

Master's thesis

**Littoral contribution to whole-lake primary production in
highly humic Lake Mekkojärvi**

Jussi Vesterinen



University of Jyväskylä

Department of Biological and Environmental Science

Aquatic Sciences

24.6.2013

University of Jyväskylä, Faculty of Mathematics and Science

Department of Biological and Environmental Science
Aquatic Sciences

Vesterinen Jussi: Littoral contribution to whole-lake primary production in highly humic Lake Mekkojärvi
Master's thesis: 42 p.
Supervisors: Prof. Roger I. Jones, Dr. Shawn Devlin
Reviewers: Dr., Doc. Jari Syväranta, Dr. Shawn Devlin
June 2013

Keywords: DOC, dystrophic, littoral, periphyton, phytoplankton, whole-lake experiment

ABSTRACT

Most studies investigating whole-lake PP have focused on oligotrophic or eutrophic lakes and little attention to dystrophic lakes have been paid. The aim of this thesis was to determine the contribution of littoral PP to whole-lake PP in dystrophic Mekkojärvi in 2012. Lake Mekkojärvi has high dissolved organic carbon (DOC) and humic acid concentrations, and as a result, very low light penetration. Mekkojärvi is kettle-shaped and has relatively low nutrient availability. Models based on these chemical and physical factors predict that benthic algal contributions to whole-lake primary production (PP) in Mekkojärvi could be as low as less than 5 %. Pelagic PP was measured from each strata by using ^{14}C incorporation method. Epiphytic algae was sampled and incubated *in situ* in both dark and light, and littoral PP values were measured from the difference of DIC in light and dark vials and in water. Daily rates for littoral PP were calculated. Clear spring maximum in pelagic PP was observed in early-June, followed by sharp decline. Pelagic PP remained low through the remained of summer. Littoral PP was relatively high throughout the summer. Whole-lake estimates indicate that littoral contribution to whole-lake PP can be over 90 % in summer, while pelagic contributions are higher in spring and in autumn.

JYVÄSKYLÄN YLIOPISTO, Matemaattis-luonnontieteellinen tiedekunta

Bio- ja ympäristötieteiden laitos
Akvaattiset tieteet

Vesterinen Jussi: Litoraalityöhykkeen osuus koko järven perustuotannossa
erittäin humuspitoisessa Mekkojärvässä
Pro gradu: 42 s.
Työn ohjaajat: Prof. Roger I. Jones, FT. Shawn Devlin
Tarkastajat: FT, Dos. Jari Syväranta, FT Shawn Devlin
Kesäkuu 2013

Hakusanat: DOC, dystrofinen, kasviplankton, koko järven mittakaavan koe, litoraali,
perifyton

TIIVISTELMÄ

Suurin osa koko järven perustuotantoa käsittelevistä tutkimuksista on tehty oligotrofisissa tai eutrofisissa järvissä ja vähän on tutkittu dystrofisten järvien toimintaa. Tämän tutkimuksen tarkoitus oli selvittää litoraalityöhykkeen perustuotannon osuutta koko järven perustuotannosta dystrofisessa Mekkojärvässä vuonna 2012. Mekkojärvässä on korkeat liuennan orgaanisen hiilen (DOC) ja humushappojen pitoisuudet ja näiden tuloksena erittäin matala valon tunkeutuvuus, suhteellisen matala ravinnepitoisuus ja suhteellisen korkea keskisyvyyden suhde maksimisyvyyteen. Mallit, jotka perustuvat edellä mainittuihin parametreihin, ennustavat pohjaleville vain 5 % osuutta kokonaisperustuotannosta Mekkojärvässä. Pelagiaalin perustuotanto mitattiin kustakin kerrostuneisuusvyöhykkeestä käyttäen ¹⁴C-menetelmää. Epifyyttistä levää poimittiin ja näytteet inkuboitiin järvessä sekä valossa että pimeässä, ja litoraalin perustuotanto mitattiin epäorgaanisen hiilen eroista valo- ja pimeänäytteissä sekä järvivedessä. Koko päivän litoraalin perustuotanto laskettiin. Pelagiaalin perustuotannossa havaittiin selvä keväthuippu, jonka jälkeen laskeva trendi syksyä kohti mentäessä. Pelagiaalin perustuotanto pysyi matalana keväthuipun jälkeen. Litoraalin perustuotanto oli suhteellisen voimakasta läpi kesän. Koko järven estimaatit ennustavat jopa yli 90 % osuutta litoraalin perustuotannolle kesällä, kun taas keväällä ja syksyllä pelagiaalin perustuotannon osuus on suurempi.

Contents

1. INTRODUCTION	5
2. BACKGROUND	6
2.1. Special characteristics of humic lakes	6
2.2. Primary production in humic lakes	7
2.2.1. Pelagic primary production	8
2.2.2. Benthic primary production.....	9
2.2.3. Primary production of macrophytes	11
2.3. Whole-lake experiment in Mekkojärvi.....	12
3. MATERIAL AND METHODS	12
3.1. Study area	12
3.2. Sampling and data collection	15
3.2.1. Physical and chemical parameters	15
3.2.2. Fish introductions	15
3.2.3. Pelagic primary production and community respiration	15
3.2.4. Littoral primary production	16
3.2.6. Littoral primary production in control lakes.....	17
3.2.7. Statistical tests	18
4. RESULTS	18
4.1. Physical and chemical parameters.....	18
4.2. Pelagic primary production	20
4.3. Littoral primary production	24
4.3.1. Littoral primary production in control lakes.....	29
4.4. Whole-lake daily estimates of primary production	29
5. DISCUSSION	31
5.1. Overview of the results.....	31
5.2. Pelagic primary production and community respiration	32
5.3. Littoral primary production	33
6. CONCLUSIONS	35
ACKNOWLEDGEMENTS	35
REFERENCES.....	36

1. INTRODUCTION

Lake primary production (PP) is often categorized based on the magnitude of phytoplankton production in the water column. However, previous studies have shown that PP of benthic algae (attached to substrata) can make substantial contributions to whole-lake PP in small- to moderate-sized lakes (Vadeboncoeur *et al.* 2003) and provide an important food resource for whole lake, since fish can be major grazers of zoobenthos (Schindler & Scheuerell, 2002). Utilization of benthic algae by consumers can be high even in very large lakes, although the total contribution to whole-lake PP may be low (Hecky & Hesslein, 1995; Hobson & Welch 1995; Vander Zanden & Vadeboncoeur 2002).

Most studies investigating whole-lake PP have focused on oligotrophic or eutrophic lakes and little attention to dystrophic lakes have been paid. Many lakes receive high loadings of allochthonous dissolved and particulate organic matter from their catchment area (Wetzel, 1995), and as a consequence, in these lakes community respiration often exceeds gross primary production (GPP) (Cole *et al.* 2000). Recent studies have provided strong evidence, that this net heterotrophy can be distinguished in most of the unproductive lakes (Duarte & Prairie, 2005). Furthermore, some studies (eg. Carpenter *et al.* 2005) have shown, that allochthony is highest in dystrophic lakes and decreases in nutrient-riched lakes. In dystrophic lakes, coloured allochthonous organic matter provides carbon source for heterotrophic bacteria and reduces light penetration, which inhibits phytoplankton PP and shifts lakes metabolisms towards heterotrophy (Kankaala *et al.* 2010).

Lake Mekkojärvi in southern Finland has high humic acid and dissolved organic carbon (DOC) concentrations (20–45 mg C L⁻¹), and as a result, very low light penetration (light attenuation coefficient, $K_d = 3-5$). The lake has relatively low nutrient availability and a relatively high depth ratio (0.65). Mekkojärvi is net heterotrophic lake (Salonen *et al.* 2005) with high microbial activity. Models based on these chemical and physical factors (eg. Vadeboncoeur *et al.* 2008) predict that benthic contributions to whole-lake PP in Mekkojärvi would be less than 5 %. Although Mekkojärvi has virtually no illuminated benthic surface, no clearly defined littoral zone, the lake is surrounded by floating moss and macrophyte bed, which provides well-illuminated surface for epiphytic algae. A previous study in 2011 revealed that the benthic rates of PP on floating vegetation in Mekkojärvi are much higher than pelagic rates on a volumetric basis and the benthic contribution to whole lake PP is much higher than models would predict, on average 78 % (S. Devlin, unpublished). What was lacking in the previous study is a fundamental understanding what is the temporal variation of littoral contribution to whole-lake PP throughout the growing season.

This thesis is a part of a whole-lake experiment, where Mekkojärvi has been divided in two basins (from inflow to outflow) with plastic curtain that extends down to the bottom of the water body, providing replicate sides for successive experimental manipulations. In 2012, to evaluate the responses of lake processes to a gradient of consumer size structure (see 2.3.), both planktivorous young of the year perch (*Perca fluviatilis*) and larger perch (50–100 g individual weight) were introduced to Mekkojärvi. The main aim of this thesis was to determine the contribution of littoral PP to whole-lake PP and its temporal variation in Mekkojärvi in 2012. The aim was also to compare the possible differences in PP between the two basins, caused by possible differences in physical and chemical conditions and/or trophic cascade, triggered by fish.

2. BACKGROUND

2.1. Special characteristics of humic lakes

Globally, non-calcareous humic substance (HS) -rich freshwaters predominate over calcareous, HS-poor water bodies (Steinberg, 2008). Among freshwaters, as much as 80 % of the total dissolved organic carbon (DOM) present may be humic in origin (Steinberg & Münster, 1985). Humic substances are defined as refractory decomposition products of biological material. Due to their complex structure, scientists have been unable to describe the exact structure of HS molecules. Although it is known, that HS exceed the organic carbon in all living organisms by roughly one order of magnitude (Wetzel, 2001), the knowledge of ecological function and quantitative significance of HS is still small (Steinberg *et al.* 2008). It is necessary to reconcile the classical food-web theory – with its implicit assumption that energy flow in aquatic ecosystems can be quantified by measuring rates of photosynthesis and rates of food ingestion by consumers – into a deepening knowledge of the system regulating functions of dissolved humic matter (Steinberg, 2004). In many instances, the fraction of humic substances in solution (dissolved humic matter, DHM) makes largest part of dissolved organic matter (DOM) present in inland waters (Steinberg, 2004). Different descriptions of organic matter (OM) and HS are used according to their origin and research interests: dissolved organic matter (DOM), particulate organic matter (POM), natural organic matter (NOM), soil organic matter (SOM) and refractory organic substances (ROS) – or according to their fractionation procedures: fulvic acids (FA; acid and base soluble), humic acids (HA; base soluble), humin (insoluble in either acid or base, available only in soil samples) or non-humic substances (NHS) (Abbt-Braun *et al.* 2003). Acidification of the alkaline extract precipitates humic acids, leaving the fulvic acids in solution (Steinberg, 2004).

DOM is a common major component in carbon, energy and nutrient budgets for freshwater ecosystems, and effects food webs, heterotrophy, nutrient retention and nutrient release (Findlay, 2003). Phytoplankton-derived, autochthonous DOM has traditionally been regarded as the most important carbon source in pelagic systems and food webs. Already in 1927 Birge & Juday (1927) distinguished between autotrophic lakes, which derive their organic matter from internal sources, and allotrophic lakes, which derive a large portion of their organic matter from their catchment areas. Allochthonous organic carbon often makes up a significant share of consumer biomass in unproductive lake ecosystems (Berggren *et al.* 2010). In addition to carbon source, allochthonous DOM is a source of nitrogen, phosphorus and sulfur (Aitkenhead-Peterson *et al.* 2003). Highly brown coloured northern boreal lakes, which receive high amounts of terrestrial organic matter from their catchments and have low nutrient contents and productivity, have long been classified as dystrophic (Thienemann, 1925; Järnefelt, 1935). However, correlation between trophic state and water colour is not always obvious, since many humic lakes are mesotrophic or even eutrophic (Jones, 1992).

Nauwerck (1963) was among the first to find that phytoplankton production was insufficient to support zooplankton secondary production in the mesotrophic Lake Erken. He suggested additional contributions from allochthonous sources via bacteria and detritus. The first evidences of many humic lakes being net heterotrophic, i.e., the total respiratory production of CO₂ exceeds the fixation of CO₂ by primary production, came from Finland (Sarvala *et al.* 1981; Salonen *et al.* 1992), and the same phenomenon has been reported from many other lakes all over the world as well (e.g. Cole *et al.* 1994; Coveney & Wetzel 1995; Jansson *et al.* 2000; Jasser *et al.* 2009). Azam *et al.* (1983) worked on marine environments and were the first to adopt the concept of microbial loop, which describes the

pathway of microbial food web where DOC is incorporated to higher trophical levels via its incorporation into bacterial biomass. Jones (1992) showed that microbial loop plays significant role in many humic lakes transferring allochthonous organic carbon to metazoans. Jones (1992) summarized the influence of HS on lacustrine planktonic food web and suggested that HS might influence planktonic food webs in lakes in two ways: (1) either altering the physical and chemical environment and thus modifying autotrophic primary production or (2) acting as a direct carbon and energy source for the pelagic food web.

In humic waters ca. 90% of the total carbon pool is in dissolved form, consisting mostly of allochthonous organic carbon (Münster *et al.* 1999), from which a large part is transported to upper food chains via microbial links (Salonen & Hammar, 1986; Jones, 1992; Tranvik, 1992). Currie & Kalff (1984) found that bacteria have higher phosphorus-binding capacity compared to algae, even though both can produce extracellular enzymes to hydrolyze organic phosphorus compounds to phosphate. The availability of dissolved inorganic nutrients (DIN) has significant effects on bacterial growth (Findlay, 2003), and Findlay (2003) suggested that the role of DIN in affecting bacterial growth is a function of DOM carbon composition, nutrient content, ambient inorganic nutrient concentration or prevalence of grazers. Tulonen *et al.* (2000) found a clear response of bacterial production (BP) to changes in the phytoplankton in mesohumic Lake Pääjärvi, but high BP:PP ratio indicated that phytoplankton production alone was insufficient to sustain the total bacterial production, and that the bacteria relied on allochthonous carbon. In highly humic lakes with anoxic hypolimnion, *Archaea* usually dominates the anaerobic carbon mineralization (Canfield *et al.* 2005) and provides both carbon and energy sources for methane-oxidizing bacteria (MOB) (Sundh *et al.* 2005). Taipale *et al.* (2009) and Kankaala *et al.* (2010a) suggested that in small highly humic lakes, ca. 50% of zooplankton (*Daphnia*) biomass may be derived from CH₄ via MOB.

Because of the ubiquity and variety of functional groups of HS, they have the potential to affect several biochemical and biogeochemical pathways in both freshwater organisms and ecosystems (Steinberg *et al.* 2006). HS have potential to act as external electron acceptors for microbial respiration (Lovely *et al.* 1996) and may directly quench electrons or bind to the bioquinones in photosystem II (PS II) and thereby block the electron transfer – thus interfering within the photosynthetic oxygen production (Steinberg *et al.* 2008). Recent findings indicate possible hormone-like effects of HS and their capability to interfere male-female reproductive physiology and behaviour (Hubbard *et al.* 2002). Even though the ecological significance of HS remains obscure, HS should be considered an abiotic ecological driving force – obviously more cryptic than light, nutrients and temperature – which can interfere within almost any biotic structure and biochemical pathway in freshwater organisms (Steinberg *et al.* 2008).

2.2. Primary production in humic lakes

Primary production (PP) is defined by Odum & Odum (1959) as the rate at which energy is stored by photosynthetic activity in the form of organic substances, which can be used as food. In lakes, this consists of photosynthesis of phytoplanktonic algae, benthic algae and large aquatic plants (macrophytes) (Verduin, 1956). Special abiotic features of humic lakes – which affect primary production – are high water colour, low irradiation and deeper penetration of red part of the spectrum (620...700 nm) to water (Eloranta, 1999), low pH and alkalinity (Kortelainen, 1999) and low concentrations of inorganic nutrients, such as phosphorus, nitrogen, silica and calcium (Münster, 1999).

2.2.1. Pelagic primary production

Small and sheltered humic lakes tend to have very steep thermal and oxygen gradients, since they are not prone to wind mixing, and incoming solar radiation is rapidly absorbed by the surface waters. The latter affects the underwater light climate by reducing the depth of euphotic zone, which limits photosynthesis to uppermost water layers. Nutrient inputs in unproductive humic lakes are often associated with allochthonous organic matter, which reduces light penetration and limits photosynthesis of pelagic – often nutrient-limited – phytoplankton, overriding possible positive effects of nutrients on pelagic primary production (Karlsson *et al.* 2009). At low nutrient concentrations, bacteria seem to take up phosphorus more effectively than phytoplankton, which thus stimulates their production (Currie & Kalff, 1984). Under those circumstances, when bacteria are no longer phosphorus limited, nutrient additions may increase primary production more than bacterial production (Arvola *et al.* 1999). DOC contributes to plankton community respiration (Hessen, 1992) and the ratio of photosynthesis and respiration (P:R ratio) should be generally lower in coloured lakes than in clearwater lakes (Jones, 1992).

Although humic lakes can have large pool sizes of allochthonous DOM in their pelagic zones, this carbon from terrestrial origin is mostly composed of high molecular weight (HMW) compounds and consequently has often been considered biologically refractory (Münster *et al.* 1999). Solar radiation mineralizes DOM abiotically, producing low molecular weight carbon (LMW-DOM) and nutrients, which may explain stable bacterial populations, independent of phytoplankton primary production, in fresh waters (Salonen *et al.* 1992). However, liberation of phosphates from humus-iron-phosphate complex by solar radiation may radically assist phytoplankton in the uppermost epilimnion to uptake nutrients and compete with bacteria (Münster *et al.* 1999). Salonen & Jokinen (1988), Bird & Kalff (1989) and Jansson *et al.* (1996) found significant contributions of mixotrophic phagotrophic flagellates to phytoplankton production in humic lakes. Jones (2000) hypothesized that under low light conditions and relatively high DOC concentrations obligate phototrophs suffer unfavourable conditions and then mixotrophy might be advantageous.

