IMPACTS OF NUTRIENTS ON PERIPHYTON GROWTH AND PERIPHYTON-MACROINVERTEBRATES INTERACTIONS IN SHALLOW LAKES: A MESOCOSM EXPERIMENT

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ABSTRACT

IMPACTS OF NUTRIENTS ON PERIPHYTON GROWTH AND PERIPHYTON-MACROINVERTEBRATES INTERACTIONS IN SHALLOW LAKES: A MESOCOSM EXPERIMENT

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Periphyton biomass on artificial strips was observed monthly to see the impacts of nutrient differences on periphyton and periphyton-macroinvertebrates interaction. The experiment was conducted for four months in a mesocosm which were runned at six countries at the same time and with the same steps. Eight enclosures at two meters depth were used that four of them had high nutrient level and the other four had low nutrient level. Sediment, macrophyte, fish, plankton, benthic invertebrates and water were added at the same time and with the same way in all of the countries. Periphyton growth which formed on artificial 32 cm^2 strips for June, July, August and September were brushed to filtered mesocosm water and dry mass, ash free dry mass, phosphorus content and chlorophyl-a concentrations were measured. Grazer pressure on the periphyton was observed with a laboratory experiment for July, August and

September months. At the end of the mesocosm experiment macrophytes and fish were harvested. Macrophytes' dry mass and fish' abundance were measured. Moreover at the end of the experiment epiphyton was also measured. Three kajak cores were taken from sediment for macroinvertebrates at the end of the experiment and identified. All physical features of mesocosm enclosures and PVI data were recorded for every 2 weeks.

Periphyton biomass was higher concentrations in HN enclosures than LN tanks. Only dry mass of periphyton biomass showed the opposite because of the marl deposition in LN tanks. This finding was also reinforced by epiphyton samples which was taken at the end of the experiment. LN enclosures had the more abundance of macroinvertebrate. The groups we found in sediment which had big grazer effect on periphyton such as gastropods and Chironomidae. Grazer experiment showed that grazer effect on periphyton increased in time. Although this raise, periphyton growth also increased in LN enclosures with nutrient increasing. This may be indicate that nutrient effect has a stronger effect than grazer pressure on periphyton.

As it is explained before in the beginning of the experiment all of the conditions were the same except nutrient level. Thus, bottom-up effect changed the top-down control and at the end of the experiment we saw the more periphyton less macroinvertebrate and more fish in HN tanks while the opposite was seen in LN tanks.

Keywords: periphyton, nutrient, grazing pressure, top-down bottom-up control, mesocosm

ÖZ

SIĞ GÖLLERDE BESİN TUZUNUN PERİFİTON VE PERİFİTON-MAKROOMURGASIZ ETKİLEŞİMLERİ ÜZERİNE ETKİSİ: MEZOKOZM DENEYİ

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Bu çalışmada yapay substrat üzerindeki perifiton gelişimi besin tuzu farklılıklarının perifiton gelişimi ve perifiton-makroomurgasız etkileşimi üzerindeki etkisini görebilmek amacıyla incelendi. Deney aylık olarak dört ay boyunca altı ülkede aynı anda başlayan ve aynı adımlarla devam eden bir mezokozmda gerçekleştirildi. Deney için dört tanesi yüksek besin tuzuna geriye kalan dört tanesi ise düşük besin tuzuna sahip iki metre derinliğindeki sekiz adet tank kullanıldı. Dip çamuru, su içi bitkisi, balık, plankton, bentik omurgasızları ve su her ülkede aynı zamanlarda aynı şekilde eklendi. Haziran, Temmuz, Ağustos ve Eylül aylarında 32 cm^2'lik yapay şeritler üzerinde oluşan perifiton filtre edilmiş mezokozm suyuna fırçalanarak kuru ağırlık, organik madde kuru ağırlığı, fosfor içeriği ve klorofil-*a* derişimi ölçüldü. Perifiton üzerindeki avlanma baskısı Temmuz, Ağustos ve Eylül aylarında bir laboratuvar deneyiyle gözlemlendi. Mezokozm deneyinin sonunda bitki ve balıklar toplandı. Bitki kuru ağırlığı ve balık miktarı hesaplandı. Ayrıca deney sonunda epifiton

miktarı da ölçüldü. Makroomurgasızlar için kajak koru ile üç adet çamur örneği alındı ve sınıflandırma yapıldı. İki haftalık sürelerle tankların fiziksel özellikleri kaydedildi ve PVI değerleri hesaplandı.

Perifiton miktarı yüksek besin tuzu derişimli tanklarda daha fazlaydı. Düşük besin tuzu derişimli tanklarda meydana gelen marl oluşumu yüzünden sadece perifiton kuru ağırlığı bunun tersi bir sonuç gösterdi. Bu bulgular deney sonunda bitkiden alınan epifiton örnekleriyle de pekiştirildi. Makroomurgasızlar LN tanklarında daha fazla miktarda bulundu. Gastropod ve Chironomidae gibi bulduğumuz gruplar perifiton üzerinde oldukça fazla avlanma etkisine sahip gruplardı. Laboratuvarda yapılan avlanma deneyi ise avlanmanın zaman içerisinde arttığını gösterdi. Fakat bu artışa rağmen son ayda LN tanklarında artan besin tuzu ile birlikte perifiton artışı görüldü. Bu belki de perifiton üzerinde besin tuzu etkisinin avlanma etkisinden daha büyük olduğunu göstermektedir.

Daha önce açıklandığı üzere deney başlangıcında besin tuzu miktarı hariç tüm koşullar aynı idi. Başlangıçta aynı olan yukardan aşağı kontrol, besin tuzunun aşağıdan yukarı etkisi ile deney sonunda değişmiştir ve HN tanklarda daha çok balık, daha az omurgasız ve daha çok perifiton görürken, LN tanklarda tam tersini gözlemledik.

Anahtar Kelimeler: perifiton, besin tuzu, otlanma baskısı, yukarıdan aşağıya aşağıdan yukarıya kontrol, mezokozm

To the 2 precious families;
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AFDM	Ash free dry mass
AM	Ash mass
ANOVA	Analysis of variance
ASS	Alternative stable state
Chl-*a*	Chlorophyll-*a*
DM	Dry mass
DO	Dissolved oxygen
HN	High nutrient
LN	Low nutrient
N	Nitrogen
NH_4	Amonia
NO_3-NO_2	Nitrite - Nitrate
P	Phosphorus
PVI	Plant volume inhabited
Rm-ANOVA	Repeated measure of ANOVA
S/W	Secchi disk depth/Water level
SRP	Soleble reactive phosphorus
SS	Suspended solids
TA	Alkalinity
TDS	Total dissolved solids
TN	Total nitrogen
TP	Total phosphorus

CHAPTER 1

INTRODUCTION

1.1 Shallow Lakes and Role of Periphyton

Although freshwaters consist a really small portion, approximately 0.01 %, of the world water resources (Dudgeon *et al.*, 2006; Wetzel, 2001), this tiny portion has an essential role for all organisms via rich biodiversity and habitats (Bailey *et al.*, 2004; Naiman *et al.*, 1995). Moreover freshwaters have fundamental places in our lives in terms of providing us many goods, materials and services. Lakes with rivers and wetlands are estimated to comprise over 25% of the total requirements of human societies and survival (Constanza *et al.*, 1997).

Considering the lakes in two main groups as shallow and deep, it is seen that shallow lakes have not had the scientific attention until the second half of the 1980s as much as deep lakes. Deep lakes had the concentration of freshwater ecology with their large basins containing a considerable volume of freshwater and thermal stratification during summer (Wetzel, 2001; Meerhoff, 2010). However almost 95% of world freshwater source is small (surface area <1 km) and relatively shallow (mean depth <10 m) (Moss, 2010; Wetzel, 2001).

In contrast to deep lakes, shallow lakes have wider littoral zones with dense submerged macrophytes and usually do not have thermal stratification

(Jeppesen *et al.*, 1998). They have a larger littoral area for sediment-water coupling which serve a rich habitat for organisms (Scheffer, 1998). Their depths are sufficiently shallow to permit the light penetration from surface to the bottom and reinforce photosynthesis of aquatic plants over the entire column (Wetzel, 2001). Moreover they have a higher overall productivity of organisms (Downing *et al.*, 1990; Gasith and Hoyer, 1998).

Philips *et al.* (1978) revealed that nutrient amount changes linearly with turbid water state and there is one possible community structure which is either phytoplankton dominated clear water conditions or macrophyte dominated turbid water conditions. However this hypothesis conflict with some observations. Scheffer *et al.* (1993, 2001) showed that many ecosystems may comprise more than one structures which are both phytoplankton dominated turbid water state and macrophyte dominated clear water states and also switches between those conditions based on stochastic events mediated by some buffer mechanisms. These switchs named by alternative stable state (ASS).

Figure 1.1 (Jones and Sayer 2003) showed the phytoplankton or macrophyte dominated communities and the alternatives between them. Under low nutrient conditions the lake will be macrophyte dominated, with increasing nutrients plant loss may not be seen and this lead to the alternative equilibria. However when the nutrient levels reach a threshold it will result with plant loss eventually and lake will be phytoplankton dominated.

Periphyton refers to the entire community of sessile or fixed organisms on any hard substrata (Azim *et al.*, 2005). Van Dam *et al.*, (2002) defined it as composing of attached plant and animal organisms embedded in a mucopolysaccharide matrix. 'Attached algae' or 'attached microorganisms' are used by some authors as well however these terms are unsufficient to explain the many other forms that lived on periphyton community. Moreover some synonyms are used for periphyton based on the substrates (e.g epiphyton for aquatic plant, epipelon for sediment, epixylon for wood etc., Azim *et al.*, 2005; Goldsborough, 2005) In this study, the term periphyton is used to refer to the total complex of attached aquatic biota on plastic substrates.

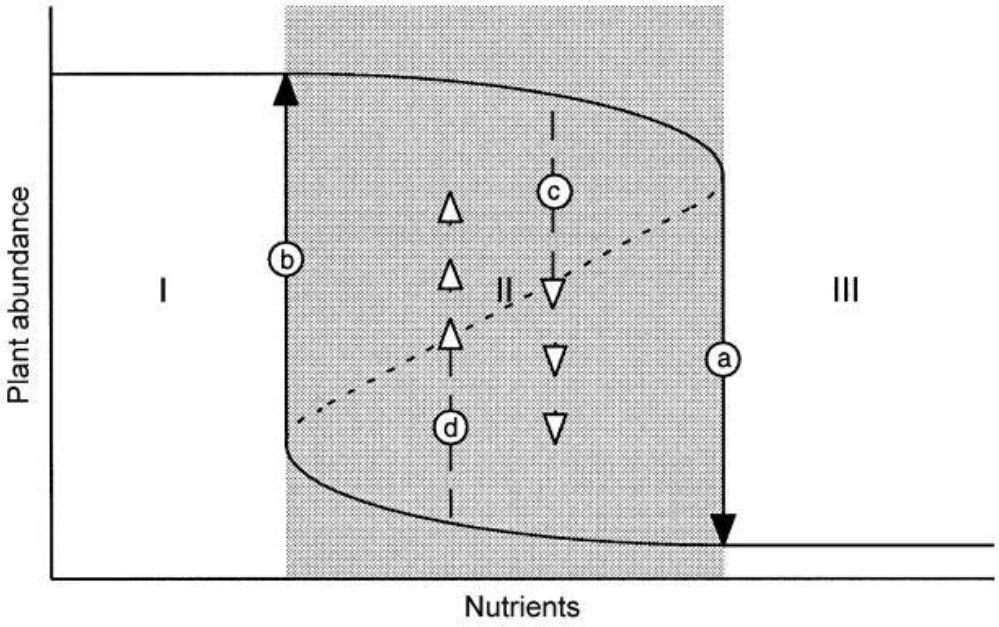


Figure 1.1: Altervative stable state; Response of plant abundance to nutrient availability. One community state is exist under low and high nutrient conditions; plants dominated (I) or phytoplankton dominated (III). Between these conditions (II) alternative communities are possible; the dashed line illustrates an unstable equilibrium returning either to plant or phytoplankton dominance. Two routes are possible for shifts between these alternative states (c, d). Until a threshold plants can maintain at high abundance under increasing nutrients, any later increase will inescapably result in plant loss (a). If nutrients are subsequently removed the community can reach the alternative equilibria and plants inevitably return (b), (Jones and Sayer, 2003).

Basically periphyton is formed by plankton. Therefore the morphology of periphyton resembles plankton morphology but with additional features inherently that make periphyton attach to substrata and adapt to periphytic environment. There are different adaptations changing with different taxonomic compositon such as stalks with sticky ends, sticky capsules, muscular suction pads, glue or simply clinging to the substrate (Reid and Wood, 1976). The size of periphyton structure can be a single cell and also can be 60 m in length. Cells forming the periphyton can be of various types such as non-motile or motile, uni or multi-cellular and filamentous structures (Hoagland *et al.*, 1982). Hudon and Bourget (1981) classified to three groups the periphyton

morphology according to their movement abilities while Maltais and Vincent (1997) classified to four groups based on their colours and growth form.

