trend became a dramatical catastrophe with unwise water management policies employed in the last half of the 20th century by Turkish authorities. Furthermore, effect of global warming would expected to be disastereous for surface waters as more drier conditions with higher temperatures and evaporation have been anticipated (ITU regional climate models). As the present study aimed to monitor submerged vegetation growth for long enough time to detect possible direct and indirect effects of nutrient enrichment in experimental *mesocosms* throughout the growing season a lake with moderate water level fluctuation should have been employed.

İğneada forest swamp ecosystem (longoz forests) consists of several freswater lakes with moderate water level fluctuations. Throughout an ongoing monitoring study on İğneada lakes we recorded a water level drop of 0.2 - 0.3 m at the end of summer for two subsequent years (Özkan and Beklioğlu, 2007). This was the basic motivation to carry out the experimental *mesocosm* study in Lake Pedina. As Lake Pedina is

away from pronounced human impact and in pristine ecological state with extensive macrophyte coverage, the lake is convenient to test the influence of nutrient loading on submerged macrophyte growth and lake ecosystem dynamics.

However, 2007 summer was exceptionally dry and resulted in 0.6 m water level drop in three and a half months (Figure 3.1). High water temperature might have resulted in early senescence of vegetation, as Lake Pedina was covered with dense and healty macrophyte cover at the end of September 2006, while majority of *T. natans* found in the lake was already decaying at the beginning of September 2007 (one month earlier). Same summer conditions resulted in water level drops in magnitudes of meters in Lakes Mogan and Eymir. Therefore, we managed to employ comperatively moderate water level fluctuation in our region for the experiment. Such an interference may also be regarded as a realistic component of the shallow lake functioning of Turkey.

There may be two possible approaches for nutrient treatments in such experiments. For example, it is possible to determine specific loads for each treatment and apply those doses with fixed amounts and intervals. This aproach may be employed if there is comprehensive knowledge on catchment level processes in specific ecosystems with realistic estimates of nutrient input and resulting ecological conditions in lakes. Unfortunaltely, we lack such data for Turkish wetlands. Rather, we determined a range of nitrogen and phosphorus concentrations that is realistic for natural lakes or lakes under antropogenic pressure in our region and in which we may test a possible response of macrophyte growth. To provide those concentrations in each treatment, we determined the nutrient level in each enclosure after each nutrient addition and added necessary amounts of nutrients to reach the targeted concentrations with a one week lag (monitor and add approach).

Four and ten mgl^{-1} TN concentrations were aimed in MN and HN treatments, respectively while performing nutrient additions. Table 4.1 shows that three distinct levels of TN were achieved in the experiment. Nutrient chemistry samples were taken one week after the last and one day before the following nutrient addition. Therefore, nutrient additions were assimilated for one week in enclosures prior to

Table 4.1: Nitrogen concentrations sustained through and at the end of the experiment (mean ± SD). For legend details, see Table 2.2.

	CN	MN	HN
Through the experiment	0.52 ± 0.17	1.99 ± 1.05	8.07 ± 5.88
At the end of the experiment	0.40 ± 0.09	1.36 ± 0.45	6.48 ± 3.00

the sampling and the lowest nutrient concentrations in the enclosures were recorded at each sampling. Thus, average concentrations sustained in treatmets were to be higher than those lowest concentrations. In conclusion, targeted concentrations were achieved in the experiment for nitrogen treatment (Figure 3.8). On the other hand, 0.10 and 0.25 mgl^{-1} TP concentrations were aimed in the treatments. Although, there was a significant difference between MP and HP treatments, both of them converged close concentrations below 0.10 mgl^{-1} (Figure 3.11). This is a clear indication that phosphorus assimilation rate in enclosures was high enough to override the effect of nutrient additions based on the calculations with targeted values, phosphorus concentrations in water column an aproximate weekly assimilation rate of phosphorus.