Lake Mekkojärvi has been an object of many plankton studies over past decades (e.g. Salonen & Jokinen, 1988; Kankaala, 1988; Arvola & Kankaala, 1989; Kankaala *et al.* 2006). Salonen *et al.* (2005) published the results from pelagic primary production of Mekkojärvi from early May until mid-September in 1984 (Fig. 1). Clear peak of PP in May can be explained by increased nutrients from catchment inflow and anoxic hypolimnion (Salonen *et al.* 2005). After that, PP remained consistently low (11–61 mg C m⁻² d⁻¹) in June-August. Meanwhile, the median respiration in the uppermost epilimnion was estimated 5.2 times higher than phytoplankton primary production, indicating the importance of heterotrophic processes. However, community respiration (CR) may have been underestimated, since it was estimated only from oxic epilimnion. Cole & Pace (1995) stated that community respiration in anoxic hypolimnion can be similar to that in epilimnion and contribute significantly to whole lake community respiration. Salonen *et al.* (2005) contemplated that CR in Mekkojärvi could possibly have been three times higher than they estimated.

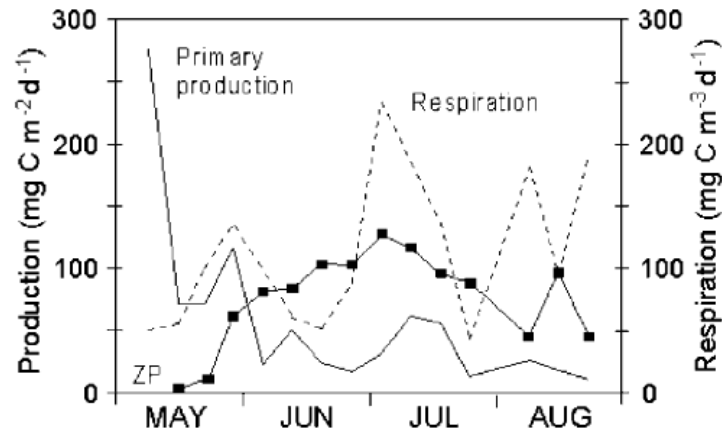


Figure 1. Pelagic phyto- and zooplankton production and community respiration in 0–1 m (epilimnion) water column in Lake Mekkajärvi during summer 1984 (modified from Salonen *et al.* 2005).

2.2.2. Benthic primary production

In the past decade the knowledge of benthic algae contributions to whole lake primary production and food webs in lakes has widely increased (e.g. Vadeboncoeur *et al.* 2001; Vadeboncoeur & Steinman, 2002; Vander Zanden *et al.* 2005; Vadeboncoeur *et al.* 2006; Ask *et al.* 2009). 10 years ago Vadeboncoeur *et al.* (2002) demonstrated that pelagic primary production had been measured about 10 times more often in limnological research compared to benthic primary production (Fig. 2). However, at low and intermediate levels of phytoplankton production, periphyton production can be similar or exceed phytoplankton production (Vadeboncoeur & Steinman, 2002).

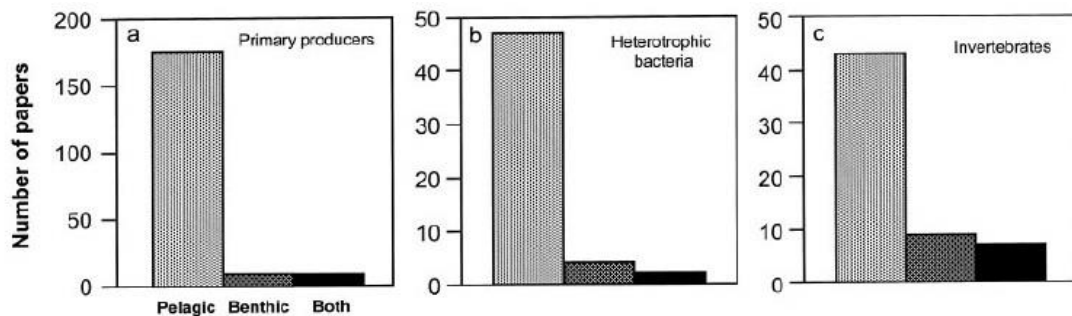


Figure 2. Frequency of publications on benthic versus pelagic habitats for (a) primary producers, (b) heterotrophic bacteria, and (c) invertebrates during years 1990–1999 (modified from Vadeboncoeur *et al.* 2002).

Vadeboncoeur *et al.* (2008) modeled the contribution of periphyton to whole-ecosystem primary production across lake size, shape and nutrient gradients. They used the ratio of mean depth to maximum depth to determine the available benthic habitats for periphyton. Light, mean depth and nutrient availability were used to model total phytoplankton production, whereas periphyton production was a function of light-saturated photosynthesis and light availability at depth. The model was developed to explore the effects of basin shape, nutrient concentration, and light attenuation by phytoplankton

biomass on potential contributions of periphyton to whole-lake autochthonous production (Vadeboncoeur *et al.* 2008). Although the depth ratio of lakes describes well the morphometry and potential habitat availability for benthic algae, the relationship with depth is extremely variable, since shallow lakes can be clear and periphyton dominated, or have limited transparency because of phytoplankton or high concentration of dissolved organic matter (DOM) (Vadeboncoeur & Steinman, 2002).

In Mekkojärvi, which is a steep sided lake with a high depth ratio, mean depth of 3 m, tot-P content between 10 and 15 $\mu\text{g L}^{-1}$ and tot-DOC concentration from 20 to 45 mg C L^{-1} (Taipale, 2007), the model predicts as low as less than 5 % benthic fraction of whole-lake PP (Fig. 3). K_b of 2.0 represents the (non-phytoplankton) light attenuation coefficient for turbid lakes with relatively high DOC and suspended solid concentrations (Vadeboncoeur *et al.* 2008).

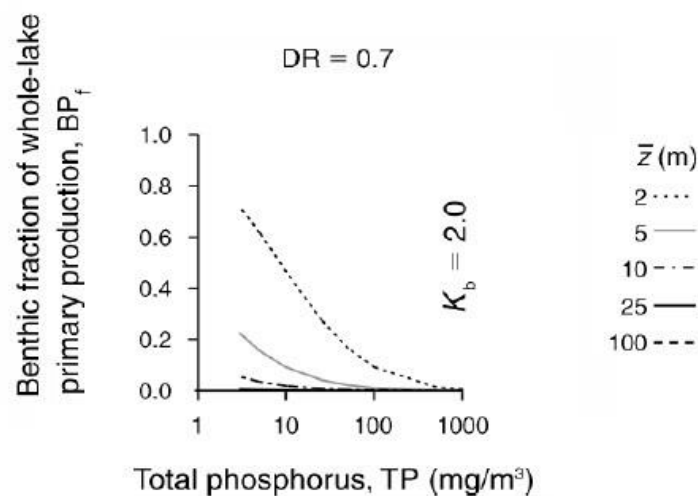


Figure 3. Effects of mean depth (\bar{z}), non-phytoplankton turbidity (K_b) and depth ratio (DR) to relative contributions of periphyton to whole-lake primary production (modified from Vadeboncoeur *et al.* 2008).

Periphyton grows on many different substrates; on macrophytes (epiphyton), rocks (epilithic), mud sediments (epipelon), sand sediments (epipsammon) and wood (epixylon) (Stevenson, 1996). Wetzel (1996) stated, that periphyton have the potential to obtain their nutrients from water column, the substratum to which they are attached or from internal cycling within the periphytic mat. However, in humic lakes with relative high concentrations of DOM, reduced light penetration to the water column is often limiting periphyton growth and benthic primary production, overriding the possible positive effects of increased nutrients (Vadeboncoeur & Steinman, 2002; Vadeboncoeur, 2006).

Ask *et al.* (2012) studied benthic and pelagic production in 15 lakes in northern Sweden, including both clear and humic lakes. They found evidence for increasing net heterotrophy along the increasing DOC gradient; benthic primary production decreased with increasing DOC concentration, while pelagic respiration increased and was consistently higher than pelagic gross primary production, resulting in a negative pelagic net ecosystem production in all lakes (Fig. 4). Ask *et al.* (2012) stated that the strong correlation between benthic primary production and respiration indicates dependence of respiration in benthic habitats on the autochthonous carbon (produced by benthic algae), and not on allochthonous (terrestrial) carbon.

Kairesalo *et al.* (1992) studied the littoral-pelagic interactions of Mekkojärvi, investigating the effects of moss vegetation on the metabolism of bacterioplankton, phytoplankton and zooplankton in laboratory throughflow system. Mekkojärvi is surrounded by the moss bed, formed mainly by *Sphagnum* species in the inner zone and *Warnstorfia* species in the outer zone, which can extend 5–10 m out from the shoreline (Kairesalo *et al.* 1992). In the inner part of the vegetation, some rooted plants, such as *Carex lasiocarpa* and *Phragmites australis*, emerge in narrow stands. These plants provide the only available substratum for periphyton in such highly humic lake with anoxic and non-illuminated sediments. Kairesalo *et al.* (1992) found several other zooplankton species from littoral vegetation zone compared to pelagic area, as well as high diversity of macroinvertebrates, indicating high primary and secondary production. Added nutrients to community bottles during the experiment, which doubled the production of moss community, while phytoplankton production was suppressed, demonstrated the capability of moss community (moss + epiphytes) to compete with phytoplankton for nutrients (Kairesalo *et al.* 1992). They stated that the peaty moss bed in littoral zone represents a large store of organic matter, and water currents may release substantial amounts of particulate organic matter from littoral zone into the pelagic area, thus providing an energy pathway between littoral and pelagic zone.

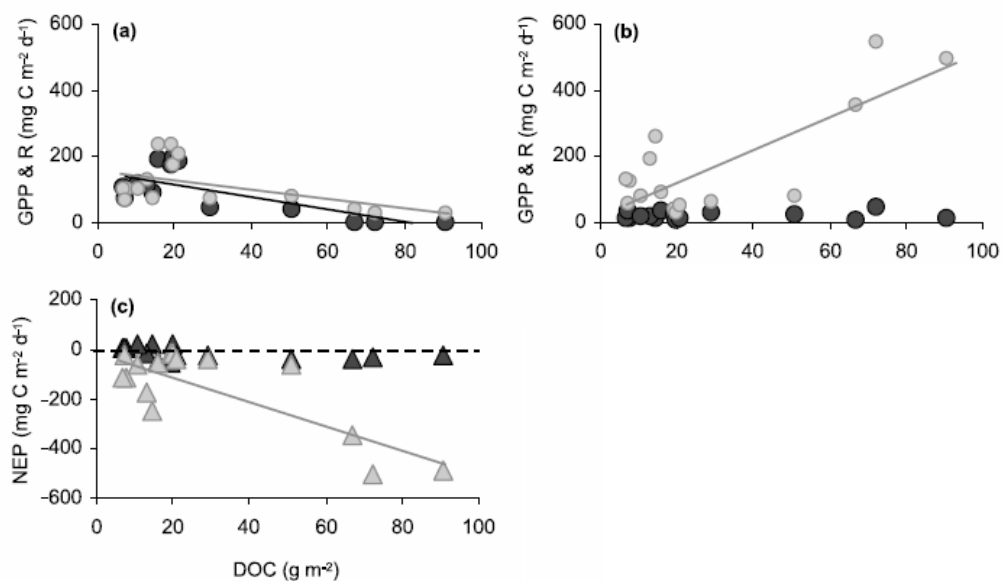


Figure 4. Gross primary production (GPP, dark gray circles) and respiration (R, light gray circles) in (a) benthic and (b) pelagic habitats, and (c) net ecosystem production (NEP) in benthic (dark gray triangles) and pelagic (light gray triangles) habitats, as a function of dissolved organic carbon (DOC) concentration in the lake water. The dotted line in Figure 4c shows NEP = 0 indicating metabolic balance. All regressions were significant. (modified from Ask *et al.* 2012)

2.2.3. Primary production of macrophytes

In highly humic waters, littoral vegetation zone is often narrow and submerged vegetation is sparse, due to the strong light attenuation, but dense beds of floating-leaved and emergent macrophyte species often exist (Estlander *et al.* 2009). The light absorption by non-photosynthetic particles (background attenuation), e.g. humic substances, restricts the integral photosynthesis of macrophytes (Sand-Jensen, 1989). Highly humic lakes tend to be naturally acidic, and several studies have shown a decline of macrophytic plants in acidifying lakes, indicating the replacement of Isoetid macrophytes by *Sphagnum* spp. species (e.g. Almer *et al.* 1974; Abrahamsen *et al.* 1976; Grahn, 1985). Grahn (1985)

hypothesized, that *Sphagnum* spp. species can contribute to acidified waters due to their capability to effectively absorb cations, such as Mg and Ca, for which they exchange H⁺. Köhler *et al.* (1984) found, that the photosynthesis of *Sphagnum girgensohnii* was stimulated 30% in pH at 4.0. Grahn (1986) found significant increase of *Sphagnum* spp. biomass along the gradient of decreasing pH in Swedish lakes. In Mekkojärvi, with pH 4–6, the vegetation zone consists mostly of moss species, which is typical for naturally acidic humic lakes.

2.3. Whole-lake experiment in Mekkojärvi

This thesis is part of the ongoing whole-lake experiment of the research group lead by Professor Roger Jones from University of Jyväskylä. The main aim of this whole-lake experiment is to test how differences in consumer intraspecific body size affect the energy flow pathways between pelagic and littoral zone and how high amounts of allochthonous organic matter modify the food web responses to trophic cascade effect. During the past decade, Jones's group has studied the impacts of increased loading of DOC on lake metabolism and lake food webs and used stable isotope enrichments as a tool to indicate, that allochthonous organic matter contributes to lake food webs. They have also used molecular tools to the analysis of functional diversity of methane-cycle bacteria in lakes.

In this on-going experiment, Mekkojärvi has been taken as a model system, since it has been widely studied during the past decades and its background ecology is well understood. Furthermore, the lake regularly becomes anoxic under winter ice cover, lacking the usual fish community, so it provides a basin for fish introductions and possibility to research the top-down control of prey by predatory fish. Mekkojärvi has been divided in two (from inflow to outflow) with plastic curtain that extends down to the bottom of the water body, providing replicate sides for successive experimental design for annual treatments. European perch was selected as the treatment consumer since its ubiquity in Finland and chance to undergo an ontogenetic (size-related) shift in diet from zooplankton to littoral macroinvertebrates to piscivory. To evaluate the responses of lake processes to a gradient of consumer size structure, both planktivorous young of the year perch and larger, more benthivorous perch (50–100 g individual weight) was introduced to Mekkojärvi.

3. MATERIAL AND METHODS

3.1. Study area

Mekkojärvi (61°13'N 25°3'E) is a small (0.35 ha) polyhumic (water colour 300–800 mg Pt l⁻¹) headwater water lake in Evo forest area in southern Finland (Fig. 5) with maximum depth of 4.3 m, mean depth of 2 m and steep temperature stratification gradient in summer. Thermocline lies between 0.5–1.0 m and total anoxia generally occurs below 1.0 m. The lake has a steep chemocline as well, i.e. hydrogen sulphide concentration below 1.0 m increases rapidly. Mekkojärvi receives high loadings of terrestrial carbon from the surrounding catchment area. Dissolved organic carbon varies between 20 and 45 mg C L⁻¹, dissolved inorganic carbon (DIC) between 3 and 12 mg C L⁻¹ and tot-P content between 10 and 15 µg L⁻¹ (Taipale, 2007). Total N in the epilimnion varies from 500 to 1000 µg N L⁻¹ and from 800 to 1100 µg N L⁻¹ in the hypolimnion (Taipale, 2007). Common physical and chemical parameters in 2012 are shown in Table 1.

The lake is naturally acidic (pH from 4 to 6) and surrounded by pine (*Picea abies*) and Scots pine (*Pinea sylvestris*) forest. Normally it is ice-free from the beginning of May

to mid-November, and during ice-cover it becomes completely anoxic. Due to the anoxia, the lake cannot maintenance planktivorous fish populations. Therefore, Mekkojärvi has very dense population of a large-bodied cladoceran *Daphnia longispina*, which can almost exclusively contribute to the whole lake zooplankton biomass in summer (Salonen & Lehtovaara, 1992).

Mekkojärvi does not have a readily defined littoral zone. It is surrounded by a floating *Sphagnum* and *Warnstorfia* moss bed (Fig. 5) and the lake has a relatively high depth ratio ($DR = \bar{z}/z_{\max} = 0.65$), so the majority of benthic surface occur in deep areas. Due to the high water colour, light can penetrate only to the uppermost water layers, so the photic zone is very narrow (0–60 cm). In this study, littoral denotes horizontally the narrow vegetation zone, which is generally consisted of floating macrophytes and moss. Vertically it does not extend to deeper than ca. 0.5 m.