Periphyton colonization starts by bacteria rapidly within hours. Organic matrix of bacteria make attachment of algae easier. Earliest algal colonization cells excreted musilage then organized into pads, stalks with contrubition of other organisms (Goldsborough, 2005). Later chemical and physical factors developed the assemblage and make it denser. In moving water, dislocation is prevented by musilage connection (Peterson, 1987). At some level the assemblage of periphyton reaches to a point that it dissociates and a process of secondary succession starts (Goldsborough, 2005).

As it is said before periphyton structure is formed by not only algae but also animal organisms. There are findings indicating that heterotrophic component of periphyton is greater than the autotrophic component. However heterotrophic ingredient has less attention and most of the studies explains the taxonomic diversity of algae. Cyanobacteria (*Cyanophyta*), diatoms (*Bacillariophyta*) and green algae (*Chlorophyta*) are frequently encountered groups in studies. Even further algae diversity of periphyton can be limited to only diatom species in some researches (Azim *et al.*, 2005), since periphytic communities are often dominated by diatoms (Vermaat, 2005).

There are plenty of reasons which make periphyton important for wetlands. Firstly; periphyton structure affects nutrient and carbon cycling significantly because it is an important contributor to nutrient and carbon fixation. Periphyton is also important for food web structure of lakes because it influences invertebrate compositions via providing food for them and their larvae (Boston and Hill, 1991). Besides it is easy grazed by small and macro invertebrates and fish (Azim *et al.*, 2005). Second; periphyton is used as an indicator which can show us the changing in aquatic enviroments. For example; some diatom species in periphyton structure can be an indicator for physical changes in wetlands such as acidity, oxygen amount (Van Dam at. al., 1994; Sharifi and Gafori, 2005) or phosphorus content of periphyton can be used for determining the phosphorus level of wetlands (Gaiser *et al.*, 2004).

Thirdly; periphyton is used for treating freshwaters and improve the water quality (Azim *et al.*, 2005). Milstein (2005) explains that introduction of hard substrates to different habitats is resulted with periphyton development. This enhances production of species and affects water quality. Periphyton is also used for fish production management in fish ponds (e. g. van Dam *et al.*, 2002; van Dam and Verdegem, 2005; e. g. Azim 2004) or natural waters (Welcomme R. L., 2005).

Lastly periphyton community contribute to primary production even as big as phytoplankton do especially in lakes which are shallow and with large littoral zones (Liboriussen and Jeppesen, 2003; Goldsborough and Robinson, 1996). However it is generally taught that phytoplankton has the most important portion for primary productivity. Researchs show that the significant and often dominant contributers to the primary production are macrophytes and periphyton (Loeb *et al.*, 1983; Azim, 2001; Eminson ve Moss, 2007). Studies in arctic, temperate and tropical regions show that periphyton is an important contributor not only to primary production but also to higher trophic levels (Hecky and Hesslein, 1995). Unfortunately there are not many studies on perihyton-based food web (Lowe, 1996; Vadeboncoeur *et al.*, 2002; Azim *et al.*, 2005) substantially the reason for that a number of methodological problems (Goldsborough *et al.*, 2005). According to Vadeboncoeur *et al.* (2002) from 91% of 193 studies measured only phytoplankton productivity, 4.5% measured only periphyton productivity while 4.5% measured both of them. Moreover there are wide studies on plant productivity but less is known about periphyton effects to those systems (Goldsbourgh *et al.*, 2005; Hecky and Hesslein, 1995).

1.2 Factors Affecting Periphyton

Abundance, diversity and productivity of periphytic community are affected by several abiotic and biotic features. Understanding of the factors which contribute to periphyton structure is critical for a full consideration of aquatic ecosystem function. Here light, temperature, depth, water level and nutrient in

abiotic factors will take place and as biotic factors grazer effect and macrophyte will be explained.

1.2.1 Abiotic Factors

Even though light had no important effect on abundance of periphyton, different light intensities form different taxa in periphyton structure (Vermaat J. E., 1995). For example if sufficient light is available, community will be microalgae dominated. If there is light penetration, periphytic community will be heterotroph dominated (Goldsborough, 1993). Although in very low light regimes (12 µmol) periphytic growth is minimal (Hill and Fanta, 2008), periphyton structure can support high irradiance (800 µmol) exposure (Nofdianto, 2010). Photosynthesis occurs at levels far below maximum daily irradiance and photoinhibition is typically rare (Goldsborough *et al.*, 2005). Temperature has effects on periphyton taxonomic structure like light regime (Vermaat J. E., 2005). For example, while high temperatures make *Scenedesmus* dominate, low temperatures favoured *Navicula* in Vermmat and Hootsmans (1994)' and Bothwell's (1988) study. Besides it is same for season differences that diatoms are dominant in spring and green algae or cyanobacteria are dominant in summer (Meulemans and Roos, 1985).

Temperature and light often show strong parallelism because if there is sunlight, it will provide light for phytosynthesis of macrophytes and warm up the environment (Vermaat J. E., 2005). Therefore separation of temperature and light interaction and impacts on periphyton growth is not very common (Bothwell, 1988). Still there are some studies (Vermaat and Hootsmans, 1994; Nofdianto, 2010) which showed the interaction of low temperature and high irradiance or vice versa. Both of these studies determined the maximum growth at 20°C and 200-225 µmol. This degree of temperature and light is optimum for all organisms groups. Above or below these levels taxa and abundance is changing.

Water level generally affects the periphyton structure because it affects the light intensity directly and may cause turbidity indirectly by increasing the wind impacts for wetlands (Goldsborough *et al.*, 1995). Liboriussen and Jeppesen (2006) studied the periphyton at different depths (0.1, 0.5, 0.9) and they did not find a linear relationship between depth and periphyton abundance.

Nutrient effect on periphyton development has been studied many times especially in streams (Vermaat, 2005). Biomass is the most used data for periphyton growth among several methods (dry mass, ash free dry mass, chlorophyll concentration, total phosphorus concentration and biovolume). Goldsborough (2005) critisized the methods with their pros and cons (Table 1.1). These studies (Marcus, 1980; Hansson, 1992; Jones and Sayer, 2003; Liboriussen *et al.*, 2005, 2006; Smith and Lee, 2006; Becares *et al.*, 2008; Özkan *et al.*, 2010; Rosemond *et al.*, 1993, 2000; Chételat *et al.*, 1999; Sharifi and Ghafori, 2005; Greenwood and Rosemond, 2005; Bowes *et al.*, 2010) generally concluded that higher nutrient caused higher periphyton biomass in both lakes and rivers.

Nutrient features of ecosystems, generally nitrogen and phosphorus, are also determining the dominant algal taxa. For example like silicon for diatoms, some goups need special requirements and some species can supply their need via producing phosphatase (Kahlert and Pettersson, 2002) or nitrogenase (Goldsborough *et al.*, 2005) enzymes when the inorganic nutrients are scarse in the environment.

1.2.2 Biotic Factors

Macrophyte and periphyton interaction is a unique relationship that has been the focus of many studies in shallow lakes and wetlands. Their results can be summarized to positive interactions (symbosis or mutualism), negative interactions (competition and allelopathy) and no interaction (neutrality) (Goldsborough *et al.*, 2005). Symbosis interaction is explained as; macrophyte

provide food by excreting and photosynthesising for periphyton and periphyton provide protection against predation covering it (Burkholder and Wetzel, 1990; Wetzel, 2001). If there is not sufficient nutrients, macrophyte and periphyton compete each other for the nutrient resources which refer to negative interaction (Goldsborough *et al.*, 2005). Allelopathy is also a negative interaction that means by excreting some substances preventing the growth of

Table 1.1: Pros and cons of various methods of periphyton biomass (Goldsborough, 2005).

Method	**Pros**	**Cons**
Dry mass, Ash Free Dry Mass	inexpensive, large body of existing data for comparison, directly comparable to metrics for other organisms	not spesific to algae; can include variable contributions by plant, heterotroph, dead and abiotic constituents
Chlorophyll-*a* Concentration	may be inexpensive (depending on method), large body of existing data for comprison, unique to algae and plants	cellular chlorophyll content varies with growth conditions
TP Concentration	method sensitive to low levels, useful in examining trophic structure of an assemblage (trophic index)	not spesific to alge, includes variable contributions by heterotrophs, cellular TP content varies with growth conditions
Species counts, Biovolume	detailed information on algal assemblage structure	time-consuming, accuracy depends on analyst skill (species identification) and estimates of cell volume

Others. Macrophytes or periphyton try to inhibit each other by using allelopathy (Gross *et al.*, 2003). However this process has not been proved with experiments. Only it is found that some macrophytes have compounds to inhibit periphyton growth but it is not known that macrophyte excrete it for interacting with periphyton (Blindow *et al.*, 2002). Finally, some arguments point that

macrophytes just have the surface periphyton need thus their relationship for the most part is biologically neutral (Goldsborough *et al.*, 2005).

Several researchers showing the interaction of macrophyte and periphyton shading such as Neundorfer and Kemp (1993), Goldsborough *et al.* (2005), Gross *et al.* (2003), Roberts *et al.* (2003), Phillips *et al.* (1978), and Köhler *et al.* (2010) revealed that periphyton shading has a negative impact on macrophyte abundance when nutrients increase in water. In these studies water conditions were turbid, thus phytoplankton abundance and suspended solids were also effective for the limitation of the light. Hillebrand and Kahlert (2001) reported a linear relationship between periphyton and macrophyte. While grazers are decreasing the periphyton biomass, they are increasing the nutrient content of periphyton, significantly. This is caused with excreation of nutrients, removal of older cells and finally more turbid water and plant loss.

Nevertheless it is accepted by some reseachers macrophyte may shade the periphyton structure (Becares *et al*., 2008; Liboriussen and Jeppesen, 2003).

There are a broad variety of animals in freshwater which graze on periphyton. The most important ones are gastropods, crustaceans, insect larvae and other small size invertebrates (Vermaat, 2005; Jones *et al.*, 2002). Besides invertebrates, vertebrates can also feed on periphyton such as fish and tadpoles. Grazers can be highly selective and like the other affecting factors may alter the spatial pattern and structure of the periphyton community (Vermaat, 2005). Hillebrand and Kahlert (2001) revealed that with nutrient addition an increase occurs in grazer effects on periphyton composition.

Many studies (Mazumder *et al.*, 1989; Liboriussen *et al.*, 2003; Vadeboncoeur *et al.*, 2002; Jones and Sayer, 2003; Hillebrand, 2002) reported that macroinvertebrates reduce periphyton biomass. Cattaneo and Mousseau (1995) found that the most important impact on periphyton-macroinvertebrate interaction is the grazer body size. Taxon of the grazer and the periphyton algae composition are less important.

1.3 Aim of The Study

Periphyton growth has an important role in freshwater ecosystems and it is a good indicator for changing conditions. Therefore it is crucial to understand its interaction with biotic and abiotic factors in the shallow lakes. This study aimed to reveal the relationships between nutrient and macroinvertebrates grazing effects on periphyton. Also since this mesocosm study targeted to compare the results with other countries which made the same experiment with the same steps and which are at different latitudes, this study also aimed to provide data for this comparison.

CHAPTER 2

MATERIALS and METHODS

2.1 Mesocosm Experimental Design

A mesocosm experiment was carried out in 6 EU-REFRESH participating countries (Sweden, Estonia, Germany, the Czech Republic, Greece and Turkey) at the same time with applying the same protocol to reveal the effects of water level changes and nutrients on trophic structure, function and metabolisms in a latitudinal gradient to show the relative importance of the benthic and pelagic communities for production, respiration and nutrient dynamics in lakes (Figure 2.1).

The experimental set-up was consisted of a floating stage (made of wooden boards and floating devices like plastic barrels) which contained 16 enclosures (two rows divided by a boardwalk). A platform and a boardwalk were built to make working easier. Platform also took part as a wave breaker. The stage was anchored from one side in order to limit its movement (Figure 2.2).

The enclosures were in cylindrical shape (R = 1.2 m), made of fibreglass (4 mm). Fibreglass is a strong material that prevents diffusion of O_2 and CO_2. The enclosures were produced in İzmit, Turkey and were sent off the other countries which were involved in the study.

Figure 2.1: Joint countries to the mesocosm experiments in along an eastern latitude gradient in Europe as part of a REFRESH project; Adaptive strategies to Mitigate the Impacts of Climate Change on European Freshwater Ecosystems: 1) Sweden, 2) Estonia, 3) Germany, 4) the Czech Republic, 5) Turkey and 6) Greece.