Similar nutrient loads to present study was employed in González Sagrario et al. (2005). Nutrient loads of 8.7 mg P, 25 mg N and 127 mg N day^{-1} m^{-2} were applied to experimental mesocosms in González Sagrario et al. (2005) and these nutrient additions achieved nutrient concentrations above 0.3 mgl^{-1} TP, 2 and 4 mgl^{-1} TN, respectively at the end of the experiment . However, nutrient additions of 7 mg P, 70 mg N and 120 mg N day^{-1} m^{-2} in present study, achieved nutrient concentrations below 0.1 mgl^{-1} TP, 2 and 6.5 mgl^{-1} TN, respectively at the end of the experiment. It is apparent that similar nutrient loads both for phosphorus and nitrogen resulted in lower water column nutrient concentrations in subtemperate Lake Pedina, compared to northern temperate Lake Stigsholm. Higher summer nutrient concentrations in Lake Stigsholm with 0.1 mgl^{-1} TP and 2 mgl^{-1} TN compared to lower summer nutrient concentrations in Lake Pedina with 0.03 mgl^{-1} TP and 1.4 mgl^{-1} TN might have been a reason for achieving higher nutrient concentrations in González Sagrario et al. (2005). However, differences in initial

conditions of lakes may not be enough to explain the lower concentrations achieved in Lake Pedina and there might have been higher assimilation rates in Lake Pedina.

İğneada region is historically known for iron beds in its geology (Maden Tetkik Arama, 2008), therefore it is reasonable to speculate about possibility of high iron content in lake sediment. Provided that extensive rooted submerged macrophyte growth occured as in the present study, oxygen transport to the sediment may take place and oxidized conditions in sediment layer and pore water may result in effective binding of phosphorus to available iron. Such an interaction may result in retention of phosphorus in the sediment and may account for the readily assimilation of phosphorus additions (Søndergaard, 2007). Low SS and chl *a* concentrations indicated reasonably good underwater light climate in enclosures (Figure 3.15, Figure 3.14), this thus might have led to an increase in benthic primary prodution. Growing benthic algea might have taken up the nutrients at the sediment surface and decreased the total amount of nutrients reaching water phase. Benthic production might have probably increased oxidation potential in the sediment and enhanced phosphorus retention (Hansson, 1989; Van Luijn et al., 1995; Woodruff et al., 1999).

On the other hand, nitrogen denitrification probably the main factor responsible for the assimilation of nitrogen additions. Undetectable levels of NO_3-N in the enclosures prior to the nutrient additions and aproximately 1:1 ratio of NO_3-N and NH_4-N is a clear indication of very low NO_3 input to the lake and extreme denitrification taking place in enclosures (Wetzel, 1975). Denitrification may take place under oxidized conditions but intensity increases to larger extend under anoxic environments, especially on sediment surface (Vitousek and Howarth, 1991). High macrophyte growth occured in each enclosure might have also enhanced denitrification (Weisner et al., 1994). Oxygen concentrations may fluctuate significantly through the day and night due to photosynthetic and respiratory activities. Such fluctuation in oxygen availability may enhance P assimilation through day, while enhancing denitrification through night (Frodge et al., 1990). High temperatures recorded in enclosures probably enhanced denitrification (Tomaszek and Czerwieniec, 2003), in particular compared to temperate lakes (Talling and Lamolle, 1998). Lastly, probable high biological uptake and sedimentation rates occured in the enclosures might have also

accounted for both nitrogen and phosphorus assimilation.

Both TDS and conductivity were significantly higher in HN treatments (Figure 3.3, Figure 3.4), which is consistent with the fact that additional Na and Ca ions were dissolved in nutrient additions, mostly dominated by nitrogen treatments as nitrogen additions were much greater than phosphorus additions in amount. However, the differences of both TDS and conductivity between nitrogen treatments were small and concentrations for these parameters were compatible with previous observations on Lake Pedina (Table 2.3). Moreover, there was no change in salinity results (3.1). Therefore, no treatment effect is expected due to the ions dissolved with nutrient additions. TDS and conductivity increased slightly for all treatments through time, most probably due to high temperature and evaporation, as CN treatment had the same pattern with no nitrogen addition.