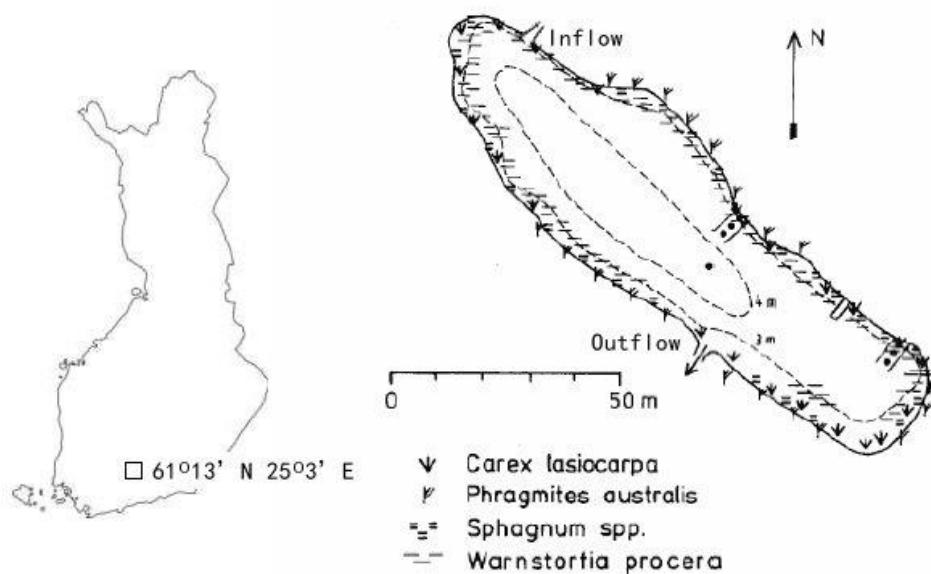


Figure 5. Location and map of Lake Mekkojärvi (modified from Kairesalo *et al.* 1992).

Table 1. Physical and chemical parameters (mean \pm S.D.) in Lake Mekkojärvi in 2012.

2012	May–June			July–August			September		
	0–0.5 m	0.5–1 m	1–3 m	0–0.5 m	0.5–1 m	1–3 m	0–0.5 m	0.5–1 m	1–3 m
pH	5.0 \pm 0.2	5.4 \pm 0.2	6.0 \pm 0.0	5.1 \pm 0.3	5.2 \pm 0.3	5.6 \pm 0.1	5.4 \pm 0.1	5.3 \pm 0.3	5.7 \pm 0.0
Chl <i>a</i> ($\mu\text{g L}^{-1}$)	6.8 \pm 5.5	26.0 \pm 40.0	46.7 \pm 18.9	8.8 \pm 5.1	34.3 \pm 23.2	68.3 \pm 11.8	8.1 \pm 5.6	10.7 \pm 7.8	36.0 \pm 16.0
DO (mg O L^{-1})	6.5 \pm 1.8	3.0 \pm 3.1	0.8 \pm 0.2	4.3 \pm 2.4	0.7 \pm 0.8	0.4 \pm 0.2	5.4 \pm 0.1*	5.3 \pm 0.3*	5.7 \pm 0.0*
DOC (mg C L^{-1})	28.3 \pm 5.7	29.3 \pm 3.1	28.1 \pm 2.1	34.7 \pm 6.3	34.3 \pm 4.9	30.3 \pm 1.8	30.5 \pm 2.7	34.6 \pm 1.3	31.5 \pm 2.3
DIC (mg C L^{-1})	3.4 \pm 0.9	3.3 \pm 1.2	8.5 \pm 1.1	3.5 \pm 1.5	5.2 \pm 1.2	8.7 \pm 1.2	4.7 \pm 1.1*	5.0 \pm 1.0*	10.5 \pm 2.8*
POC (mg C L^{-1})	0.6 \pm 0.1	0.9 \pm 0.5	1.2 \pm 0.3	0.5 \pm 0.1	0.8 \pm 0.3	1.1 \pm 0.1	0.6 \pm 0.0	0.6 \pm 0.0	0.9 \pm 0.1
Tot-P ($\mu\text{g P L}^{-1}$)	9.8 \pm 1.0	10.0 \pm 3.0	64.0 \pm 6.7	12.0 \pm 3.0	17.0 \pm 9.7	55.0 \pm 22.9	14.0 \pm 4.9	20.0 \pm 8.0	60.0 \pm 2.0
NH ₄ ($\mu\text{g L}^{-1}$)	27.0 \pm 10.4	38.0 \pm 21.9	491.0 \pm 59.5	35.0 \pm 17.5	62.0 \pm 44.6	338.0 \pm 152.7	44.0 \pm 27.6	66.0 \pm 45.3	344.0 \pm 8.5
NO ₂ + NO ₃ ($\mu\text{g L}^{-1}$)	28.0 \pm 19.8	44.0 \pm 9.3	37.0 \pm 18.5	32.0 \pm 13.0	71.0 \pm 28.8	88.0 \pm 36.7	76.0 \pm 78.8	89.0 \pm 36.0	99.0 \pm 27.2

* = measured in late-September (27.9.) during autumn overturn

3.2. Sampling and data collection

3.2.1. Physical and chemical parameters

Temperature and oxygen concentration were measured during every sampling with an automatic oxygen and temperature sensor (YSI 55 probe, Yellow Springs Instruments). Light penetration to water was measured *in situ* during every sampling with an automatic sensor (LI-193, LI-COR).

Phosphorus (total P), nitrogen (NO_2^- -N + NO_3^- -N), chlorophyll *a*, DOC and pH samples were carried out in the laboratory of Lammi Biological Station for every sampling by the technical staff by using standard analytical methods (<http://www.sfs.fi>).

3.2.2. Fish introductions

A total 100 adult perch, caught from nearby Lake Horkkajärvi with traps, were introduced to the west side of Mekkojärvi on 4.7. According to the original study plan (see 2.3.), young of the year perch were introduced to the east side of the lake, but due to high precipitation during July and strong runoff of organic matter and water from the catchment, the chemical and physical conditions in Mekkojärvi changed: pH decreased to near 4 and epilimnion oxygen decreased to near zero. This was lethal for young of the year perch and was expected to be lethal to adult fish, too. Therefore, additional 80 adult perch were introduced to the west side of Mekkojärvi in 10.8., which changed the original study plan for whole-lake experiment in 2012. Afterwards was found, that the first amount of perch did survive in the lake, so the total amount of perch was consequently doubled.

3.2.3. Pelagic primary production and community respiration

Pelagic primary production (PP) was measured 8 times through summer (from May to late-September) from integrated water samples from each strata (surface, epi-, meta- and hypolimnion) using ^{14}C incorporation method described by Schindler *et al.* (1972). Sub-samples were taken from acrylic tube sampler (Limnos) into 20 mL glass vials: 4 replicates from each depth. One replicate from each depth was a dark sample and covered with foil. One replicate from both epilimnion and hypolimnion was "killed", being a dark control, by adding 4 drops of glutaraldehyde. 20 mL glass vials contained 20 μL of ^{14}C -bicarbonate with activity of 10 $\mu\text{Ci mL}^{-1}$. Samples were incubated *in situ* at 4 depths (0.25 m, 0.5 m, 1 m and 2 m) for 24 h and then killed by adding 8 drops of gultaraldehyde. Pelagic PP samples were taken from both sides of the lake, from Fish + and Fish –.

After the photosynthesis by the phytoplankton had proceeded for 24 hours, and when the samples were "killed", 0.5 mL of sample water from each glass vials was added into plastic scintillation vials with 0.5 mL ethanalamine-ethanol solution for total activity measurement. Then those plastic vials were filled by 9 mL of OptiPhase3 scintillation liquid. Then 6 mL of sample water from each glass vials were added into empty plastic scintillation vials for PP measurement, and two drops of strong phosphoric acid (H_3PO_4) were added into each plastic vial to remove the excess DI^{14}C . After 2 days (at earliest) 9 mL of OptiPhase3 was added into each of the plastic scintillation vials. The ^{14}C activity was measured with a Packard Tri-Carb liquid scintillation counter.

^{14}C incorporation method is based on the ability of phytoplankton to take up and incorporate tracer amounts of radioactive isotopes into organic matter during photosynthesis, which allows measurements of the *in situ* rates of PP. The uptake of ^{14}C , which is a fraction of initial whole, is assumed to measure the assimilation of total DIC, as a fraction of the whole, over the time period (Wetzel & Likens, 2000). When the total CO_2

content of the water is known, as well as the amount of ^{14}C -added before the incubation and ^{14}C retained in particulate matter (^{14}C -POC) at the end of the incubation, the total amount of carbon assimilated can be calculated by the proportional relationship (Steemann Nielsen, 1951, 1952):

$$\frac{{}^{14}\text{C available}}{{}^{14}\text{C assimilated}} = \frac{{}^{12}\text{C available}}{{}^{12}\text{C assimilated}} \quad (1)$$

And the ^{14}C uptake of phytoplankton can be measured by the following equation:

$$C_{\text{uptake}} = \frac{(\text{naturally occurring DIC})({}^{14}\text{C POC})(1.05)}{{}^{14}\text{C DIC added}} \quad (2)$$

where 1.05 is a 5% metabolic discrimination factor, since phytoplankton preferentially take up lighter isotopes.

Liquid scintillation counting is widely used and sophisticated laboratory counting system, which is defined by the incorporation of the radiolabeled analyte into uniform distribution with liquid chemical medium capable of converting kinetic energy of nuclear emission into light energy. It is used to quantify the activity of particulate emitting radioactive samples. In a relatively dense liquid, β -particle travels short distance dissipating all of its kinetic energy. The energy of excited solvent is emitted as UV light, and the total number of photons from the excited molecule constitutes the scintillation. The amplitude of the electrical pulse is then converted into a digital value. A spectrum can be plotted to provide information about the energy of the radiation or the amount of radioactive material dissolved in the cocktail. (University of Wisconsin-Milwaukee, 2012)

Community respiration and initial DIC was measured from surface, epi-, meta- and hypolimnion by taking 16 samples from Limnos into 50 ml glass vials (4 replicates from each depth) for $t_{0\text{h}}$ and another 16 for $t_{24\text{h}}$. Half of the samples ($t_{24\text{h}}$) were incubated *in situ* for 24 h and another half ($t_{0\text{h}}$) were taken immediately to laboratory. DIC was measured on DIC-analyzer (Salonen, 1981) in the laboratory at Lammi's Biological Station, respectively.

3.2.4. Littoral primary production

Littoral PP was measured 8 times through summer (from late-May to late-September) concurrently with pelagic PP. Littoral PP was measured from *in situ* incubations of periphyton samples in both transparent and dark vials, where the consumption or production of DIC over time period indicates either production or respiration. Since the lake has very narrow photic zone, it lacks benthic algae but has epiphytic algae on surrounding macrophytes and moss vegetation. Preliminary measurements in 2011 showed, that PP of moss was almost equal to periphyton PP on macrophytes (S. Devlin, unpublished). Therefore, and due to inconvenient sampling of moss, macrophytes were sampled and treated in this study.

The lake was sampled from 8 sites around the lake: 4 sites from both treatment "Fish" and "No fish" sides. Pristine sampling sites were selected and pieces of macrophytes were clipped into 20 mL glass vials with overlying water from each sampling site. 4 replicates were taken from each site: 2 for light and 2 for dark incubations. Initial DIC was measured from each sampling site by filling 20 mL glass vials up with overlying water, putting them into ice and determining the DIC concentration in laboratory. "Light

samples” were incubated *in situ* in surface water for 2 h and “dark samples”, covered with foil, were incubated in cool box, filled up with lake water, at the same time. After 2 h, samples were put into ice and taken to the laboratory. DIC was measured on DIC-analyzer (Salonen, 1981) in the laboratory at Lammi's Biological Station, respectively. After that, periphyton was scraped off from the substrates and filtered onto glass-fiber filters (Whatman GF/G). Filters were then frozen and chlorophyll *a* was later measured on UV-spectrophotometer (Shimadzu UV-1800). The substrates were dried in oven in 60 °C for 24 h and weighted out.

Net primary production (NPP) was calculated as $NPP = DIC_{initial} - \Delta DIC_{light}$. Community respiration was calculated as $CR = \Delta DIC_{dark} - DIC_{initial}$. Littoral values were then normalized to mg C (g dry-weighted substrate)⁻¹. To estimate whole-lake NPP, littoral habitats were quantified, which consisted primarily of two substrata, macrophytes and moss. Since PP of both moss and periphyton (on macrophytes) is similar (S. Devlin, unpublished), the whole-lake extrapolation of littoral NPP was based on average weight of littoral substrate m⁻¹ lake shore (S. Devlin, unpublished). The weight of littoral substrate m⁻¹ was divided by two to get an estimate for illuminated substrate m⁻². Daily irradiation values (measured with Kipp & Zonen B.V., model CM11) and day lengths were received from Finnish Meteorological Institute's measurement station in Jokioinen, which is the nearest observatory to the Evo area.

Whole-lake pelagic primary production was estimated by using the model described by McBride (1992):

Daily whole-lake
phytoplankton production,
TPP (mg C m⁻²):

$$TPP = \frac{\sum_{z=0}^{z_{epi}} PP_z}{A_0} \quad (3)$$

Whole-lake littoral primary production was estimated by using the models described by Vadeboncouer *et al.* (2001):

Daily littoral primary
production, LitPP, at depth *z*
(mg C):

$$LitPP_z = \Delta t \sum_{sunrise}^{sunset} LitPP_{max} \tanh(I_{zt}/I_k) (A_{z-\Delta z} - A_z) \quad (4)$$

Daily whole-lake littoral
primary production, TLitPP
(mg C):

$$TLitPP = LitPP_z W_s L_s \quad (5)$$

, where Δt is a time increment, Δz is a depth interval, V_z is lake volume above depth z , A_z is lake surface area at depth z , I_{zt} is the light at depth z and time t ($\mu\text{mol m}^{-2} \text{s}^{-1}$), I_k is the light intensity at onset of saturation ($\mu\text{mol m}^{-2} \text{s}^{-1}$), W_s is littoral substrate weight m⁻¹ lake shore and L_s is the lake's shoreline length (m).

3.2.6. Littoral primary production in control lakes

In addition to Mekkojärvi, littoral primary production in two nearby lakes (Table 2) was measured. Sampling was done in smaller scale than in Mekkojärvi, merely to compare the maximum photosynthetic (P_{max}) rates between the three lakes. The first lake was

Alinen-Mustajärvi (61°12'N 25°06'E), sampled once on July 27 in 2012, and the second lake was Halsjärvi (61°13'N 25°08'E), sampled once on September 5 in 2012. Littoral primary production was measured by using the same methods as in Mekkojärvi (see 3.2.4.).

Table 2. Physical and chemical parameters in Lake Alinen-Mustajärvi and Lake Halsjärvi. Values of Alinen-Mustajärvi are from Kankaala *et al.* (2010b), and the values of Halsjärvi are from Verta *et al.* (2010).

	Alinen-Mustajärvi	Halsjärvi
Lake area (ha)	0.8	4.7
Maximum depth (m)	6.5	5.9
pH	5.3	6.5
Colour (mg Pt L ⁻¹)	120–150	175–237
OC (mg L ⁻¹)	11–15 ¹	9.6–10.8 ²

¹ = OC as DOC, ² = OC as TOC

3.2.7. Statistical tests

The models for whole-lake estimations were performed by using R (R project for Statistical Computing). Other tests were performed by using IBM SPSS Statistics 20.0 for Macintosh. Both parametric and nonparametric test were used to compare daily values of primary production and chlorophyll *a* values, depending on if the assumptions for parametric tests were met. Chlorophyll *a* values between basins were tested by independent samples t-tests. Correlations of pelagic NPP to chl *a*, phosphorus and nitrogen, or littoral NPP to chl *a*, were tested by Pearson's correlation or Spearman's rank correlation, depending on the assumptions met. Since the PP data was heteroscedastic despite of various transformations, nonparametric Kruskal-Wallis one-way analysis of variance was chosen to test for differences in mean values.

4. RESULTS

4.1. Physical and chemical parameters

Temperature profiles displayed similar patterns at both sides of the lake throughout all seasons of the study period, while oxygen concentrations in epi- and metalimnion on Fish – were lower than on Fish + (Fig 6). Very steep temperature and oxygen gradients formed already in May, when temperature difference from surface to 1 m was about 10°C and oxygen difference from 5 to 7 mg L⁻¹.