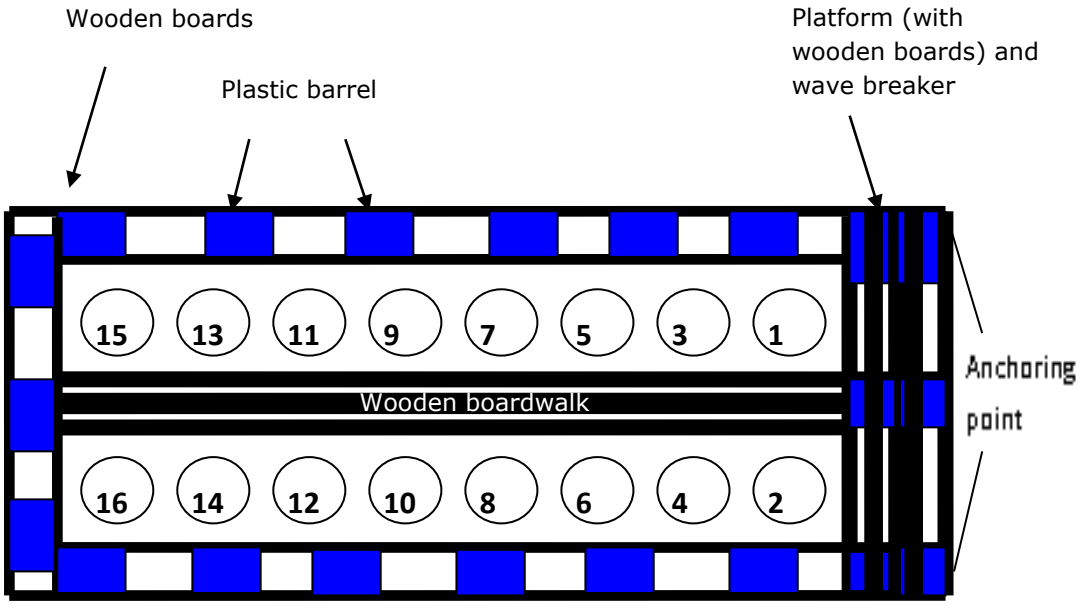


Figure 2.2: Floating stage

Enclosures encompassed a 2x2x4 matrix (2 nutrients, 2 water levels, 4 replicates). Eight of them were 1.2 meters; four of these enclosures had high (200 µg TP l^{-1}) nutrient and the other four had low (25 µg TP l^{-1}) nutrient level. The other eight enclosures were 2.2 meters; similarly four of them had high nutrient and the other four had low nutrient (Table 2.1).

The bottom of the tanks were covered by 10 cm lake sediment. The upper edge of all tanks was attached to the stage 20 cm above the water surface in order to provide a water depth in the 16 enclosures as 1 meter in shallows and 2 meters in high ones, respectively. A starting water volume (10 cm sediment, 90 cm water) was 1020 liters for 1.2 meters enclosures and (10 cm sediment, 190 cm water) was 2150 liters for 2,2 meters enclosures.

Ten percent (by volume) of the sediment that covered bottom of the enclosures was oligotrophic local lake sediment and the remaining 90% was sand with a grain size less than 1 mm. The local sediment was collected from 5 oligotrophic lakes (Poyrazlar, Abant, Çubuk, İznik, Beyşehir) of Turkey. The collected mud from five lakes was mixed, homogenised and sieved through a 10 mm mesh. Large particles (>10 mm like plant fragments, mussels, stones etc.) were removed.

Sediment was equilibrated to the desired nutrient level to adapt the mud to the experimental conditions, enable easier creation and maintenance of phosphorus (P) concentrations at two levels [25 (low tanks) and 250 (high tanks) µg TP l^{-1}] that took four months.

After establishment of placing the sediment to the tanks, enclosures were filled with filtered (500 µm) lake water by the help of a water-pump. The date was recorded as "day 0" and refered to May 9th 2011. To reduce the stirring of the sediment a wooden disc was placed on top of the sediment during the addition of water.

Table 2.1: Random distribution of the enclosures in the floating stage. Red ones were used for the periphyton experiment and DH enclosures were labeled as high nutrient (HN), DL enclosures were labeled low nutrient (LN). Shallow = 1 m, Deep = 2 m.

Enclosure number	Depth	Nutrient	Enclosure name
1	Shallow	High	SH 1
2	Deep	High	DH 1 (HN 1)
3	Shallow	High	SH 2
4	Deep	Low	DL 1 (LN 1)
5	Shallow	Low	SL 1
6	Shallow	High	SH 3
7	Deep	High	DH 2 (HN 2)
8	Shallow	Low	SL 2
9	Shallow	High	SH 4
10	Deep	Low	DL 2 (LN 2)
11	Shallow	Low	SL 3
12	Deep	High	DH 3 (HN 3)
13	Deep	High	DH 4 (HN 4)
14	Deep	Low	DL 3 (LN 3)
15	Deep	Low	DL 4 (LN 4)
16	Shallow	Low	SL 4

Figure 2.3: Before addition of the water to the tanks (METU limnology lab photo archive).

Stratification of the water column in the enclosures was avoided by water pumps during the experiment. This process needed a continuous power suply. Therefore a power cord from the shore was designed. Since there was no electricity in study site exchangeable batteries were used as a power suply due to make water pumps work and these batteries were changed every two or three days, regularly. For circulation RS Electrical (RS-072A) 3 W filters were used.

In addition a bird protecting net was used to prevent the birds to rest and forage over the tanks.

Figure 2.4: After addition of water and sediment to the tanks (METU limnology lab photo archive).

2.1.1 Study Site

Yalıncak DSİ dam lake located at METU campus was selected for setting up mesocosm experiment as it fullfilled some physical and chemical requirements (Table 2.2) of the mesocosm experiment protocol according to which the mesocosm experiments were set up and run in the 6 countries. Other morphological and hydrological characterictics of the dam lake is summarized in Table 2.3.

According to these conditions we selected Yalıncak Dam Lake which was made by DSI in METU campus (39º52'N 32º46' E, Figure 2.5). The closeness of the lake to our department was also another reason for our choice. It's building started in 2002 however because of some financial problems ended in 2004. It was made to prevent the flooding downstream and provide water for irrigation.

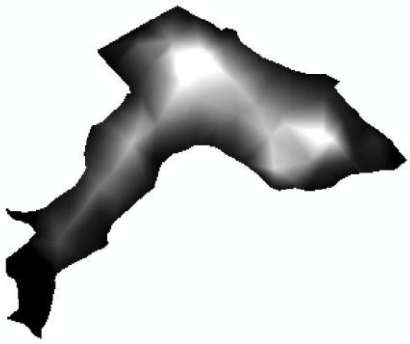
Table 2.2: Lake selection criteria.

Feature	Requested Values	Dam Lake Yalıncak Values
Lake mean depth	< 4 – 5 m	4.7 m
Lake alkalinity (TA)	1< TA < 4 meq/l	2.87 meq/l
Lake salinity	< 1 ‰	0.22 ‰

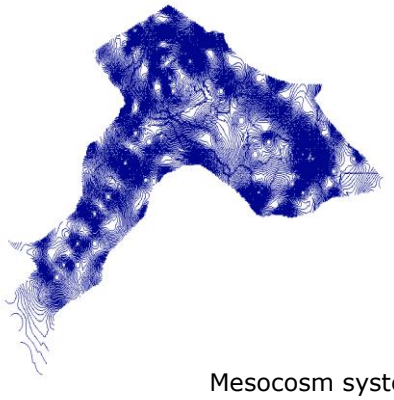
Table 2.3: Morphometric and hydrological characteristics of Dam Lake Yalıncak.

Feature	Values	Feature	Values
Area (ha)	1.96	TP (µg/l)	22.02
Max Depth (m)	11.3	Conductivity (µS/cm)	457.2
Oxygen (mg/l)	7.58	Secchi Depth (m)	168.75
TDS g/l	0.29	SRP (µg/l)	4.32
Chl-*a* µg/l	3.29	SS (mg)	3.34
pH	6.9		

a) A photo from the dam lake



b) The bathymetry map of the dam lake



c) The contour map of the dam lake

d) Google Earth scene of the dam lake

Figure 2.5: Different scenes from the Dam Lake Yalıncak at METU campus

2.1.2 Inoculums and Additions

On the 4th day of the experiment (May 13rd 2011), in every countries, plankton and a mixed sample of sediment collected from five other lakes (Poyrazlar, Küçük Akgöl, Taşkısığı, Gölcük, Yeniçağ) were inoculated. They were collected from these five lakes in order to enable potential development of a diverse flora and fauna. Each of the five lakes was covering a nutrient gradient of 25 - 200 µg TP l^{-1}. The sediment was collected from a low slope area and at a depth corresponding to approximately mean depth of the lakes. In order to avoid fish and large mussels sediment was filtered with a 10 mm mesh. The sediment was mixed firmly and one litre of the inoculums sediment was added to each enclosure by dispersing it evenly on top of the 10 cm sediment layer.

Zooplankton was collected from these five lakes through five vertical zooplankton hauls covering the entire water column. Samples were kept separately in 5 lt barrels filled with lake water from the sample lake. On the addition day these five samples of plankton were mixed and a litre of the plankton inoculated to each enclosure.

On the 7th day of the experiment, submerged macrophytes and fish were added to the enclosures. As a plant *Myriophyllum spicatum* L. (Eurasian watermilfoil), (Figure 2.6a) was used. Because we needed a submerged macrophyte and this plant could be found in every country easily. Eight plants of *Myriophyllum spicatum* L. were planted into each enclosure. The length of plants were 5-10 cm. They were bought from an aquarium center. Before addition of the plants they were placed in soda mineral water for 15 minutes to remove snails and invertebrates on the plants. The plants were sinked with stones which tied to their bases. To ensure that they anchor to the sediment and start growing a lot of trials were done before sinking.

As a fish, *Gasterosteus aculeatus* L. (three-spined stickleback) was chosen (Figure 2.6b). Despite our huge efforts to collect the fish in Turkey, we failed. Hence we imported the fish in Germany, a fish farm called Fischzucht

Rhönforelle GmbH & Co. KG. Before addition of the fish, they were placed in a big aquarium for acclimatization. Some of them died but we managed to add 96 fish (6 fish x 16 enclosures) into the tanks. The remianing fish were taken care in the aquariums for replacing dead fish in the mesocosms.

Six sticklebacks with a length of 2-4 cm were introduced to the enclosures. Male and female abundance was divided on the purpose of being equal in each enclosures (+/- one fish) based on Baggerman *et al*. (1989).

a) *Gasterosteus aculeatus*

b) *Myriophyllum spicatum*

Figure 2.6: Macrophyte and fish which were added to the enclosures

Two levels of nutrient concentrations were established in the enclosures: low (25 µg TP l^{-1}) and high (200 µg TP l^{-1}) with four replicates of each per water depth. Since it is imposible to create exactly 25 and 200 µg TP l^{-1}, + / - 25% differences were accepted.

The experiment tanks were filled up with the dam lake water that had oligotrophic conditions and whose TP concentrations was below 25 µg (TP l^{-1}), thus initial conditions were assumed to be 25 µg TP l^{-1} and 0.5 mg N l^{-1} for all

tanks. So, there wasn't any problem in the low nutrient enclosures but for the high nutrient enclosures an initial addition was needed of both Phosphorus (P) and Nitrogen (N) to provide the requirement of high TP and TN levels (200 µg TP l^{-1}, 2 mg N l^{-1}). Since every country should have a standart for addition of nutrients, eutrophic Danish Lakes were used as a base.

Moreover, because of the natural removal (like denitrification, sedimentation) both N and P would decrease during the experiment. Hence, N and P were added to all of the tanks every four weeks (+/- 2 days) in order to counteract the natural removal and maintain the relative difference between low and high nutrient levels in the enclosures. Na_2HPO_4 (4.60 g l^{-1}) and $Ca(NO_3)_2$ (117.2 g l^{-1}) were used as P and N sources, respectively. The montly dosing of these nutrients were carried out.

2.1.3 Sampling

Following the addition of fish and macrophytes the first sampling was made on May 16th 2011. Thereafter we took samples from enclosures every two weeks. A total of 2.75 lt were collected as 0.5 lt for water chemistry, 1.5 lt for chrophyl-a, 0.5 lt for suspended solids, 0.25 lt for total nitrogen.

Temperature, dissolved oxygen, conductivity, total dissolved solids, salinity and pH were measured by using YSI 556 MPS sensor from surface and 0.5 m intervals through the water column monthly. In each sampling, water depth and Secchi disc depth were recorded. Light also was measured at regular dates by using LI-COR LI250A light meter.

2.1.4 Laboratory Analyses

Total phosphorus (TP), soluble reactive phosphorus (SRP) and alkalinity (TA) were analysed on water chemistry samples. For determination of TP in water sample, acid hydrolysis method was used (Mackereth *et al*., 1978). For SRP, filtered water was processed with molybdate reaction method. TA analysis was done with acid titration with phenolphtelene and BDH indicator (Mackereth *et al.*, 1978). Nitrogen analysis including total nitrogen (TN), ammonia (NH_4) and Nitrite-Nitrate ($NO_{2,3}$) analysis were carried out using Scalar Autoanalyzer Standart Methods (Houba *et al.*, 1987; Krom, 1980; Kroon, 1993; Searle, 1984). Ethanol extraction method (Jespersen and Christoersen, 1987) was used for determining the clorophyll-a with three replicates and measured at 663 and 750 nm spectrophotometer concentration.

2.2 Periphyton Experiment

In this mesocosm experiment, effects of nutrient levels on periphyton growth was studied on an artificial substrate just in deep enclosures. As the water level was assumed to have less effect in our set up, the artifical substrate periphyton experiment was only carried out in the deep water enclosures. The tanks which were deep and had the high nutrient level (DH) in the mesocosm experiment will be hereafter regarded as high nutrient (HN) and the tanks which were deep and had the low nutrient level (DL) will be regarded as low nutrient (LN).

2.2.1 Periphyton Experimental Design

Periphyton growth experiment was carried out on an artificial substrate consisting of 16 cm x 2 cm transparent polypropylene strips with a slightly textured surface (GBC, PolyClearView) to allow attachment of periphyton.

