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# in Highly Humic Boreal Lakes

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# ABSTRACT

Littoral zones in lakes are among the most productive habitats in the world, but have been seriously understudied, as most limnological studies have concerned only pelagic habitats. The likely importance of littoral zones in clear lakes is widely acknowledged, but in contrast their role in dystrophic humic lakes has generally been assumed to be minor. In this thesis, littoral importance in whole-lake metabolism was studied in small and highly humic lakes in Southern Finland, where Lake Mekkojärvi was the principal study lake. Primary production (PP) measurements revealed that littoral periphyton, growing on surrounding aquatic vegetation, can dominate the whole-lake PP, and the highly autotrophic littoral can help balance the whole-lake metabolism or even shift these lakes to occasional net autotrophy. Measurements of bacterial production (BP) within the littoral epiphytic biofilms and in the pelagic zone revealed overall pelagic dominance of BP in Mekkojärvi. However, epiphytic bacteria made a strong contribution, and their highest production rates were almost equal to those of pelagic bacterioplankton. Stable isotope analysis revealed that periphyton can be an important food resource for the consumers in humic lakes. The naturally fishless Mekkojärvi was divided into two basins by a plastic curtain and perch (*Perca fluviatilis*) were introduced to one basin while the other remained fishless. The results indicate that the pelagic zooplankton do not appreciably utilize littoral prey under the fishless state, but that fish can promote coupling of the pelagic and littoral food webs by directly utilizing littoral prey or by inducing predation pressure on zooplankton, which drives them to the littoral to seek refuge from predation. This study emphasizes the important role of the littoral in whole-lake processes in humic lakes, and suggests that when the littoral is taken into account many small humic lakes are actually more productive and not as heterotrophic as is commonly supposed.

Keywords: Autotrophy; bacterial production; heterotrophy; periphyton; stable isotopes; whole-lake primary production

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# CONTENTS

**LIST OF ORIGINAL PUBLICATIONS**

This thesis is based on the following original papers, which will be referred to in the text by their Roman numerals I–IV.

JV, SPD, JS and RIJ designed all the studies together. JV, SPD and JS conducted study I and JV and SPD analysed the data. JV and JS conducted study II and JV analysed the data. JV conducted studies III and IV and analysed all the data. SPD and JS assisted in all the data analysis. JV, SPD, JS and RIJ wrote the papers.

I    Vesterinen J., Devlin S.P., Syväranta J. & Jones R.I. 2016. Accounting for littoral primary production by periphyton shifts a highly humic boreal lake towards net autotrophy. *Freshwater Biology* 61: 265–276.

II   Vesterinen J., Syväranta J., Devlin S.P. & Jones R.I. 2016. Periphyton support for littoral secondary production in a highly humic boreal lake. *Freshwater Science* 35: 1235–1247.

III  Vesterinen J., Devlin S.P., Syväranta J. & Jones R.I. 2017. Epiphytic bacteria make an important contribution to heterotrophic bacterial production in a humic boreal lake. Submitted manuscript.

IV   Vesterinen J., Devlin S.P., Syväranta J. & Jones R.I. 2017. Influence of littoral periphyton on whole-lake metabolism relates to littoral  vegetation in humic lakes. Submitted manuscript.

**ABBREVIATIONS**

BP          bacterial production
Chl *a*     chlorophyll *a*
CR          community respiration
DIC         dissolved inorganic carbon
DOC         dissolved organic carbon
DR          depth ratio, mean depth:maximum depth
GPP         gross primary production
MOB         methane-oxidizing bacteria
NPP         net primary production
OM          organic matter
P:L ratio   pelagic:littoral ratio by area
POM         particulate organic matter
PP          primary production
SIA         stable isotope analysis
TCA         trichloroacetic acid

# 1    INTRODUCTION

## 1.1   Littoral zones in lake ecosystems

The littoral zone is commonly defined as that part of a lake that is close to the shore extending from the point at the shoreline where influence of wave action ends to the depth at which light penetration is no longer sufficient for photosynthesis (e.g. Wetzel 2001). The extent of development of the littoral varies greatly among lakes depending on geomorphology of the basin, sedimentation accumulation since its formation and water clarity (Wetzel 1990). As an interface between the land of the catchment area and the open water of the lake (the pelagic zone) littoral zones are considered "critical transition zones", being dynamic habitats controlling or influencing the movement of organisms, nutrients, materials and energy within and across landscapes through linked ecosystem processes (Wall *et al.* 2001). Most lakes in the world are small (Downing *et al.* 2006, Verpoorter *et al.* 2014), and their morphometry is such that the ratio of the pelagic zone to the littoral zone by area (P:L ratio) is low, making the littoral the dominant habitat over the pelagic on a global basis (Wetzel 2001). Besides being abundant, littoral zones are also among the most productive habitats in the world (Wetzel 1990).

In addition to abiotic factors, the physical structure of the littoral is modified by highly variable emergent and submerged macrophytes and the associated periphytic communities, which maintain diverse life forms, from those attached to substrata to freely swimming organisms. High primary production (PP) by major primary producers in the littoral (macrophytes, periphyton and phytoplankton) results in high heterotrophic activity and complex trophic interactions, not only within the littoral food webs themselves but also between different lake habitats through habitat coupling, occurring particularly via fish (Schindler and Scheuerell 2002) but also via zooplankton (Van de Meutter *et al.* 2005). High habitat heterogeneity, which is a common characteristic of littoral zones, increases the availability of refuges and their spatial separation, limiting populations and affecting trophic interactions by

weakening the foraging efficiency of predators (e.g. Diehl 1992, Beukers and Jones 1998, Shurin *et al.* 2002, Grabowski 2004). The inherent structure and trophic interactions are particularly complex in the macrophyte-associated littoral food webs (Jeppesen *et al.* 1998). Macrophytes have been demonstrated to increase food chain length in shallow lakes (Ziegler *et al.* 2015) and they potentially have a significant effect on chemical and physical conditions in the water and sediment, on internal nutrient loading, and on lake productivity, as well as on biotic interactions (Gasith and Hoyer 1998). Littoral slope is a morphometric variable which can be used as a predictor for submerged macrophyte biomass in lakes, except in highly turbid conditions, where light penetration is pre-eminent rather than slope (Duarte and Kalff 1986). A typical distribution of macrophytes in a small, oligotrophic temperate lake includes, from the upper part of the littoral to the lowest part, helophytes, nymphaeids, elodeids and isoetids (Pokorný and Björk 2010). With decreasing light availability elodeids are the first to disappear, followed by isoetids (Pokorný and Björk 2010). However, in many small humic boreal lakes, where the poor light penetration constrains development of this typical zonation of littoral vegetation, bryophytes can develop extended and dense floating mats along the lake shoreline (Rintanen 1996).

### 1.1.1 Dominant habitats in biomass production

In aquatic ecosystems phototrophic organisms occupy three different habitats: open water, illuminated solid surfaces and the water surfaces (Sand-Jensen and Borum 1990). Algae attached to different substrata (periphyton), together with macrophytes, form a major photosynthetic component in the littoral (Kalff 2002). PP by periphyton can be a substantial energy source for lake food webs particularly under conditions such as shallow depth, low water nutrient concentrations or high water-column transparency (Hecky and Hesslein 1995, Vadeboncoeur and Steinman 2002, Vadeboncoeur *et al.* 2002). Macrophytes determine the distribution and abundance of epiphytic algae (attached to plants) while the distribution of benthic algae (attached to sediments, rocks and wood) is largely determined by the basin slope and water transparency (Kalff 2002, Vadeboncoeur *et al.* 2008). The availability and relative abundance of different substrata also affects the chlorophyll *a* (chl *a*) content and production rates of periphyton in lakes (Vadeboncoeur *et al.* 2006). Particularly in shallow oligotrophic lakes, benthic algae can be almost completely responsible for whole-lake PP, whereas in eutrophic lakes phytoplankton usually dominates the whole-lake PP and inhibits the PP by benthic algae due to shading (Vadeboncoeur *et al.* 2003). PP in large and deep oligotrophic lakes tends to be dominated by phytoplankton with a low benthic fraction of the whole-lake PP (Vadeboncoeur *et al.* 2008), but evidence from stable isotopes has revealed that periphyton may still be an important energy source for consumers including many fish species (Hecky and Hesslein 1995, Bootsma *et al.* 1996, Yoshii 1999, Devlin *et al.* 2013). In turbid and humic lakes, low light availability effectively limits both pelagic and benthic PP (Vadeboncoeur *et al.* 2008, Ask *et al.* 2009),

and light has been proposed to be a major limiting factor of overall lake productivity in a large proportion of unproductive lake ecosystems (Karlsson *et al.* 2009).

As transition zones between the catchment and the lake, littoral zones potentially provide considerable buffer capacity for inflowing nutrients and are prone to pollution (Wetzel 1990). Littoral habitats are hotspots of metabolism and recycling of particulate and dissolved carbon from both autochthonous and allochthonous sources, and heterotrophic bacteria are the most important organisms in this process despite their low biomass (Buesing 2002, Steinberg 2003). Bacterial production (BP) in the littoral can apparently be of a similar magnitude to, or even higher than that in the pelagic, although it has only rarely been measured in limnological studies (Vadeboncoeur *et al.* 2002). The littoral zone with its vegetation and associated periphytic communities may act as a net sink for autochthonous and allochthonous dissolved organic carbon (DOC), being simultaneously a source of refractory C compounds from autochthonous production, which may be released to pelagic zone (Steinberg 2003). Particularly in small humic boreal lakes with surrounding peaty littoral moss beds, part of the refractory humic substances (HS) in the pelagic may originate from autochthonous sources within the littoral (Kairesalo *et al.* 1992).

### 1.1.2 Littoral has been understudied in humic lakes

Limnological research has historically had an overwhelmingly pelagic focus (Sand-Jensen and Borum 1991, Vadeboncoeur *et al*. 2002). In 2002, only 3–12 % of studies of PP, heterotrophic BP and invertebrate production in lakes concerned both pelagic and benthic habitats (Vadeboncoeur *et al*. 2002). Thus, most limnologists have studied either the benthic or, more often, the pelagic habitat, with the exception of the role of macrophytes in shallow lakes (Scheffer *et al.* 1993, Jeppesen *et al.* 1998, Vakkilainen 2005). Probable reasons for this continued skewness in lake research include a strong tradition of phytoplankton ecology and the relative ease by which phytoplankton biomass (often estimated as chl *a*) and productivity ($^{14}$C-fixation) can be measured *in situ*, in contrast to the very heterogeneously distributed attached algae in littoral zones (Sand-Jensen and Borum 1991). Littoral zones have received more attention during recent years, but most studies including PP, BP and invertebrate production in both pelagic and littoral habitats have been conducted either in clear oligotrophic lakes or in eutrophic lakes, and very rarely in humic dystrophic lakes.

Humic lakes, which are abundant in the boreal zone, receive high loadings of allochthonous (terrestrially derived) organic matter (OM) from their catchment areas, which affects several fundamental functions in lake ecosystems. Incorporation of allochthonous OM into lake food webs through microbial processes increases heterotrophy along a humic gradient (Jones 1992, Jansson *et al.* 2000, Carpenter *et al.* 2005, Jansson *et al.* 2007) and leads to community respiration (CR) which exceeds gross primary production (GPP) resulting in an overall lake net heterotrophy (Del Giorgio and Peters 1994,

Duarte and Agusti 1998). There is strong evidence that allochthonous OM particularly negatively affects both the primary and secondary production in boreal lakes by reducing light availability (Ask *et al.* 2009, Karlsson *et al.* 2009, Jones *et al.* 2012, Seekell *et al.* 2015). In nutrient-poor humic lakes the littoral PP is expected to be almost negligible due to the lack of illuminated benthic areas (Vadeboncoeur *et al.* 2008). Thus, the littoral has been assumed to have an unimportant role in the metabolism and biomass production in humic boreal lakes. This, together with the much more demanding challenge of measuring production in highly complex littoral environments, has probably led to under-representation of the littoral zone in studies of humic lakes.

The benthic (littoral) fraction of whole-lake PP has been estimated with a model using parameters such as light attenuation, nutrient concentrations and depth ratio (DR = $z_{mean}:z_{max}$) (Vadeboncoeur *et al.* 2008). Both high light attenuation and high DR (steep-sided lake basin) result in a reduction of illuminated benthic areas and leads to low benthic contribution to whole-lake PP. Many small boreal lakes are highly humic and surrounded by aquatic vegetation, which leads to a cylindrical lake morphometry and thus a lack of illuminated benthic areas. However, the surrounding aquatic vegetation can be very extensive, sometimes covering a large fraction of the lake surface area, and provides substrata for colonization by periphyton close to the water surface under relatively well-lit conditions. Thus the littoral in such lakes can be better defined as the area covered by aquatic vegetation. Bryophytes (mosses) in particular can form extensive floating beds in humic lakes and show a trend of expanding and perhaps eventually filling entire water bodies (Rintanen 1996). This littoral vegetation can also sustain rich invertebrate communities in humic lakes by providing shelter and potentially abundant food resources (Kairesalo *et al.* 1992). How productive these periphytic communities are and how much they contribute to whole-lake PP in humic lakes is still poorly understood, although there is some evidence of their appreciable contribution to invertebrate food webs in highly humic lakes in Sweden (Lau *et al.* 2014) and in bog pools in Estonia (van Duinen *et al.* 2013). While most of these humic lakes in the boreal zone are considered net heterotrophic and net emitters of $CO_2$ to the atmosphere (Cole *et al.* 1994, Del Giorgio *et al.* 1997), this concept is too often based on measurements of metabolic rates in pelagic zones alone. Therefore, it is crucial to investigate the role of littoral zones in lake metabolism and food web dynamics to understand better the function of these humic lake ecosystems.

## 1.2 Aims of the study

The aim of this thesis was to investigate the role of the littoral zone in biomass production and metabolism in highly humic boreal lakes. The principal hypothesis was that the periphyton colonizing the surrounding aquatic littoral vegetation (epiphyton) can be highly productive and contribute strongly to the whole-lake PP, and that consequently many of these unproductive humic

(dystrophic) lakes can be more productive than previously assumed. The study encompassed eight boreal lakes, located near each other in southern Finland. All the lakes are small, highly humic and mostly headwater lakes, but show varying development of the littoral zone, which in most of the lakes can be defined as the area occupied by floating aquatic vegetation.