Alkalinity in enclosures were dominated by bicarbonate alkalinity, which is common pattern for majority of fresh water lakes (Wetzel, 1975). However, frequent ocurrances of carbonate alkalinity up to 10% might have been an indication of high intensity photosynthesis observed in enclosures (Wetzel, 1975). Higher pH values for HN treatment was also due to higher photosynthetic activities (Wetzel, 1975) and pH values above 8 for HN treatment was higher than the values observed in Lake Pedina prior to the experiment (Table 2.3). There was a pattern of decrease in pH through time, indicating a seasonal pattern with decreasing photosyntetic acivity and increasing respiratory activity through the end of summer.

Dissolved oxygen concentrations in enclosures had a trend of decrease, which was most probably seasonal, resulting from senescence and increasing respiration (Figure 3.7). Dissolved oxygen concentrations in enclosures were between 2-10 mgl^{-1} and were not limiting for aerobic respiration for aquatic life; although, singular measurements with biweekly periods on dissolved oxygen were not adequate for understanding daily cycles.

This three month study of dual nutrient addition revealed no significant effect on macrophyte development. Submerged macrophytes extensively grew in all the treatments with a minimum average biomass of 60 DW m^{-2} in dual HNHP treatment

(Figure 3.20). Moreover, all of three species of submerged macrophytes found in the lake prior to the study were recorded in majority of enclosures. In addition to stocked *M. spicatum*; *C. demersum* recorded in all enclosures other than one and *P. crispus* recorded at least in one enclosure for each treatment with smaller biomassses and a disposition for being more frequent in the low nutrient treatments.

Present study seems to fail to validate the findings of Barker et al. (2008) as they have found a decline in species richness with increasing nitrogen loading in a two year *mesocosm* experiment in England. They sustained four stable TN concentrations (1, 2, 5 and 10 mgl^{-1}) with a TP concentration centered around 0.05 mgl^{-1} for all treatments. However, the rooted macrophytes included in present study were also included in Barker et al. (2008) (*M. spicatum*, *C. demersum* and *Potomageton sp.*) and remained indifferent to nutrient treatments through 2 year period. Species from *Chara*, *Enteromorpha* and *Elodea* genus were the responding macrophytes to nutrient additions resulted in species richness decline. Therefore, the results of the present study is compatible with the findings of Barker et al. (2008) for rooted macrophytes and for the first year of their experiment, after which they have found significant response of macrophyte.

There may be direct and indirect influence of nutrient treatments on submerged macrophyte community. High nutrient availabilty in water column may enhance macrophyte growth but nutrient limitation is not intense for rooted macrophytes as they have a reach of relatively abundant nutrient resources from the sediment (Moss, 1998). However, nitrogen limitation may be likely for macrophytes as high rates of denitrification may result in available nitrogen deficiency in sediment (Hameed et al., 1999; Vitousek and Howarth, 1991; Moss, 1998). Contrary, phytoplankton and periphyton are bound to the nutrient availability in water column. The indirect effect of nutrient treatments on submerged macrophyte development is caused by increasing abundances of phytoplankton and periphyton and resulting shading effect (Jeppesen, 1998). This indirect effect can be intense and might result in total macrophyte loss in a lake.

However, top-down effects cascading through the lake ecosystem may prevent phy-

toplankton or periphyton from responding to nutrient additions. High zooplankton density dominated by large-sized cladocerans may exert high grazing pressure and thus they may keep the phytoplankton density low (Jeppesen et al., 1997a). This in turn may override the effect of nutrients on phytoplankton community. However, zooplankton communities are composed of small-sized members and do not have strong control over phytoplankton abundance in (sub)tropical ecosystems due to abundant fish population dominated by small size classes even in high macrophyte densities (Meerhoff et al., 2006a, 2007).

Zooplankton community in all treatments in present experiment were dominated by cyclopoida and smaller members of other groups, excluding the larger cladocerans; most probably due to the high fish density stocked inside the enclosures. The zooplankton : phytoplankton ratio was estimated below one and even lower through the middle of the experiment. The ratio in present study was relatively high compared to ratios derived in Danish lakes with low zooplankton grazing pressure on phytoplankton community (Jeppesen, 1998). However, lack of large cladocerans and abundant cyclopoid copepods in addition to zooplankton:phytoplankton ratios below one implied that there was no strong zooplankton control over phytoplankton community. Thus lack of strong top-down control on phytoplankton enables us to assess the direct and indirect effect of nutrient treatments on primary producers and their interactions.