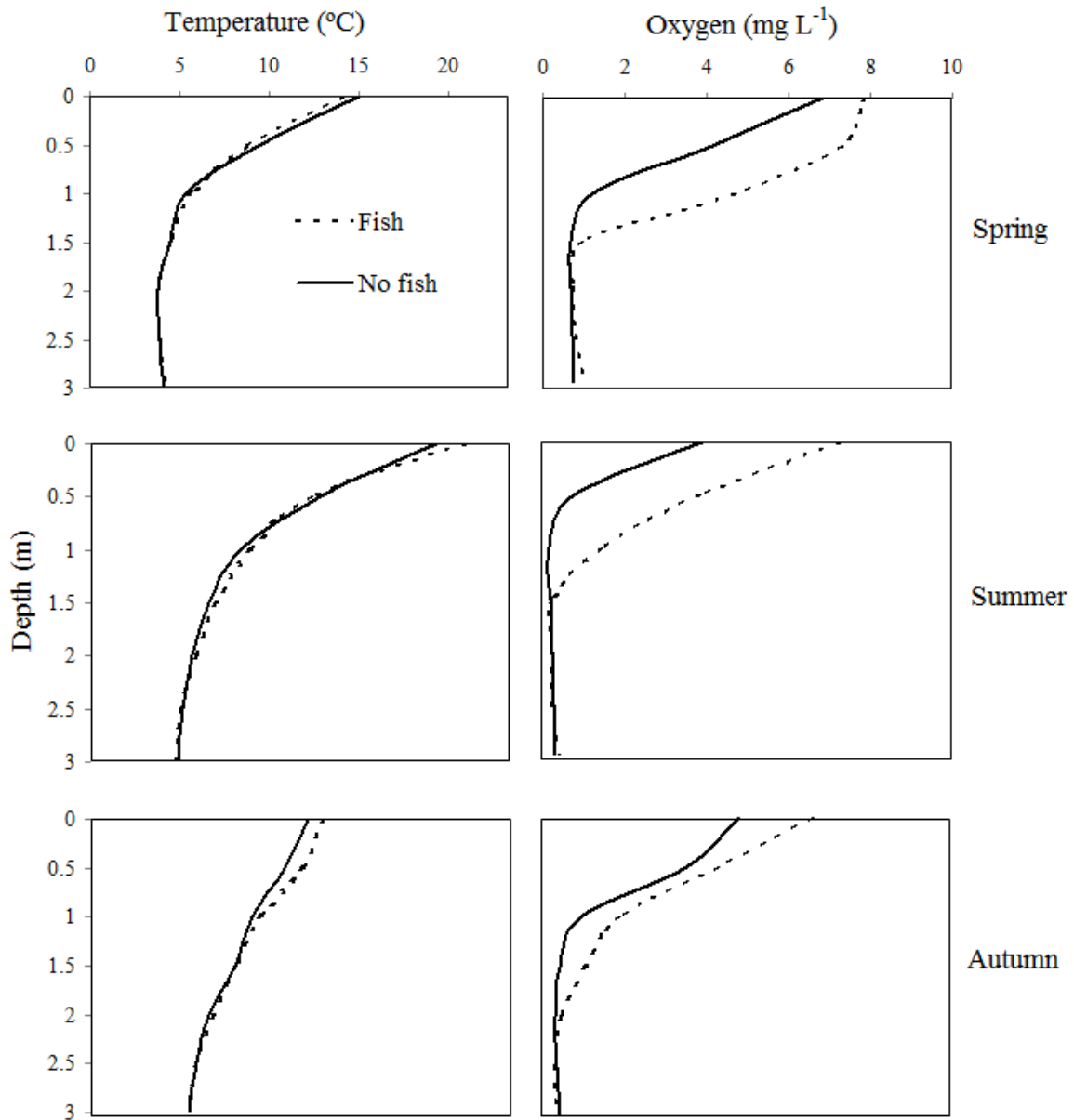


Figure 6. Vertical profiles of temperature and oxygen concentration in Mekkojärvi during spring (May), summer (June–early-August) and autumn (late-August–late September). Profiles represent the average of all temperature and oxygen measurements made during all seasons.

Light attenuation and photosynthetic photon flux (PPF) in Mekkojärvi was measured 7 times in 2012 (Fig 7). When examining the temporal variation of PPF, clearly higher surface values were obtained in spring than in autumn due to brighter days. However, the light attenuation was very rapid and almost equal throughout the year. Almost all the light was absorbed within first 50 cm. Low surface values in autumn are due to cloudy days, which proportion increased from summer towards autumn.

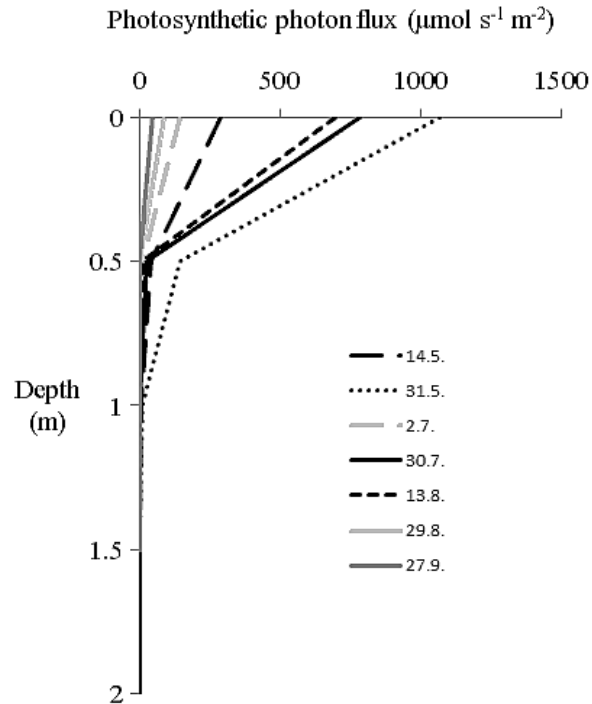


Figure 7. Photosynthetic photon flux (PPF) in Mekkojärvi from 7 sampling periods in 2012.

4.2. Pelagic primary production

The highest mean (\pm S.E.) value for pelagic primary production was 605.4 ± 129.3 $\text{mg C m}^{-2} \text{ day}^{-1}$, observed in late-May on Fish +, indicating a clear spring maxima (Fig. 8). Pelagic PP values varied substantially on Fish – in 29.5. and 18.6., and the data from two days (19.7. and 1.8.) were left out from the Fish –, showing clearly incorrect values. In 19.7., mean (\pm S.E.) PP value in Fish + was still 117.3 ± 45.8 $\text{mg C m}^{-2} \text{ day}^{-1}$, but after the spring maxima, the pelagic PP pattern decreased towards autumn. From August to late September, the mean (\pm S.E.) values ranged from 7.3 ± 1.0 to 47.0 ± 6.5 $\text{mg C m}^{-2} \text{ day}^{-1}$.

The highest mean (\pm S.E.) value for pelagic community respiration (CR) was 1493.6 ± 140.0 $\text{mg C m}^{-3} \text{ day}^{-1}$, observed in 18.6. in Fish – (Fig 8.). CR was also high in Fish + at the same time, where the mean (\pm S.E.) value was 1132.0 ± 187.0 $\text{mg C m}^{-3} \text{ day}^{-1}$. After the peak in mid-June, CR decreased rapidly to 38.3 ± 55.5 $\text{mg C m}^{-3} \text{ day}^{-1}$ on Fish + and 36.5 ± 50.3 $\text{mg C m}^{-3} \text{ day}^{-1}$ on Fish –. Mid-August values are missing, and the values in late-August varied substantially, but were relatively low: 82.5 ± 138.5 $\text{mg C m}^{-3} \text{ day}^{-1}$ on Fish + and 26.9 ± 170.1 $\text{mg C m}^{-3} \text{ day}^{-1}$ on Fish – (mean \pm S.E.).

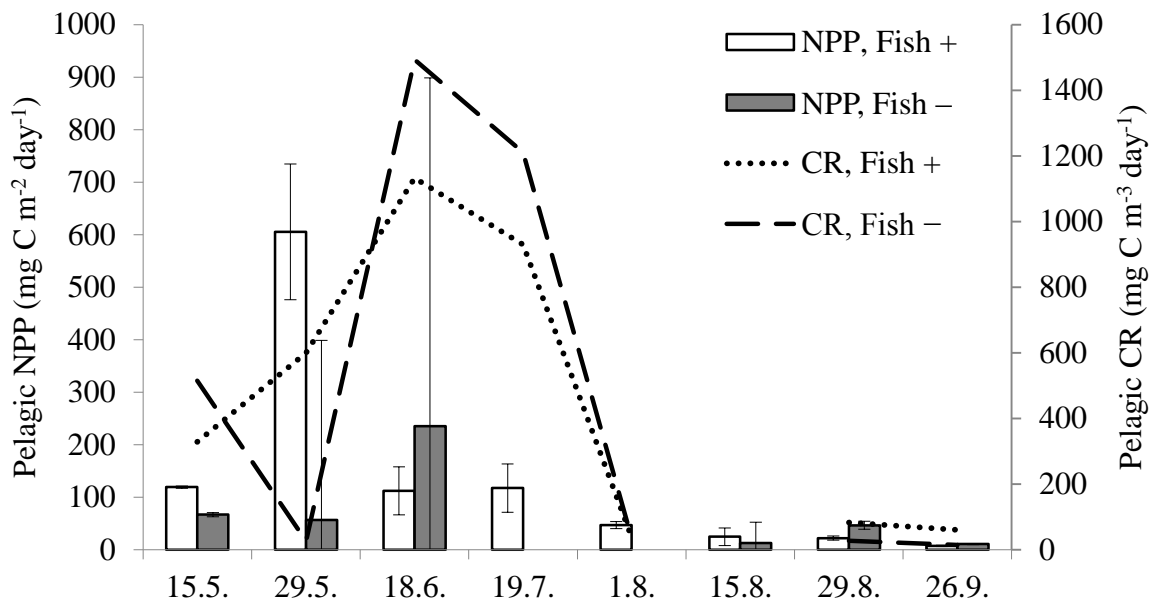


Figure 8. Daily depth-integrated pelagic net primary production (mean \pm S.E.) and mean community respiration in Mekkojärvi from 8 sampling periods in 2012. CR data is missing in 15.8.

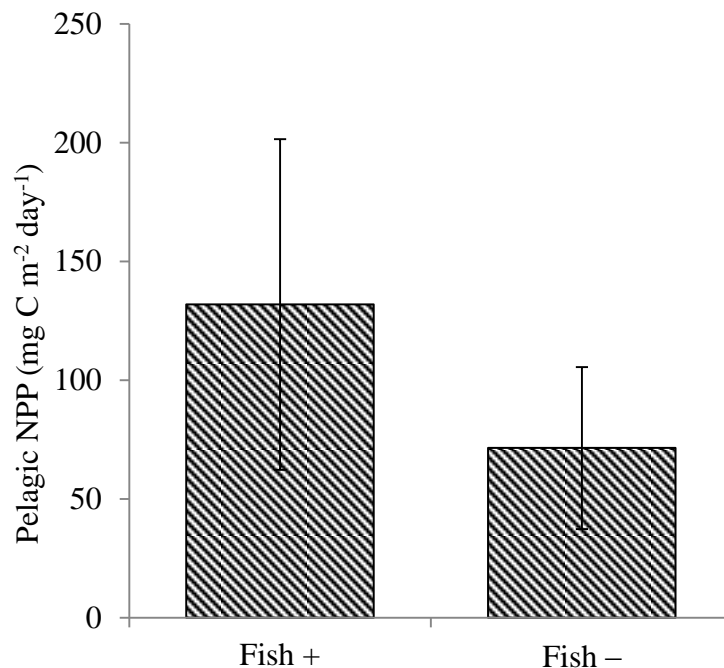


Figure 9. Mean \pm S.E. net pelagic primary production on both basins in Mekkojärvi from all the sampling periods in 2012.

Depth-integrated pelagic chlorophyll *a* (chl *a*) concentration on Fish - (mean \pm S.E.: $37.8 \pm 5.4 \mu\text{g L}^{-1}$) was significantly higher than on Fish + (mean \pm S.E.: $22.6 \pm 4.3 \mu\text{g L}^{-1}$), ($t_{(58)} = 2.19$, $p < 0.05$) (Fig 10.). Chl *a* concentration showed similar pattern on both basins until June. Then the relative contributions of chl *a* changed within strata, when most of the chl *a* on Fish + was in hypolimnion. Metalimnetic chl *a* had higher contribution on Fish - than on Fish + side. Epilimnetic chl *a* had similar low contributions on both basins.

The highest epilimnetic chl *a* concentration on Fish + was 12.2 $\mu\text{g L}^{-1}$, occurred in late-July, while the lowest value was 0.7 $\mu\text{g L}^{-1}$, occurred in mid-July. On Fish – the highest epilimnetic chl *a* concentration was 17.1 $\mu\text{g L}^{-1}$, occurred in late-June, while the lowest value was 2.7 $\mu\text{g L}^{-1}$, occurred in mid-May. Mean (\pm S.E.) epilimnetic chl *a* on Fish + side was $5.17 \pm 1.0 \mu\text{g L}^{-1}$ and $10.9 \pm 1.6 \mu\text{g L}^{-1}$ on Fish –, and the difference was statistically significant ($t_{(15.9)} = 3.0, p < 0.05$).

The highest metalimnetic chl *a* concentration was 28.0 $\mu\text{g L}^{-1}$ on Fish +, occurred in late-June, and 106.1 $\mu\text{g L}^{-1}$ on Fish –, occurred on the same day. The lowest metalimnetic value was 1.1 $\mu\text{g L}^{-1}$ on Fish +, occurred in late-June, and 5.7 $\mu\text{g L}^{-1}$ on Fish –, occurred in late-May. Mean (\pm S.E.) metalimnetic chl *a* on Fish + was $12.0 \pm 3.2 \mu\text{g L}^{-1}$ and $42.4 \pm 10.0 \mu\text{g L}^{-1}$ and the difference was statistically significant ($t_{(10.8)} = 2.9, p < 0.05$).

The highest hypolimnetic chl *a* concentration on Fish + was 71.5 $\mu\text{g L}^{-1}$ and occurred on both sampling periods in August, whereas on Fish – the highest value was 84.4 $\mu\text{g L}^{-1}$, occurred on early-August. Mean (\pm S.E.) hypolimnetic chl *a* on Fish + was $50.6 \pm 5.7 \mu\text{g L}^{-1}$ and $60.1 \pm 6.7 \mu\text{g L}^{-1}$ on Fish –, and the difference was not statistically significant ($t_{(18)} = 1.0, p = 0.30$).

There was no statistically significant correlation between NPP and total phosphorus concentration, Spearman's rho, $R^2 = 0.151, p = 0.623$, nor between NPP and $\text{NO}_2^- + \text{NO}_3^-$ (Pearson, $R^2 = -0.263, p = 0.386$), nor between NPP and chl *a* concentration (Spearman's rho, $R^2 = -0.367, p = 0.197$) (Fig. 11).

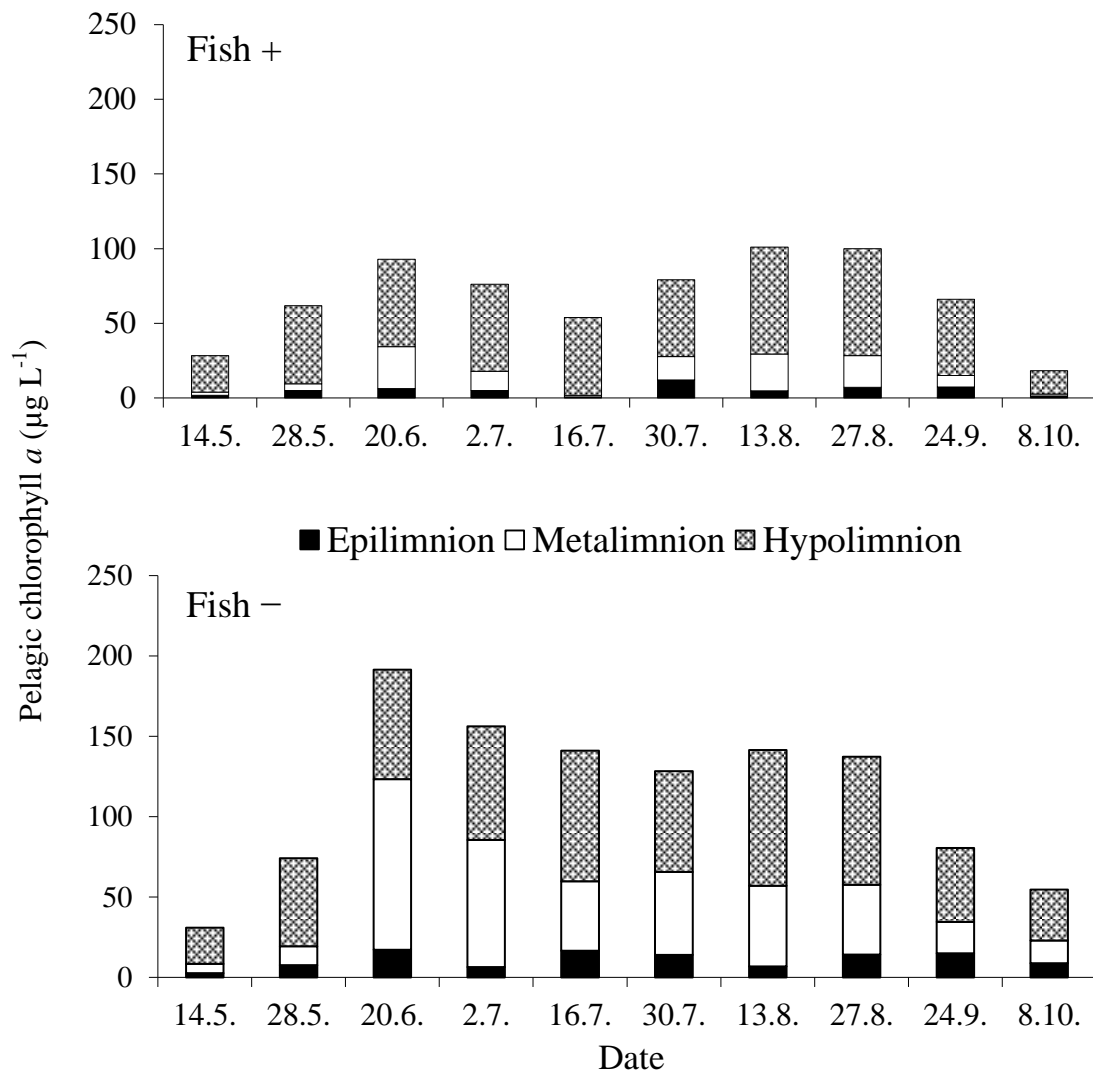


Figure 10. Temporal variation of mean pelagic chlorophyll *a* concentrations in each strata of both basins in Mekkojärvi in 2012.