The strips were placed with a stainless steel to 50 cm below the surface and 30 cm away from the tank walls. The strips were tied comblike to stainless steel, firmly. The backside of the strips were covered with plaster to prevent periphyton growth on both sides (Figure 2.7).

Figure 2.7: Strips before taking place in tanks.

2.2.2 Sampling

For four months periphyton strip samples were collected and strips were changed with new ones monthly (Table 2.4).

Table 2.4: Periphyton Strips Exposure Times

1. month	18.06.2011 - 14.07.2011
2. month	15.07.2011 - 16.08.2011
3. month	16.08.2011 - 15.09.2011
4. month	16.09.2011 - 17.10.2011

After taking the strips out of the water, they were brushed with a toothbrush to dislocate the sticky organisms into 150 ml filtered mesocosm water, mixed firmly and taken to the laboratory for analyses.

2.2.3 Laboratory Analyses

The samples were used to determine of dry mass (DM), ash free dry mass (AFDM), total phosphorus (TP) and chlorophyll-*a* (chl-*a*). Whatman 25 mm GF/C filters were used to filter and analyse the suspension. Before the analyses filters were always passed over pre-washed, pre-combusted, pre-weighed.

Firstly, 25 mg of suspension was filtered as two replicates then dried at 60ºC overnight to determine dry mass (DM). Secondly, after measuring the DM filters were combusted at 500ºC for 5 hours to determine ash mass (AM). After filters

were combusted, they placed in a desicator and waited for cooling off before measuring the AM. AFDM was calculated by subtraction DM from AM (Roberts *et al.* 2003).

For determination of total phosphorus (TP) in periphyton samples, acid hydrolysis method was used (Mackereth *et al.*, 1978). Since for this method 25 ml of sample is needed, 5 ml dense periphyton suspension diluted up to 25 ml with distilled water and then analysed.

Lastly, 20 ml periphyton suspension with two replicates were filtered for chlorophyl-*a* (chl-*a*) determination. Chl-*a* pigment content was determined with ethanol extraction method (Jespersen and Christoersen, 1987) and the absorbance was measured at 410, 430, 480, 663, 665 and 750 nm.

2.2.4 Grazer Experiment

A grazing laboratory experiment was also conducted to determine the effect of invertebrate grazing pressure on periphyton biomass. Extra two strips (16 cm x 2 cm) were placed to high nutrient enclosures (HN) for the last three months of the periphyton sampling (Table 2.4).

After these two strips were taken out of the mesocosms, they were cut into two halves and transported to the lab immediately in a dark humid box. Thus there were 4 piece (8 cm x 2 cm) of strips from each of HN enclosures (Figure 2.8).

In order to remove potential grazers which might have attached to the strips, two strips from each enclosures were treated with CO_2 for 3 - 5 minutes (control strips). The other two were remained untreated. All strips were kept in 0.5 lt beakers under a light regime of 12 hours dark and 12 hours light and constant temperature of 18ºC in the climate room. Throughout the incubation of the strips the amount of water which was lost through evaporation was replaced with filtered (Whatman GF/C filters) mesocosm water of the tank.

A nutrient solution which was composed of macroelements, trace elements, $NaHCO_3$ and Fe-EDTA solutions was added to all treatments at day one and proceed in 5 days intervals due to ensure non-limiting nutrient conditions in all treatments.

After two weeks, from each HN tank one control and one untreated strip were sampled. The untreated strips were transported into carbonated water for 3 - 5 minutes to remove the grazers and these grazers were fixed in formaldehyde for further examination with microscope and compare the grazers before and after the experiment. Periphyton itself was brushed to 50 ml of filtered mesocosm water and DM, AFDM, chl-*a* and TP were analysed like others.

If the differences in measured variables (AFDM, DM, chl-*a*, TP) between control and untreated strips were large enough (about 30%) after two weeks of incubation period, experiment was terminated. If not, 2 weeks later the other control and untreated strips were processed like before. Even if there was not 30% difference end of the 4 weeks incubation period, the experiment was nevertheless terminated.

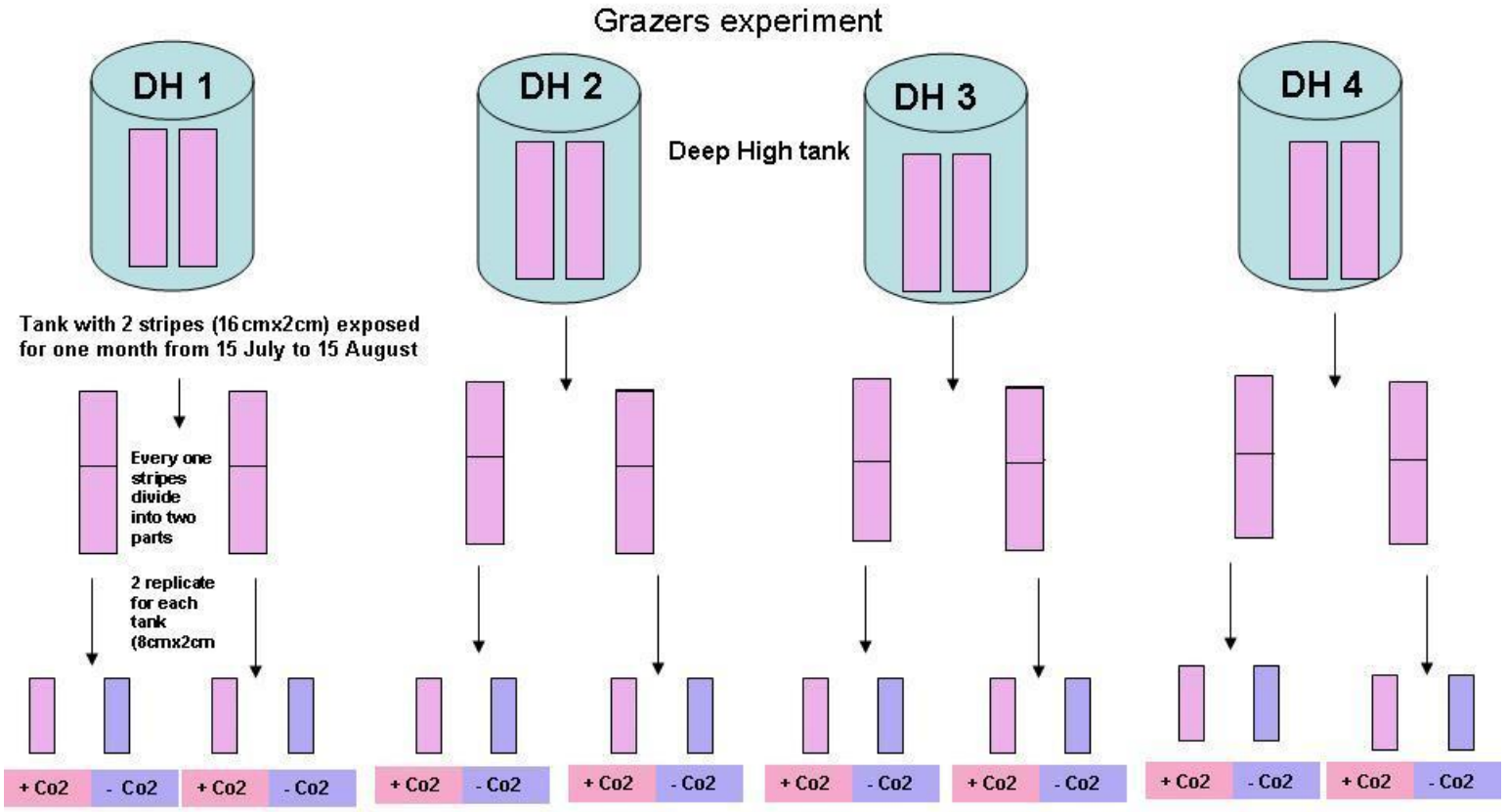


Figure 2.8: Grazer Experiment

2.3 Macrophyte and Macroinvertebrate Sampling and Analyses

On each sampling date and for each enclosure a species list was made for macrophytes. Macrophyte percent plant volume inhabited (%PVI) was calculated by visually estimating percentage coverage and measuring macrophyte average plant height using the formula:

PVI = %coverage x average height / water depth (Canfield *et al.*, 1984)

Coverage estimation was be performed by dividing the into quarters the enclosures and estimating the area which covered by macrophyte by using the scale:

0: no plants
1: 0-5% coverage
2: 5-25% coverage
3: 25-50% coverage
4: 25-75% coverage
5: 75-95% coverage
6: 95-100% coverage

If present, filamentous algae was included as part of the total macrophyte coverage.

At the end of the experiment a piece of macrophyte were harvested from enclosures in order to determine the periphyton content on the real plants. Dry mass, ash free dry mass, cholorophyll-a and total phosphorus were measured. Remaining of the macrophytes were all harvested, washed and dried at 105ºC overnight to measure the dry weight of them.

For identification of macroinvertebrates three separate kajak cores were taken from sediments of each enclosures at the end of the experiment. Samples are pooled, rinsed, filtered on a 500 mm mesh and preserved in 96% ethanol. Their identification was performed to familia level except Chironomidae. Since the range of diversity was narrow and Chironomidae is a good indicator for nutrient levels, Chironomidae familia was further identified to the species level with the help from a specialist at the University of Pamukkale (Webb and Scholl 1985;

Şahin 1991; Epler, 2001 and Pilot and Vallenduk, 2002 keys were followed for identification).

2.4 Statistical Analyses

SPSS 15.0 was used for all the statistics. Initial conditions tested with one-way ANOVA to see if any difference existed among enclosures. Ln or sqrt transformations were used to provide normality of data if necessary. Repeated measure one-way ANOVA was used to see the changing nutrient and time interaction. Bonferroni test was used to see the significant differences between time periods. 95% confidence level was used for all statistical tests to show statistical difference. For comparing the low and high nutrient level enclosures of macrophyte dry weight, epiphyton parameters, macroinvertebrates and fish abundance which was collected at the end of the experiment one way ANOVA was used. Finally, linear regression analysis was used to see if there is any important impact of physical conditions on periphyton biomass.

CHAPTER 3

RESULTS

3.1 Physico-chemical Parameters

A major water level drop was observed for all of the enclosures throughout the experiment. Only at the end of June water levels increased slightly because of the precipitation. While water levels were 190 cm for all enclosures in the begining, there were 0.51 ± 0.2 meter reduction in high nutrient level (HN) and 0.46 ± 0.1 meter reduction in low nutrient level (LN) enclosures at the end of the experiment (Figure 3.1). A sharp decrease was observed after 11th July when the enclosures' water temparature reached to 25.57 ± 0.36 ºC. (Figure 3.2). Repeated measures of one-way ANOVA showed that there was not any difference between HN and LN enclosures in terms of water temperature ($p=0.998$) throughout the experiment (Table 3.1). In order to see how temperature changing affected periphyton growth, linear regression analysis was carried out. It showed that temperature did not have significant effect neither on periphyton ash free dry mass, chlorophyll-*a* nor phosphorus amount. Furthermore, stratification did not occur since we used water pumps to stimulate mixing of water.

Secchi disc depth/water depth ratio (S/W) was used due to measure the clarity of water which remained the same with the water depth in the LN tanks whereas it significantly decreased through time in the HN ones (Table 3.1).

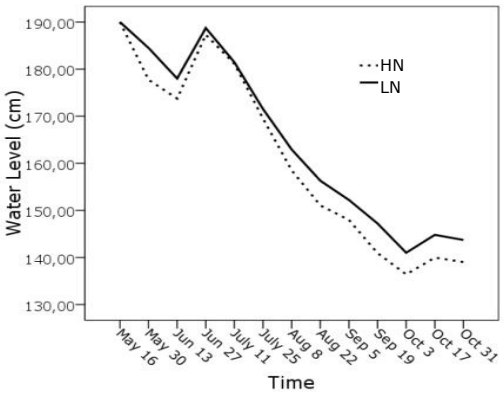


Figure 3.1: Water levels of the enclosures during the experiment.

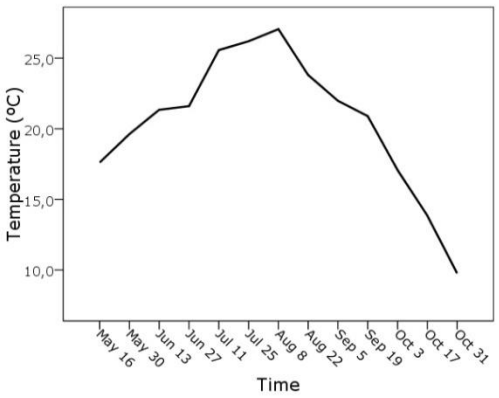


Figure 3.2: Temperature degrees of the enclosures during the experiment.

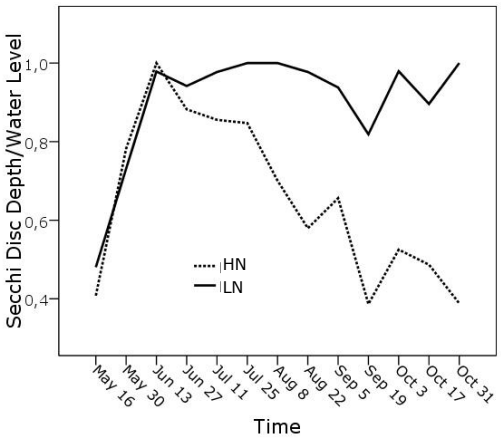


Figure 3.3: Changes of secchi depth/water level ratio in high and low nutrient level tanks in time.