There was a negative relation between *A. harpae* and nutrient additions at the end of the experiment. *A. harpae* is known to be associated with submerged macrophytes (Alonzo, 1991). Although there is no significant difference in submerged macrophyte biomass for treatments at the end of the experiment, this decline may be an implication of a possible qualty loss in macrophytes as a habitat for *A. harpae*.

There was a significant response of epiphyton chl *a* to treatments. The average epiphyton biomass (chl *a*) for HNHP treatment was twice as much as the others (Figure 3.17). Water level drop through the experiment affected the sample quality of periphyton abundance as the strips hung down water column with an attached weight and especially through the end of the experiment the strips were not kept

straight in the water column. Thus they twisted around and this in turn created mechanical damage to periphyton film. However, the trend in epiphyton abundance was also recorded for periphyton, as average values for HNHP treatment were always higher than the others and periphyton abundance differed significantly for nitrogen treatments (Figure 3.16). Both periphyton an epiphyton abundance shows that the dual treatment for HNHP resulted in a significant increase in periphyton abundance.

Periphyton abundance in Barker et al. (2008) fluctuated in a range of 0 - 150 mg chl *a* m^{-2} in two year duration and these are in accordance with the periphyton abundance observed in the present study, as the nitrogen and phosphorus concentrations employed in both of the studies were similar. Vadeboncoeur et al. (2006) compiled periphyton biomass data on natural hard substrata in three geographic regions. These lakes had TP concentrations in a range of 0 - 0.04 mgl^{-1} and periphyton abundance in a range of 0 - 100 mg chl *a* m^{-2}. Periphyton biomass in the present study was at the highest TP range for HNHP treatment with an average of 120 mg chl *a* m^{-2} at the third sampling compared to Vadeboncoeur et al. (2006). Periphyton abundance observed through the course of the experiment were similar to the concentrations observed in Vadeboncoeur et al. (2006). (Vadeboncoeur et al., 2006) also recorded epiphyton abundance on *M. spicatum* in Lake Memphremagog as 0.184 μg chl *a* per mg DW macrophyte. The abundance of epiphyton in Lake Memphremagog was lower than the control treatments of the present study and the mean of HNHP treatment quadrupled it (Figure 4.1).

On the other hand; Liboriussen and Jeppesen (2006) introduced artificial substrata for periphyton colonization in 13 lakes with a TP range of 0.01 - 0.54 mgl^{-1}. They found a peak in periphyton biomass in a range of 0.05 - 0.28 mgl^{-1} TP with a maximum periphyton abundance around 100 mg chl *a* m^{-2}. They found significant relationships between TP and periphyton abundance at 0.1 m depth after eight weeks of incubation and at 0.5 m depth after 13 weeks of incubation. When these regression were used to estimate periphyton abundance for mean TP concentrations of MP and HP treatments in the present study (0.055 and 0.072 mgl^{-1} TP, respectively), estimated periphyton abundances with these regressions were 9.9, 11.8 mg chl *a* m^{-2} at 0,1 m depth for eight week incubation and 37.9, 45.4 mg chl *a* m^{-2} at 0.5 m

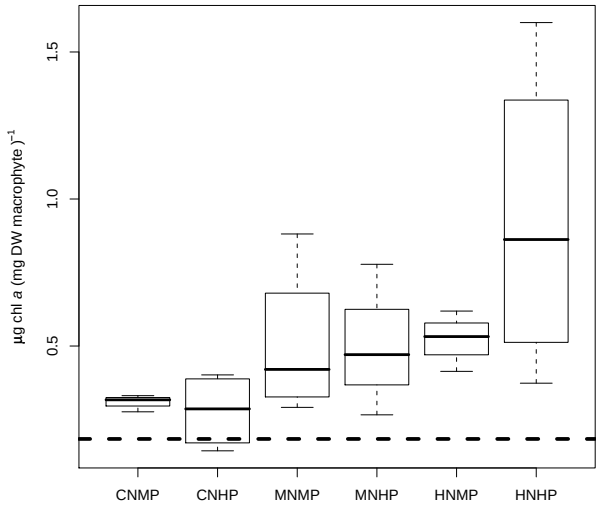

Figure 4.1: Epiphyton concentrations in treatments compared to Lake Memphremagog. Dashed line represents the epiphyton concentration found in Lake Memphremagog.For legend details, see Table 2.2.

depth for 13 week incubation, for MP and HP treatments, respectively. Periphyton abundances in the present study both for the third sampling and through the course of the experiment were higher than the estimated concentrations derived from these regressions (Figure 4.2).