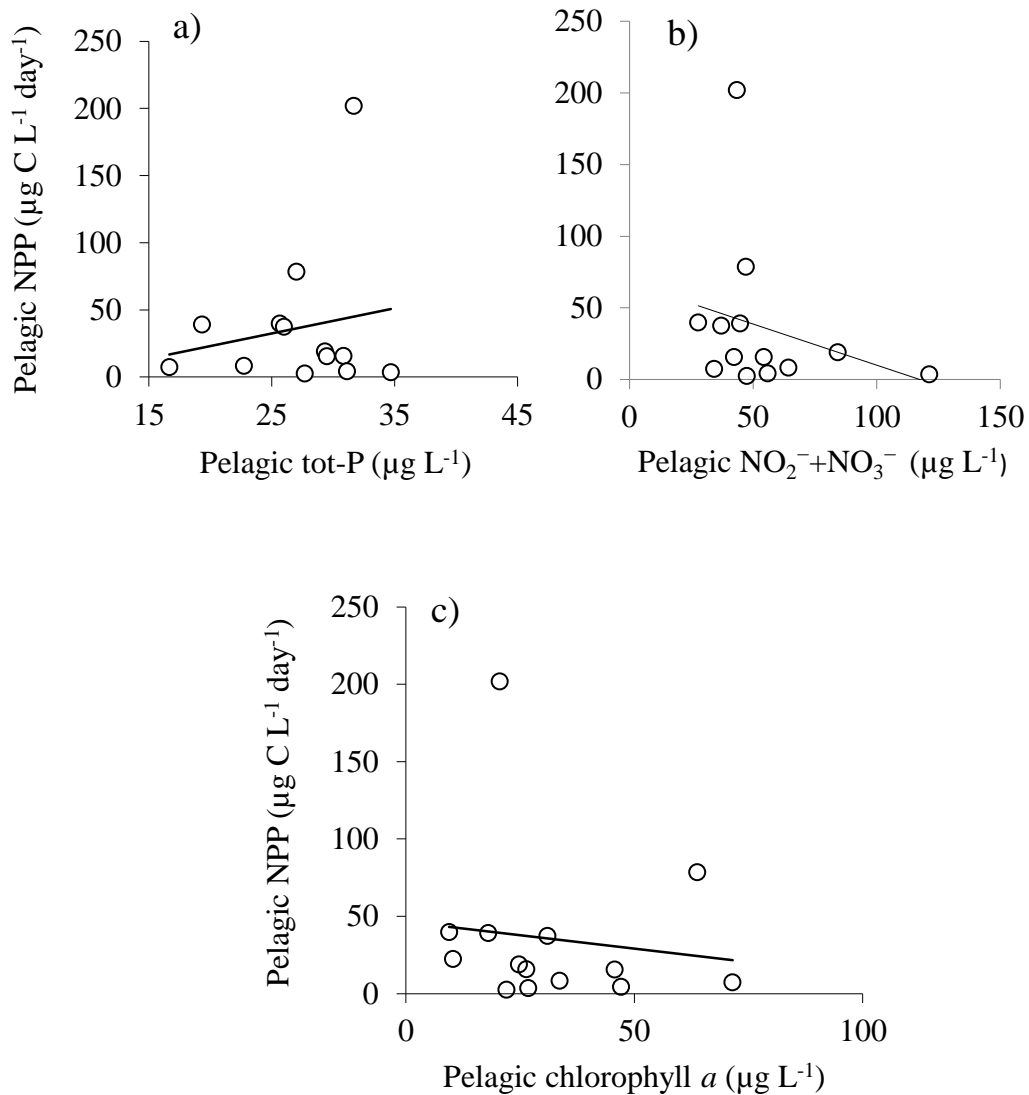


Figure 11. Plot of depth-integrated mean pelagic net primary production to depth-integrated total phosphorus (a) (Spearman, $R^2 = 0.151$, $p = 0.623$), chlorophyll *a* (b) (Spearman, $R^2 = -0.367$, $p = 0.197$) and nitrite and nitrate (c) (Pearson, $R^2 = -0.263$, $p = 0.386$) in Mekkojärvi in 2012.

4.3. Littoral primary production

Littoral PP was relatively high throughout the whole sampling period in 2012 (Fig. 12). Since littoral PP was measured at midday, the values represent the maximum photosynthetic rate (P_{max}) (mean \pm S.E). The highest value of NPP was $172.3 \text{ mg C g DW substrate}^{-1} \text{ h}^{-1}$, measured in early-August on Fish -, while the lowest value was $0.17 \text{ mg C g DW substrate}^{-1} \text{ h}^{-1}$, measured in late-September from the same side. There was no clear seasonal trend of NPP on Fish -. The lowest values were measured in late-September and late-May, and between these, the rates were rather constant. On Fish +, NPP decreased almost two thirds between late-July and early-August, and remained low. Meanwhile on the Fish -, NPP was high throughout the August, but had high variation until late-August.

Kruskal-Wallis statistics revealed a statistically significant difference in NPP between the sampling days ($\chi^2 = 33.7$, $p < 0.01$), when basins were grouped together. When comparing the basins separately, statistical significant difference in NPP between basins was found in 30.8. ($\chi^2 = 8.7$, $p < 0.01$), and 27.9. ($\chi^2 = 4.0$, $p < 0.05$). On the other

days, the differences were statistically nonsignificant. Mean NPP throughout the whole sampling period (Fig. 13) was higher on Fish – than on Fish + ($t_{(121)} = 2.78, p < 0.01$).

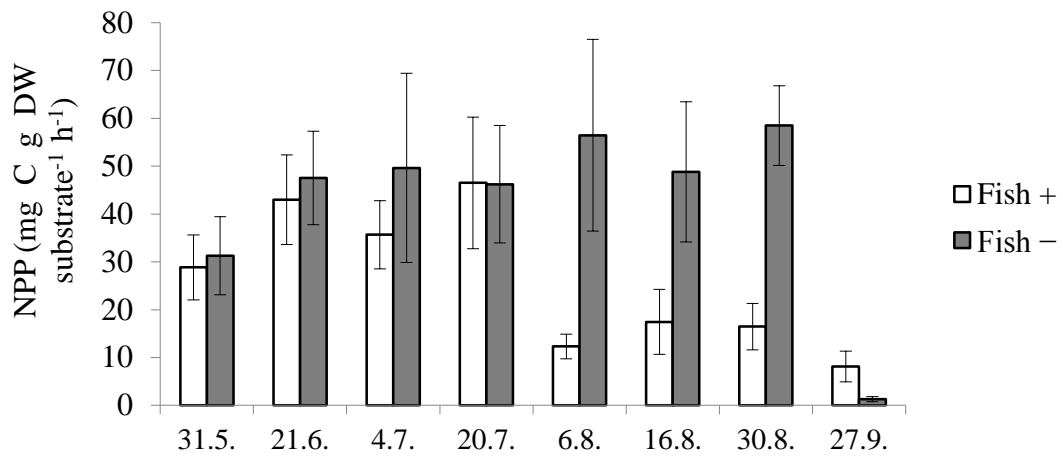


Figure 12. Littoral net primary production of periphyton per hour (mean \pm S.E.) on both basins in Mekkojärvi from 8 sampling periods in 2012. The values are normalized to g dry-weighted substrate.

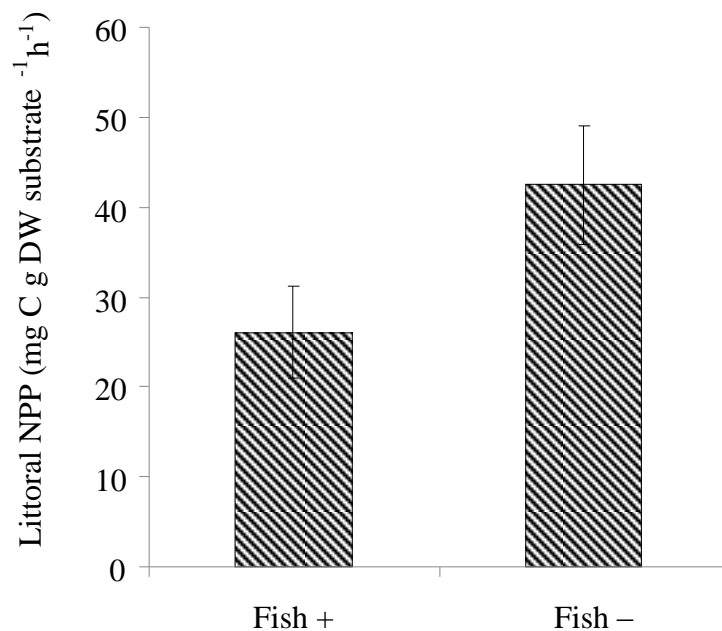


Figure 13. Mean \pm S.E. net littoral primary production of periphyton on both basins in Mekkojärvi from all the sampling periods in 2012.

Littoral community respiration did not show clear seasonal trend, and the variation was high throughout the summer (Fig 14). The highest value was 93.07 mg C g DW substrate⁻¹ h⁻¹, measured in early-July on Fish +, while the lowest value was 0.11 mg C g DW substrate⁻¹ h⁻¹, measured in late-July, revealing the high variation in the data.

When comparing the mean values of the two basins, Kruskal-Wallis statistics revealed a significant difference in CR only in 31.5. ($\chi^2 = 3.9$, $p < 0.05$), while on the other days, the difference in CR between basins was nonsignificant. When examining the basins separately, statistically significant difference between days was found on Fish + ($\chi^2 = 22.7$, $p < 0.01$), but on Fish – the difference was nonsignificant ($\chi^2 = 9.4$, $p = 0.22$). There was no statistically significant difference in mean CR between the basins throughout the whole sampling period ($t_{(76,8)} = 1.18$, $p = 0.24$).

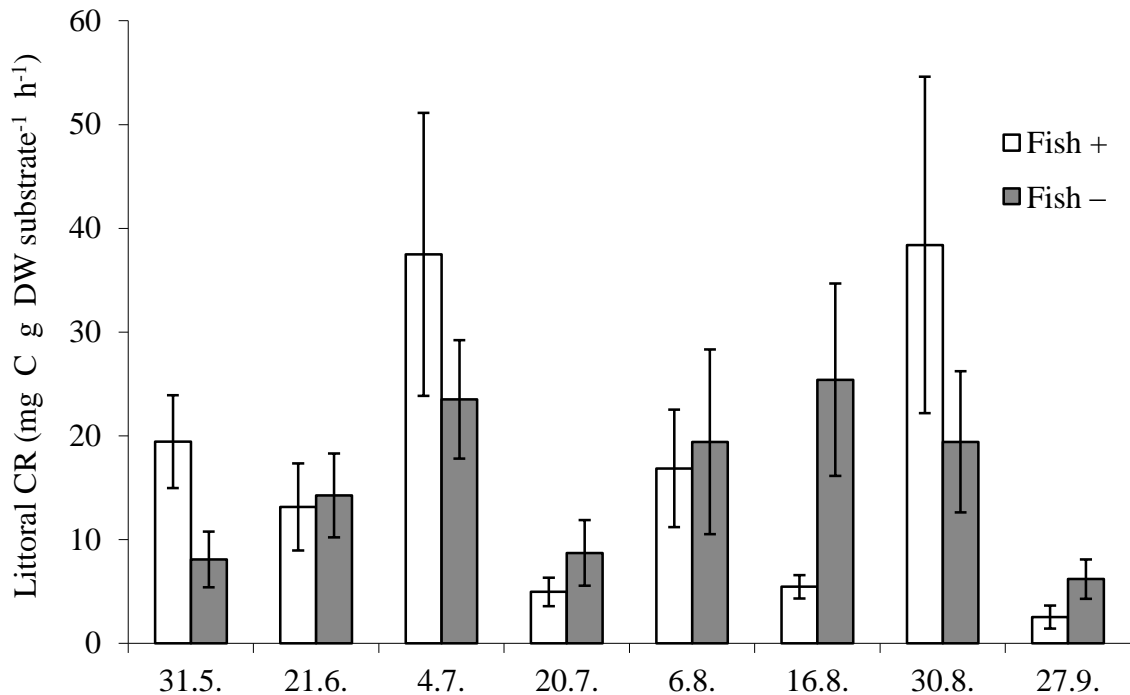


Fig 14. Littoral periphyton community respiration per g dry-weighted substrate per hour (mean \pm S.E.) on both basins in Mekkojärvi from 8 sampling periods in 2012. The values are normalized to g dry-weighted substrate.

Littoral chlorophyll *a* content in periphyton did not show clear seasonal trend (Fig 15). The highest value was 4.9 mg g DW substrate⁻¹, measured in late-August, while the lowest value was 0.01 mg g DW substrate⁻¹, measured in late-September. When comparing the two basins, Kruskal-Wallis statistics revealed a significant difference in chl *a* in 21.6. ($\chi^2 = 6.6$, $p = 0.01$), and in 4.7. ($\chi^2 = 6.6$, $p < 0.05$), while on the other days, the difference in chl *a* between basins was nonsignificant. There was no statistically significant difference in mean chl *a* between the basins throughout the whole sampling period, $t_{(108)} = 1.5$, $p = 0.124$.

To compare how many milligrams of carbon one milligram of chl *a* produces, the biomass specific littoral NPP was calculated, and it is shown in Figure 16. The highest value was 305.8 mg C mg chl *a*⁻¹ h⁻¹ in Fish + in early-July, while the lowest value was 0.2 mg C mg chl *a*⁻¹ h⁻¹ in Fish – in late-September. When comparing the two basins, Kruskal-Wallis statistics revealed a significant difference in biomass specific NPP in 6.8. ($\chi^2 = 10.6$, $p < 0.01$), while on the other days, the difference in biomass specific NPP between basins was nonsignificant. There was no statistically significant difference in biomass specific NPP between the basins throughout the whole sampling period ($t_{(99)} = 0.70$, $p = 0.48$).

There was a statistically significant positive correlation between littoral chlorophyll *a* and littoral net primary production (Pearson, $R^2 = 0.524$, $p < 0.01$) (Fig 17.).

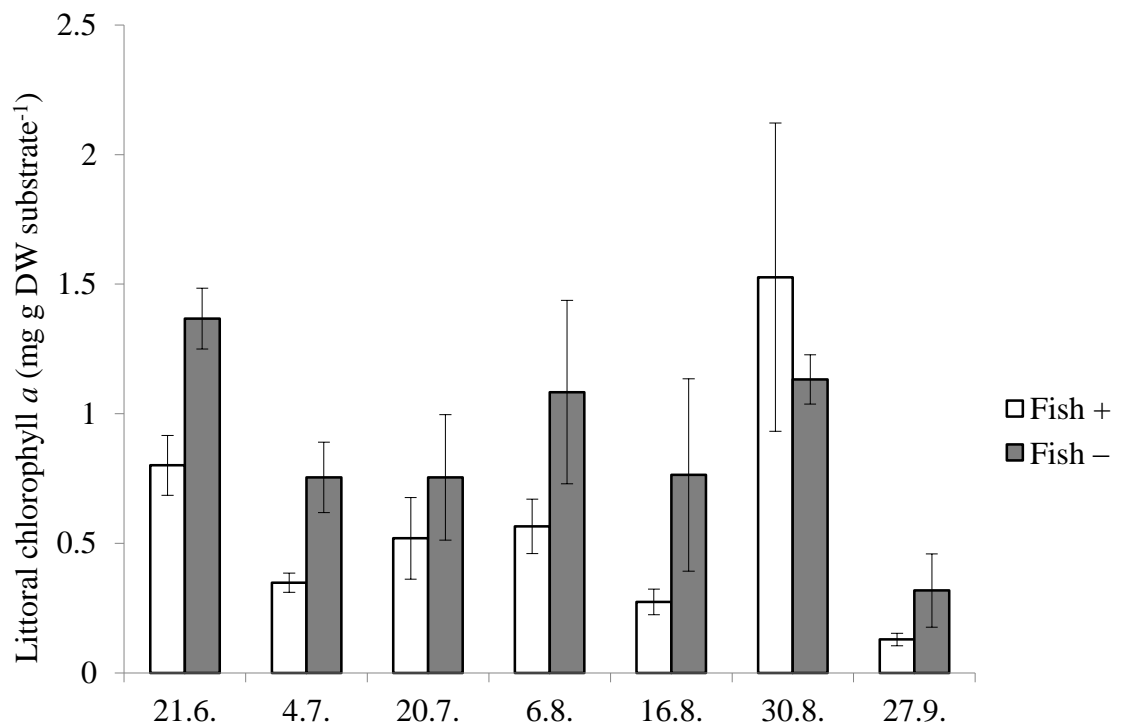


Figure 15. Littoral chlorophyll *a* content per g dry-weighted substrate per hour (mean \pm S.E.) in periphyton in Mekkojärvi from 8 sampling periods in 2012. The values are normalized to g dry-weighted substrate.

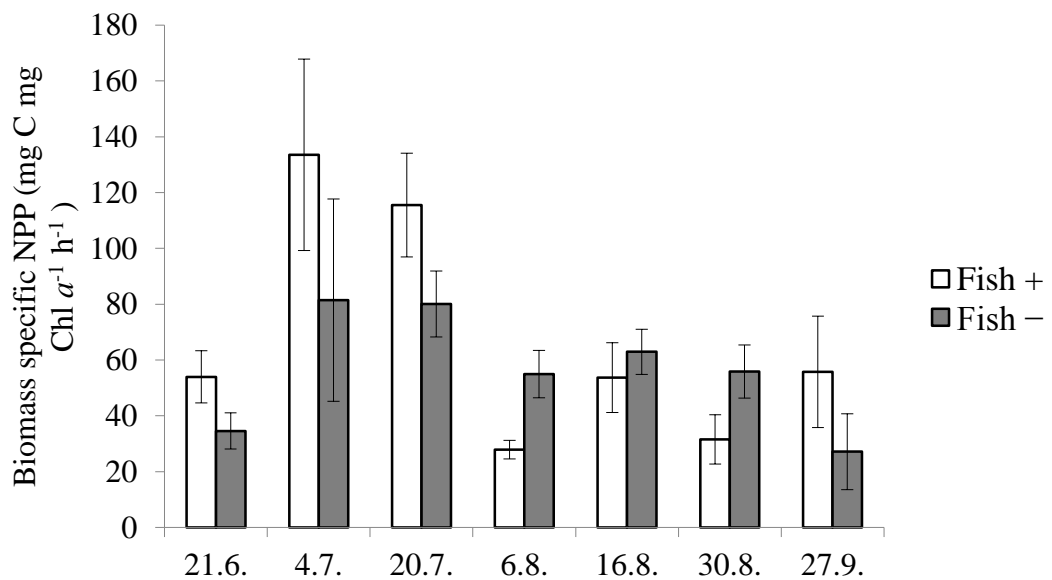


Figure 16. Littoral net primary production (mean \pm S.E.) per unit chlorophyll *a* per hour in Mekkojärvi from 8 sampling periods in 2012.