The principal study lake for this thesis was Mekkojärvi, which was the subject in three of the four papers. In the first paper I PP and CR were investigated from both pelagic planktonic and littoral periphytic communities in Mekkojärvi several times during an open-water period and the rates were extrapolated to the whole-lake scale. Then, in II the importance of periphyton as a food resource in Mekkojärvi and potential coupling of pelagic and littoral habitats in the presence and absence of planktivorous fish were studied using stable isotope analysis (SIA). In III pelagic planktonic and littoral epiphytic BP was investigated during an open-water period and the rates were compared at the whole-lake scale. In IV a lake survey was conducted in which both PP and CR were measured from eight lakes during one month in summer as in I to test the wider generality of littoral importance in whole-lake PP of highly humic lakes. Within these general objectives, testable hypothesis were formulated as follows:

i.   Periphyton in the littoral zone is highly productive and contributes strongly to whole-lake PP in Lake Mekkojärvi. The littoral zone is expected to be net autotrophic to the extent of being able to balance the whole-lake metabolism in Mekkojärvi in summer. (I)

ii.  Periphyton is an important food resource for littoral invertebrates in Mekkojärvi. The Littoral and pelagic zones are not expected to be strongly coupled in the absence of planktivorous fish, but introduction of European perch (*Perca fluviatilis*) will drive pelagic zooplankton towards the littoral to seek refuge from predation. Zooplankton is then expected to rely more on littoral food sources and thereby enhance the coupling of the pelagic and littoral habitats. (II)

iii. Littoral epiphytic BP can significantly contribute to the whole-lake BP in Mekkojärvi. (III)

iv.  Highly productive littoral epiphytic communities can be found from many small humic lakes similar to Mekkojärvi and can contribute strongly to whole-lake metabolism of such lakes. The contribution of littoral PP by periphyton to whole-lake PP is expected to be dependent on the area occupied by aquatic vegetation. (IV)

# 2 MATERIAL AND METHODS

## 2.1 Study lakes

This study encompasses eight humic lakes located in the Evo forest area in southern Finland, an area of ca. 18 km² between 61° 10′ and 61° 13′ N and 25° 5′ and 25° 12′ E (Fig. 1). The area contains mainly small humic headwater lakes, which are inter-connected by brooks forming a network of lakes and are predominantly fed by surface runoff having one or more inflows and one outflow (Järvinen *et al.* 2002, Arvola *et al.* 2010). Lakes in Evo are mainly surrounded by coniferous forests with patches of deciduous trees, and the forest development has been strongly influenced by forestry. There is no agricultural activity remaining in the study area.

Mekkojärvi was the subject of I–III and was also included with other lakes in IV. Mekkojärvi has an area of 0.35 ha, a mean depth of 2.0 m and a maximum depth of 4.3 m. The lake is highly humic (water colour 300–800 mg Pt l$^{-1}$) with high DOC concentration (around 30 mg C l$^{-1}$) and low pH (5–6), and it develops very steep temperature and $O_2$ gradients rapidly after ice-off in spring. The thermocline lies between 0.5 and 1.0 m and anoxia normally occurs below 1.0 m during summer. Mekkojärvi has ice cover usually from early November until the beginning of May. The lake cannot usually sustain overwintering fish populations due to complete winter anoxia under ice, and the absence of planktivorous fish has led to very dense summer populations of zooplankton dominated by the cladoceran *Daphnia longispina*. Mekkojärvi does not have a clearly defined littoral zone, as the lake is mostly surrounded by a floating moss bed (mainly *Warnstorfia* and *Sphagnum* species) and submerged sedges with some patchy appearance of other macrophytes such as *Phragmites australis* and *Menyanthes trifoliata*. This surrounding vegetation does not extend further than ca. 1 m from the lake edge and not deeper than ca. 0.5 m, but covers ca. 25 % of the lake surface area and, due to its highly complex structure, provides extensive substrata for epiphytic algae. Periphyton forms relatively thick growths, rich in pennate diatoms (Bacillariophyceae), already early in summer

and these achieve their highest biomass in July–August. During the studies I–II in 2012 and 2014, as part of a larger research project Mekkojärvi was divided in two with a plastic curtain and adult perch (*Perca fluviatilis*) were introduced to one basin in early July in both years while the other basin remained fishless. The biomass of introduced perch was intended to mimic the natural biomass of perch in local lakes (1–2 g m$^{-2}$, Natural Resource Institute Finland). During III and IV the curtain had been removed from Mekkojärvi.

The study IV was conducted in eight small and highly humic lakes, including Mekkojärvi, which are all located nearby each other in the Evo forest area (Fig. 1, Table 1). All the lakes are small (mean ± SE surface area 1.0 ± 0.2 ha) and highly humic (mean ± SE water colour 280 ± 57 mg Pt l$^{-1}$) and have ice cover usually from early November until the beginning of May. Light penetration is restricted to the uppermost water layers, and all the lakes develop steep $O_2$ and temperature gradients early in spring and have an anoxic hypolimnion throughout the summer. The lakes are mostly steep-sided and lack illuminated benthic areas, but a few have some shallow areas close to the shoreline where light can reach the bottom. Littoral areas consist mainly of surrounding aquatic vegetation and associated periphyton. Lakes Mekkojärvi, Möläkkä and Nimetön have similar characteristics in their littoral: surrounded by a floating moss bed with partly submerged sedges and patches of some other floating, emergent and submerged macrophytes. Lakes Horkkajärvi and Tavilammi have the most bare rocky shore (lacking vegetation) and the least vegetation with some patches of emergent and floating macrophytes along the shoreline. Lakes Haukijärvi, Huhmari and Keskinen Rajajärvi can be considered as intermediate in terms of vegetation cover, having more macrophytes than Horkkajärvi and Nimetön but lacking as extensive a moss bed as in Mekkojärvi and Nimetön.
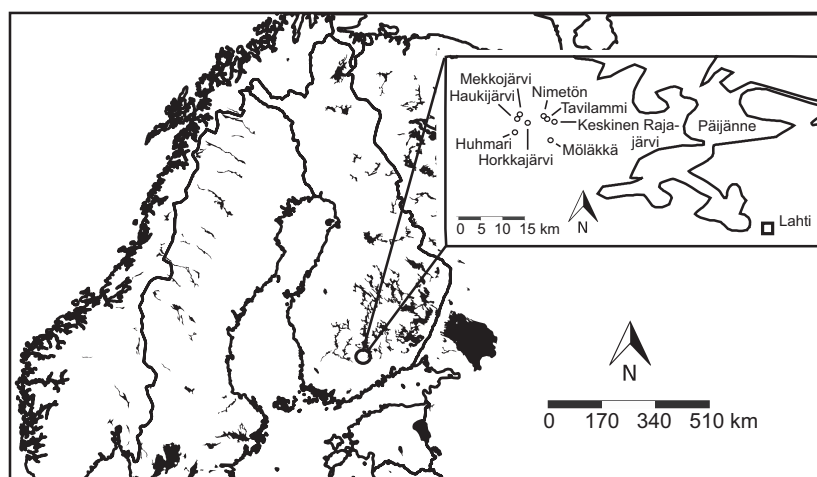


FIGURE 1     Location of the eight study lakes in the Evo forest area in southern Finland (map derived following Shorthouse 2010).

TABLE 1    Some characteristics of the eight study lakes in 2015. All the presented chemical parameters except chl *a* were provided by Lammi Biological Station. SA = Surface area, CA = Catchment area, P:L ratio = Pelagic:Littoral ratio by area, SW = mean ± SE substratum dry-weight per m lake shore (g), [*] = sampled on 3.11.2015, [**] = sampled on 27.-28.11.2014, [***] = sampled from epilimnion.

| Lake | Mek-ko-järvi | Hork-ka-järvi | Huh-mari | Mö-läkkä | Nime-tön | Tavi-lammi | Keski-nen Ra-jajärvi | Hau-ki-järvi |
|---|---|---|---|---|---|---|---|---|
| Sampling date | 8.7. | 9.7. | 13.7. | 14.7. | 15.7. | 16.7. | 21.7. | 22.7. |
| SA (ha) | 0.4 | 1.1 | 1.1 | 0.7 | 0.4 | 0.8 | 1.5 | 2.3 |
| CA (ha) | 24.8 | 62.1 | 102.0 | 4.4 | 32.3 | 18.1 | 109.9 | 661.3 |
| Max. depth (m) | 4.3 | 12.0 | 8.0 | 15.0 | 11.0 | 7.0 | 12.0 | 8.5 |
| Mean depth (m) | 2.0 | 7.7 | 4.2 | 6.0 | 8.6 | 5.2 | 6.6 | 3.8 |
| P:L ratio | 3.0 | 19.8 | 5.5 | 5.4 | 2.9 | 8.9 | 4.0 | 8.4 |
| pH[1] | 5.9 | 5.9 | 6.4 | 4.6 | 4.6 | 4.6 | n/a | 6.5 |
| Colour (mg Pt $l^{-1}$)[*] | 429.0 | 402.0 | 34.0 | 233.0 | 449.0 | 199.0 | n/a | 216.0 |
| Tot-N ($\mu$g $l^{-1}$)[*] | 635.0 | 745.0 | 265.0 | 593.0 | 758.0 | 680.0 | n/a | 524.0 |
| Tot-P ($\mu$g $l^{-1}$)[*] | 16.0 | 21.0 | 8.0 | 23.0 | 18.0 | 15.0 | n/a | 13.0 |
| DOC (mg $l^{-1}$)[**] | 24.0 | 27.0 | 6.0 | 17.0 | 30.0 | 14.0 | n/a | 22.0 |
| Chl *a* (mg $m^{-3}$)[***] | 3.0 | 4.9 | 6.6 | 5.4 | 1.5 | 10.7 | 15.1 | 7.1 |
| SW | 42.6 ± 3.4 | 11.0 ± 1.8 | 22.1 ± 5.1 | 53.1 ± 9.8 | 19.2 ± 2.9 | 11.4 ± 1.7 | 38.9 ± 8.6 | 45.4 ± 8.7 |

## 2.2   Sampling procedures

### 2.2.1   Physical and chemical parameters (I, II, III, IV)

Light attenuation was measured from Mekkojärvi during eight sampling occasions in 2012 (I) using a single planar LI-COR® (Li-192) Underwater Quantum light sensor (LI-COR Inc., Lincoln, Nebraska, USA). Light was measured at 0.5 m intervals from the surface to the bottom of the lake. In 2015, light was measured similarly from the eight study lakes (IV). Temperature and $O_2$ concentrations were measured with YSI 55 probe (YSI Inc., Yellow Springs,

Ohio, USA) at 0.5 m intervals from the surface to the bottom from Mekkojärvi during every sampling occasion in 2012 (I) and 2015 (III) and once from the other seven study lakes in summer 2015 (IV). Temperature and $O_2$ were also measured with the YSI 55 at six randomly selected sites from the littoral areas in each of the eight study lakes concurrently with pelagic measurements (IV) and also five times during the open water period from the littoral surface water in Mekkojärvi in 2015 (III). Two miniDO$_2$T data loggers (PME Inc., Vista, California, USA) were used to record pelagic and littoral $O_2$ concentrations and temperatures in Mekkojärvi during July in 2015 (IV). One logger was placed just above the littoral moss mat under the water surface and the other was in the pelagic ca. 1 m from the edge of the littoral moss mat. Daily irradiance values (measured with a Kipp and Zonen B.V., model CM11; Delft, Netherlands) and day lengths were obtained from the Finnish Meteorological Institute Observatory in Jokioinen, ca. 110 km west from the Evo study area (I, IV). The area of littoral vegetation cover in each study lake was calculated from a satellite image using an area calculation tool (in Retkikartta.fi, © Metsähallitus) and was used for estimating P:L ratios by area for each lake (I, IV).

Nutrients, particulate organic matter (POM) and DOC samples were taken from Mekkojärvi during every sampling occasion in 2012 and analyzed by staff at Lammi Biological Station (I). POM samples were taken 3–4 times from Mekkojärvi in June–August 2014 after the isotope additions (see 2.2.3). 500 ml of lake water was filtered through pre-ignited Whatman GF/F glass fibre filters (nominal pore-size 0.7 µm) and then dried. Retained material was scraped into Sn capsules for stable isotope analysis (see 2.2.3).

## 2.2.2 Pelagic primary production, community respiration and phytoplankton biomass (I, IV)

Pelagic PP was measured from Mekkojärvi eight times during the 2012 open water period between the end of May and the end of October (I) and from the eight study lakes (Table 1) during July 2015 (IV) using the $^{14}C$ incorporation method described by Schindler *et al.* (1972). Composite water samples were taken with a Limnos acrylic tube sampler (Limnos Ltd., Turku, Finland) by combining three pulls from four strata corresponding to surface, epi-, meta- and hypolimnion in each lake, which were determined according to $O_2$ measurements. Subsamples were pipetted into 20 ml glass vials containing NaH$^{14}CO_3$ with four replicates (one dark sample in each depth). Subsamples were then incubated *in situ* in each stratum for 24 h and then killed with glutaraldehyde. $^{14}C$ activity was measured with a Packard Tri-Carb® liquid scintillation counter (PerkinElmer, Waltham, Massachusetts, USA), and these 24 h determinations were assumed to approximate to net primary production (NPP). Volumetric values were converted to areal basis by multiplying the values from each stratum by the total depth of the stratum and then summing over the mean depth. Whole-lake daily PP estimates were calculated by multiplying the daily areal values by the lake surface area.

Pelagic CR was measured eight times from Mekkojärvi in 2012 (I) and once from the eight study lakes during July 2015 (IV) concurrently with PP. CR was determined as the increase in dissolved inorganic carbon (DIC) concentration during dark *in situ* incubations. Subsamples (two initials and two dark samples) were taken from the composite water samples from each stratum (surface, epi-, meta- and hypolimnion) into 50 ml BOD bottles. Half of the bottles were wrapped in aluminium foil, put into light-impermeable tubes (filled with water) and incubated *in situ* in each stratum for 24 h. The other half ($t_{0h}$) were put into ice in the dark and taken immediately to the laboratory, where DIC was measured with a DIC-analyser (Salonen 1981). The same was done for the $t_{24h}$ sample the next day. Areal values and whole-lake estimates were calculated similarly as for PP. Pelagic NPP was converted to GPP as the sum of NPP and CR values over the photic zone assuming that most of the CR was attributable to primary producers (I, IV).