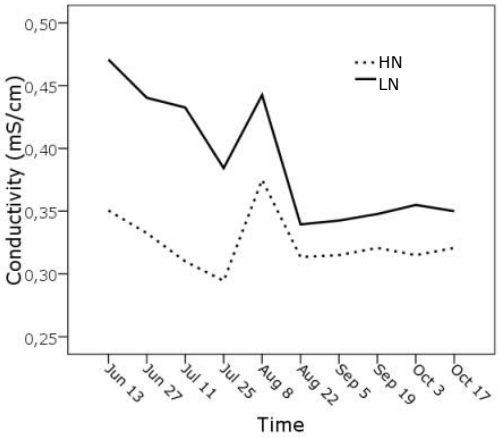


Figure 3.4: Conductivity changes in time and with nutrient level.

In the begining of the experiment, the S/W ratio increased in both nutrient levels because the disturbed sediment was settling down after the filling up enclosures with the lake water (Figure 3.3).

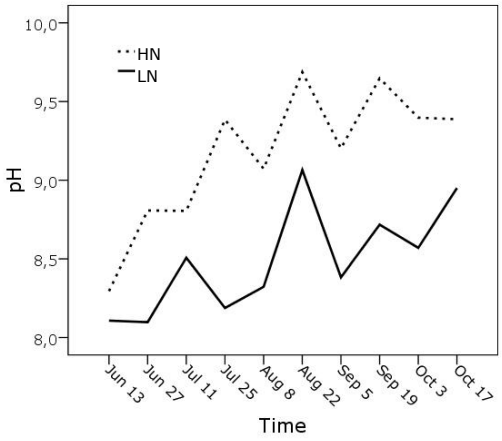


Figure 3.5: pH changes in time and with nutrient level.

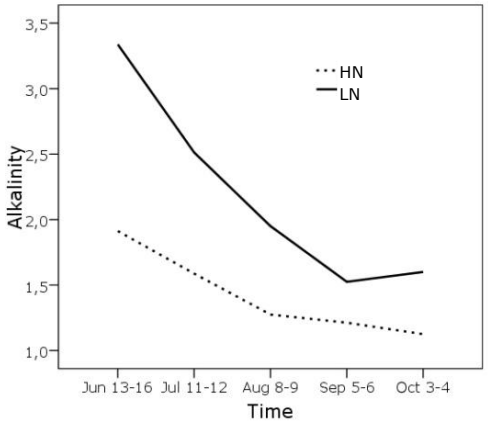


Figure 3.6: Changes of alkalinity in time and with nutrient level.

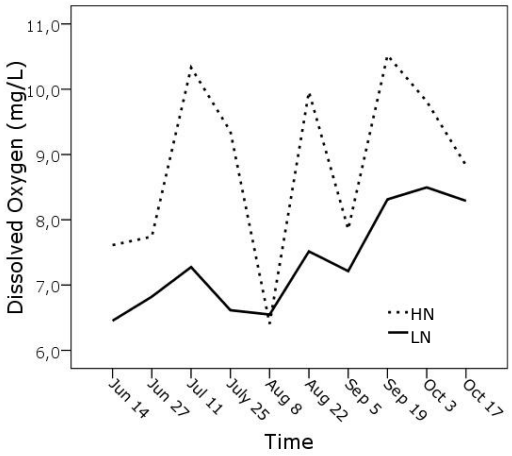


Figure 3.7: Dissolved oxygen change in time and with nutrient level.

One-way ANOVA was used if there was any difference between HN and LN tanks for initial conditions. While water depth, temperature, Secchi disc depth/water level ratio, pH, dissolved oxygen, nitrite-nitrate and amonnium concentrations were no different initially; conductivity, alkalinity, total

phosphorus, soluble reactive phosphorus and total nitrogen concentrations were different for the beginning (Table 3.1).

Comparing the conductivity through all treatments repeated measure of ANOVA (Rm-ANOVA) revealed that nutrient levels-time interaction was important on conductivity ($p<0.0001$). It increased through time ($p=0.001$) and there were significant differences between 4th (Jul 25) - 5th (Aug 8) and 5th - 6th (Aug 22) samplings. Conductivity was higher in LN treatments than in HN tanks (Figure 3.4, Table 3.1).

Rm-ANOVA showed that nutrient levels significantly affected pH, ($p<0.0001$). pH of HN enclosures was greater than LN ones. Time also had important effect on pH ($p=0.007$). It changed between 5th (Aug 8) - 6th (Aug 22) and 6th - 7th (Sep 5) samplings (Figure 3.5, Table 3.1).

Rm-ANOVA indicated that alkalinity significantly changed with nutrient level and time effect interaction ($p=0.02$). LN enclosures had higher alkalinity (Figure 3.6, Table 3.1).

Time and nutrient level interaction significantly affected ($p<0.0001$) dissolved oxygen (DO). In the LN enclosures DO was lower than HN ones. However on 8th August they were coming closer. 5th (Aug 8) and 6th (Aug 22) samplings were different from each other, statistically ($p=0.010$, Figure 3.7, Table 3.1).

Naturally, total phosphorus (TP) concentrations significantly differed between the low and high nutrients tanks ($p=0.003$, Figure 3.8, Table 3.1). Time also had an affect on TP, there was an important different between 3rd (Aug8) and 4th (Sep 5) sampling ($p=0.04$). Rm-ANOVA indicated that nutrient treatments and time affected soluble reactive phosphorus (SRP), significantly (Figure 3.9, Table 3.1).

Neither nutrient level differences nor time had effects on nitrite-nitrate (NO_3-NO_2) concentrations (Table 3.1). Total nitrogen (TN, $p=0.009$) and amonnium (NH_4, $p=0.002$) were affected by nutrient treatment-time interaction (Table 3.1). TN concentrations increased until 8th August in both nutrient levels but after that while HN enclosures were increasing LN enclosures started to

decrease (Figure 3.10). HN and LN enclosures' amonnium concentrations changed differently during the experiment (Figure 3.11, Table 3.1).

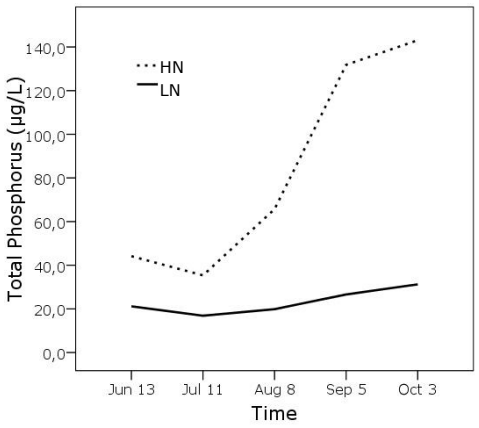


Figure 3.8: TP changes in time and with nutrient level.

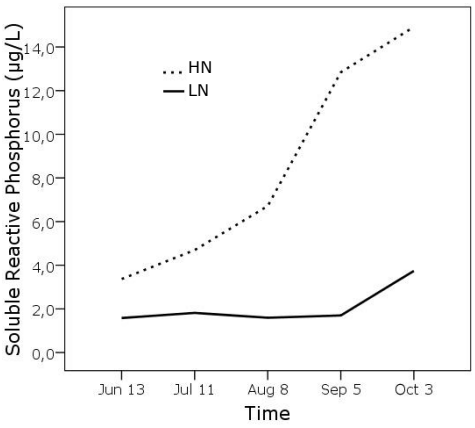


Figure 3.9: SRP changes in time and with nutrient level.

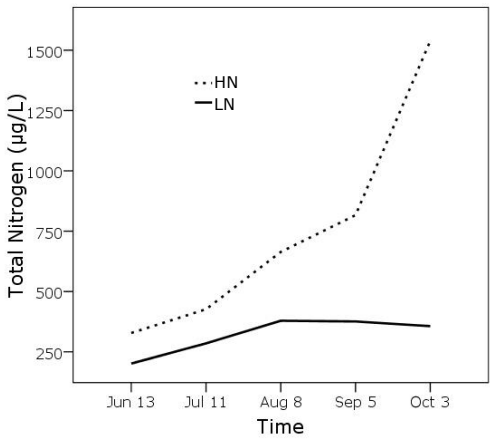


Figure 3.10: TN changes in time and nutrient level.

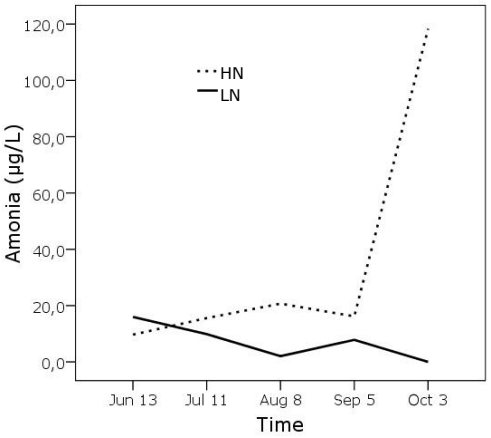


Figure 3.11: Amonia changes in time and nutrient level.

Table 3.1: Mean value and standart deviation of phico-chemical parameters (conductivity, pH, alkalinity, dissolved oxygen, total phosphorus, soluble reactive phosphorus, total nitrogen, nitrite-nitrate, dissolved nitrogen and amonia). These values were calculated for only from June 13 to October 17 which is the time periphyton experiment was runned. While initial conditions column shows the results of One-Way ANOVA, the last three columns show that results of repeated measure of ANOVA.

Variables	HN	LN	Initial Conditions (June 13)	Nutrient Level	Time	Time x Nutrient Level
Water Depth (cm)	165.19 ± 19.44	168.47 ± 18.14	ns	ns	<0.0001	ns
Temperature (ºC)	20.49 ± 4.86	20.49 ± 4.80	ns	ns	<0.0001	ns
Secchi Disc Depth/Water Level	0.69 ± 0.27	0.95 ± 0.10	ns	0.043	<0.0001	ns
Conductivity (mS/cm)	0.3255 ± 0.03	0.3879 ± 0.05	0.023	<0.0001	0.001	<0.0001
pH	9.21 ± 0.60	8.46 ± 0.36	ns	<0.0001	0.008	ns
Alkalinity	1.42 ± 0.40	2.19 ± 0.73	<0.0001	<0.0001	<0.0001	0.002
Dissolved Oxygen (mg/L)	8.70 ± 1.94	7.32 ± 1.07	ns	0.001	<0.0001	0.022
Total Phosphorus (µg/L)	84.04 ± 17.28*	23.16 ± 9.30	0.001	0.003	<0.0001	ns
Soluble Reactive Phosphorus (µg/L)	13.01 ± 5.09*	2.07 ± 0.29*	0.003	<0.0001	0.023	ns
Total Nitrogen (µg/L)	754.92 ± 140.69*	319.54 ± 87.57	0.010	0.001	<0.0001	0.009
NO_3 - NO_2 (µg/L)	21.34 ± 5.29*	11.23 ± 1.57*	ns	ns	ns	ns
Amonia (µg/L)	36.08 ± 20.66*	7.14 ± 2.05*	ns	0.039	ns	0.002

*Standart error of mean was given instead of standart deviation.

3.2 Biological Parameters

3.2.1 Periphyton

Since the water depth decreased in time, the water above periphyton strips decreased and light intensity increased. The changes through time were significant (Table 3.2).

Dry mass (DM) of periphyton was compared within low and high nutrient enclosures by Rm-ANOVA. Time had a significant effect on DM ($p<0.0001$). 1st (18 Jun - 14 Jul) - 2nd (15 Jul – 16 Aug) ($p<0.0001$) and 3rd (16 Aug- 15 Sep) - 4th (16 Sep – 17 Oct) sampling ($p=0.012$) were significantly different from each other. (Figure 3.12, Table 3.2). Rm-ANOVA showed that time and nutrient level interaction had significant effect ($p=0.070$) on ash free dry mass (AFDM). 1st and 2nd sampling values were significantly different ($p=0.002$) (Figure 3.13, Table 3.2).

Nutrient levels and time had an important effect on TP concentration of periphyton. 1st and 2nd sampling were significantly different ($p=0.013$) from each other (Figure 3.14, Table 3.2). Chlorophyll-*a* concentrations of periphyton changed with nutrient, significantly ($p<0.0001$, Figure 3.15, Table 3.2).

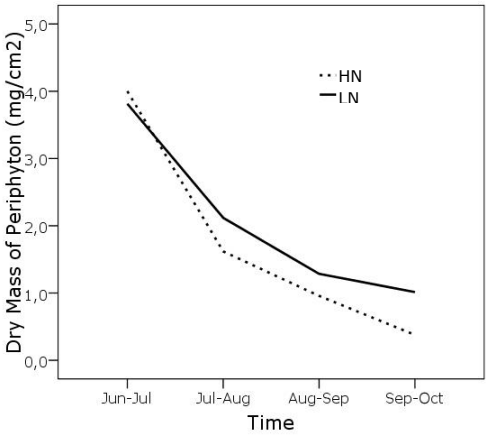


Figure 3.12: Periphyton dry mass changings in time and with nutrient level.