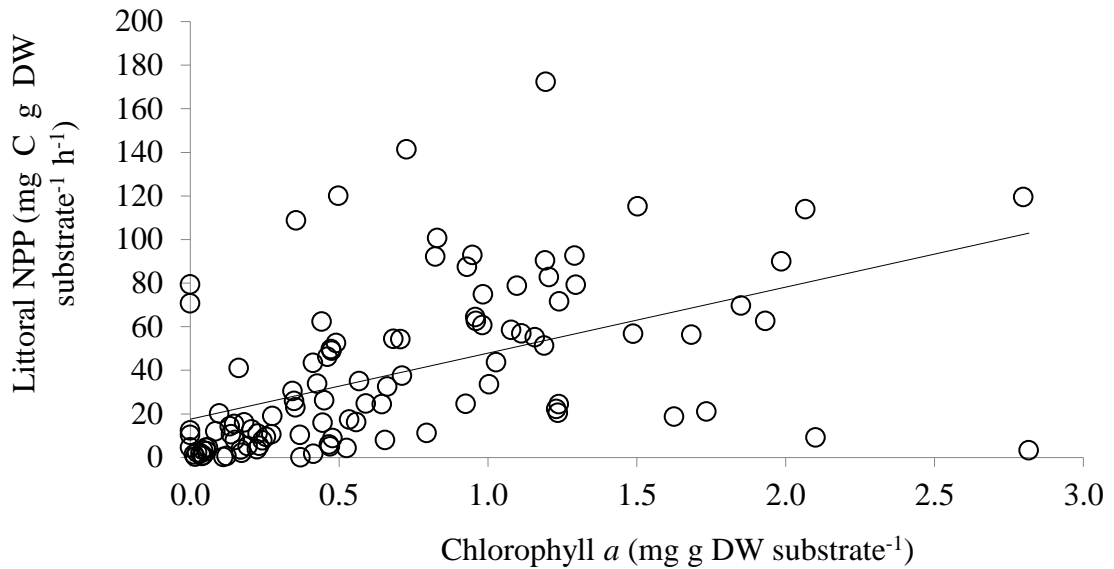


Figure 17. Plot of mean littoral net primary production to littoral chlorophyll *a* in Mekkojärvi in 2012. Pearson, $R^2 = 0.524$, $p < 0.01$.

Daily estimates of littoral NPP per g dry-weighted substrate per day (Fig. 18) are the outcome of equation 3 (see 2.3.5.). P_{\max} values in late August were relatively high on fishless side (Fig. 12), but due to shorter day length and lower irradiation, the model gave low estimates (Fig. 18). Also, the lower values in 4.7. are due to rather low irradiation on that day.

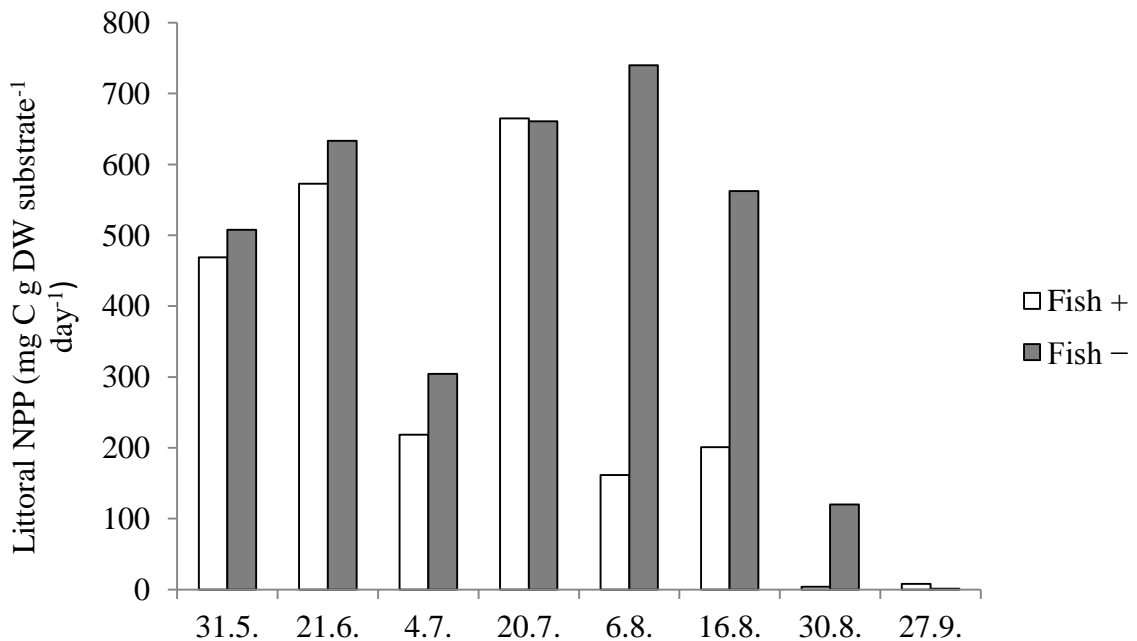


Figure 18. Estimated daily littoral net primary production on both basins in Mekkojärvi from 8 sampling periods in 2012.

4.3.1. Littoral primary production in control lakes

Littoral PP values were substantially lower in control lakes than in Mekkojärvi (Fig. 19). The highest value obtained in Alinen-Mustajärvi was $6.83 \text{ mg C g DW substrate}^{-1}$, while the lowest was $0.75 \text{ mg C g DW substrate}^{-1}$. In Halsjärvi, the highest value was $11.0 \text{ mg C g DW substrate}^{-1}$, while the lowest was $0.23 \text{ mg C g DW substrate}^{-1}$. Chlorophyll *a* values correspond well to the values in Mekkojärvi (Fig. 15).

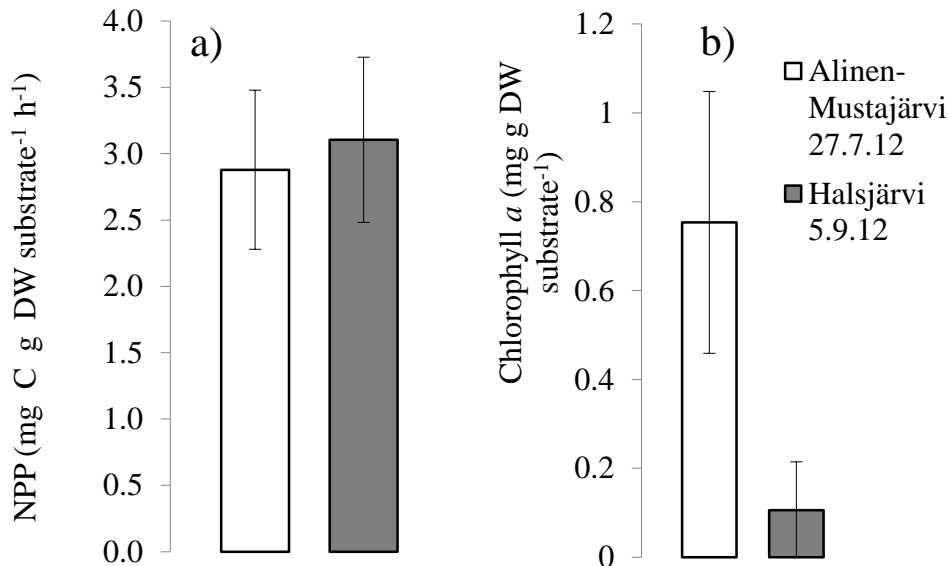


Figure 19. Littoral net primary production (mean + S.E.) per g dry-weighted substrate per hour (a) and chlorophyll *a* content (mean + S.E.) per dry-weighted in the control lakes in 2012.

4.4. Whole-lake daily estimates of primary production

Whole-lake estimates of primary production indicate the importance of pelagic PP in early spring and late autumn (Fig. 21). This can be clearly seen on Fish +, where 70 % of daily PP in 31.5. was estimated to be pelagic (Fig. 20). In 30.8., pelagic contribution to whole-lake production was estimated as 91 %. On Fish -, littoral primary production dominated already in spring and still in late-August. In late-September, 94 % of the whole-lake production was estimated as pelagic on Fish -, while on Fish +, the contribution was 63 %. However, the total production rates in late autumn were much lower than in spring (Fig. 20). On Fish -, littoral PP showed highest values (747 g C day^{-1}) in early-August and was still high in mid-August, while on Fish + littoral PP rates were under 200 g C day^{-1} already in early-August.

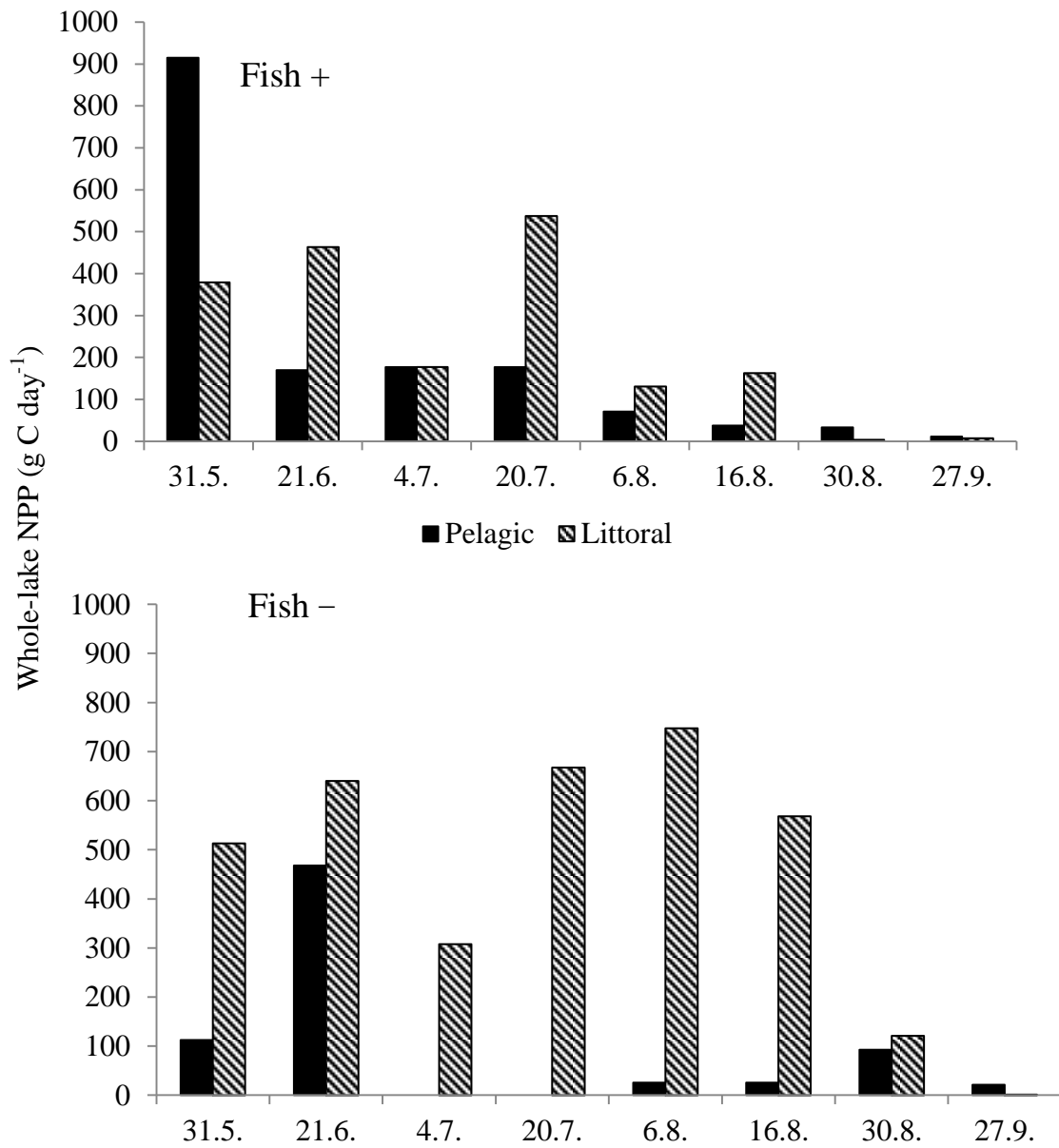


Figure 20. Whole-lake daily net primary production in both littoral and pelagial in Mekkojärvi from 8 sampling periods in 2012. Two days from Fish - are missing the pelagic PP data.

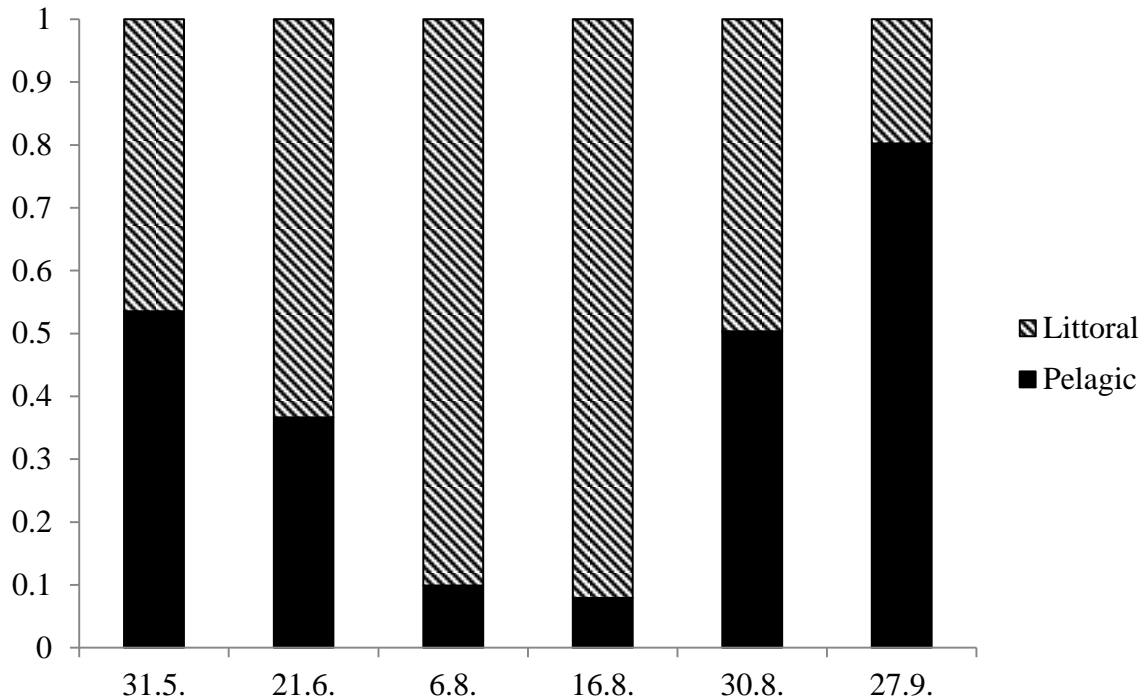


Figure 21. Proportions of littoral and pelagic net primary production in whole-lake (both basins included) net primary production in Mekkojärvi from 8 sampling periods in 2012. Two days from fishless side are missing the pelagic PP data.

5. DISCUSSION

5.1. Overview of the results

Spring bloom in phytoplankton PP in Mekkojärvi was observed early in the summer when the highest observed mean values on Fish + were over $600 \text{ mg C m}^{-2} \text{ day}^{-1}$ (Fig. 8). Due to substantial variation in the data, statistically significant differences in pelagic PP between the two basins were not observed. High peak in CR, which exceeded the PP, was observed about the same time as phytoplankton PP peak (Fig. 8). CR decreased rapidly in mid-July and was low in early-August. CR values in mid-August were missed. Chl *a* concentrations in epilimnion were rather low and stable on both basins, mean values ranging between $7\text{--}8 \mu\text{g L}^{-1}$, whereas the chl *a* concentrations in meta- and hypolimnion were high, $14\text{--}106 \mu\text{g L}^{-1}$ (Fig. 10). Mean chl *a* concentrations were higher in Fish – than in Fish +. Littoral PP was higher on Fish – than on Fish + (Fig. 13) and was relatively high and stable throughout the summer, except the decrease on Fish + on early August (Fig. 12). CR in periphyton assemblages was generally lower than NPP, except on Fish + in early-July and late-August (Fig. 14). No statistically significant differences in biomass specific littoral NPP between the basins were found, but highest biomass specific rates were observed in July due to rather low chl *a* contents relative to high NPP rates (Fig. 16). However, the observed NPP values should be considered as P_{max} values, since the samples were incubated for 2 h at noon under the most favourable illumination conditions. Estimated daily littoral PP values (Fig. 18) were rather stable until August, except the decrease in early July. Similar collapse in daily littoral NPP rates (Fig. 18) were observed on Fish + as in P_{max} values in August (Fig. 12). Whole-lake estimates revealed pelagic dominance in PP on Fish + in spring, while on Fish – littoral PP dominated already in

spring (Fig. 20). Overall, pelagic PP had highest contributions in autumn and littoral PP had over 90 % contribution in whole-lake PP on Fish – in August (Fig. 21). Littoral PP rates on control lakes were approximately one order of magnitude lower than in Mekkojärvi (Fig. 19).