Phytoplankton biomass in Mekkojärvi in 2012 (I) was measured 10 times between May and October as chl *a* concentration in the composite water samples from epi-, meta- and hypolimnion. Samples were filtered onto Whatman GF/C glass fibre filters (nominal pore size 1.2 μm) and chl *a* was measured by UV-spectrophotometry (Shimadzu UV-1800; Shimadzu Corporation, Kyoto, Japan) according to a Finnish standard SFS 5772 (Finnish Standards Associations, Helsinki, Finland). In 2015 (IV), chl *a* samples were taken from 0.2–0.5 m (representing the epilimnion) in each of the eight study lakes (Table 1) during the other sampling.

### 2.2.3 Littoral periphyton primary production, community respiration and periphyton biomass (I, IV)

PP in the littoral zone in Mekkojärvi was measured concurrently with pelagic PP in 2012 (I) and 2015 (IV) from *in situ* incubations of periphyton in both dark and light 20 ml glass vials where the consumption or production of DIC over time indicates either respiration or production. Submerged sedges instead of highly complex mosses were routinely sampled since they were metabolically inert (tested in 2012) and easier to process. Before the study in 2012, periphyton PP on moss and on sedges was compared in Mekkojärvi and no statistically significant difference was found. In 2012 (I), Mekkojärvi was sampled from eight sites around the shoreline by clipping random sedge pieces into 20 ml glass vials (four replicate samples per site) containing surrounding littoral lake water. Trial incubations of the surrounding littoral water without any periphyton or substratum did not reveal any substantive changes in DIC over time. In 2015 (IV), the samples were collected from six random sites around each study lake. Samples of surrounding littoral lake water were taken from each sampling site to obtain initial DIC concentration. Samples containing periphyton were incubated *in situ* for 2 h at noon, half of the samples in darkness and the other half in the surface water under the same light conditions as at the depth from which the periphyton originated. After that the samples were put into ice and dark, and taken immediately to the laboratory, where DIC

was measured with a DIC-analyser (Salonen 1981). Periphyton was then entirely removed from the collected substratum by scraping with a spatula and filtered onto Whatman GF/F glass fibre filters. In 2015 (IV), this was done for six randomly selected samples from each lake. Periphyton biomass was measured as chl *a* as described for phytoplankton, and the values were normalized to chl *a* per g dry weight (DW) of substratum. Substrata were dried at 60 ºC for 24 h and the dry mass was recorded.

Periphyton GPP was calculated from the difference in DIC between dark and light samples (I, IV) and CR from the difference between the dark and initial samples (I, IV). NPP was then calculated as NPP = GPP − CR, assuming that most of the CR was attributable to primary producers. Values for each sample were normalized to mg C (g DW substratum)$^{-1}$. Daily estimates for periphyton PP were calculated using a slightly modified version of the equation described by Vadeboncoeur *et al.* (2008) as follows:

$$\text{Periphyton PP d}^{-1} = \Delta t \sum_{\text{sunrise}}^{\text{sunset}}[\text{PP}_{\text{max}} \quad \tanh(I_{zt}/I_k)]$$

where $\Delta t$ is the time increment (h), $\text{PP}_{\text{max}}$ is the light-saturated primary production right under the water surface at noon (mg C g DW substratum$^{-1}$ h$^{-1}$), tanh is the hyperbolic tangent function, $I_{zt}$ is the light at depth z (µmol m$^{-2}$ s$^{-1}$) and time t (h) and $I_k$ is the light intensity for onset of saturation, which was set to 300 µmol m$^{-2}$ s$^{-1}$ according to Hill (1996). The irradiance data and day lengths were obtained from the Finnish Meteorological Institute Observatory in Jokioinen. To define $I_z$, the photosynthetically active radiation (PAR) on the surface was multiplied by the fraction of light at the incubation depth in each lake.

The availability of substrata along the littoral zone of each lake was quantified in order to estimate whole-lake littoral PP by periphyton. In Mekkojärvi in 2012 (I), littoral substratum along 40 cm of lakeshore was entirely removed from 24 sites along the lake and dried at 60 ºC for 48 h. The average DW of littoral substratum per metre of lake shore was calculated and, as the substratum consists mainly of flat and horizontally aggregated plant leaves, the weight was divided by two in order to conservatively estimate the mass of the illuminated side of the substratum. In 2015 (IV), 50 cm of littoral substratum was removed from six sites around each study lake and processed as described for 2012 samples. PP per m of lake shore was calculated, and those values were multiplied by the total shoreline lengths to estimate the whole-lake littoral PP by periphyton.

### 2.2.4 Isotope additions and sampling of periphyton and invertebrates (II)

To investigate the possible consumption of periphyton in Mekkojärvi, $^{15}$N-enriched ammonium nitrate ($^{15}$NH$_4$$^{15}$NO$_3$) was added evenly around the littoral vegetation during summer 2014 in order to give the periphyton a $^{15}$N label distinct from other basal resources (phytoplankton and allochthonous organic matter) (II). Periphyton was sampled during 2011–2013 open water seasons to

determine the natural abundance (no isotope additions) $\delta^{15}N$ values. In 2014, periphyton was sampled twice before the isotope additions to determine the initial $\delta^{15}N$ values. Later during the summer, 200 to 250 mg of $^{15}NH_4{}^{15}NO_3$ (diluted into water) was spread evenly around the lake littoral zone with a watering can from a rowboat four times between 24 June and 1 September. Periphyton was sampled before each isotope addition and few days later to measure the increase in $\delta^{15}N$, and then ca. 1 week later to measure the decrease of the label. Eight subsamples of littoral vegetation were randomly selected around the lake and associated periphyton was scraped into cryogenic vials and taken into the laboratory, where they were cleaned of any remaining plant pieces and then frozen and later freeze-dried with a Christ Alpha 1-4 LD plus (Martin Christ, Osterode, Germany). Littoral invertebrates were sampled several times during the study period using a hand net (mesh size 500 μm) from a rowboat by rowing around the lake shoreline and placing all the hand-net hauls into plastic buckets. In the laboratory all the littoral macroinvertebrate and zooplankton taxa that were sufficiently abundant for SIA were picked out. Pelagic zooplankton was sampled several times during the study period with a standard plankton net (mesh size 50 μm; Limnos, Komorów, Poland) either by vertical hauls or by rowing around the pelagic area and pulling the plankton net behind the boat. All the invertebrates were identified to family or genus level and oven-dried at 60 ºC for 48 h. Perch that were introduced into the one basin were recaptured with traps during August–October, and a small piece of dorsal muscle from each fish was removed and freeze-dried.

### 2.2.5  Bacterial production in the pelagic and littoral zone (III)

Pelagic BP was measured five times from Mekkojärvi during the open water period between June and October in 2015 (III) using a slightly modified version of the [$^{14}C$]-leucine incorporation method (Kirchman *et al.* 1985) described by Tulonen (1993). Composite water samples from three strata (epi-, meta- and hypolimnion, depending on the prevailing stratification in the lake) were collected with a Limnos sampler (Limnos Ltd., Turku, Finland) combining three pulls, and triplicate subsamples of 5 ml were pipetted into 20 ml pre-ignited glass vials containing 30 nM of [$^{14}C$]-leucine and incubated for 60 min *in situ* in the stratum from which they originated. Glutaraldehyde-killed controls were run in parallel. After the incubations, all the samples were killed, taken to the laboratory and processed according to Tulonen (1993). Leucine incorporation rates were converted to biovolume and to carbon production according to Tulonen (1993). Daily BP rates were calculated by multiplying the hourly rates by 24. Volumetric values were converted to an areal basis by multiplying by the fraction of the depth of each stratum and summing over the mean depth. These were multiplied by the surface area of the lake to derive whole-lake estimates for pelagic BP.

Littoral BP was measured from the epiphytic biofilms in Mekkojärvi concurrently with the pelagic measurements using a slightly modified version of the [$^3H$]-leucine incorporation method described by Ask *et al.* (2009) which is

based on the methods originally developed by Smith and Azam (1992). Littoral vegetation was collected from six randomly selected sites around the littoral zone in Mekkojärvi into plastic buckets filled with lake water from each site. Samples were taken immediately to the laboratory, where six randomly selected 1 cm long subsamples of plant substratum were clipped and put into Eppendorf tubes containing 30 µl of [$^3$H]-leucine and 70 µl of distilled water with the final concentration of 300 nM. That was the concentration determined from a saturation experiment of the maximum leucine incorporation into protein in epiphytic biofilms, which was conducted in spring before the actual BP measurements. Samples were incubated for 60 min outside in a cool box containing lake water, submerged to the same depth from which they originated. The water temperature during the incubation was recorded and no marked change was found. The incubation was then terminated with trichloroacetic acid (TCA) and the samples were processed according to the leucine method described by Smith and Azam (1992). The mean DW of the substratum was calculated and BP was normalized to mg C (g DW substratum)$^{-1}$ h$^{-1}$. Daily rates were calculated by multiplying the hourly rates by 24. Whole-lake BP estimates were calculated as described for the littoral PP by first calculating the BP per m lake shore and then multiplying that by the total shoreline length of the lake.

## 2.3   Data analyses

### 2.3.1   Stable isotope analysis (II)

Each freeze-dried sample for stable isotope analysis (SIA) was ground to a fine powder with a mortar and pestle, and ca. 0.6 mg (invertebrates and fish) or ca. 1.5 mg (periphyton and POM) was accurately weighted into a tin capsule. Sample C and N isotopic composition was analysed at the University of Jyväskylä with a FlashEA 1112 elemental analyser (Thermo Electron Corporation, Waltham, Massachusetts, USA) coupled to a Thermo Finnigan DELTAplus Advantage mass spectrometer (Thermo Electron Corporation). Pulverized pike muscle tissue for macroinvertebrates and fish and pulverized potato leaves for periphyton and POM were used as internal laboratory working standards. Replicate standards were run repeatedly in every analysis (standard deviation for both $\delta^{13}$C and $\delta^{15}$N was always < 0.2 ‰) to ensure accurate analysis and correction for possible drift and linearity.

The relative reliance of invertebrates on periphyton, allochthonous OM and on methane-oxidizing bacteria (MOB) in Mekkojärvi was estimated using the Bayesian mixing model SIAR (Parnell *et al.* 2010). The model was run for combined nonpredatory taxa (herbivorous or detritivorous taxa [herbi-detritivores]), since these are the primary consumers of the basal resources. Models were compared for the invertebrate isotope data from years with (2014) and without (2013) isotope additions to the littoral zone. Isotope values for

allochthonous OM were derived from POM, which corresponds to the values of allochthonous DOC in Mekkojärvi (Taipale *et al.* 2007). For MOB, the isotope values published by Taipale *et al.* (2008) were used. Trophic fractionation factors used were those recommended by Vander Zanden and Rasmussen (2001). Before the SIAR model was run, model uncertainty for both 2013 and 2014 data was evaluated with Monte Carlo simulations for mixing polygons defined by the putative food sources (Smith *et al.* 2013) to resolve whether the consumer values lay within the 95 % mixing regions. Both SIAR mixing model and Monte Carlo simulations of mixing polygons were run in R (version 3.2.3; R Project for Statistical Computing, Vienna, Austria).

### 2.3.2   Statistical analyses (I, II, III, IV)

All the statistical tests in this thesis (Table 2) were conducted with IBM SPSS Statistics (version 20.0.0.2; IBM, Armonk, New York, USA). All the descriptive statistics are means ± SE, if not expressly noted, except the isotope values (II), which are means ± SD.

TABLE 2      Statistical analyses used in this thesis.

| Analysis | Description |
| --- | --- |
| Durbin-Watson statistics | II |
| Levene's test | I, III, IV |
| Linear regression analysis | III, IV |
| One-way analysis of variance (ANOVA) | II |
| Repeated measures of analysis of variance (RMA) | I, III |
| *t*-test | I, III |
| Welch's *t*-test | II, IV |

# 3   RESULTS AND DISCUSSION

## 3.1   The littoral as an integral part of the function of highly humic Lake Mekkojärvi (I, II, III)

### 3.1.1   Littoral and pelagic primary production and whole-lake metabolism

Primary production measurements in both littoral and pelagic zones in Mekkojärvi revealed an overwhelming littoral dominance of whole-lake autotrophic production, with the mean littoral contribution to whole-lake NPP over 80 % throughout the study period in 2012 (I Fig. 4). Pelagic NPP followed the pattern reported from previous studies in Mekkojärvi (Salonen *et al.* 2005, Taipale *et al.* 2007): a clear spring maximum of $331 \pm 274$ mg C m$^{-2}$ d$^{-1}$ followed by a rapid decrease towards the autumn with the mean rates under 100 mg C m$^{-2}$ d$^{-1}$. Pelagic CR exhibited rates over one order of magnitude higher than those for NPP (highest rates of $3210 \pm 1655$ mg C m$^{-2}$ d$^{-1}$ observed in June) indicating intense respiration of allochthonous OC and heterotrophy in the pelagic. Indeed, the calculated mean GPP:CR ratio in the pelagic was $0.4 \pm 0.1$ ($n = 5$) indicating strong net heterotrophy. In contrast, the well-developed and highly productive periphyton mats led to net autotrophy in the littoral with a mean GPP:CR ratio of $2.2 \pm 0.4$ ($n = 8$), significantly higher than in the pelagic (*t*-test, $t_{11} = 3.19$, $P < 0.01$). The mean daily periphyton NPP in the littoral throughout the study period was $364 \pm 66$ mg C (g DW substratum)$^{-1}$ d$^{-1}$, while the mean littoral CR throughout the period was $395 \pm 65$ mg C (g DW substratum)$^{-1}$ d$^{-1}$. Periphyton NPP was rather constant throughout the summer until it decreased rapidly from late-August to the end of September, and RMA revealed significant change over time ($F_{7,98} = 6.94$, $P < 0.01$), but no significant interactions between the time and the two basins ($F_{1,14} = 3.98$, $P = 0.066$). Thus, the ongoing experiment with lake division and fish introduction did not significantly affect periphyton PP. Periphyton CR showed greater fluctuation throughout the study period, but the differences were not statistically significant (RMA, $F_{2.88,40.36} = 1.53$, $P = 0.222$).