In another study, Liboriussen et al. (2005a) recorded periphyton biomasses up to 200 and 300 mg chl *a* m^{-2} on artificial substratum in two lakes with mean summer TP of 0.102 and 0.421 mgl^{-1} and TN of 1.95 and 1.52 mgl^{-1}, respectively. The results in Liboriussen et al. (2005a) were higher than the biomasses observed in this study probably owing to the high TP concentrations found in the study lakes.

In contrast to periphyton abundance, phytoplankton chl *a* concentrations in water column did not show any strong response to the treatments although they differed significantly for NP treatments (Figure 3.14). Phytoplankton abundance increased with nutrient treatments up to 0.04 mgl^{-1} and then dropped to the beginning conditions through the end of the experiment in September, suggesting a seasonal effect. A similar result was observed in Barker et al. (2008) at the first year of their study; however, phytoplankton abundance differed significantly in the following

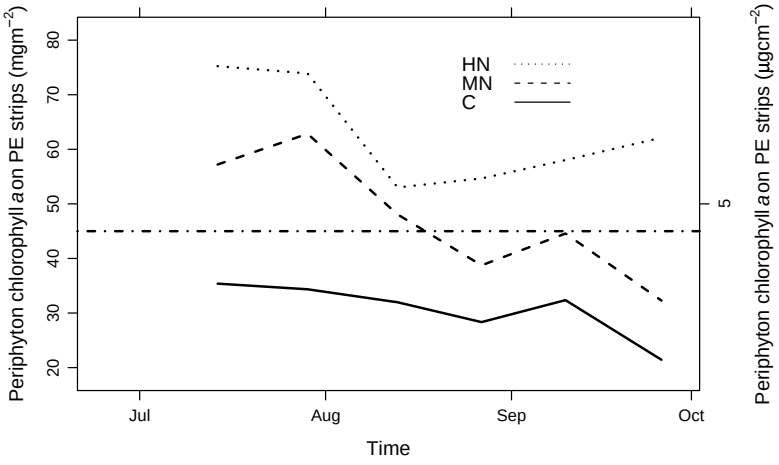

Figure 4.2: Periphyton concentrations in N treatments compared to concentrations observed in Liboriussen and Jeppesen (2006). Dashed line represents the periphyton chl a concentration derived from the regression equation (13 week inqubation of PE strips at 0.5 m depth in 13 lakes) for HP treatment mean TP concentration (0.072 mgl^{-1}). For legend details, see Table 2.2.

year.

Using the regression equation derived for the interaction between phytoplankton chl *a* and TP found for 13 temperate lakes in Liboriussen and Jeppesen (2006) for our MP and HP treatment TP means (0.055 and 0.072 mgl^{-1} TP, respectively) estimated phytoplankton concentrations of 59.6 and 78.4 mgl^{-1} chl *a*, respectively. The chl *a* concentrations in the present study were lower than estimations based on TP and phytoplankton chl *a* regression in Liboriussen and Jeppesen (2006).

Bécares et al. (2007) compiled data on several *mesocosm* experiments conducted in a lattitudinal gradient through western Europe for macrophyte, phytoplankton and periphyton growth and interactions among them. They found a clear lattitudinal gradient as northern lakes had higher periphyton abundance and lower phytoplankton concentrations while southern lakes had the opposite. They also estimated the critical levels of TP, phytoplankton and periphyton concentrations for 50% reduction in macrophyte biomass. A range of 0.27-0.90 mgl^{-1} TP was estimated for 50% macrophyte reduction in study lakes with a lattitudinal gradient, while southern Spain had the highest concentration. A range of 0.03-0.15 mgl^{-1} chl