5.2. Pelagic primary production and community respiration

Pelagic primary production in Mekkojärvi was relatively low throughout the growing season, since mean pelagic PP was $131.8 \text{ mg C m}^{-2} \text{ day}^{-1}$ on Fish + and $71.5 \text{ mg C m}^{-2} \text{ day}^{-1}$ on Fish –. This is common in high latitude humic lakes, where water colour and low irradiance contributes to low effective light climate. These PP rates differ substantially from eutrophic low latitude lakes, where areal rates can be several thousands of milligrams of carbon per square meter in day, or even $30,000 \text{ mg C m}^{-2} \text{ day}^{-1}$ in extreme cases (Kalff, 2002). Nürnberg & Shaw (1998) found, that PP in coloured lakes differs greatly, from 4 to $4131 \text{ mg C m}^{-3} \text{ day}^{-1}$ in volumetric basis and is even higher for same amount of TP and chl than in clear lakes. In turn, when PP was expressed on areal basis, humic lakes had lower PP rates. Nürnberg & Shaw (1998) suggested that volumetric measures of PP and chl are not negatively affected by colour or DOC, since mixing depths in humic lakes are shallow and optimal in terms of light for algae, contribution of mixotrophs is high and food path is different. V.-Balogh & Vörös (1997) found very high PP production rates in humic-rich hypertrophic lake reservoirs in Hungary, ranging between $2.9\text{--}6.1 \text{ g C m}^{-2} \text{ day}^{-1}$. Jackson & Hecky (1980) studied reservoirs in Canada and found that PP was depressed due to humic matter, which made iron unavailable to phytoplankton. Rodríguez *et al.* (2012) studied two shallow and highly humic (DOC between $28\text{--}50 \text{ mg L}^{-1}$) lakes in Argentina and found that other factors than light, such as nutrients, limited the phytoplankton PP. These findings demonstrate, that light is not always the limiting factor for pelagic PP in humic lakes and humic substances can interfere the phytoplankton uptake of e.g. nutrients. Jasser *et al.* (2009) studied three small humic lakes in Poland with different water colours and DOC concentrations and found high PP rates in polyhumic lake (max. value of $851 \text{ mg C m}^{-3} \text{ day}^{-1}$) and low BP:PP ratio (bacterial production : primary production). They doubted that high PP contribution to lake carbon budget should have been attributed to autotrophic picoplankton, which occurred there in high numbers.

The pattern of pelagic PP in Mekkojärvi in Figure 8 corresponds to previous primary production measurements in Mekkojärvi (Salonen *et al.* 2005; Taipale, 2007): high peak in early summer and decreasing pattern towards autumn. The high peak in early summer can most likely be explained by inputs of hypolimnetic water with rather high concentrations of nutrients (Table 1) to epilimnion, when the lake was near isothermal, or by spring snowmelt runoff from the catchment to the lake. Peak in CR (Fig. 8) fit well to the peak found by Salonen *et al.* (2005): CR was highest in late-June and during July and decreased towards autumn. Furthermore, Salonen *et al.* (2005) found another CR peak in August 1984 (Fig. 1), which might have happened in 2012 as well, but was missed in this study. CR showed high values, which is usual for net heterotrophic humic lakes (see 2.1.). Bacterial production was not included in this study, but bacterial biomass can be even 23 times higher than phytoplankton biomass in Mekkojärvi (Kuuppo-Leinikki & Salonen, 1992). Approximately 90 % of bacterial production can be based on allochthonous carbon (Jansson *et al.* 1999). Therefore, in highly humic waters nutrients more likely limits bacterial production than carbon, inducing competition for limiting nutrients with phytoplankton as bacteria can be efficient competitors for the same inorganic nutrients that limit phytoplankton growth (Currie & Kalff, 1984).

Pelagic primary production in Mekkojärvi varied substantially early in the summer, and two sampling days (19.7. and 1.8.) from the Fish – treatment were omitted due to incorrect preparations of the samples. High variation in pelagic PP in early summer was observed on Fish –, on the same basin with no data from July. It is unsure, whether those problems were caused by different chemical conditions on Fish – than on Fish +. On July, due to high runoff from the catchment, oxygen decreased to near 0 and pH to near 4 on Fish –, which killed all the YOY perch and may have affected negatively to primary production as well. Kwiatkowski & Roff (1976) found drastic decrease of PP in Northern Ontario lakes, when pH decreased below 4.4. Yan & Stokes (1978) found no clear response of algae biomass to decreased pH in Carlyle Lake, Canada, but found drastic change in phytoplankton community structure, when pH decreased below 5.6. Extreme conditions on Fish – may thus explain the high variation in PP and odd values.

There was no relationship between depth-integrated pelagic NPP and P, $\text{NO}_2^- + \text{NO}_3^-$ and chl *a* (Fig. 12). This was most likely due to the fact that volumetric measures and their relationships with other variables differ from areal measures with range of colour, since the euphotic zone changes with colour (Jones, 1992). In Mekkojärvi, relatively high nutrient concentrations in hypolimnion (Table 1) do not reflect to PP, since the euphotic zone is so narrow (50 cm) and the lake is so steeply stratified (Fig. 6), that algae cannot uptake nutrients effectively. However, mixotrophy of algae in Mekkojärvi enable vertical migrations from epilimnion to nutrient rich meta- and hypolimnion (Jones, 2000).

5.3. Littoral primary production

Littoral PP rates were high in Mekkojärvi throughout the whole summer in 2012 and decreased towards autumn. That was the hypothesis on the basis of preliminary measurements in 2011. It has long been recognized that benthic algae can have a substantial contribution to whole-lake primary and secondary production (Lindeman, 1942), and studies of their importance in energy flows have increased recently (see 2.2.2.). Benthic secondary production can play significant role in supporting higher trophic level production (e.g. Strayer & Likens, 1986; Jónasson *et al.* 1990; Lindegaard 1994; James *et al.* 1998; Vander Zanden & Vadeboncoeur, 2002). However, in lakes with high concentrations of coloured organic matter, light is usually the principal factor controlling benthic PP. Karlsson *et al.* (2009) showed, that small and unproductive lakes with high DOC concentration are usually light limited. Ask *et al.* (2012) studied benthic and pelagic PP in 15 lakes in northern Sweden, including both clear and humic lakes. They found that both benthic PP and respiration decreased with increasing DOC concentration. The same phenomenon is widely noted: high inputs of coloured OC decrease light penetration to water, reducing PP and narrowing suitable, illuminated substrates for benthic algae. Generally littoral zones are heterogeneous and very complex with high chemical and structural variability, changing markedly over spatial scales of centimeters to meters (Vadeboncoeur *et al.* 2006). In Mekkojärvi, the only available habitats for adnate algae are floating and submerged macrophytes near the surface, which are well illuminated. This complex structure can provide relatively large area of illuminated substrata for benthic algae. Rodríguez *et al.* (2012) found periphyton dominance in humic-rich shallow lake in Argentina in winter (530 vs. 23 $\text{mg C m}^{-2} \text{ h}^{-1}$, periphyton and phytoplankton, respectively), while in summer phytoplankton production dominated. They suggested, that periphyton was able to outcompete phytoplankton in winter, when nutrient and light competition was less important. In another shallow humic-rich lake with dense macrophyte cover available as a substrate for periphyton, Rodríguez *et al.* (2012) found one or two orders of magnitude higher periphyton PP rates than those of phytoplankton (86 vs. 2.7 $\text{mg C m}^{-2} \text{ h}^{-1}$ in winter

and 250 vs. 1 mg C m⁻² h⁻¹ in summer, periphyton, phytoplankton, respectively). These results support the findings from Mekkojärvi in this study and demonstrate, that adnate algae may dominate PP even in humic lakes if suitable substrates stand near well illuminated surface.

There was clearly visible difference in periphyton biomass between the two basins in Mekkojärvi in August: clearly less periphyton on Fish + than on Fish -. This may explain the decrease in littoral PP on Fish + in August (Fig. 12). Secondly, it may have caused some overestimation of littoral PP on Fish + on that time. In total, littoral NPP was higher on Fish - than on Fish + (Fig. 13). Although the knowledge of the importance of allochthonous OC in heterotrophic lake's food-webs has widely increased, it is not clear how the importance of allochthonous OC varies among consumers and among lake trophic states (Carpenter *et al.* 2005). In Mekkojärvi, most of the catchment runoff comes from east and northeast due to greater catchment area and its higher elevation on that side. Therefore, most of the runoff is first available for the periphyton growing on Fish -, which may explain thick periphyton growth on that basin throughout the year; periphyton might have been taken up nutrients faster on Fish - than on Fish +. Reasons for decrease in periphyton biomass on Fish + are unknown, but that may be associated with high precipitation in July, which increased the surface level in Mekkojärvi and changed the light climate. There could have been differences in littoral zone chemical conditions as well. It is possible, that there was fish effect on periphyton. Top-down effect on zooplankton was strong within the first few weeks since the early-July fish introductions: large bodied *Daphnia longispina* was almost disappeared from Fish + already in August and zooplankton composed mainly of small individuals. It is unlikely that adult perch would have had direct effect on periphyton, since adult perch was expected to prey on macroinvertebrates beyond zooplankton. However, it is possible that perch was primarily preying on zooplankton in July, and after that resource was utilized, shifted their diet to macroinvertebrates and were actively preying in the littoral zone. The biomass of perch was so high for such a small basin, when the biomass was doubled in early-August in Fish +, that it might have caused physical disturbance on relatively sensitive periphyton assemblages.

Littoral PP on control lakes was approximately one order of magnitude lower than in Mekkojärvi (Fig. 19). Both lakes were sampled similarly as Mekkojärvi, so only epiphytic algae on macrophyte vegetation were sampled. Both lakes were less coloured with lower depth ratio than Mekkojärvi and had epipelon, epipsammon and epixylon growth as well. Especially in Lake Alinen-Mustajärvi, which was sampled on 27.7.2012, rather high benthic algae biomass was visible. Therefore, the understanding of total benthic PP rates on those lakes was not received. Temporally, these results represent only extremely short P_{max} rates. Sampling date for Lake Halsjärvi was 5.9., which was late. Even in Mekkojärvi littoral PP was low at that time. Both of the control lakes have fish naturally, so a possible trophic cascade effect on periphyton might explain low PP rates in those lakes – similarly as on Fish + on Mekkojärvi.

Periphyton in lakes is usually less affected by shading of terrestrial vegetation, but in small headwaters, leaf canopies can intercept 95 % or more of incident radiation, reducing maximum photon flux densities (Hill, 1996). Heterogeneity of shoreline vegetation can form small sunflecks and larger sunpatches, which can contribute from 10 to 85% of total daily irradiance (Chazdon & Pearcy, 1991). This could cause some overestimation to the whole-lake estimates of PP, which has been modelled by using P_{max} values. Mekkojärvi does not have overhanging canopy, since the surrounding moss or reed bed extends far enough to the lake and prevents conifers and deciduous trees to grow close to the

shoreline. However, the fishless eastern side has some reed vegetation on its shoreline, which can cause sunflecks in the littoral zone. It is still likely, that sunflecks are too brief to contribute much to PP, especially in lentic ecosystems.

In most of the periphyton studies only autotrophic component, the algae, is considered, and much less have been focused on heterotrophic component, the fungi and microbes, which can play significant role in the response of algae to changes in physical and chemical conditions (Planas, 1996). Fungi and bacteria can have effect on nutrient cycling within the periphyton mat and they can compete for inorganic nutrients with autotrophic organisms (Wetzel, 1993). Wetzel (1993) stated that rapid recycling of nutrients and OC is mandatory to high sustained productivity of benthic algae. These relationships between autotrophs and heterotrophs may explain high PP rates of periphyton in Mekkojärvi. It is likely that heterotrophs play significant role in littoral zone in Mekkojärvi, causing high metabolism and recycling of nutrients in biofilms. This is what Kairesalo *et al.* (1992) stated as well: high densities of organisms (animals, algae, fungi and microbes) in littoral zone in Mekkojärvi indicate that their production likely exceeds that of pelagic community, which requires high littoral PP. Peaty moss bed in Mekkojärvi represents a large storage of OM (Kairesalo *et al.* 1992), which can be major energy source for littoral zone. Perhaps different relationship between auto- and heterotrophs in epiphytic biofilms in control lakes, especially in Alinen-Mustajärvi, explain low PP rates.

6. CONCLUSIONS

This study showed that simplified models, based on rather small number of parameters, can be misleading and do not apply to all lakes. Littoral PP in Mekkojärvi was high and contributions to whole-lake PP were estimated as high as over 90 % in summer, while model predictions were less than 5 %. Humic lakes have variety of different characteristics and functions. Littoral areas are complex and have variety of functional groups in biota, and their relationships are still poorly understood. Benthic algal contributions are generally light limited in coloured lakes, but epiphytic algae near surface are under well-lit conditions. In small lakes, macrophytes and bryophytes can provide large areas of well-lit substrate, which can have high contribution to whole lake area. Besides, periphyton can effectively compete algae for nutrients and possibly receive them from anoxic hypolimnion.

Mekkojärvi is an example of a small and highly humic headwater lake which lacks illuminated sediments but has highly productive epiphytic community on macro- and bryophytes. This study provides a background for further studies. Since the littoral PP seems to be high, more interesting questions are associated with utilization and transport of that autochthonous OM in food webs. What are energy flow pathways between littoral and pelagic zone? How much the littoral autochthonous OM supports the pelagic food webs? How much of the periphyton deposits and how much it is utilized?

ACKNOWLEDGEMENTS

I want to thank my supervisor Dr. Shawn Devlin for technical help in the field and laboratory and for all his guidance and valuable discussions during the data analysis. I also want to thank my supervisor Prof. Roger Jones for offering this chance to work in his group. Many thanks also to Lammi Biological Station's Environmental Research Foundation, Maa- ja vesitekniikan tuki ry and Olvi Foundation for their financial support.

REFERENCES

- Abbt-Braun, G., Lankes, U. & Frimmel, F. 2004. Structural characterization of aquatic humic substances - The need for a multiple method approach. *Aquat Sci* **66**: 151–170.
- Abrahamsen, G., Dovland, H., Bjor, K., Horntvedt, R., Tveite, B., Joranger E. & Semb A. 1976. Impact of Acid Precipitation on Forest and Freshwater Ecosystems in Norway. *Agric Res Counc of Norway Report 6/76*.
- Aitkenhead-Peterson, J. A., McDowell, W. D. & Neff, J. C. 2003. Sources, production and regulation of allochthonous dissolved organic matter inputs to surface waters. In: Stuart, E., Findlay, G. & Sinsabaugh, R. L. (edit.). *Aquatic Ecosystems, Interactivity of dissolved organic matter*. Academic Press.
- Almer, B., Dickson, W., Ekström, C., Hornström, E. & Miller, U. 1974. Effects of acidification of Swedish lakes. *Ambio*, vol 3, no 1, P 30–36, 1974, Illus.
- Arvola, L. & Kankaala, P. 1989. Winter and spring variability in phyto- and bacterioplankton in lakes with different water colour. - *Aqua Fennica* **19**: 29–39.
- Arvola, L., Eloranta, P., Järvinen, M., Keskitalo, J. & Holopainen, A.-L. 1999. In: Keskitalo, J. & Eloranta, P (ed.). *Limnology of Humic Waters*. Backhuys Publishers, Leiden, Netherlands.
- Ask J., Karlsson, J., Persson, L., Ask, P., Byström, P. & Jansson, M. 2009. Whole-lake estimates of carbon flux through algae and bacteria in benthic and pelagic habitats of clear-water lakes. *Ecology* **90**: 1923–1932.
- Ask, J., Karlsson, J. & Jansson, M. 2012. Net ecosystem production in clear-water and brown-water lakes. *Global Biogeochemical Cycles* **26**: GB1017.
- Azam, F., Fenchel, T., Field, J.G., Gray, J.S., Meyer-Reil, L.A. & Thingstad, F. 1983. The ecological role of water-column microbes in the sea. *Marine Ecology Progress Series* **10**: 257–263.
- Berggren, M., Ström, L., Laudon, H., Karlsson J., Jonsson, A., Giesler R., Bergström, A. & Jansson, M. 2010. Lake secondary production fueled by rapid transfer of low molecular weight organic carbon from terrestrial sources to aquatic consumers. *Ecol Lett* **13**: 870–880.
- Birge, E. A. & Juday, C. 1927. The organic content of the water of small lakes. *Proc. Am. Phil. Soc.* **66**: 357–372.
- Canfield, D., Kristensen, E. & Thamdrup, B. 2005. Microbial Ecosystems. *Adv Mar Biol* **48**: 465–506.
- Carpenter, S., Cole, J., Pace, M., Van De Bogert, M., Bade, D., Bastviken, D., Gille, C., Hodgson, J., Kitchell, J. & Kratzberg, E. 2005. Ecosystem subsidies: Terrestrial support of aquatic food webs from ¹³C addition to contrasting lake. *Ecology*, **86**(10): 2737–2750.
- Chazdon, R.L. & Pearcy, R.W. 1991. The importance of sunflecks for forest understory plants. *Bioscience* **41**: 760–766.
- Cole, J., Caraco, N., Kling, G. & Kratz, T. 1994. Carbon dioxide supersaturation in the surface waters of lakes. *Science (Wash)* **265**: 1568–1570.
- Cole, J. & Pace, M. 1995. Why measure bacterial production? A reply to the comment by Jahnke and Craven. *Limnology and Oceanography* **40** (2), 441–444.