Pelagic chl *a* concentrations were rather constant in the photic epilimnion ($8.0 \pm 1.0$ mg m$^{-3}$) although substantially lower than in the meta- and hypolimnion ($27.2 \pm 6.2$ mg m$^{-3}$, $55.4 \pm 4.4$ mg m$^{-3}$, respectively) throughout the study period. It is common in these steeply stratified humic lakes to find a chl *a* maximum in deeper anoxic water layers, as motile components of the phytoplankton undertake diurnal vertical migrations (DVM) to avoid predation by zooplankton and also to take up inorganic nutrients from the meta- and hypolimnion (e.g. Jones 1991, Arvola *et al.* 1991, 1992). However, as the chl *a* samples were taken during the day when phytoplankton is expected to migrate to the photic zone, it is unlikely that DVM explains the high hypolimnetic chl *a* concentrations in this case. The high chl *a* concentrations in the meta- and hypolimnion also presumably contains appreciable quantities of bacteriochlorophyll (BChl), which is abundant in Mekkojärvi and has been shown to overlap with chl *a* in spectrophotometric determination (Karhunen *et al.* 2013). Periphyton chl *a* was constant throughout the summer, but decreased significantly in September (RMA, $F_{2.52,35.36} = 5.70$, $P < 0.01$), with a mean value of $0.7 \pm 0.1$ mg (g DW substratum)$^{-1}$ over the whole sampling period. There was also no effect of fish treatment on periphyton chl *a* (RMA, $F_{2.52,35.46} = 1.19$, $P = 0.323$).

Combined GPP:CR ratios, including both pelagic and littoral zones, revealed overall net autotrophy in Mekkojärvi with a mean value of $1.6 \pm 0.5$ (*n* = 5). This demonstrates that the remarkably productive periphyton on aquatic vegetation in the littoral can have a significant effect on whole-lake metabolism in highly humic lakes. The littoral PP can dominate whole-lake PP and even occasionally shift these lakes to net autotrophy. However, the respiration of allochthonous OC proceeds throughout the year, while photosynthesis is restricted mainly to summer months, so these lakes are still likely to be net heterotrophic on an annual basis. Nevertheless, these findings challenge the current theoretical framework regarding the whole-lake metabolism in unproductive humic lakes during the open-water period, as they are considered strongly net heterotrophic due to high allochtonous DOC, high colour and low phytoplankton PP (Del Giorgio *et al.* 1997, Cole *et al.* 2000). This concept is, however, often based on pelagic measurements alone. Although many factors that regulate the littoral contribution to whole-lake PP would appear to seriously constrain the potential significance of periphyton (Vadeboncoeur *et al.* 2008), an important variable, surrounding aquatic vegetation, has been previously disregarded. Aquatic vegetation is highly variable among lakes and its extent is challenging to measure. Satellite imagery used in this study appeared to be a reasonably useful tool. The estimated P:L ratio for Mekkojärvi was 3.0, which demonstrates that littoral vegetation covers a relatively large fraction of the lake surface area. As this vegetation is mainly a floating moss bed, it provides extensive substrata for colonization by periphyton under well-lit conditions, where light, which is a major limiter for overall productivity in humic lakes, is no longer so strongly limiting.

### 3.1.2 Littoral periphyton as a food resource for consumers

The additions of $^{15}NH_4^{15}NO_3$ to Mekkojärvi during summer 2014 (II) increased the $\delta^{15}N$ values of periphyton and most littoral invertebrates well above the natural abundance levels, although the variation was high and the decrease was rather fast after every addition. Periphyton $\delta^{15}N$ values before the isotope additions varied between $-0.3 \pm 1.0$ and $0.9 \pm 1.9$ ‰, whereas the mean value over the whole sampling period including four isotope additions was $32.7 \pm 47.0$ ‰. The increase in $\delta^{15}N$ of periphyton was consistent across the basin with fish (Fish+) and the basin without fish (Fish−). RMA revealed a significant change over time ($F_{11,66} = 8.35$, $P < 0.01$), which was not affected by the basin treatment ($F_{11,66} = 0.83$, $P = 0.46$). Hand net and plankton net samples revealed 20 repeatedly occurring invertebrate taxa from Mekkojärvi. The pelagic was dominated by the cladoceran *D. longispina*, while the littoral appeared to be dominated by predatory taxa, with high proportions of anisopterans and cyclopoids, as reported in previous studies (e.g. Kairesalo *et al.* 1992, Zúñiga 2011).

The added $\delta^{15}N$ label transferred effectively to the littoral consumers, indicating their reliance on periphyton as a food source. The highest $\delta^{15}N$ values of macroinvertebrates were observed rather late in September–October, which was particularly evident for predators, reflecting slower accumulation of the isotope label into predators than into herbi-detritivores. The mean $\delta^{15}N$ of herbi-detritivores over the study period was $20.5 \pm 16.0$ in the Fish+ and $13.0 \pm 8.0$ ‰ in the Fish− basin, and the respective values for predators were $20.3 \pm 17.9$ and $14.0 \pm 14.2$ ‰. The increase in $\delta^{15}N$ above the initial values was statistically significant for both predators (Welch's *t*-test, $t_{1,112.4} = 58.1$, $P < 0.01$) and herbi-detritivores (Welch's *t*-test, $t_{1,110.8} = 73.3$, $P < 0.01$).

The $\delta^{15}N$ value of *Daphnia* in the pelagic was ca. 3 ‰ before the isotope additions. Within one month of the introduction of perch to the Fish+ basin, *Daphnia* had declined to such low abundance that it was impossible to obtain enough material for SIA. Meanwhile the *Daphnia* biomass in the Fish− remained high. The $\delta^{15}N$ of *Daphnia* in Fish− remained mostly < 10 ‰, indicating no marked labelling. In contrast, a much higher value of 23.6 ‰ for *Daphnia* was recorded on 17 July from Fish+, ca. two weeks after the fish were introduced to the basin, which then decreased to 11.8 ‰ in the last sample from Fish+. That last sample consisted mainly of very small individuals, likely from the next generation with lower $\delta^{15}N$ values. Perch did not show markedly increased $\delta^{15}N$ values. Of the 33 recaptured perch only one individual with a value of 23.2 ‰ had clearly utilized labelled food resources. It is likely that the exceptional increase in $\delta^{15}N$ of *Daphnia* in the Fish+ basin was caused by the introduced perch which forced the daphnids to relocate towards the littoral to seek refuge from predation, where they were locally exposed to $^{15}N$-enriched food sources. Such behaviourally induced horizontal migration due to predation pressure has been reported for *Daphnia* populations and can occur from littoral to pelagic and vice versa (Van de Meutter *et al.* 2005, Meerhoff *et al.*

2007). The one strongly labelled perch also indicates some possible food web coupling by fish between the pelagic and the littoral. In turn, it implies an effective capacity of the moss bed to shelter invertebrates from fish predation in humic lakes, as only one individual of 33 recaptured perch was markedly labelled. These results indicate that the pelagic and littoral in Mekkojärvi are not strongly coupled via zooplankton under the natural state lacking zooplanktivorous fish, but that introduction of such fish can promote the coupling. This is also supported by the data from 2013 when $^{15}NH_4^{15}NO_3$ additions were made only to the pelagic and no increase was seen in the $\delta^{15}N$ values of periphyton and littoral invertebrates.

An isotope biplot for $\delta^{13}C$ and $\delta^{15}N$ from 2014 demonstrated considerable separation of food-web components (II Fig. 6). *Daphnia* and the rotifer *Polyarthra* had clearly the lowest $\delta^{13}C$ values, while most other invertebrate taxa, except ephmeropterans and chironomids, had $\delta^{13}C$ values closer to the potential food sources of periphyton and POM. Most invertebrates were rather $^{13}C$-depleted, which is common in lakes with high DOC and respired inorganic C (Premke *et al.* 2010). However, as chironomids had the lowest values of the littoral invertebrates and are well-documented utilizers of MOB, it appears likely that MOB contributes to their diets. Taipale *et al.* (2008) derived a very low estimate for the $\delta^{13}C$ of MOB in Mekkojärvi (−69.8 ± 10.0‰) and demonstrated how pelagic *Daphnia* fed extensively on MOB in the lake, which can account for the highly $^{13}C$-depleted *Daphnia* values found in this study. It is also likely that MOB contributes strongly to the diet of the rotifer *Polyarthra*.

The SIAR mixing model was run for both 2013 (without isotope additions to the littoral) and 2014 (isotopes added to the littoral) herbi-detritivore data (II fig. 7). When the model uncertainty was evaluated with mixing polygons, the model appeared to fit the 2014 data better than the 2013 data, as all the consumer values in 2014 lay within the 95 % mixing region. In contrast, several values in 2013 data fell outside this region. The model output for 2014 data was reasonable, and highlighted the importance of periphyton as a food source for *Asellus*, Trichoptera and Ephemeroptera, all of which are well-known grazers of periphyton. The SIAR model also indicated higher proportions of allochthonous OM in the diets of Corixidae, which tend to feed on terrestrial plant material from the water surface. For chironomids, the model gave higher proportions of MOB and allochthonous OM. However, these results should be considered only indicative due to the rapid turnover of $\delta^{15}N$ in periphyton and the rather slow accumulation of $^{15}N$ label into consumer tissue, creating uncertainties in distinguishing $\delta^{15}N$ values of allochthonous OM and periphyton. Also, older unlabelled autochthonous detrital periphyton, which can significantly support invertebrate production in lakes (Solomon *et al.* 2008), may have contributed to the invertebrate diets in Mekkojärvi with isotope values close to that of allochthonous OM. Thus, the SIAR mixing model may have overestimated the proportion of allochthonous OM in the invertebrate diets.

Overall this study demonstrates that highly productive periphyton in the littoral in Mekkojärvi (I) is an important food resource for the littoral consumers.

The results indicate that the pelagic and littoral food-webs in humic lakes lacking zooplanktivorous fish are not strongly coupled, but fish as ecosystem integrators may change that and promote coupling of the two habitats.

### 3.1.3   Littoral and pelagic bacterial production in Mekkojärvi

The comparison of pelagic and littoral epiphytic BP (III) revealed the overall dominance of pelagic BP during the sampling period in 2015, although epiphytic BP made an appreciable (24 %) contribution to the combined BP (III Fig. 6). Pelagic BP was highest in early summer with a value of 34.9 mg C m$^{-2}$ d$^{-1}$ and then decreased steadily towards the autumn with the mean value of 11.6 ± 2.0 mg C m$^{-2}$ d$^{-1}$ over the sampling period. There was a statistically significant change in the pelagic BP over time (RMA, $F_{4,24}$ = 6.0, $P$ < 0.01). Epi- and metalimnion constituted the largest fraction of the pelagic BP during the early-summer maximum and hypolimnion the largest fraction in late-summer and autumn. A phytoplankton PP maximum in early summer was also seen in 2012 (I), as in earlier study in Mekkojärvi (Salonen *et al.* 2005), so phytoplankton PP and pelagic BP are presumably interconnected and likely reflect a response by both groups to a pulse of nutrients from the catchment with snow-melt in spring. Alternatively, or in addition, BP may be stimulated by labile autochthonous OM released during the phytoplankton PP maximum. Such positive relationship between bacterio-, and phytoplankton is well-documented (e.g. Cole *et al.* 1988, White *et al.* 1991, Kritzberg *et al.* 2005). Littoral epiphytic BP was highest in summer (June, July) and decreased towards the autumn (August, September, October), and this change was statistically significant (RMA, $F_{4,68}$ = 17.6, $P$ < 0.01). The mean daily epiphytic BP was 1.52 ± 1.36 mg C (g DW substratum)$^{-1}$ d$^{-1}$. Temperature has often been considered the major factor affecting seasonal variation in BP rates (White *et al.* 1991) and positive correlations between BP and temperature have been found particularly at temperatures < 12 ºC (Wigner and Hagström 1991, Tulonen *et al.* 1994). There was a statistically significant positive relationship between the littoral epiphytic BP and the littoral surface temperatures (exponential regression, $F_{1,3}$ =21.7, R$^2$ = 0.878, $P$ < 0.05). A positive relationship between the epilimnetic BP and the pelagic surface temperature was apparent, but a very high value in early-summer, presumably related to the above-mentioned relationships with phytoplankton and nutrients, prevented a statistically significant correlation (exponential regression, $F_{1,3}$ = 6.320, R$^2$ = 0.678, $P$ = 0.087).

Whole-lake estimates revealed that the pelagic dominated the combined BP with a mean proportion of 76 % over the sampling period (III fig. 6). The littoral made its highest contribution of 45 % in July. These results show that a large fraction of BP will be overlooked in Mekkojärvi if the littoral epiphytic bacteria were not taken into account. Although this study did not account for sediment bacteria, bacteria in the surrounding littoral water, or bacteria requiring dark and anoxic conditions (such as green sulphur bacteria, Karhunen *et al.* 2003), and thus was not an actual "whole-lake study", it nevertheless demonstrates a potentially important contribution of epiphytic bacteria to

whole-lake BP in humic lakes. Comparison of the mean epiphytic PP of 364 ± 66 mg C (g DW substratum)$^{-1}$ d$^{-1}$ in Mekkojärvi in 2012 (I) and the mean epiphytic BP of 1.52 ± 1.36 mg C (g DW substratum)$^{-1}$ d$^{-1}$ in this study reveals a strong dominance of autotrophic production in the biofilms in Mekkojärvi, although comparison of PP and BP from different years should be considered only indicative. Anyway, littoral periphyton can clearly provide large pools of autochthonously produced C compounds, which can potentially fuel the BP in the littoral in Mekkojärvi. How much periphytic bacteria use autochthonous or allochthonous C remains uncertain, although it is known that epiphytic biofilms, when forming an isolated microenvironment with an extracellular polysaccharide matrix, can be "hotspots" for BP (Wetzel 1991, Theil-Nielsen and Søndergaard 1999). This positive relationship between epiphytic PP and BP in light-mediated decomposition process has been recently recognized (e.g. Francoeur *et al.* 2006, Danger *et al.* 2013, Kuehn *et al.* 2014). This study does not provide evidence of how much epiphytic BP truly use autochthonous and allochthonous C in Mekkojärvi, which merits future study.

## 3.2 Lake survey: littoral contribution to whole-lake metabolism in highly humic boreal lakes (IV)

The wider generality of the findings of highly productive littoral periphyton in Mekkojärvi (I) was tested in eight humic lakes in Evo (Table 1) during July in 2015 (IV). The study revealed that the littoral periphyton NPP exceeded pelagic NPP in five of the eight lakes, but CR was clearly dominated by the pelagic in every study lake (IV Fig. 6). In Nimetön and Mekkojärvi the littoral contributed over 90 % and in Möläkkä over 80 % to the whole-lake NPP. All these lakes have similar littoral development of a surrounding moss bed and partly submerged sedges lining the lake perimeter. In Horkkajärvi and Tavilammi the pelagic clearly dominated whole-lake NPP with an 80 % contribution, and these two lakes had the least surrounding aquatic vegetation and the highest P:L ratios (Table 1). On average, the littoral contributed 58 % to the whole-lake NPP in the study lakes.