a was estimated for 50% macrophyte reduction in study lakes with a lattitudinal gradient, while southern Spain had the highest concentration again. On the other hand, a range of 5-92 mg chl *a* m^2 periphyton abundance was estimated for 50% macrophyte reduction in study lakes without a lattitudinal gradient, while southern Spain had one of the lowest concentrations. The TP concentrations estimated for 50% macrophyte reduction in Bécares et al. (2007) was higher than the concentrations sustained in present study. Present study had chl *a* concentrations at the lower end of the range estimated for 50% macrophyte reduction in Bécares et al. (2007) with no apparent effect on macrophyte growth. Moreover, periphyton chl *a* concentrations observed in present study match with the range of estimations for 50% macrophyte reduction, although no macrophyte response was recorded. In conclusion results of present study is similar to the results of southern Mediterranean lakes in Bécares et al. (2007) with high macrophyte resilience against nutrient additions. However, the main source of turbidity was periphyton in present study in contrast to phytoplankton in southern Mediterranean lakes.

These comparisons imply that periphyton and epiphyton abundances in present study are in accordance with or relatively high than the observed concentrations on various lakes, while phytoplankton abundances remained low for the corresponding TP concentrations. This implies that periphyton may have outcompeted phytoplankton by removing the nutrients from the water column and suppresed a possible responce from phytplankton chl *a*. Moreover, periphyton and epiphyton abundances increased with increasing nitrogen availability for moderate levels of phosphorus, which is an indication of nitrogen limitation on periphyton community (Figure 4.2).

The macrophytes stocked or spontaneously grew in the enclosures were rooted macrophytes which have an access to nutrients in the sediment. Therefore, no strong direct effect was planned in the experiment. Periphyton was the main factor leading to turbidity through the course of the experiment. However, there was no significant implication for an indirect effect of periphyton or epiphyton growth on macrophyte development by shading in this study. This may be consistent with the observations on southern lakes as having a more persistent macrophyte community to turbidity (Bécares et al., 2007).

Water level drop observed through the experiment might have had positive effect on submerged macrophyte growth in the enclosures. It is probable that low water depth might have increased the light availability experienced by submerged macrophytes as a positive effect on their development (Beklioglu et al., 2006; Bécares et al., 2007). Low water depth might have also increased the interaction between the water colunm and sediment, which could increase the internal loading (Romo et al., 2005). However, the TP levels prior to the experiment was lower than the treatments and no effective P release from the sediment was expected. Low chl *a* and SS concentrations also indicated good underwater light climate. High periphyton biomass and spontaneous occurrance of filamentous algea were two important stress factors on macrophyte growth in present study; however, their effect was insignificant.

It should also be noted that the nutrient treatments were applied in enclosures stocked with healthy *M. spicatum* upto 7% PVI. Therefore, the effects of the nutrients were tested among primary producers in an environment containing already grown macrophytes. This might have increased the resistance capacity of macrophytes against the shading effect of phytoplankton or periphyton and increased their competitive capacity over other primary producers. However, macrophytes might have had experienced difficulty to regenerate at the beginning of the season, if the nutrient treatments had been applied well before the beginning of the growth of submerged macrophytes. Phytoplankton may start to proliferate prior to the submerged macrophytes in early spring, use the available nutrients effectively and prevent macrophyte or periphyton growth by shading as submerged macrophytes may be prone to adverse effects while regenerating. Under such circumstances phytoplankton may reach and sustain higher abundances in water column and effectively suppress macrophyte and associated periphyton growth.

Barker et al. (2008) found no significant response of phytoplankton, periphyton or macrophyte, while recording continuous growth of macrophytes with lower phytoplankton abundance in the first year of their experiment with pre-stocked submerged macrophytes. In contrast, they found significant response in all three primary producers in the consequent year. Although rooted macrophytes remained

indifferent in the second year, total PVI was lower in higher nutrient treatments probably because phytoplankton might have gained competitive advantage over other primary producers. Thus, it is possible to observe no significant adverse effect of nutrient additions on stocked submerged macrophytes, while observing pronounced response of submerged macrophytes if they are to regenerate in pre-treated enclosures.