- Cole, J. J., Pace, M., Carpenter, S. & Kitchell, J. 2000. Persistence of net heterotrophy in lakes during nutrient addition and food web manipulation. *Limnology and Oceanography* **45**:1718–1730.
- Coveney M. & Wetzel R. 1995. Biomass, production, and specific growth rate of bacterioplankton and coupling to phytoplankton in an oligotrophic lake. *Limnol Oceanogr* **40**: 1187–1200.
- Currie, D. & Kalff, J. 1984. Can bacteria outcompete phytoplankton for phosphorus - a chemostat test. *Microb Ecol* **10**: 205–216.
- Duarte, C. & Prairie, Y. 2005. Prevalence of heterotrophy and atmospheric CO₂ emissions from aquatic ecosystems. *Ecosystems* **8**: 862–870.
- Eloranta, P. 1999. In: Keskitalo, J. & Eloranta, P (ed.). *Limnology of Humic Waters*. Backhuys Publishers, Leiden, Netherlands.
- Estlander, S., Nurminen, L., Olin, M., Vinni, M. & Horppila, J. 2009. Seasonal fluctuations in macrophyte cover and water transparency of four brown-water lakes: implications for crustacean zooplankton in littoral and pelagic habitats. *Hydrobiologia* **620**: 109–120.
- Findlay, S. 2003. In: *Aquatic Ecosystems: Interactivity of Dissolved Organic Matter*. 2003. Elsevier Science Ltd., The Boulevard Langford Lane Kidlington Oxford OX5 1GB UK.
- Grahn, O. 1985. Macrophyte biomass and production in Lake Gardsjon - an acidified lake in southwestern Sweden. *Ecological Bulletins* **37**: 203–2012. Stockholm.
- Grahn, O. 1986. Vegetation structure and primary production in acidified lakes in southwestern Sweden. *Expertia* **42**. 5. 465–570
- Hecky, R. & Hesslein, R. 1995. Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. *Journal of the North American Benthological Society* **14**:631–653.
- Hessen, D. 1992. Dissolved organic carbon in a humic lake: effects on bacterial production and respiration. *Hydrobiologia* **229**: 115–123.
- Hill, W. 1996. Effect of Light. In: *Algal Ecology, Freshwater Benthic Ecosystems*. San Diego: Academic-Press inc.
- Hubbard, P., Barata E. & Canario, A. 2002. Possible disruption of pheromonal communication by humic acid in the goldfish, *Carassius auratus*. *Aquat Toxicol* **60**, 169–183
- Hobson, K. & Welch, H. 1995. Cannibalism and trophic structure in a high Arctic lake: Insights from stable-isotope analysis. *Can. J. Fish. Aquat. Sci.* **52**: 1195–1201.
- Jackson, T. & Hecky, R. 1980. Depression of primary productivity by humic matter in lake and reservoir waters of the boreal forest zone. *Can. J. Fish. Aquat. Sci.*, **37**(12)
- James, M., Weatherhead, M., Stanger, C. & Graynoth, E. 1998. Macroinvertebrate distribution in the littoral zone of Lake Coleridge, South Island, New Zealand: Effects of habitat stability, wind exposure, and macrophytes. *New Zealand Journal of Marine and Freshwater Research* **32**: 287–305.
- Jansson, M., Blomqvist, P., Jonsson, A. & Bergström, A. 1996. Nutrient limitation of bacterioplankton, autotrophic and mixotrophic phytoplankton, and heterotrophic nanofagellates in Lake Öträsket. *Limnology and Oceanography*, **41**, 1552–1559.

- Jansson, M., Bergström, A. K., Blomqvist, P. & Drakare, S. 2000. Allochthonous organic carbon and phytoplankton/bacterioplankton production relationships in lakes. *Ecology* **81**: 3250–3255
- Jansson, M., Karlsson, J. & Blomqvist, P. 2003. Allochthonous organic carbon decreases pelagic energy mobilization in lakes. *Limnol Oceanogr* **48**: 1711–1716.
- Jasser I., Kostrzevska-Szlakowska I., Ejsmont-Karabin J., Kalinowska K. & Weglenska T. 2009. Autotrophic versus heterotrophic production and components of trophic chain in humic lakes: the role of microbial communities. *Pol J Ecol* **57**: 423–439.
- Jónasson P., Lindegaard, C., Dall, P., Hamburger, K. & Adalsteinsson, H. 1990. Ecosystem studies on temperate Lake Esrom and the subarctic Lakes Myvatn and Thingvallavatn. *Limnologica* **20**: 259–266.
- Jones, R. I. 1992. The influence of humic substances on lacustrine planktonic food chains. *Hydrobiologia* **229**: 73–91
- Jones, R. I. 2000. Mixotrophy in planktonic protists: an overview. *Freshwater Biology* **45**, 219–226
- Järnefelt, H. 1935. Die regionale Verteilung der Gewässertypen in Finnland. *Verh. Internat. Verein. Limnol*: **7**: 653–656.
- Kairesalo, T., Lehtovaara, A. & Saukkonen, P. 1992. Littoral-pelagial interchange and the decomposition of dissolved organic matter in a polyhumic lake. *Hydrobiologia* **229**: 199–224
- Kalff, J. 2002. Limnology. Prentice Hall. ISBN: 0-13-033775-7
- Kankaala, P., Taipale, S., Li, L. & Jones, R.I. 2010a. Diets of crustacean zooplankton, inferred from stable carbon and nitrogen isotope analyses, in lakes with varying allochthonous dissolved organic carbon content. *Aquat Ecol* **44**: 781–795.
- Kankaala, P., Peura, S., Nykänen, H., Sonninen, E., Taipale, S., Tiirola, M. & Jones, R.I. 2010b. Impacts of dissolved organic carbon on boreal freshwater metabolism and foodwebs in mesocosm experiments. *Fundam. Appl. Limnol, Arch. Hydrobiol.* Vol **177/3**, 161–176.
- Kankaala, P. 1988. The relative importance of algae and bacteria as food for *Daphnia longispina* (Cladocera) in a polyhumic lake. *Freshwater Biology* **19**: 285–296.
- Kankaala, P., Huotari, J., Peltomaa, E., Saloranta T. & Ojala, A. 2006. Methanotrophic activity in Relation to methane efflux and total heterotrophic bacterial production in a stratified, humic, boreal lake. *Limnol. Oceanogr.* **51**: 1195–1204.
- Karlsson, J., Byström, P., Ask, J., Ask, P., Persson, L. & Jansson, M. 2009. Light limitation of nutrient-poor lake ecosystems. *Nature* **460**: 506–509.
- Kuoppo-Leinikki, P. & Salonen, K. 1992. Bacterioplankton in a small polyhumic lake with anoxic hypolimnion. *Hydrobiologia* **229**: 159–168.
- Kwiatkowski, R. & Roff, J. 1976. Effects of Acidity on the Phytoplankton and Primary Productivity of Selected Northern Ontario Lakes. *Canadian Journal of Botany* Vol **54**, No 22, p 2546–2561.

- Köhler, A., Schön, R., Umweltbundesamt, Berlin (FRG). 1984. (Resistance of submerged macrophytes to acidification – preliminary results.)
- Lindegaard, C. 1994. The role of zoobenthos in energy flow in two shallow lakes. *Hydrobiologia* **275–276**: 313–322.
- Lindeman, R. L. 1942. The trophic-dynamic aspect of ecology. *Ecology* **23**: 399.
- Lovley, D., Coates, J., Blunt-Harris, E., Phillips, E. & Woodward, J. 1996. Humic substances as electron acceptors for microbial respiration. *Nature* **382**, 445–448
- McBride, G. 1992. Simple calculation of daily photosynthesis by means of five photosynthesis-light equations. *Limnol Oceanogr* **37**: 1796–1808.
- Münster, U. 1999. In: Keskitalo, J. & Eloranta, P (ed.). Limnology of Humic Waters. Backhuys Publishers, Leiden, Netherlands.
- Münster, U., Salonen, K. & Tulonen, T. 1999. In: Keskitalo, J. & Eloranta, P (ed.). Limnology of Humic Waters. Backhuys Publishers, Leiden, Netherlands.
- Nauwerck, A. 1963. Die Beziehungen zwischen Zooplankton und Phytoplankton in see Erken. *Symb.Bot. Upsal.*, **17**, 163.
- Nürnberg, G. & Shaw, M. 1998. Productivity of clear and humic lakes: nutrients, phytoplankton, bacteria. *Hydrobiologia* **382**: 97–112.
- Odum, E. P. & Odum, H. T. 1959. Fundamentals of Ecology. 2nd Ed. W. B. Saunders Co. Philadelphia and London.
- Planas, D. 1996. Acidification effects. In: Algal Ecology, Freshwater Benthic Ecosystems. San Diego: Academic-Press inc.
- Rodríguez, P., Solange Vera, M. & Pizarro, H. 2012. Primary production of phytoplankton and periphyton in two humic lakes in a South American wetland. *Limnology* **13**:281–287
- Salonen, K. 1981. Rapid and precise determination of total inorganic carbon and some gases in aqueous solutions. *Wat. Res.* **15**: 403–406.
- Salonen, K. & Hammar, T. 1986. On the importance of dissolved organic matter in the nutrition of zooplankton in some lake waters. *Oecologia* **68**: 246–253.
- Salonen, K. & Jokinen, S. 1988. Flagellate grazing on bacteria in a small dystrophic lake. *Hydrobiologia* **161**: 203–209
- Salonen K. & Lehtovaara A. 1992. Migrations of haemoglobin-rich *Daphnia longispina* in a small, steeply stratified, humic lake with an anoxic hypolimnion. *Hydrobiologia* **229**: 271–288.
- Salonen, K., Hammar, K., Kuuppo, P., Smolander, U. & Ojala, A. 2005. Robust parameters confirm predominance of heterotrophic processes in plankton of a highly humic pond. *Hydrobiologia* **543**: 181–189
- Sand-Jensen, K. 1989. Environmental Variables and their Effect on Photosynthesis of Aquatic Communities. *Aquatic Botany AQBODS* **38** (1-3): 5–25
- Sarvala, J., Ilmavirta, V., Paasivirta, L. & Salonen, K. 1981. The ecosystem of the oligotrophic Lake Pääjärvi 3. Secondary production and an ecological energy budget of the lake.

Verhandlungen der Internationalen Vereinigung für Limnologie, **21**, 422–427.

- Schindler D., Schmidt, R. & Reid, R. 1972. Acidification and bubbling as an alternative to filtration in determining phytoplankton production by the ^{14}C method. *Journal of the Fisheries Research Board of Canada* **29**: 1627–1631.
- Schindler, D. & M. Scheuerell, D.. 2002. Habitat coupling in lake ecosystems. *Oikos* **98**: 177–189.
- Steemann Nielsen, E. 1951. Measurements of the production of organic matter in the sea by means of carbon-14. *Nature*: **167**:846–685
- Steemann Nielsen, E. 1952. The use of radioactive carbon (^{14}C) for measuring organic production in the sea. *J. Cons. Int. Expl. Mer* **18**:117–140
- Steinberg, C. & Münster, U. 1985. Geochemistry and Ecological Role of Humic Substances in Lakewater. In: Aiken G.R.,McKnight D.M., Wershaw R.L. & MacCarthy P, editors. Humic Substances in Soil, Sediment and Water. New York: Wiley, p. 105–145.
- Steinberg, C. 2004. Regulatory Impacts of Humic Substances in Lakes. In: The Lakes Handbook Volume 1, Limnology and Limnetic Ecology. O’Sullivan, P.E. & Reynolds, C.S., editors. USA: Blackwell Publishing.
- Steinberg, C., Kamara, S., Prokhotskaya, V.Y., Manusadzianas, L., Karasyova, T.A., Timofeyev, M.A., Jie Z., Paul, A., Meinelt, T., Farjalla, V.F., Matsuo, A.Y., Kent Burnison, B. & Menzel, R. 2006. Special review: Dissolved humic substances – ecological driving forces from the individual to the ecosystem level? *Freshwat Biol* **51**: 1189–1210.
- Steinberg, C., Meinelt, T., Timofeyev, M., Bittner, M. & Menzel, R. 2008. Humic Substances, Part 2: Interactions with Organisms. *Environ Sci Pollut Res Int* **15**: 128–135.
- Stevenson, R., Bothwell, M. & Lowe, R. 1996. Algal ecology : freshwater benthic ecosystems / edited by R. Jan Stevenson, Max L. Bothwell, Rex L. Lowe. Academic Press.
- Strayer, D. & Likens, G. 1986. An energy budget for the zoobenthos of Mirror Lake, New Hampshire. *Ecology* **67**: 303–313.
- Sundh I. & Bell R. 1992. Extracellular dissolved organic carbon released from phytoplankton as a source of carbon for heterotrophic bacteria in lakes of different humic content. *Hydrobiologia* **229**: 93–106
- Sundh I., Bastviken D. & Tranvik L.J. 2005. Abundance, Activity, and Community Structure of Pelagic Methane-Oxidizing Bacteria in Temperate Lakes. *Appl Environ Microbiol* **71**: 6746–6752.
- Taipale. S. 2007. Bacterial-Mediated Terrestrial Carbon in the Foodwebs of Humic Lakes. PhD thesis. University of Jyväskylä, Jyväskylä.
- Taipale S., Kankaala P., Hämäläinen, H. & Jones R. I. 2009. Seasonal shifts in the diet of lake zooplankton revealed by phospholipid fatty acid analysis. *Freshwat Biol* **54**: 90–104.
- Thienemann, A. 1925. Die Binnengewässer Mitteleuropas. *Die Binnengewässer* **1**: 1–255.
- Tranvik L. 1992. Rapid microbial production and degradation of humic-like substances in lake water. Schweizerbatsche Verlagsbuchhandlung, Stuttgart (FGR)

- Tulonen T., Kankaala P., Arvola L. & Ojala A. 2000. Growth and production of bacterioplankton in a deep mesohumic boreal lake. *Arch Hydrobiol* **147**: 311–325.
- University of Wisconsin-Milwaukee. 2012. Liquid scintillation counting handout. Available at: <https://pantherfile.uwm.edu/groups/sa/usa/public/RAD/HANDOUT.pdf>
- Vadeboncoeur, Y., Lodge, D. & Carpenter, S. 2001. Whole-lake fertilization effects on distribution of primary production between benthic and pelagic habitats. *Ecology* **82**: 1065–1077.
- Vadeboncoeur, Y., Vander Zanden, M. & Lodge, D. 2002. Putting the lake back together: Reintegrating benthic pathways into lake food web models. *Bioscience* **52**: 44–54.
- Vadeboncoeur, Y. & Steinman, A. 2002. Periphyton Function in Lake Ecosystems. *The Scientific World Journal* **2**: 1449–1468.
- Vadeboncoeur, Y., Jeppesen, E., Vander Zanden, J., Schierup, H.-H., Christoffersen, K. & Lodge, D. 2003. From Greenland to green lakes: Cultural eutrophication and the loss of benthic energy pathways in lakes. *Limnology and Oceanography* **48**: 1408–1418.
- Vadeboncoeur, Y., Kalff, J., Christoffersen K. & Jeppesen E. 2006. Substratum as a driver of variation in periphyton chlorophyll and productivity in lakes. *J N Am Benthol Soc* **25**: 379–392.
- Vadeboncoeur, Y., Peterson, G., Zanden M. & Kalff J. 2008. Benthic algal production across lake size gradients: Interactions among morphometry, nutrients, and light. *Ecology* **89**: 2542–2552.
- Vander Zanden, M. & Vadeboncoeur, Y. 2002. Fishes as integrators of benthic and pelagic food webs in lakes. *Ecology* **83**: 2152–2161.
- Vander Zanden, M., Essington, T. & Vadeboncoeur, Y. 2005. Is pelagic top-down control in lakes augmented by benthic energy pathways? *Can J Fish Aquat Sci* **62**: 1422–1431.
- V.-Balogh, K. & Vörös, L. 1997. High bacterial production in shallow reservoirs rich in humic substances. *Hydrobiologia* **342/343**: 63–70.
- Verduin, J. 1956. Primary production in lakes. *Limnol Oceanogr* **1**: 85–91.
- Verta, M., Salo, S., Korhonen, M., Porvari, P., Paloheimo, A. & Munthe, J. 2010. Climate induced thermocline change has an effect on the methyl mercury cycle in small boreal lakes. *Sci Total Environ.* **1**:408(17) 3639-47
- Wetzel R. G. 1993. Microcommunities and microgradients: Linking nutrient regeneration, microbial mutualism, and high sustained aquatic primary production. *Neth J Aquat Ecol* **27**: 3–9.
- Wetzel, R. G. 1995. Death, detritus and energy flow in aquatic ecosystems. *Freshwater Biology* **33**: 83–89.
- Wetzel, R. G. 1996. Benthic algae and nutrient cycling in lentic freshwater ecosystems. Modeling benthic algal communities: an example from stream ecology. In: *Algal Ecology: Freshwater Benthic Ecosystems*. Stevenson.
- Wetzel, R. G. & Likens, G. 2000. *Limnological analyses*. Third edition. New York: Springer Science+Business Media, Inc.

Wetzel, R. G. 2001. Limnology, Third edition. Lake and River Ecosystems. Academic Press, 525 B Street, Ste. 1900, San Diego, CA 92101, USA.

Yan, N. & Stokes, P. 1978. Phytoplankton of an acidic lake, and its responses to experimental alterations of pH. *Environ.Conserv.*, **5**(2), 93–100.