Pelagic PP was rather low in every study lake with a mean value of 55.6 ± 8.5 mg C m$^{-3}$ d$^{-1}$. No statistically significant differences were found between the lakes (Welch's *t*-test, $t_{7,20.8}$ = 2.2, $P$ = 0.079). The mean areal NPP was 50.5 ± 7.6 mg C m$^{-2}$ d$^{-1}$. Instead, pelagic CR was relatively high with a mean value of 363 ± 137 mg C m$^{-3}$ d$^{-1}$, and the clearly highest volumetric rates were observed in Mekkojärvi, which also differed significantly from several other lakes (Welch's *t*-test, $t_{7,22.0}$ = 4.4, $P$ < 0.01). The mean areal CR value in the pelagic was 1375 ± 364 mg C m$^{-2}$ d$^{-1}$. GPP:CR ratios, calculated from the whole-lake estimates, revealed strong net heterotrophy in the pelagic in all the lakes with the mean value of 0.35 ± 0.10 (IV Fig. 7).

The mean littoral NPP by periphyton at noon was 22.9 ± 4.4 mg C (g DW substratum)$^{-1}$ h$^{-1}$, and the highest values were observed in Mekkojärvi and Nimetön and the lowest in Horkkajärvi. There were statistically significant differences between the lakes (Welch's *t*-test, $t_{7,23.2}$ = 9.810, *P* < 0.001). The mean daily littoral NPP was 251 ± 99 mg C (g DW substratum)$^{-1}$ d$^{-1}$ and the highest daily values were also observed in Mekkojärvi and Nimetön. The mean littoral CR at noon was 14.5 ± 2.1 mg C (g DW substratum)$^{-1}$ h$^{-1}$, and some statistically significant differences were observed between the lakes (Welch's *t*-test, $t_{7,33.2}$ = 8.862, *P* < 0.001). GPP:CR ratios revealed net autotrophy in the littoral with a mean value of 1.59 ± 0.17 (IV Fig. 7). When the pelagic and littoral GPP:CR ratios were combined, the mean whole-lake GPP:CR was 0.63 ± 0.13, indicating overall net heterotrophy, although littoral balanced the metabolism to some extent in every lake. Huhmari was the only lake showing net autotrophy with a value of 1.29 and also having the lowest water colour of the study lakes. These results demonstrate that in Mekkojärvi, which is the shallowest of the study lakes, respiration of allochthonous OM in the pelagic takes place in a smaller volume than in the other lakes. Thus, despite very high pelagic respiration rates in Mekkojärvi, the autotrophic littoral can occasionally shift the lake to net autotrophy (I). There is also remarkable temporal variation in the metabolic rates, as the respiration on the day Mekkojärvi was sampled in 2015 clearly exceeded the GPP.

The mean epilimnetic chl *a* in the lakes was 6.8 ± 1.5 mg m$^{-3}$, and there was no significant relationship observed between the chl *a* and NPP (linear regression, $F_{1,6}$ = 0.54, R$^2$ = 0.083, *P* = 0.489). This is presumably for similar reasons as described for Mekkojärvi in 2012 (see 3.1.1). The mean periphyton chl *a* was 0.55 ± 0.11 mg (g DW substratum)$^{-1}$ and there was a significant positive relationship between the periphyton NPP and chl *a* (linear regression, R$^2$ = 0.7451, $F_{1,7}$ = 17.7, *P* < 0.01).

O$_2$ data loggers, which were placed into the littoral and pelagic in Mekkojärvi during July 2015, revealed substantial differences in diel O$_2$ rates. The littoral logger showed substantial variation in O$_2$ from supersaturation at noon to almost zero during night. The pelagic logger, which was only ca. 1 m from the edge of the littoral moss mat, showed a similar diel pattern of O$_2$ saturation, which, however, remained between 80 % at noon and 20 % at night. The O$_2$ measurements, which were made simultaneously with the PP measurements at noon, revealed supersaturation in the littoral in every lake except Horkkajärvi and Haukijärvi. In contrast, O$_2$ saturation in the pelagic remained mostly between 80–90 %, and pelagic O$_2$ supersaturation was only seen in Tavilammi. These O$_2$ measurements give support to the high littoral PP found in most of the study lakes. Logger data in Mekkojärvi indicated substantial and quite small-scale spatial heterogeneity in PP rates in the lake, as also reported by Van de Bogert *et al.* (2007), and demonstrates very low horizontal O$_2$ fluxes in the lake, as the distance between the two loggers was only ca. 1 m.

Whole-lake littoral NPP estimates were plotted against different environmental variables (IV Fig. 8). There was a significant positive relationship between the littoral NPP and P:L ratios by area (inverse linear regression, $F_{1,6}$ = 14.01, $R^2$ = 0.700, $P$ = 0.01, and logarithmic regression, $F_{1,6}$ = 13.10, $R^2$ = 0.686, $P$ = 0.011). Also, a strong negative relationship was observed between the littoral proportion of the whole-lake littoral NPP and P:L ratio (logarithmic regression, $F_{1,6}$ = 18.416, $R^2$ = 0.754, $P$ < 0.01), as well as between the whole-lake NPP and the fraction of the lake surface area covered by macrophytes (linear regression, $F_{1,6}$ = 14.433, $R^2$ = 0.706, $P$ < 0.01). A strong relationship was also observed between the littoral $O_2$ saturation and the littoral NPP, when Tavilammi was excluded (linear regression, $F_{1,5}$ = 34.885, $R^2$ = 0.875, $P$ < 0.001). Periphyton NPP was low in Tavilammi, but the pelagic was supersaturated with $O_2$ as was the littoral, presumably due to high PP by phytoplankton present within the littoral. Altogether, these relationships demonstrate an increase in littoral NPP by periphyton along with increasing relative potential vegetation cover in highly humic lakes. This study shows that the high littoral PP Mekkojärvi is not exceptional; similar production rates can be found from other humic lakes. However, littoral PP varies considerably among the lakes, and this variability appears to be strongly associated with the available plant substrata in these lakes.

## 3.3 Limitations of the study and proposals for future research

There are always uncertainties and limitations involved when upscaling rate estimates made in bottle incubations to the ecosystem level (Pace 2001, Hanson *et al.* 2015). Estimation of PP, BP and CR at the whole-lake scale (I, II, III) for the structurally highly complex littoral environment is particularly challenging, and these estimates undoubtedly have higher uncertainty than whole-lake scale estimates for the more homogeneous pelagic environment, although there can also be high spatial heterogeneity in the pelagic (Van de Bogert *et al.* 2007). However, as the study lakes are all small and have rather uniform basin morphometry, whole-lake extrapolations are likely to yield better constrained estimates than for larger lakes with highly variable morphometry. The estimation of error for littoral whole-lake estimates is difficult due to the spatial heterogeneity of both the periphyton and the available substratum (vegetation) along the lake. In Mekkojärvi, substrata were collected from 12 sites from both basins to quantify the availability of substrata, which in a very small lake with rather evenly distributed littoral vegetation can be considered rather high number of replicates, yielding a comprehensive representation of different vegetation in the sample. In the other study lakes, substrata were collected from only six sites because of the need to keep the amount of work manageable. Although these estimates were likely not as accurate as for Mekkojärvi, the variation was not particularly high (data not shown).

Considering that the littoral NPP was obtained by subtracting CR from GPP, and that the true autotrophic respiration must be less than the CR, littoral NPP values are actually underestimates. In turn, the pelagic GPP values, which were obtained by summing the pelagic NPP and CR in the photic zone, assuming that most of the respiration in the photic zone is attributable to primary producers, are overestimated to some extent. There has also been debate about what is actually measured with the $^{14}$C incorporation technique, GPP, NPP or something between (Marra 2009). This technique has been widely used in the studies of humic lakes and with 24 h incubations has been considered to approximate NPP (Salonen and Holopainen 1979, Keskitalo and Salonen 1993). Hence these associated uncertainties do not alter the essential message of high littoral PP and its potential to contribute strongly to whole-lake metabolism in most of the study lakes.

In this study, littoral PP and BP were normalized to unit of substratum mass rather than to unit of area. The latter is actually the more commonly used method, as PP takes place on a planar substratum. However, in this study the highly complex structure of the moss substratum made such normalization particularly difficult, and therefore mass was used in this study. To estimate the PP on the illuminated side of the substratum, the mass was divided by two. Before the study in 2012 (I), a simple comparison between area- and mass-specific extrapolations was done and the results were within 20 % deviation from each other. Thus using mass rather than area was considered justified.

The green sulphur bacterium *Chlorobium* is abundant in Mekkojärvi. This bacterium is strictly anaerobic and can have an important role in pelagic food webs and lake respiration (Taipale *et al.* 2009, Karhunen *et al.* 2013). BP samples in this study were exposed to $O_2$, which was likely lethal for *Chlorobium* and led to their underrepresentation in the BP measurements. Although the effect of $O_2$ contamination on the BP in the hypolimnion was tested and no significant effect was found (III), it is still reasonable to suppose that, considering their abundance and demand for anaerobic conditions, *Chlorobium* contribution to the measured pelagic BP in this study was not well represented in those tested samples.

The SIAR mixing model was run for the autumn data to mitigate the problem of temporal variation of $\delta^{15}$N, which was high within the data. The limitations of this approach are associated with the use of static mean values to represent a dynamic situation. A more sophisticated method to handle such temporally dynamic isotope data would be to fit an autoregressive model (e.g. Carpenter *et al.* 2005), but since this study lacked adequate sampling frequency of all the end members, such modelling was considered inappropriate. Considering these limitations, the SIAR model outputs should be considered only indicative.

In the light of these limitations of this study, development of more sophisticated methods measuring production and respiration rates as well as the food web dynamics within the pelagic and littoral is clearly desirable. Being a novel study demonstrating the potential of littoral to dominate autotrophic

production in highly humic lakes, this can hopefully lead to future studies addressing these questions and providing more robust evidence for the findings that have emerged from this study. The thesis work encompassed only small lakes, which are all highly coloured, so there is certainly a need for similar approaches to study littoral and pelagic relationships along lake size and light attenuation gradients. The use of P:L ratio as a variable to predict littoral contribution to whole-lake PP in lakes merits exploration. Fatty acid and lipids as biomarkers have been used to study the food web interactions in both marine and freshwater environments (e.g. Dalsgaard *et al.* 2003, Brett *et al.* 2009, Taipale *et al.* 2009) and could be useful tools to provide more insights about periphyton as a food resource and potential pelagic–littoral food web coupling in highly humic lakes. The $^{15}$N-label used in this study presumably labelled the whole biofilm with associated microbes and protozoa. Fatty acid and lipid techniques could potentially better distinguish these different food web components from periphyton. Isotope additions with more frequent and complete sampling of all the possible end members would allow the use of more sophisticated models to study the food web relationships. Since the evidence for possible pelagic–littoral food web coupling via zooplankton was not particularly strong, it certainly merits future study. Also, the question of how much bacteria use allochthonous and autochthonous C in these lakes merits future study. As these small humic lakes are very abundant globally and are important in C sequestration (Cole 2013), it is crucial to understand their whole-lake function and metabolism (Hanson *et al.* 2015). Moreover, predicting how these lakes are likely to change under changing environmental conditions (Solomon *et al.* 2016) is challenging, but if efforts to address this are to be robust they clearly should take littoral zones into account.

# 4 CONCLUSIONS

This thesis has demonstrated the highly important (but previously often overlooked) role of the littoral in the metabolism of highly humic lakes. Historically limnology has had an overwhelmingly pelagic focus, and lakes have usually been classified based on pelagic measurements taken at the deepest point of the lake. While the importance of the littoral to biomass production in oligotrophic, mesotrophic and eutrophic lakes is recognized, its role in dystrophic humic lakes has been minimised based on an assumption of low benthic PP due to very low light penetration into water. This study shows that littoral zones in highly humic lakes lacking illuminated benthic areas should better be viewed and defined as the areas covered by aquatic vegetation, since periphyton can colonize these and be productive under well-lit conditions near the water surface. Also, these littoral vegetation zones can sustain rich aquatic invertebrate communities. A strong positive relationship between the extent of surrounding aquatic vegetation and periphyton PP was found in this study, indicating that the P:L ratio could be a useful variable for modelling littoral contribution to whole-lake PP. Including the P:L ratio in existing models (e.g. Vadeboncoeur *et al.* 2008) would likely yield better estimates.

This study found unexpectedly high littoral PP by periphyton in Mekkojärvi and several other highly humic lakes during summer. The magnitudes of production of new biomass were high, and inclusion of the net autotrophic littoral into whole-lake metabolism shifted some of these lakes to net autotrophy. This is an important finding considering that these lakes are widely considered to be strongly net heterotrophic and net emitters of $CO_2$ to the atmosphere. However, this study also shows how variable whole-lake metabolism can be within and between years. These lakes have high quantities of allochthonous OM which enters the lake food webs via microbial pathways and is respired. As respiration can proceed throughout the year, while PP is mainly restricted to months from spring to mid-autumn, these lakes are presumably still net heterotrophic on an annual basis. Moreover, respiration also proceeds throughout low light conditions, when PP is strongly limited. Lakes in this study were all very small so the surrounding littoral vegetation

covered a relatively large proportion of the lake surface area. Whether the P:L ratio used in this study is as applicable to larger lakes remains to be tested. Larger brown boreal lakes presumably have much higher P:L ratios, although they also tend to be less humic due to relatively smaller allochthonous OM load and faster net sedimentation rate (Eloranta 1999), and thus have more illuminated benthic (littoral) areas. Considering that lake morphometry also affects the distribution of macrophytes in lakes, P:L ratio is controlled by several variables, which should be quantified also in larger humic lakes.