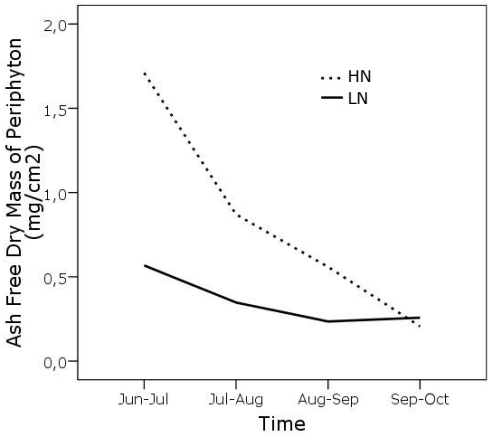


Figure 3.13: Periphyton ash free dry mass changings in time and with nutrient level.

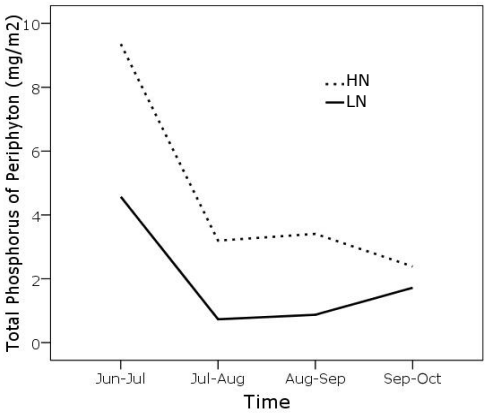


Figure 3.14: Periphyton TP changings in time and with nutrient level.

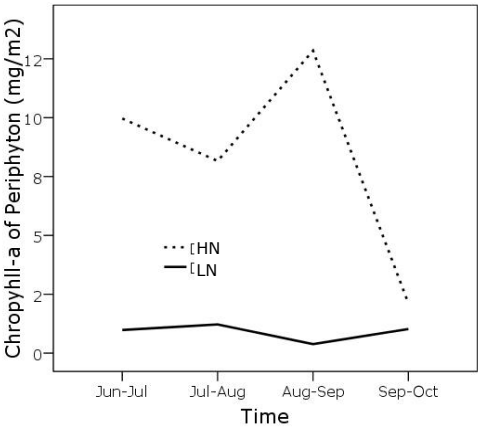


Figure 3.15: Periphyton chlorophyll-*a* changings in time and with nutrient level.

Table 3.2: Mean value and standart errors of periphyton parameters (dry mass, ash free dry mass, total phosphorus, chrophyll-*a*, water levels above periphyton strips and light intensity) and PVI%. The last three columns showed the results of Rm-ANOVA.

Variables	**HN**	**LN**	**Nutrient Level**	**Time**	**Time x Nutrient Level**
Water Levels Above Periphyton Strips	20.48 ± 2.92	23.46 ± 2.76	ns	<0.0001	ns
Light Intensity Above Periphyton Strips	983.9 ± 104.5	883.6 ± 75.7	ns	<0.0001	ns
Dry Mass of Periphyton (mg/cm^2)	1.74 ± 0.22	2.06 ± 0.29	ns	<0.0001	ns
Ash Free Dry Mass of Periphyton (mg/cm^2)	0.84 ± 0.15	0.35 ± 0.05	0.015	<0.0001	0.007
Total Phosphorus of Periphyton (mg/m^2)	4.59 ± 1.12	1.97 ± 0.65	0.041	0.002	ns
Chrophyll-*a* of Periphyton (mg/m^2)	8.47 ± 2.32	0.95 ± 0.18	<0.0001	0.046	ns
PVI%	25.29 ± 5.6	8.40 ± 1.29	0.010	<0.0001	0.005

3.2.1.1 Grazer Experiment

Thirty percent difference between control and untreated strips were seen for chl-*a* concentrations, ash free dry mass and dry mass but not for total phosphorus content. At the first two months the 30% difference of total phophorus content between control and untreated strips was not observed. Only at the last month it reached to 50% so it could be said that grazer pressure incereased with time in terms of total phosphorus like the other parameters. Dry mass and ash free dry mass showed that grazer pressure on periphyton increased with time. On the other hand chlorophyl-*a* content of periphyton showed that grazer effect decreased in August and increased in September (Figure 3.16).

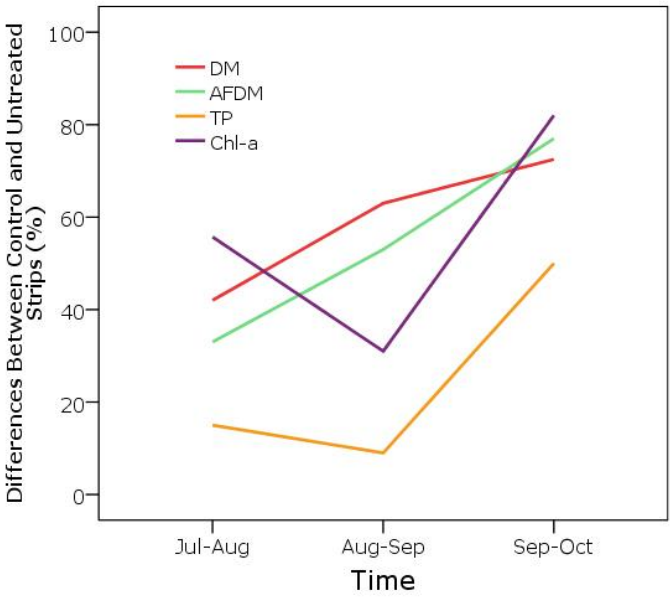


Figure 3.16: Changes in grazer pressure on periphyton during the grazing experiment.

3.2.2 Macrophytes

Plant Volume Inhabited percentage (PVI%) significantly changed with nutrient levels and time (Table 3.2). Despite the fact that *Myriophyllum spicatum* only originally introduced to the enclosures, *Chara contraria* (A. Braun ex Kützing 1845) was also harvested. *M. spicatum* was much more in HN tanks than LN tanks (Figure 3.18, Table 3.3).

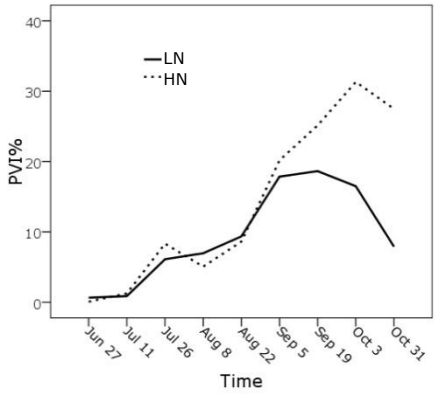


Figure 3.17: PVI% changes in time and with nutrient level.

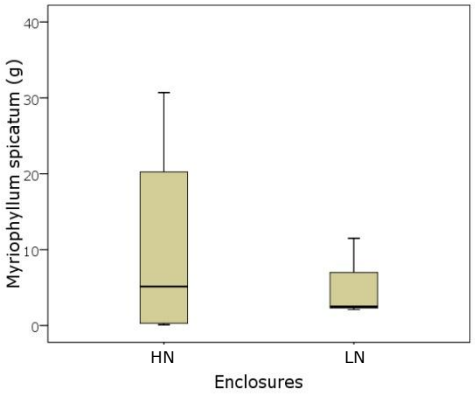


Figure 3.18: *Myriophyllum spicatum* abundance in the HN and LN tanks at the end of the experiment

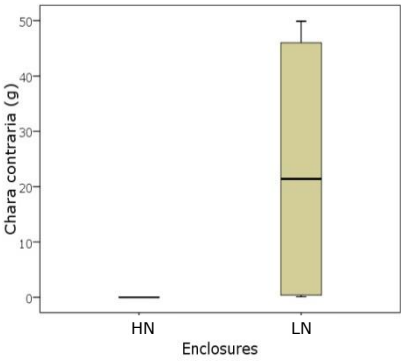


Figure 3.19: *Chara contraria* abundance in the HN and LN tanks at the end of the experiment

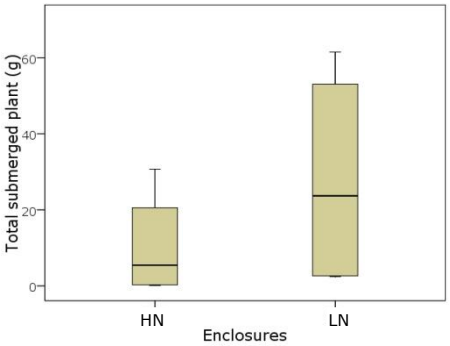


Figure 3.20: Total plant abundance in the HN and LN tanks at the end of the experiment

None of the HN enclosures had *C. contraria* while LN tanks had considerable amount which provided that LN enclosures had much more macrophyte at total (Figure 3.19 and 3.20, Table 3.3). However PVI% graph showed us HN enclosures had higher PVI% value. Since *C. contraria* was at the bottom of the tanks they did not reflected the PVI% (Figure 3.17). The 4th high nutrient enclosure (DH_4 or HN_4) had neither macrophyte nor filamenteous algae at the end of the experiment.

One-way ANOVA showed that only cholorophyll-a content of the epiphyton was significantly different between HN and LN enclosures (Table 3.3, Figure 3.21).

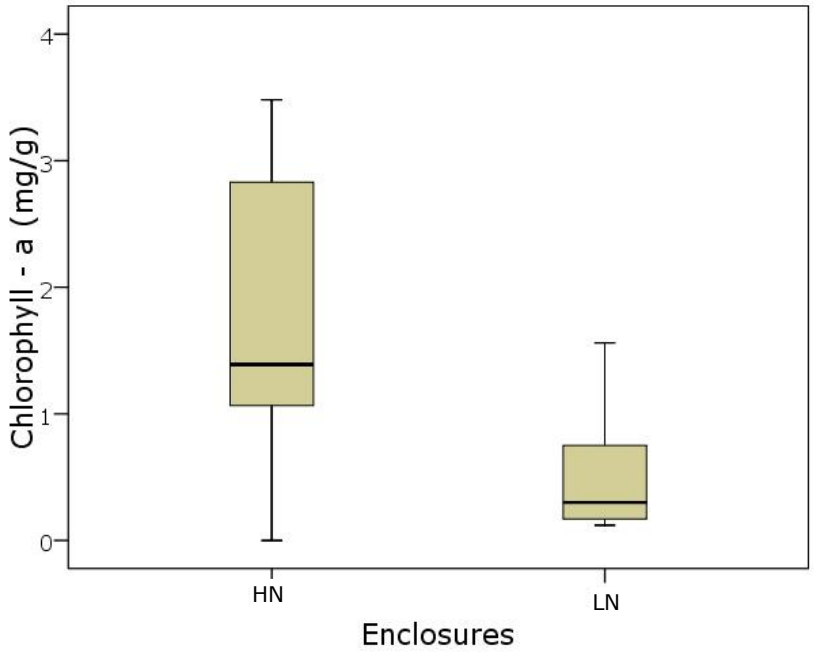


Figure 3.21: Chlorophyll-*a* concentrations of the epiphyton.

Table 3.3: Mean value and standart error of macrophyte dry weight and periphyton of macrophyte. The last column showed the results of One-way ANOVA.

Variables	**HN**	**LN**	**The difference between HN and LN**
Macrophyte Dry Weight (g):			
Myriophyllum spicatum	10.24 ± 7.19	4.67 ± 2.29	ns
Chara contraria	0	23.17 ± 13.28	<0.0001
Total dry weight	10.40 ± 7.19	27.83 ± 14.95	0.031
Macrophyte Periphyton:			
Dry Mass (mg/g macrophyte dry weight)	98.8 ± 46.10	69.33 ± 16.81	ns
Ash Free Dry Mass (mg/g macrophyte dry weight)	34.79 ± 13.53	15.32 ± 3.20	ns
Clorophyll-a (mg/g macrophyte dry weight)	1.81 ± 0.48	0.52 ± 0.18	0.002
Total phosphorus (mg/g macrophyte dry weight)	7.05 ± 3.75	3.57 ± 1.21	ns
Macroinvertebrate (total abundance)	6.50 ± 1.50	19.00 ± 9.31	ns
Fish (total abundance)	5.75 ± 3.77	2.75 ± 1.18	ns

3.2.3 Macroinvertebrates

Three calsses, which were identified from the sediments were taken at the end of the experiment included Gastropoda, Oligocheta and Chronomidae. Total macroinvertabrate abundance were compared through nutrient level differences with One way ANOVA which did not show any significant difference (Table 3.4). Gastropoda was divided into family groups while Chironomidae was into species level (Table 3.4).

Table 3.4: Macroinvertebrates community of 3 kajak cores which was taken at the end of the experiment from enclosures.