Longer plant growing-season, higher light intensities and temperature and strong water level fluctuations are the characteristics of Mediterranean lakes and lead to higher resilience capacity in macrophytes against higher turbidities and nutrient concentrations than northern temperate lakes (Bécares et al., 2007). Those mechanisms were possibly functioning in the enclosures as macrophytes remained indifferent to increasing nutrients and high periphyton abundance.

4.2 Conclusion

A *mesocosm* experiment was performed in Lake Pedina, Turkey in 2007 summer to elucidate the effect of increasing nitrogen concentrations with moderate phosphorus availability on macrophyte development. Enclosures were kept open to sediment and atmosphere and stocked with high densities of fish to provide necessary ecological conditions for assesing the direct and indirect effects of nutrient treatments on and among primary producers. Healthy *M. spicatum* shoots were stocked inside the enclosured prior to nutrient treatments. High temperatures and evaporation resulted in a water level drop of 0.6 m throughout the three and a half month duration of the study, which had some influence on sampling eficiency and observed parameters. Three distict levels of concentrations were achieved for nitrogen treatment throughout the study; whereas, phosphorus treatments failed to reach the aimed concetration levels and converged to close concentrations. In comparison to other studies, nutrient additons resulted in lower nutrient concentrations in water column, indicating higher assimilation rates of nutrients in Lake Pedina.

Total macrophyte biomass had a strong pattern of increase and two more sub-

merged macrophyte found in the lake previously spontaneously grew in majority of enclosures in addition to stocked *M. spicatum*. Submerged macrophyte biomass did not significantly respond to treatments and failed to validate any direct or indirect effect of increasing nutrient concentrations upto a mean level of 8 and 0.07 mgl^{-1} for TN and TP respectively. In comparison to other studies phytoplankton reached lower and periphyton reached higher abundances for referance TP concentrations, indicating a competitive advantage of periphyton over phytoplankton on nutrient utilization. Periphyton abundance differed significantly only for nitrogen treatment with a positive relationship, revealing a possible nitrogen limitation on periphyton for given phosphorus concentration. Pre-stocked, rooted macrophytes were indifferent to nutrient treatments and resistant to indirect effects over periphyton and phytoplankton in present study. However, it should also be noted that they might be still prone to possible effects of increasing nitrogen concentrations in their regenerative stage at the beginning of growing season.

Global warming predictions state an increase in denitrification and assimilation of nitrogen resulting in decreasing nitrogen load on lakes for our region, which may increase the resistance and resiliance capacity of macrophyte communities to direct and indirect effects of nutrient loading. However, hotter and drier climatic predictions for south of 45° nitrogen may result in cascading effects on lake ecosystems by changing the abundance and periodicity of water. The possible overriding effects of such changes is still in debate and may complicate the situation for macrophyte communities by alterating water level fluctuations, major ion balance and intrenal nutrient recycling in lakes.

4.3 Perspectives

Water level fluctuation is an important component of shallow lake functioning in our region. However, its influence on the dynamics of primary producers is not clear. Apparently, more research on the effects of water level fluctuation especially on submerged macrophyte growth would fill an important gap. Present study found high submerged macrophyte resistance to possible adverse effects of phytoplankton

and periphyton growth under increasing nutrient concentrations. However, another experiment incorporating the effect of similar nutrient addition on both stocked and naturally regenerating submerged macrophte over a duration from early spring to late autumn would be appropriate. Futhermore, such experiment over two growth seasons may reveal the effect of nutrient addition better on submerged macrophyte development, especially on the second year.

There are also several methodological points arose in present experiment for similar studies:

- More care should be taken for better management of water level fluctuation in *in situ mesocosm* experiments in our region. Choosing deeper sites for construction or employing *ex situ mesocosms* could be an option.
- Periphyton abundance on hard substrate should be sampled with a setup independent of water level. Floating sampling surfaces for different water depths could be employed.
- Secchi disc has no use in small *mesocosms* to assess water clarity without disturbance. Making use of a turbidity meter or downward light measurements may provide better assessment.
- Nylon net became insufficient to prevent water snakes that may also predate fish in enclosures. Another method with a better fit should be seeked.
- PVC ring and attached bricks at the lower end of the enclosures need someone working in water for a proper setup. Premanifactured metal rings may provide the necessary sink with easier manipulation.

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