Stable isotope analysis revealed that periphyton can be an important food resource for consumers in highly humic lakes. This supports the recent findings of periphyton contributing strongly to invertebrate diets in other humic lakes (van Duinen *et al.* 2013, Lau *et al.* 2014). As those two studies did not measure PP by periphyton, it is reasonably to suppose, according to this study, that periphyton contributes appreciably to the whole-lake metabolism also in those lakes. Stable isotope analysis also indicates that littoral and pelagic food webs are not strongly coupled via zooplankton in fishless Mekkojärvi, but that introduction of fish may promote such coupling (Fig. 2). According to the data from stable isotope analysis and $O_2$ loggers, it appears that littoral zones with peaty moss beds are rather isolated from the pelagic environment. Small and sheltered humic lakes are not exposed to heavy wind stress, which, together with the high heat absorbing capacity of the coloured water, promotes steep stratification and prevents horizontal fluxes. These boundaries break down during the mixing periods, which are very short in spring but can last for longer in autumn depending on ice formation. The autumnal mixing period is presumably a more opportune time for horizontal fluxes between littoral and pelagic zones. How much there is transportation of materials between littoral and pelagic during that period compared to the stratification period, remains unresolved and speculative. These lakes are mainly fed by surface runoff and can have several inflows, which transport large amounts of allochthonous material to the lakes. Extensive littoral vegetation is potentially a powerful buffer for allochthonous material, which may explain the high production rates in the littoral as well. Part of that material may be synthetized within the littoral and then released to the pelagic (Kairesalo *et al.* 1992). Periphyton appeared to be lost during the autumn in Mekkojärvi and littoral PP and BP showed similar decreasing trends. Presumably only part of that periphyton biomass is being grazed by invertebrates, and most of it is decomposed in the littoral. Potentially part of that old periphyton may washed out to the pelagic zone where it may be mineralised.

Several studies during the last decades have investigated from where the abundant *Daphnia* populations in Mekkojärvi receive their energy. In addition to phytoplankton, heterotrophic bacteria and MOB, which derive their energy from allochthonous sources, are major food resources for the high zooplankton density in the unproductive Mekkojärvi (e.g. Kankaala 1988, Taipale *et al.* 2008). However, this concept is challenged by the fact that allochthonous OM is not particularly nutrient rich and is therefore not supposed to be able to support all

the zooplankton production in many lakes (Brett *et al.* 2009). Although this study indicates, that *Daphnia* do not much utilize littoral food sources in the absence of fish, some increase in $\delta^{15}N$ values was also observed in the fishless basin. Therefore, it is possible that littoral periphyton does make some contribution as a food resource for the high *Daphnia* production in the pelagic in Mekkojärvi. As presence of fish appears to promote the coupling of littoral and pelagic habitats, periphyton may contribute more to the diets of pelagic zooplankton in many other humic lakes where fish are present.
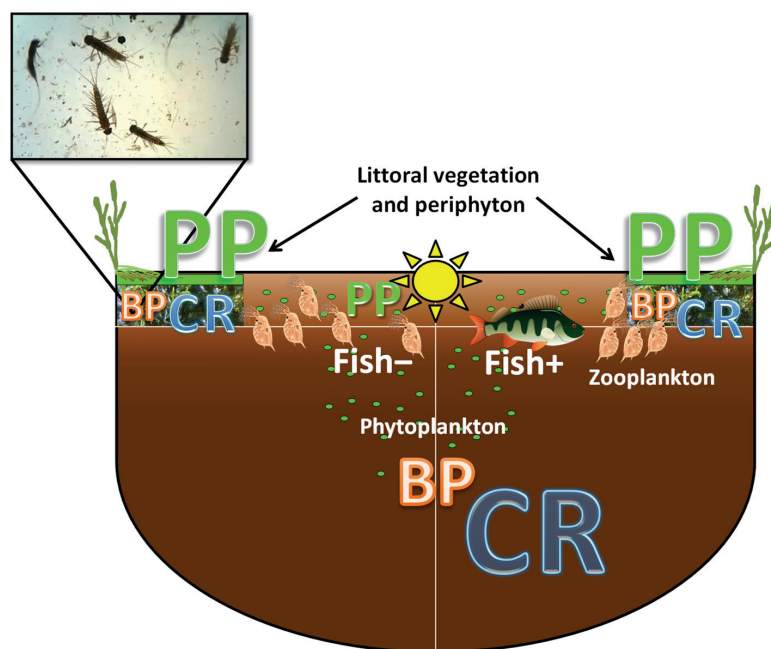


FIGURE 2    Illustration of the main findings of this thesis in the steep-sided, steeply stratified and highly humic Lake Mekkojärvi (and potentially in other similar lakes), which was divided into two basins: with fish (Fish+) and without fish (Fish−). The littoral, with periphyton dominating the whole-lake PP (primary production), contributes strongly to whole-lake metabolism in summer (I, III, IV). In the absence of fish, zooplankton (*Daphnia*) are abundant in the pelagic and do not much utilize littoral food sources. In contrast, invertebrates in the littoral are strongly supported by periphyton. Introduction of perch forces *Daphnia* into the littoral to seek refuge from predation, which promotes the coupling of the pelagic and littoral food webs (II). BP = bacterial production, CR = community respiration. Perch and *Daphnia* drawings by ClipartFest.

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# YHTEENVETO (RÉSUMÉ IN FINNISH)

## Litoraalivyöhykkeen merkitys erittäin humuspitoisten järvien toiminnassa

Litoraalilla eli rantavyöhykkeellä tarkoitetaan sitä osaa järveä, jossa pohjalevät ja makrofyyttiset vesikasvit kykenevät yhteyttämään. Litoraalissa kasvavat vesikasvit ja niiden pintaan sekä järven pohjaan kiinnittyneet levät tekevät litoraalista erittäin monipuolisen ja tuottavan ympäristön. Perifyton (litoraalin pohjalevät) on tärkeä pohjaeläinten ja kalojen ravintoresurssi, ja vesikasvillisuus tarjoaa järven eliöstölle suojaa sekä lisääntymisalueita. Litoraalin laajuus riippuu vahvasti veden kirkkaudesta sekä järvialtaan muodoista. Kirkkaissa ja vähäravinteisissa järvissä se voi kattaa koko järvialtaan, jolloin litoraalin pohjalevät voivat olla vastuussa suurimmasta osasta koko järven perustuotantoa. Erittäin rehevissä järvissä runsas kasviplankton usein varjostaa litoraalin pohjaleviä ja haittaa niiden perustuotantoa. Tummavetisissä humusjärvissä taas valon läpäisykyky on hyvin heikko ja litoraalin perifytonin perustuotanto oletetaan hyvin vähäiseksi, sillä yhteyttämiseen tarvittava valomäärä ei ulotu järven pohjaan asti. Osittain tästä syystä litoraalia on tutkittu erittäin vähän humusjärvissä. Näiden järvien on todettu olevan voimakkaan nettoheterotrofisia, eli järven oma perustuotanto ei kykene ylläpitämään kaikkea toisenvaraista tuotantoa. On osoitettu, että alloktoninen (järven ulkopuolelta kulkeutunut) orgaaninen aines (mm. humus) yhtäältä haittaa järven omaa perustuotantoa estämällä valon tunkeutumista mutta on toisaalta mikrobien käytettävissä energiaksi. Sen vuoksi monet järvet ovat nettoheterotrofisia. Lähes kaikki humusvesiä koskevat tutkimukset käsittelevät ainoastaan pelagiaalia (ulappaa), ja litoraali oletetun vähäisen merkityksensä vuoksi on jätetty huomiotta. Monissa boreaalisten alueiden humuspitoisissa järvissä lähellä pintaa kasvaa kuitenkin runsas vesikasvillisuus, jonka tiedetään olevan tärkeä elinympäristö monille selkärangattomille ja tarjoavan runsaasti tarttumapintaa perifytonille. Tällaisten järvien litoraalivyöhykkeen perustuotantoa ei ole aikaisemmin tutkittu.

Tässä väitöskirjassa tutkittiin litoraalin merkitystä erittäin humuspitoisten järvien perustuotannossa ja bakteerituotannossa sekä järviekosysteemin aineenvaihdunnassa. Tutkimuskohteena oli kahdeksan pientä, erittäin humuksista järveä Evon alueella Etelä-Suomessa. Päätutkimuskohde oli luontaisesti lähes kalaton Mekkojärvi. Mekkojärven perustuotanto ja yhteisöhengitys mitattiin useita kertoja sekä litoraalista että pelagiaalista ja näistä tuloksista laskettiin koko järven kattavat nettotuotantoarviot. Tulosten perusteella litoraalin perifyton hallitsi koko järven perustuotantoa suurimmillaan yli 90 %:n osuudella kesällä, jolloin voimakkaasti autotrofinen litoraali tasapainotti koko järven aineenvaihdunnan tai jopa muutti järven nettoautotrofiseksi joinain päivinä. Tämän mahdollistivat litoraalia ympäröivät makrofyyttiset vesikasvit, joiden pinnalla kasvava perifyton oli hyvissä valaistusolosuhteissa, jolloin erittäin tumma veden väri ei päässyt rajoittamaan perustuotantoa. Myös bakteerituotantoa mitattiin sekä litoraalissa että pelagiaalissa, ja tuloksista laskettiin vastaavasti koko järveä koskevat estimaatit. Bakteerituotannon osalta pelagiaali hallitsi koko jär-

ven tuotantoa, mutta litoraalin perifytonin biofilmin bakteerituotanto oli suurimmillaan lähes samansuuruista kuin pelagiaalin. Tämä osoittaa yhdessä perustuotantomittausten kanssa, että järven tuotantoa, aineenvaihduntaa ja hiilen kiertoa arvioitaessa tulisi litoraali aina huomioida pelagiaalin ohella.

Voimakas litoraalin perustuotanto herätti kysymyksiä sen merkityksestä järven toisenvaraiselle eliöstölle, mitä tässä työssä tutkittiin käyttäen vakaita isotooppeja. Litoraali leimattiin pienellä määrällä $^{15}$N-ammoniumnitraattia, jonka perifyton käytti ravinteena hyväkseen ja sai vahvan isotooppileiman. Tämän leiman kulkeutumista järven ravintoverkossa seurattiin analysoimalla näytteitä ravintoverkon pohjalta aina huipulle asti. Tulosten perustella perifyton on tärkeä ravintoresurssi etenkin litoraalissa eläville selkärangattomille. Leiman ei merkittävästi havaittu levinneen pelagiaalissa runsaita populaatioita muodostaviin *Daphnia*-suvun vesikirppuihin, mikä viittaa siihen, että Mekkojärven pelagiaalin ja litoraalin ravintoverkot eivät ole vesikirppujen välittäminä juurikaan kytkeytyneet toisiinsa. Järvi oli tutkimuksen aikana jaettu kahteen osaan muoviverholla, ja ahvenia istutettiin kesällä yhteen osaan toisen pysyessä kalattomana. Kaloja sisältävän osan vesikirpuissa huomattiin voimakas isotooppileima hieman ennen kuin kalat olivat ehtineet syödä suurimman osan vesikirpuista. Tämä viittaa siihen, että kalojen aiheuttama saalistuspaine ajoi vesikirput suojaan litoraaliin, jossa ne saivat isotooppileiman hyödyntäessään litoraalissa leiman saaneita ravintokohteita. Kuitenkin vain yksi myöhemmin näytteiksi pyydetyistä 33 kalasta oli voimakkaasti leimautunut, mikä osoittaa sen, että vaikka kalat voivat yhdistää pelagiaalin ja litoraalin ravintoverkot, litoraalin vesikasvillisuus on selkärangattomille eläimille tehokas suoja saalistusta vastaan.

Jotta tulokset olisivat yleistettävissä koskemaan laajempaa järvijoukkoa, litoraalin ja pelagiaalin perustuotanto mitattiin kertaluontoisesti kahdeksalta Evon alueen pieneltä, erittäin humuspitoiselta järveltä heinäkuun aikana, ja järvikohtaiset tuotantoestimaatit laskettiin kuten edellä. Tulosten perusteella litoraali hallitsi koko järven perustuotantoa viidessä järvessä kahdeksasta. Litoraalin vesikasvillisuustiheysarviot korreloivat positiivisesti litoraalin perustuotannon kanssa. Mitä suuremman osan vesikasvit peittivät järven pinta-alasta, sitä suurempi oli litoraalin osuus koko järven perustuotannosta. Vain yksi järvi kahdeksasta osoittautui tutkimushetkellä nettoautotrofiseksi muiden ollessa nettoheterotrofisia, eli niissä yhteisöhengitys ylitti järven bruttoperustuotannon. Litoraalin tuotannon sisällyttäminen perustuotantoarvioon vaikutti aineenvaihdunta-arvioihin tasapainottaen jokaisen järven aineenvaihdunta-arviota. Mikä osoittaa, että monet humusjärvet ovat siis paljon tuottavampia ja vähemmän heterotrofisia kuin on aiemmin oletettu.

Tämä väitöskirja osoittaa, että myös humusjärvissä litoraalivyöhyke on merkittävä alue järven perustuotannossa ja toisenvaraisessa tuotannossa. Tämä tulisi huomioida jatkossa koko järven prosesseja arvioitaessa. Litoraalin osuutta koko järven perustuotannosta arvioitaessa litoraalin vesikasvillisuuden runsaus, joka tässä arvioitiin muun muassa pelagiaalin ja litoraalin pinta-alojen suhteena, voi olla käyttökelpoinen muuttuja, jonka voi arvioida satelliittikuvista. Olisi

syytä tutkia, miten käyttökelpoinen tällainen muuttuja on isompien järvien kohdalla, olisi syytä tutkia. Monipuolisempia tutkimusmenetelmiä hyödyntämällä ja yhdistelemällä voidaan saada tarkempia arvioita kokonaistuotannosta sekä oikeampi arvio litoraalin ja pelagiaalin välisistä prosesseista sekä litoraalin ja pelagiaalin prosessien suuruusluokista. Pienten humusjärvien on oletettu olevan voimakkaan nettoheterotrofisia ja pinta-alaan suhteutettuna voimakkaita hiilidioksidin lähteitä. Tämä tutkimus kuitenkin osoittaa, että litoraali voi tällaisissa järvissä olla ajoittain korkean perustuotannon aikana voimakas hiilinielu. Perustuotanto ja hengitys kuitenkin vaihtelevat huomattavasti vuorokauden ja vuoden sisällä, mikä tulisi ottaa huomioon tehtäessä tarkempia arvioita näiden järvien aineenvaihdunnasta.

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# ORIGINAL PAPERS

# I

# ACCOUNTING FOR LITTORAL PRIMARY PRODUCTION BY PERIPHYTON SHIFTS A HIGHLY HUMIC BOREAL LAKE TOWARDS NET AUTOTROPHY

by

Jussi Vesterinen, Shawn P. Devlin, Jari Syväranta & Roger I. Jones 2016

Freshwater Biology 61: 265–276.

**Abstract**

1. The prevailing view that many humic lakes are net heterotrophic is commonly based on pelagic measurements alone. Poor light conditions in humic lakes are assumed to constrain littoral primary production (PP), such that the littoral zone has been considered an insignificant contributor to whole-lake PP. However, that assumption is based on models and inferences from pelagic processes which do not take littoral zone structure into account. Many lakes have an extensive ring of aquatic vegetation lying near the water surface, which provides substratum for epiphytic algae under well-illuminated conditions.