Class	**Family**	**Species**	**HN**	**LN**
Gastropoda	Physidae	-	1	1
	Lymnaeidae		-	4
	Valvatidae		-	1
	Planorbidae		2	3
Oligocheta	-	-	7	19
Insecta	Chironomidae	*Chironomus cingulatus*	2	-
		Chironomus dorsalis	-	2
		Chironomus nuditarsis	10	6
		Cryptochironomus defectus	-	1
		Glyptotendipes pallens	1	1
		Kiefferulus tendipediformis	1	9
		Polypedilum nubeculosum	2	6
		Procladius choreus	4	7
		Tanytarsus mendax	1	4

CHAPTER 4

DISCUSSION

4.1 Physico-chemical Parameters

Water temperature of tanks changed significantly during experiment. Until 8th August it increased ordinarily as considering the Mediterranean climate and the maximum degree was observed at this date as 28ºC. In the end of the September temperature decreased to approximately 20ºC. In present experiment when periphyton strips were in tanks, temperature changed between approximately 20 and 30ºC expect the last sampling (Sep 16 – Oct 17). It changed between approximately 20 - 13ºC (Table 3.2). Vermaat and Hootsmans' (1994) experiment showed that periphyton development changed at three different temperatures (10, 15, 20 ºC), since temperature affects enzymatic processes of periphyton community. At all these three level periphyton growth increased but at 20ºC it reached to the carrying capacity in a shorter time (30 days). According to DeNicola (1996) approximately a temperature range of 0-30ºC increased the biomass of periphyton and 30-40ºC decreased it. However, the temperatures that we observed in the current experiment was most of the time above the critical temperature of their experiment probably because of this we did not have major effect of temperature on periphyton. Moreover linear regression analysis showed that temperature did not have any significant effect on periphyton growth.

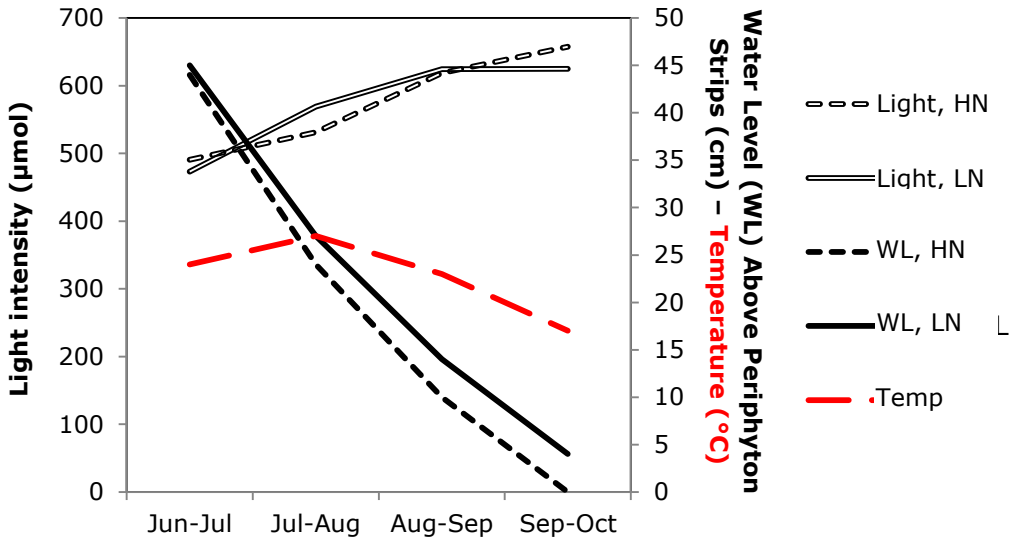


Figure 4.1: Increasing the light intensity as a result decreasing of water level above the periphyton strips with temperature.

Usually temperature did not limit biomass in natural communities but it caused an upper limit for production when other factors were optimal. If they were not, primary productivity was limited by factors such as light, nutrients and grazing depends on temperature (DeNicola, 1996).

There was a huge drop in water level at the end of the experiment because of the evaporation as a result of increasing temperature. Decreased water level had an indirect effect on periphyton strips that increased the light intensity. Periphyton strips were stayed still at 50 cm from water surface. Hence when the water level dropped, the water column on the strips reduced. Thus the light intensity on strips rose with time (Figure 4.1). Secchi disc depth was always higher than the place where strips were. Hereby there was not any limitation for light penetration to strips. Since light intensity changed with time significantly (Table 3.3) linear regression analysis was runned for revealing whether light has a significant effect on periphyton growth or not. Regression analysis showed that light was not an important effect for periphyton growth [R^2=0.331 (AFDM), R^2=0.293 (chl-*a*), ns (TP)].

Light availability is a prior abiotic effect that high or low intensity of light causes widely different taxa in periphyton structure and affects the dominant ones (Loeb and Reuter, 1981). However, different light regimes had greatly similar effects on growth curves and development of periphytic communities (Vermaat and Hootsmans, 1994). Low light is sufficient for the periphyton community but is also able to support high irradiance exposure (800 µmol; Nofdianto, 2010). Only in very low level of light regime (15 µmol) periphytic algal development will be minimal (Meulemans and Roos 1985; Liboriussen *et al.*, 2005).

4.2 Nutrient Effects

Regarding the periphyton abundance on the strips; dry mass (DM), ash free dry mass (AFDM), total phosphorus (TP) and chlorophyll-*a* (chl-*a*) content were estimated. AFDM, chl-*a* and TP were greater in high nutrient level enclosures (HN) than those of low ones (LN). On the other hand DM was greater in LN tanks than HN tanks.

There are large number of researches showing the effects of nutrients on periphyton which used chl-*a* parameter for periphyton biomass (Hansson, 1992; Jones and Sayer, 2003; Liboriussen *et al.*, 2005, 2006; Smith and Lee, 2006; Becares *et al.*, 2008; Özkan *et al.*, 2010). Many of their results were similar with the current experiment that chl-*a* content of periphyton was strongly correlated with high nutrient levels in lakes. However Sánchez *et al.* (2010) showed that periphyton chl-*a* amount was lower in the turbid lake (240 µg/L TP and 1540 µg/L TN) than that of the clear lake (70 µg/L TP and 229 µg/L TN) in Argentina. It was atributed to the light limitation imposed by planktonic phytoplankton that took advantage of the nutrient availability in the water column. Nevertheless, in our high nutrient enclosures light limitation was not encountered for the periphyton strips because secchi disc depth were always higher than 50 cm. Another experiment was concluded with an opposite result is Hillebrand and Kahlert (2002) which was conducted for sediment periphyton

construction. Their results indicated that water column nutrient enrichment affected periphyton chl-*a* on hard substrata but not sediment-dwelling algae.

Comparing the parameter used for periphyton biomass DM, AFDM and TP are generally less frequently used than chl-*a* concentration especially in lakes [Mazumder *et al.*, 1989 (PP: particulate phosphorus, lake study); Gaiser *et al.*, 2004 (TP, lake study); Sharifi and Ghafori, 2005 (DM and AFDM); Hill and Fanta 2008 (P $gAFDM^{-1}$); Greenwood and Rosemond, 2005 (AFDM); Rosemond *et al.*, 1993 (AFDM), 2000 (AFDM)]. Results of these researchers (given with periphyton biomass parameter) also the similar with the present experiment in term of nutrient effect on periphyton excluding DM. Sharifi and Ghafori (2005) conducted an experiment in a natural stream as a control and two artificial streams enriched with nitrogen (N) and phosphorus (P). They found significantly higher periphyton DM and AFDM content in artificial streams than that of control one. We also found similarly high concentrations of AFDM content of periphyton. However, in our experiment DM was higher in low nutrient level enclosures and the difference between them was not significant. This was probably due to our system; hard water with high alkalinity and $CaC0_3$ leading to marl precipitation (Figure 4.2). Moreover active photosysthesis results with marl deposits (Goldsborough *et al.*, 2005). *Chara* existed only in LN tanks relatively dense and may cause the marl development.

Figure 4.2: Marl development on the periphyton strips in LN tanks.

Hill and Fanta (2008) showed that soluble reactive phosphorus (SRP) and periphyton phosphorus content was related in a nonlinear way. Here phosphorus of periphyton reached a maximum at 82 µg l^{-1} SRP. Thereby between the SRP range of our experiment (LN: 2.07, HN: 13.01 µg l^{-1}) and further TP of periphyton rose with increasing nutrient concentrations.

DM, AFDM, TP and chl-*a* of periphyton decreased with time in both HN and LN enclosures. But, at the last sampling all periphyton parameters increased in LN tanks and Bonferroni test showed that TP and chl-*a* amount of periphyton statistically increased. Was it random or significant? Actually TP concentrations of water column in enclosures (Figure 3.8) also increased and Bonferroni test showed the raise of the last month was significant and it was coincided with the raise of periphyton biomass of LN tanks. Thus, significant phosphorus increase of enclosures might have affected the LN periphyton biomass. But then the same effect was not observed in the HN tanks. It is probably because nutrient addition to shallow lakes which had low nutrient level were more affected than the lakes had high nutrient level and also in low nutrient levels, uptake of nutrients and response to them is faster (Noe et. al.,2003; Hwang *et al.*, 1998).

Moreover decreasing of grazing pressure might cause the raise of periphyton biomass in LN tanks. Because of the decreasing temperature, chironomidae larvae could decrease and reduce the grazing pressure on the periphyton biomass (Liboriussen *et al.*, 2003).

On the other hand grazer experiment showed that grazing pressure in HN tanks increased through time. Since periphyton biomass in HN tanks decreased under high nutrient and high grazing conditions, this observation might lead to an understanding that grazing effect has more impact on periphyton growth than nutrients have. While Phillips *et al.*, (1978) revealed that nutrient effect is stronger than gazer pressure on periphyton; Jones and Sayer (2003) found a significant relationship between grazers and periphyton but not between phosphorus concentration and periphyton.

4.3 Macrophytes and Periphyton

Studies (e. g. James *et al.*, 2005; Baker, 2010) showed that macrophyte abundance decreases with the increasing nutrients. However in our experiment although the difference between LN and HN tanks was not important significantly, the plant we used *Myriophyllum spicatum* was much more in HN tanks. On the other hand while HN enclosures had only *M. spicatum* LN tanks had not only it but also *Chara contraria* that is known as an indicator of low nutrient conditions with a significant difference. Two main reasons can be accounted for both nutrient level enclosures had likely the same abundance of *M. spicatum.* Firstly; it was less in LN tanks because existence *C. contraria* could cause a competition with *M. spicatum* for nutrient supply and limit its growth, *M. spicatum* is a plant which also compete with other macrophytes for nutrients (Smith *et al.*,2002). Blindow *et al.* (2002) observed that dense *Chara* (app. half of the lake) caused a decrease in chlorophyll and phosphorus concentrations. Secondly; it did not decrease with high nutrient levels in HN tanks because the dropping water level increases light availability for macrophytes and often coincides with a raise in residence time (Beklioğlu *et al.*, 2006, 2008; Bucak, 2012). Smith *et al.* (2002) revealed that while in low nitrogen conditions autofragments and stems of *M. spicatum* developed more, in high nutrient conditions stems and root parts developed greater. This may lead to longer residence time in high nutrient levels.

In HN tanks despite the high nutrient, *Myriophyllum spicatum* grew in the beginning of the experiment because planktonic chl-*a* was low and so water was clear enough to allow early growth of the plant. However, the later growth appeared to be controlled by the periphytopn growth as the macrophyte PVI% and periphytic chl-a concentration showed the opposite trend such as in July and August but more clearly and strongly in September. Despite the high level of planktonic chl-*a* in the water column, macrophyte kept growing since *M. spicatum* already grew until August, high level of planktonic chl-*a* itself did not set a light limitation for the macrophyte (Figure 4.3).

In LN tanks, PVI% of *M. spicatum* increased to approx. 15% in September but then it started to decrease probably as a response to increased periphyton biomass. Furthermore, competition with *Chara* might have reinforced the reduction. On the other hand, in LN tanks *Chara* grew well because of the low nutrient and consequently clear water conditions during the experiment. Though it was not easy to estimate *Chara* coverage and PVI% beacuse of the depth induced visibility problem as well as the short stature of the plant unabling measurement. Harvesting the plant at the end of the experiment explicity showed that we had strong *Chara* growth (Figure 3.19, Table 3.3)). Thus it appeared to be that for chara growth, periphyton did not have a major effect prorbably because of allelopathy effect of Chara on periphyton via excreting cupper sulphate (Figure 4.3).

The results of epiphyton which was taken at the end of our experiment from *M. Spicatum* also supperted the periphyton biomass results of artificial substrates. It showed that DM, AFDM and TP were not different significantly despite they were much more in HN tanks. On the other hand chlorophyll-*a* concentrations are significantly different. It is because periphyton experiment ended in October however macrophytes were harvested in November at the end of the mesocosm experiment. Thus dead cells increased on macrophytes and as it was shown in Table 1.1, these parameters also include dead cells. On the other hand, since chl-*a* is spesific to algae, there was a significant difference between HN and LN tanks (Figure 3.21).