2. We measured both pelagic and littoral PP and community respiration (CR) in Mekkojärvi, a small, highly humic headwater lake, in southern Finland throughout the open water season in 2012. We used a $^{14}C$ incorporation technique to measure pelagic PP, while littoral PP was determined using changes in dissolved inorganic carbon (DIC) concentrations during *in situ* incubations. We then estimated whole-lake PP and CR for both the littoral and pelagic zones.

3. We found that littoral PP usually dominated whole-lake PP, contributing >90% to total PP in summer. A mean pelagic production to respiration ratio (GPP:CR) of 0.4 clearly indicated strong net heterotrophy, but a value of 2.2 for the littoral zone indicated strong autotrophy. For both habitats combined, the mean whole lake GPP:CR was 1.6, indicating net autotrophy.

4. We suggest that littoral PP can contribute significantly to whole-lake PP even in highly humic lakes, and that the littoral contribution can shift some lakes which have been considered net heterotrophic to metabolic balance or even net autotrophy during the ice-free period. Small humic lakes like Mekkojärvi with rings of littoral vegetation are widespread, especially in the boreal zone, and at least for similar lakes, evaluating lake metabolism from pelagic measurements alone is likely to be misleading.

**Introduction**

Most studies concerning primary production (PP) in lakes have included only phytoplankton and have ignored benthic algae in littoral habitats (Vadeboncoeur, Vander Zanden & Lodge, 2002). Over the last decade, a consensus has emerged that littoral and

45 pelagic food webs are linked and that a combination of the PP of phytoplankton, benthic algae and macrophytes together with allochthonous organic carbon (OC) forms the energetic base of lake food webs (Vander Zanden & Vadeboncoeur, 2002; Vadeboncoeur *et al.,* 2008; Reynolds 2008; Karlsson *et al.,* 2009; Cole *et al.,* 2011). Although the view of discrete pelagic and littoral food webs has changed, quantitative integrations of littoral processes into

50 whole-lake dynamics remain under-represented in the literature (Vadeboncoeur & Steinman, 2002). Of the few studies investigating littoral productivity and dynamics, most are from clear-water lakes, both oligotrophic (eg. Ask *et al.,* 2009) and eutrophic (eg. Van de Bogert *et al.,* 2007), and only very few from dystrophic humic lakes. There is strong evidence that benthic algae can make substantial contributions to whole-lake PP in clear-water lakes

55 (Vadeboncoeur *et al.,* 2003; Andersson & Brunberg, 2006; Ask *et al.,* 2009) and can provide a major food resource for consumers even in large lakes, despite a low benthic contribution to whole-lake PP (Hecky & Hesslein, 1995; Hobson & Welch, 1995; Vander Zanden & Vadeboncoeur, 2002; Devlin, Vander Zanden & Vadeboncoeur, 2013). In contrast, poor light penetration into humic lakes is considered to severely constrain primary production, and

60 especially benthic primary production (Karlsson *et al.,* 2009).

Allochthonous (terrestrially derived) organic matter can affect several fundamental functions in lake ecosystems (e.g. constraining primary production) and allochthony increases along a humic gradient (Jones 1992; Carpenter *et al.,* 2005; Jansson *et al.,* 2007). Incorporation of allochthonous OC into lake food webs increases heterotrophy, leading to

65 community respiration (CR) that exceeds gross primary production (GPP) and overall net

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heterotrophy (del Giorgio & Peters, 1994; Duarte & Agusti, 1998). However, most studies investigating whole-lake carbon flux concern only pelagic habitats without measurements of benthic autotrophic production or metabolism in the littoral zone. Ask *et al.* (2009) measured biomass production of algae and bacteria in both pelagic and benthic habitats in subarctic

70     clear-water lakes and found that pelagic habitats were net heterotrophic with respiration and net $CO_2$ production dominating carbon fluxes, whereas primary production was dominant in benthic habitats. Those authors also concluded that allochthonous OC supported a significant part of the total consumer biomass production in both pelagic and benthic habitats. Humic or highly turbid lakes, where light effectively limits phytoplanktonic primary production to the

75     uppermost water layers, are expected to have very low benthic contribution to whole-lake PP (Vadeboncoeur *et al.,* 2008). This is especially pronounced if a lake has a high depth ratio ($DR = \bar{z}/z_{max}$) and lacks illuminated benthic habitats. Consequently, and possibly due to more demanding sampling and measurement techniques for benthic algae and benthic PP in complex littoral habitats compared to relatively simple pelagic PP measurements, inclusion of

80     littoral habitats has been rare in studies of humic lakes.

      We measured primary production and respiration in both pelagic and littoral habitats of a small, highly humic lake, Mekkojärvi, which has been well studied (e.g. Kairesalo, Lehtovaara & Saukkonen*,* 1992; Järvinen & Salonen, 1998; Salonen *et al.,* 2005; Taipale *et al.,* 2008). There are numerous similar lakes in Finland and globally, with the estimated total

85     number of lakes with surface area from 0.1 to 1 ha being $2.77 \cdot x\ 10^8$ (Downing *et al.,* 2006). Finnish lakes are predominantly shallow due to flat topography and consequently allochthonous carbon inputs are diluted in a small volume of water resulting in higher TOC concentrations and water colour than in deeper lakes (Kortelainen, 1999). Climate change is expected to increase terrestrial exports of DOC to lakes (Tranvik & Jansson, 2002) affecting

90     the light climate in these lakes. Previous studies have revealed that carbon fluxes in the

pelagic zone of Mekkojärvi are dominated by heterotrophic processes and the lake has been

considered to be net heterotrophic. However, the littoral zone of Mekkojärvi consists of a

surrounding floating moss bed with submerged, inert, sedge stems supporting profuse

epiphytic algal mats (periphyton) under well-illuminated conditions in summer. The

95   productivity of this periphyton has not been measured before. Species diversity and biomass

of animals in the littoral zone of Mekkojärvi is much higher than in the pelagic zone, and

those animal communities have been assumed to have strong reliance on periphyton

(Kairesalo *et al.,* 1992). Our main hypothesis was that the periphyton productivity in the

littoral zone should be high in order to support the abundant macroinvertebrate consumers,

100  despite the argument that benthic PP should be low considering the trophic status, light

regime and basin shape of the lake (Vadeboncoeur *et al.,* 2008). Our second hypothesis was

that, although the pelagic zone in Mekkojärvi is strongly dominated by heterotrophic

processes, the littoral zone is more dominated by autotrophic processes and would offset

pelagic heterotrophy even to the extent of making the lake net autotrophic.

105

**Methods**

*Study site*

     The study was conducted at Mekkojärvi (61º13'N 25º3'E) in the Evo forest area in

southern Finland (Fig. 1A) during the open water season in 2012. Mekkojärvi is a small (0.35

110  ha) and highly humic (water colour 300–800 mg Pt l$^{-1}$) headwater lake with maximum depth

of 4.3 m and mean depth of 2 m. The lake receives a high loading of terrestrial organic matter

from its catchment area, and has high DOC concentrations and low pH (Table 1). Despite

being so shallow, the very high water colour and shelter from wind by the surrounding

coniferous forest cause the lake to develop a very steep temperature and oxygen stratification

115  gradient in summer, when the thermocline lies between 0.5–1.0 m and anoxia generally

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occurs below 1.0 m. The lake cannot sustain fish populations due to complete under-ice

anoxia during winter, and the absence of planktivorous fish has led to very dense populations

of the large bodied cladoceran *Daphnia longispina* in summer. Our study was conducted as

part of a whole-lake food web experiment in 2012, when Mekkojärvi was divided into two

120   basins with a plastic curtain and adult perch (*Perca fluviatilis*) (n = 100) were introduced to

one basin in early July, while the other basin remained fishless. Both lake basins were always

sampled simultaneously.

Mekkojärvi has a depth ratio of 0.47 and lacks illuminated benthic surfaces due to its

dark brown water colour and extremely low light penetration (light-attenuation coefficient,

125   $K_d$, ranges from 4.5 to 7.5). Despite its moderate DR, Mekkojärvi does not have a clearly

defined littoral zone, as the lake is surrounded by a floating moss bed with fallen submerged

terrestrial sedges, and a water column lies underneath the vegetation mat leading to a more

cylindrical lake morphometry than implied by the DR. The littoral zone is thus composed

mainly of *Sphagnum* and *Warnstorfia* moss species, with submerged and emergent

130   macrophytes such as *Menyanthes trifoliata, Phragmites australis, Carex* spp. and *Utricularia*

sp. associated with the moss mat. The littoral vegetation mat does not extend further than ca.

1 m from the lake edge and not deeper than ca. 0.5 m, but it forms a complex physical

structure, which is well-illuminated and has a large surface area for epiphytic algal

development. Relatively thick (ca. 1 cm) periphyton communities, rich in pennate diatoms

135   (Bacillariophyceae), develop early in June, and achieve their highest biomass from July to

mid-August. The area of littoral vegetation mat, estimated from a satellite image by using an

area calculator tool (in Retkikartta.fi, © Metsähallitus, MML, CGI 2015), is ca. 0.086 ha,

which covers ca. 25% of the whole lake area and gives a ratio of pelagic to littoral zone by

area of 3.0.

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*Physical and chemical parameters*

Light attenuation was measured at each sampling period using a single planar LI-COR® (Li-192) Underwater Quantum light sensor. Light was measured at 0.5 m intervals from the surface to the bottom of the lake and light attenuation coefficients were calculated (Kirk, 2003). Temperature and oxygen concentration were measured during every sampling in 2012 and once in 2014 with an oxygen and temperature sensor (YSI 55 probe, Yellow Springs Instruments). Daily irradiance values (measured with a Kipp & Zonen B.V., model CM11) and day lengths were obtained from the Finnish Meteorological Institute measurement station in Jokioinen, ca. 110 km west from Mekkojärvi. Nutrient, DOC and POM samples were taken at each sampling period and were analysed at Lammi Biological Station.

*Pelagic phytoplankton biomass, production and respiration*

Composite water samples were taken with an acrylic tube sampler (Limnos) by combining three pulls covering each of three strata (0–0.5, 0.5–1.5 and 1.5–3 m) corresponding to epi- meta- and hypolimnion in both basins of the lake. Samples were taken 10 times between May–October in 2012. Phytoplankton biomass was estimated as chlorophyll *a* (chl *a*) concentration in the composite water samples. Samples were filtered onto Whatman GF/C glass fibre filters and chl *a* was measured by UV-spectrophotometry (Shimadzu UV-1800) according to a standard spectrophotometric method (SFS 5772, Finnish Standards Association).

Primary production in the pelagic habitat was measured eight times from composite water samples from four strata (0–0.2 m, 0.2–0.5 m, 0.5–1 m and 1–3 m) from both basins according to the $^{14}$C incorporation method described by Schindler, Schmidt & Reid (1972). Subsamples were pipetted into 20 mL glass vials (containing NaH$^{14}$CO$_3$) with four replicates

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(one dark sample in each depth). Samples were then incubated *in situ* in each stratum for 24 h. The $^{14}$C activity was measured with a Packard Tri-Carb$^{®}$ liquid scintillation counter. These 24 h determinations were assumed to approximate most closely to net primary production (NPP). Primary productivity of the water column was converted from volumetric basis to areal basis by multiplying the values from each stratum by the total depth of the stratum and then summing over the whole water column. Whole-lake daily PP estimates were calculated by multiplying daily areal values by the lake surface area.

Pelagic community respiration (CR) was measured eight times, together with PP, as the increase in DIC concentration during dark incubations. Subsamples (two initials and two samples for dark incubation) were taken from composite water samples from each stratum into 50 mL glass BOD bottles. Half of the bottles were wrapped in aluminium foil and incubated *in situ* for 24 h in each stratum and the other half ($t_{0h}$) were placed on ice and taken immediately to the laboratory, where DIC concentration was measured with a DIC-analyser (Salonen, 1981). This was repeated for $t_{24h}$ samples the next day. Areal values and whole-lake estimates for CR were calculated as for pelagic PP. Pelagic NPP was converted to GPP as the sum of NPP and CR values in the photic zone, assuming that most of the CR was attributable to primary producers.

*Littoral primary production, respiration and periphyton biomass*

Primary production in the littoral habitats was measured eight times, concurrently with pelagic PP, from *in situ* incubations of periphyton samples in both dark and transparent 20 ml glass vials where the consumption or production of DIC over time indicates either respiration or production. Periphyton PP on moss and on sedges per unit dry mass of substratum was compared and there was no statistically significant difference in PP rates (*t*-test, $t_{39}$ = -0.2005, $P$ = 0.8421, data not shown). This allowed us to sample routinely from

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submerged sedges instead of from the more complex moss substratum. Furthermore, sampling of sedges was appropriate because while lying under the water surface they were metabolically inert. To insure that the sedge substratum was indeed metabolically inert, epiphyton was carefully removed from sedges and the cleaned substratum was incubated in both light and dark. No change in DIC was measured (data not shown). The lake was sampled at eight sites around the shoreline, four sites from each basin. Samples were collected by clipping random sedge pieces into 20 mL glass vials and filling with surrounding littoral water. Trial incubations of this littoral water without any epiphyton or substratum demonstrated no substantive changes in DIC concentration over time. Four replicate samples of surrounding water to obtain initial DIC concentrations were taken from each sampling site. Samples were incubated *in situ* for 2 h at noon, half of the samples in dark conditions and the other half in the surface water in the same light conditions as those from which the periphyton originated (barely submerged under the water surface). After the incubations, samples were put into ice and dark and taken immediately to the laboratory, where DIC was measured with a DIC-analyser (Salonen, 1981). Periphyton was then entirely removed from the collected substratum by scraping with a spatula and was filtered onto glass fibre filters (Whatman GF/F) and chl *a* was determined as described above for phytoplankton. Periphyton biomass was estimated as total chl *a* per dry weight of substratum in a sample. Substrata were dried at 60 ºC for 24 h and dry mass was recorded.