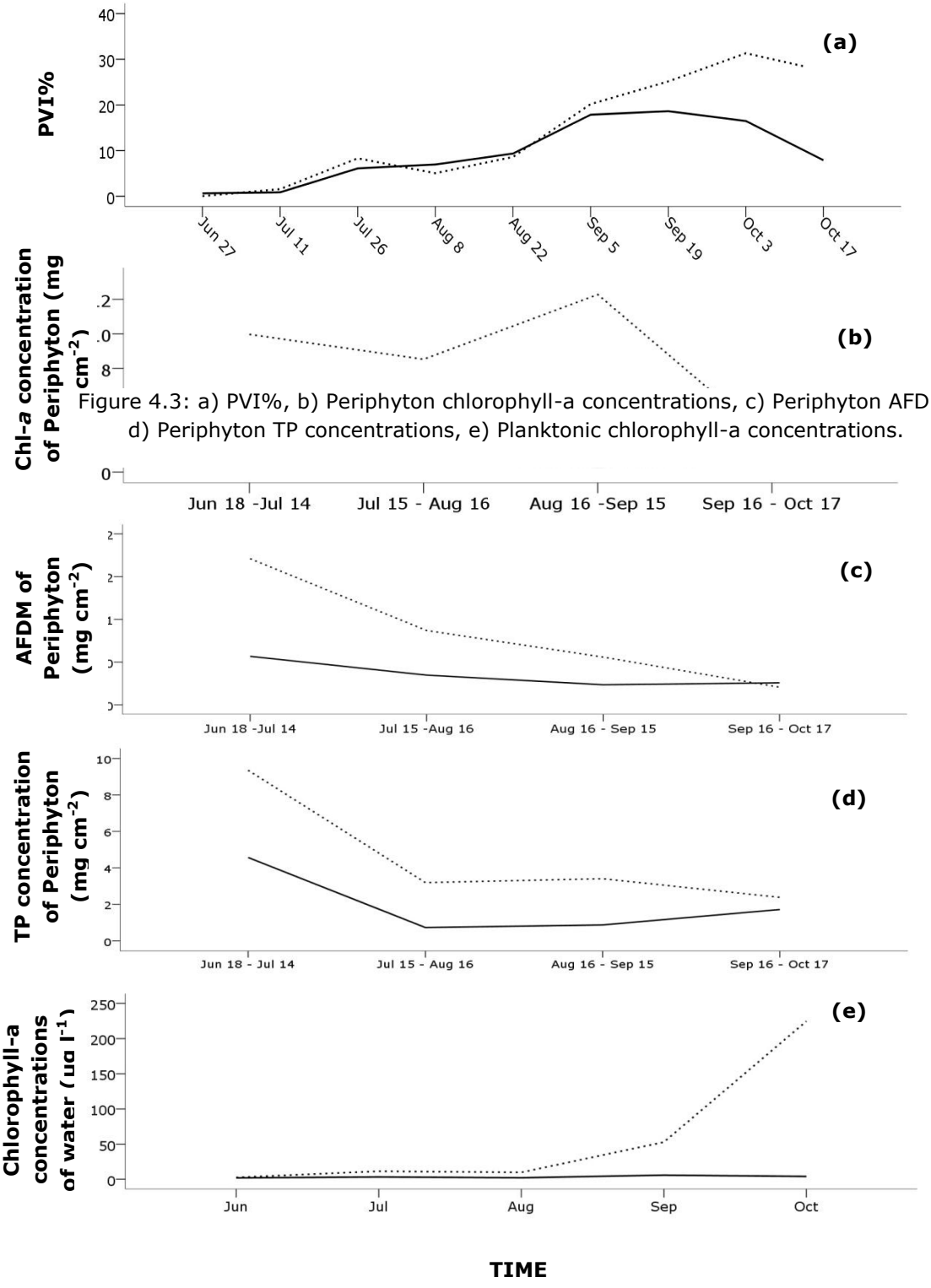


Figure 4.3: a) PVI%, b) Periphyton chlorophyll-a concentrations, c) Periphyton AFDM, d) Periphyton TP concentrations, e) Planktonic chlorophyll-a concentrations.

4.4 Macroinvertebrates and Periphyton

Although difference of macroinvertebrate abundance was not significant between HN and LN tanks, low nutrient level enclosures have more diverse and more macroinvertebrates because of the fish grazer pressure (Mazumder *et al.*, 1989) and nutrient level differences (Liboriussen *et al.*, 2005).

In our tanks we observed just three groups of macroinvertebrates which were gastropod, oligacheta and chiromidae (Table 3.1). Periphyton is an important resource for chironomids as a habitat and also as food (Liboriussen *et al.*, 2005). Also gastropods are one of the important grazers feeding on periphyton (Hillebrand and Kahlert, 2001). Researchs show that oligochaeta concentrations are increasing with increasing periphyton (Kilroy *et al.*, 2009; Friberg *et al.*, 2003). In LN tanks these three groups were more abundant and it was resulted with a lower concentrations of periphyton biomass.

There was not so many diverse macroinvertebrates in sediment samples. Therefore Chironomidae family was identified to species level to compare the nutrient levels better. However the species could not be compared with the statistical programme because of insufficient data. We should have taken more than 3 kajak cores. But considering the abundance of species, we may say that *Chironomus dorsalis, Cryptochironomus defectus* and *Kiefferulus tendipediformis* can not modify the high nutrient conditions. Besides *Chironomus nuditarsis* may be an indicator for the increasing nutrient conditions.

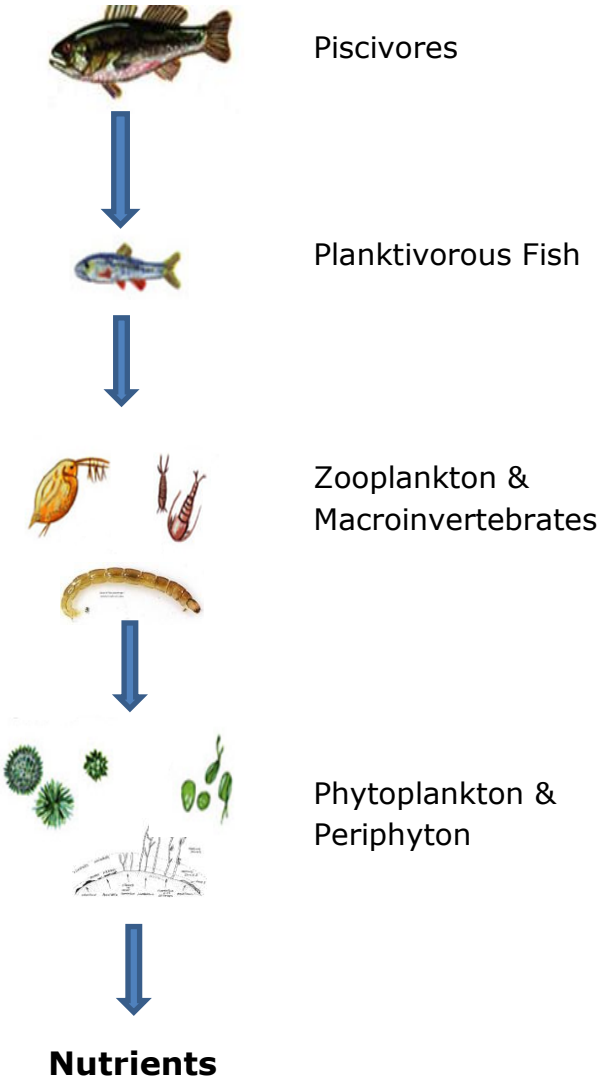


Figure 4.4. Classical food web (Taken and adapted from Molles, 2010).

4.5 Top-down, Bottom-up Effect

All periphytic algae are not affected from grazers in the same way, all grazing invertebrates are not a good prey for fish as well. Moreover invertebrates and also periphytic algae can exhibit behavioral responses to predation. After all the cascade between predators, herbivores and producers seem to be strong (Figure 4.4), (Jones and Sayer, 2003).

In the beginning of our experiment all conditions were the same except nutrient level (Figure 4.4). At the end of the experiment we saw more fish, less macroinvertebrate and more periphyton abundance in LN tanks, while less fish, more macroinvertebrates and less periphyton in HN tanks (Figure 4.5, 4.6, 3.13, 3.14, 3.15). Considering bottom-up effect high nutrient much more increased primary production in HN enclosures via rising phytoplankton and periphyton abundance than LN tanks. This raise increased macroinvertebrates which feed on periphyton and increased fish which feed on macroinvertebrates. In our enclosures there was planktivorous fish but not piscivorous fish. Hence amount of planktivorus fish much more increased in HN enclosures than LN ones (Table 3.3, 3.4). This had a strong cascading effect of fish on periphyton via grazers that included macroinvertebrates and zooplankton. Thus we had limited the grazer effect on periphyton and periphyton composition much more increased in high nutrient level enclosures. Despite same number of fish inoculum to every enclosure, HN tanks developed more fish. Hence, despite the same top-down control effect at the start of the experiment, top down control of fish via periphyton grazers also varied with nutrients reflecting the bottom-up effect (Mazumder *et al.*, 1989; Jones and Sayer, 2003).

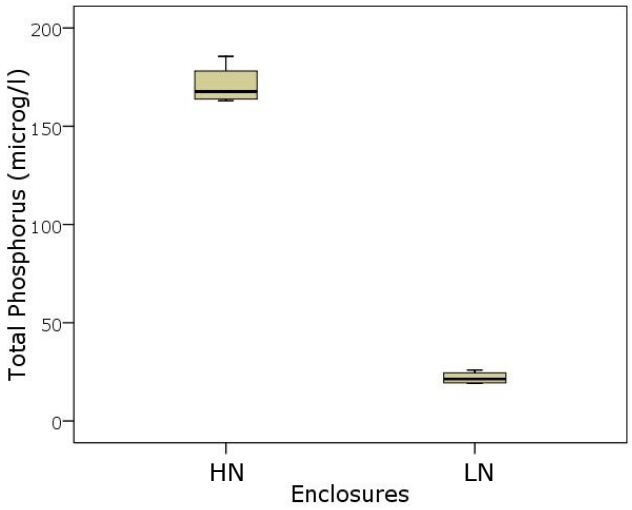


Figure 4.5: The phosphorus concentrations of the tanks in the beginning of the experiment (the only difference between them).

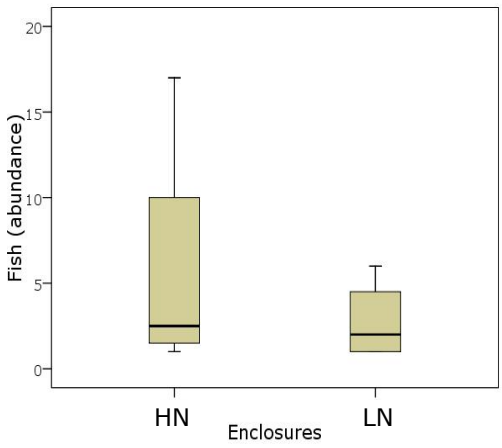


Figure 4.6: Fish abundance in HN and LN tanks at the end of the experiment.

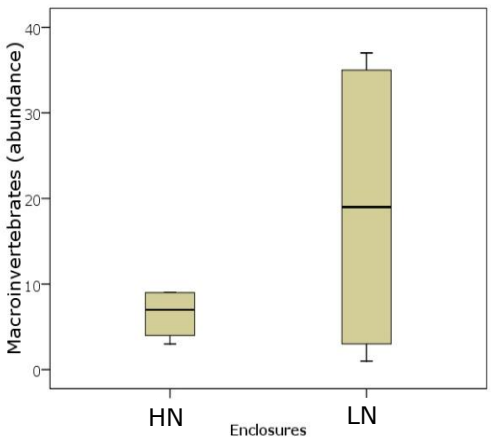


Figure 4.7: Macroinvertebrate abundance in HN and LN tanks at the end of the experiment.

CHAPTER 5

CONCLUSION

With current experiment it was aimed to determine the effects of nutrients on periphyton growth. Experiment was carried on with two different nutrient level (25 µg l^{-1} P, 0.5 mg l^{-1} N and 200 µg l^{-1} P, 2.0 mg l^{-1} N) which were regarded as high nutrient (HN) and low nutrient (LN). All of the initial conditions of the enclosures were the same except the nutrient level. Nutrient level differences had a significant effect on both physico-chemical parameters such as conductivity, pH, alkalinity, Secchi disk depth, dissolved oxygen and biological parameters.

Through the experiment water level reduction in enclosures was observed up to 0.50 m and water temperature was observed up to 26 ºC. The reduction of water level incresed the light intensity on the periphyton. However linear regression analysis showed that temperature and water level changings did not have a sihnificant effect on periphyton growth.

Periphyton biomass was higher in HN enclosures than LN tanks. Only dry mass of periphyton biomass showed the opposite because of the marl deposition in LN tanks. This finding was also supported by epiphyton samples which was taken at the end of the experiment.

LN enclosures had the more abundance of macroinvertebrate which were mainly large grazers such as gastropods and Chironomidae. Grazer experiment showed that grazing effect on periphyton increased in time. Although this raise, periphyton growth also increased in LN enclosures with nutrient increasing. This may be indicate that nutrient effect has a stronger impact than grazer pressure has on periphyton.

As it is indicated before in the beginning of the experiment all of the conditions were the same except nutrient level. Thus, bottom-up effect changed the top-down control and at the end of the experiment we saw the more periphyton less macroinvertebrate and more fish in HN tanks while the opposite was seen in LN tanks. High abundance of periphyton had a suppression effect on macrophyte in HN tanks and when periphyton decreased, PVI% of *M. spicatum* increased. Because of the low nutrient and low planktonic chl-*a* during the experiment in LN tanks *C. contraria* also grew besides *M. spicatum*.

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