Gross primary production (GPP) was calculated from the difference in DIC between dark and light samples ($GPP = DIC_{dark} - DIC_{light} /$ incubation time); CR was calculated from the difference in DIC between dark and initial samples ($CR = DIC_{dark} - DIC_{initial} /$ incubation time). Net primary production (NPP) was then calculated as $NPP = GPP - CR$, assuming that most of the CR was attributable to primary producers. Values for each incubated sample were normalized to mg C g dry weight (DW) substratum$^{-1}$. To estimate whole-lake littoral primary

production, we quantified the availability of littoral substratum, which consisted of macrophytes and moss. Littoral substratum along 40 cm of lakeshore at 12 sites within each basin was entirely physically removed, carefully collected and dried at 60º C. We calculated the average dry weight of littoral substratum per meter of lake shore (20.1 and 22.5 g DW

220    substratum $m^{-1}$, respectively) in both basins and, since the substratum consists mainly of flat and horizontally aggregated macrophytes and moss leaves, we divided the weight by two in order to conservatively estimate the mass of the illuminated side of the substratum. It should be noted that more than half the substrate collected had substantial development of epiphyton and that this approach is likely to have yielded a conservative estimate of available

225    substratum. We compared the use of this method of whole-lake extrapolation to a simple areal extrapolation method and found that the results were within 20% at the whole-lake scale. Given the vast oversimplification of defining the heterogeneous, extremely rugose and complex physical nature of the littoral substratum as a plane, we considered that extrapolation by substratum weight was more realistic. Daily estimates for periphyton PP were calculated

230    from a slightly modified version (Eq. 1) of the calculation described by Vadeboncoeur *et al.* (2008). For whole-lake estimates, we calculated PP per m of lake shore and multiplied that by the total shoreline lengths of each basin (135 and 185 m, respectively). Daily estimates for periphyton respiration were calculated by multiplying the mean values at noon by 24.


235    Periphyton $PP^{-day}$ =

$$\Delta t \sum_{sunrise}^{sunset} [PP_{max} \quad \tanh(I_{zt}/I_k)]$$

(Eq. 1)

where $\Delta t$ is the time increment (h), $PP_{max}$ is the light-saturated primary production (mg C g dry-weighted substrate$^{-1}$ h$^{-1}$) derived from incubating samples right under the surface at noon , tanh is the hyperbolic tangent function, $I_{zt}$ is the light at depth z ($\mu$mol m$^{-2}$ s$^{-1}$) and time t (h)

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240    and $I_k$ is the light intensity for onset of saturation, which was set to 300 µmol m$^{-2}$ s$^{-1}$

according to Hill (1996). We used the irradiance data and day lengths obtained from the

Finnish Meteorological Institute in Jokioinen. To define $I_z$, the photosynthetically active

radiation (PAR) on the surface was multiplied by 0.75, which was the average fraction of

light at the incubation depth over the whole sampling period (Fig. 1B).

245

*Statistical analyses*

Since the lake was divided into two basins with a plastic curtain, and one basin was

fishless and the other had fish, we used multivariate repeated measures of ANOVA (RMA) to

test if there were statistically significant differences in PP, CR and chl *a* between the

250    sampling occasions and the basins. The data were log-transformed, when necessary, to

achieve the requirements of parametric analyses. A non-significant basin effect (between-

subject factor) revealed that the fish experiment and the lake division did not affect the

production and respiration values, which allowed us to pool the data. The daily estimates for

periphyton PP (Eq. 1) were performed using R project for Statistical Computing version 3.0

255    (R Core Team, 2013). Other tests were performed using IMB SPSS Statistics 20.0.0.2. If not

expressly noted, all the descriptive statistics are means + S.E.


**Results**

*Pelagic primary productivity, respiration and phytoplankton biomass*

260    Physical and chemical parameters are reported in Table 1. Light attenuation

coefficient, $K_d$, ranged from 4.5 to 7.5. Pelagic NPP (Fig. 2A) was highest in late May (331 ±

274 mg C m$^{-2}$ d$^{-1}$), indicating a clear spring maximum of phytoplankton. PP then decreased

towards autumn, being lowest in late September (9.0 ± 1.7 mg C m$^{-2}$ d$^{-1}$). PP values for 19

July and 1 August include data from only one basin, as the data from the other basin were

265    unreliable. RMA was not executed for the pelagic PP data, since variation was substantial and

assumptions for repeated measures were not met; however, a t-test showed no differences in

mean PP for the whole sampling period between the two basins (t-test, $t_{40} = 0.254$, $P =$

0.801), or in pairwise comparisons between the basins on each date, allowing us to pool the

data from the two basins.

270         Community respiration also varied substantially (Fig. 2A), and exhibited rates over

one order of magnitude higher than those for PP. The highest mean CR on 18 June was 3210

$\pm$ 1655 mg C m$^{-2}$ d$^{-1}$, of which the hypolimnetic fraction was 78%. The mean hypolimnetic

fraction of the CR in the water column over the sampling period was 77% $\pm$ 5. CR values for

1 and 15 August include values from only one basin. RMA for CR from May to August

275    showed a significant main effect of dates ($F_{3, 48} = 11.88$, $P < 0.01$), indicating change over

time, but there was no significant interaction between the basins and the dates ($F_3 = 2.03$, $P =$

0.12), allowing us to pool the data from the two basins.

Chlorophyll $a$ concentration in the epilimnion was rather constant from spring to

autumn (8.0 $\pm$ 1.1 mg m$^{-3}$) and substantially lower than in the metalimnion (27.2 $\pm$ 6.2 mg m$^{-}$

280    $^3$) and hypolimnion (55.4 $\pm$ 4.4 mg m$^{-3}$) (Fig. 3A). However, the high chl $a$ concentrations in

both meta- and hypolimnion presumably contain appreciable quantities of

bacteriochlorophyll (BChl), which is abundant in Mekkojärvi and which overlaps with chl $a$

in spectrophotometric determination (Karhunen $et\ al.,$ 2013). Indeed BChl may also have

contributed to epilimnetic chl $a$, as chl $a$ did not follow the same pattern as pelagic PP. When

285    the basins were compared for the mean chl $a$ over the sampling period, chl $a$ was higher in

the fishless basin (37.8 $\pm$ 5.4 mg m$^{-3}$) than in the basin which had fish (22.6 $\pm$ 4.2 mg m$^{-3}$) ($t$-

test, $t_{58} = -2.200$, $P < 0.05$).

*Littoral primary productivity, respiration and periphyton biomass*

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290　　　　　　Littoral periphyton NPP was highest in spring and throughout the summer except for a

rapid decrease in early July (Fig. 2C), and showed a pattern of decline towards autumn,

decreasing substantially from late-August to late-September (Fig. 2B, C). The mean NPP at

noon was $35.8 \pm 3.2$ mg C g DW substratum$^{-1}$ h$^{-1}$ and the highest value was determined on 20

July ($47.2 \pm 11.0$ mg C g DW substratum$^{-1}$ h$^{-1}$). RMA revealed a significant main effect of

295　　time ($F_{7, 98} = 6.940$, $P < 0.01$), which was not affected by basin treatments ($F_{7, 98} = 1.098$, $P =$

0.366). The only significant difference in NPP was observed between 30 August and 27

September ($F_{1, 14} = 3.462$, $P < 0.05$), although the main effect of basin was not significant ($F_{1,}$

$_{14} = 3.982$, $P = 0.066$), allowing us to pool the data from both basins. Mean daily littoral NPP

was $364 \pm 66$ mg C g DW substratum$^{-1}$ d$^{-1}$ and the highest mean rates were observed on 20

300　　July ($663 \pm 2$ mg C g DW substratum$^{-1}$ d$^{-1}$), which was later than the highest noon rates

observed on 4 July (Fig. 2B, C). This, as well as the decrease in daily NPP values on 30

August, was caused by rapid changes in weather and light conditions, which gave lower daily

estimates. NPP rates remained constant until August, when variation increased between the

basins.

305　　　　　　Littoral CR was low relative to the highest NPP rates (Fig. 2B, C). The mean CR at

noon was $16.7 \pm 2.4$ mg C g DW substrate$^{-1}$ h$^{-1}$; the highest rates were observed on 4 July

($37.0 \pm 11.3$ mg C (g DW substratum)$^{-1}$ h$^{-1}$) and the lowest on 27 September ($4.6 \pm 1.3$ mg C

(g DW substratum)$^{-1}$ h$^{-1}$). RMA revealed no significant main effect of time ($F_{2.88, 40.36} = 1.530$,

$P = 0.222$) and no interaction between time and the basins ($F_{2.88, 40.36} = 0.252$, $P = 0.852$), or

310　　any main effect of basin ($F_{1, 14} = 0.013$, $P = 0.858$). When the daily CR values were

calculated from the hourly rates, the mean CR over the sampling periods was $395 \pm 65$ mg C

(g DW substratum)$^{-1}$ d$^{-1}$.

　　　　　The mean periphyton chl $a$ content over the sampling periods was $0.7 \pm 0.1$ mg (g

DW substratum)$^{-1}$; the highest contents were observed on 30 August ($1.3 \pm 0.3$ mg (g DW

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315    substratum)$^{-1}$) and the lowest on 27 September (0.2 ± 0.3 mg (g DW substratum)$^{-1}$) (Fig. 3B).

RMA revealed a significant main effect of time ($F_{2.52, 35.36}$ = 5.705, $P < 0.01$), but no

interactions between time and basin ($F_{2.52, 35.36}$ = 1.191, $P = 0.323$).


*Whole-lake estimates for net primary production and respiration*

320      Whole-lake estimates showed that littoral contributions dominated whole-lake NPP

from spring to early autumn (Fig. 4A, B). The mean littoral contribution to whole-lake NPP

over the sampling period was 81 ± 8%. Pelagic NPP contribution most early in the spring

(24%) and in late autumn (54%), while in the summer the pelagic contribution varied from 2

to 20%. The highest estimate for the whole-lake NPP in the littoral was 4.1 kg C d$^{-1}$ on 21

325    June and the lowest was 17.5 g C d$^{-1}$ on 27 September, while the mean was 2.4 ± 0.7 kg C d$^{-1}$.

The highest whole-lake estimate for pelagic NPP was 1.1 kg C d$^{-1}$ on 31 May and the lowest

was 32.4 g C d$^{-1}$ on 27 September, while the mean was 338 ± 165 g C d$^{-1}$. Whole-lake

estimates for July could not be calculated due to missing pelagic data from the fishless basin,

but the littoral contributions in the basin with fish remained high in July (77 and 91%).

330    Whole-lake estimates of pelagic CR showed higher values in spring when the highest

peak, observed on 21 June, was 10.4 kg C d$^{-1}$ (Fig. 4C, D). The lowest estimate was 385 g C

d$^{-1}$ on 30 August while the mean was 3.2 ± 1.9 kg C d$^{-1}$ over five sampling periods. Littoral

CR was relatively stable, ranging from 766 g C d$^{-1}$ in late September to 4.4 kg C d$^{-1}$ in late

August, while the mean was 2.5 ± 0.5 kg C d$^{-1}$ (Fig. 4C, D).

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*Production to respiration ratio (GPP:CR)*

     The mean GPP:CR ratio in the littoral zone was 2.2 ± 0.4, n = 8), but was

significantly lower in the pelagic zone (0.4 ± 0.1, n = 5) (*t*-test, $t_{11}$ = 3.185, $P < 0.01$). The

highest GPP:CR ratio in the littoral was 4.8 on 20 July, and the lowest was 1.0 on 27

340     September. For the pelagic zone, the highest GPP:CR ratio was 1.0 on 20 July, and the lowest

was 0.2 on 16 August. The whole-lake average of five sampling periods was 1.6 ± 0.5. The

results indicated net heterotrophy (P:R<1) in the pelagic zone but net autotrophy (P:R>1) in

the littoral zone (Fig. 5). When whole-lake pelagic and littoral results were combined, the

outcome indicated net autotrophy for the complete lake ecosystem (Fig. 5).

345

**Discussion**

     Our results demonstrate an overwhelming dominance of littoral PP in whole-lake

autotrophic production in Mekkojärvi. The lake is very humic, which limits light penetration,

while its depth ratio and complex littoral structure leave the majority of the lake bottom in

350     darkness. However, littoral vegetation lining the lake perimeter is well-illuminated and

supports highly productive periphyton assemblages, with mean contribution to whole-lake

NPP over 80% throughout the sampling period. Intense respiration of allochthonous OC in

the pelagic habitat substantially exceeded phytoplankton PP, indicating strong net

heterotrophy with low GPP:CR ratios in the open water of the lake. In contrast, well-

355     developed and highly productive periphyton mats led to net autotrophy in the littoral habitat,

and when incorporated into whole-lake PP  the system GPP:CR ratio was net autotrophic.

Estimating PP and CR for structurally complex littoral zones is very challenging, and our

estimates of whole-lake littoral PP and CR undoubtedly have higher uncertainty than the

pelagic values. Nevertheless, the littoral values are so high that the associated uncertainty

360     cannot alter their essential message. Moreover, the values for littoral NPP must be

underestimates, as we obtained these by subtracting CR from GPP, and the true respiration

attributable to primary producers must be less than the CR. Hence our results demonstrate

that, despite high terrestrial inputs of DOC and dominance of heterotrophic processes in the

pelagic zone, some humic lakes can be metabolically balanced or even net autotrophic when

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365    highly productive periphyton communities are included in measurements of whole-lake

metabolism. Our findings challenge the current theoretical framework regarding whole-lake

metabolism in humic systems and provide some answers to recently posed questions about

the carbon budgets in lakes (Hanson *et al.,* 2014).

Lakes with high DOC, high colour and low pelagic primary production are often

370    assumed to be heterotrophic due to the allochthonous OC (Cole *et al.,* 2000). However,

littoral primary production is often overlooked in lakes like Mekkojärvi, as many factors that

regulate possible littoral contributions to whole lake autochthony would appear to seriously

constrain the potential significance of periphyton. Indeed, based on Mekkojärvi's depth ratio,

light attenuation and P content, equations presented by Vadeboncoeur *et al.* (2008) predict

375    that the overall contribution of littoral PP to whole-lake PP should be a maximum of 10%.

However, these models do not account for sub-surface littoral vegetation lining the lake

perimeter that provides substantial substratum for colonization by epiphytic algae.

Previous work demonstrating that benthic algal contribution to whole-lake PP

declines rapidly with increasing DOC concentration in northern boreal lakes, offers a

380    perspective for autotrophy in humic lakes when epiphytic benthic algae is not measured and

included as a source of PP (e.g. Ask *et al.,* 2009). When metabolism on sediment and by

periphyton colonizing only the lake bottom are measured, logically the reduced light

penetration caused by high DOC concentrations explains why benthic algae could not

contribute substantially to humic lake autotrophy. However, to fully account for and evaluate

385    the contribution of benthic algae in humic lakes, the extremely productive, albeit easily

overlooked, PP from shallow periphyton needs to be included.

It is still likely that large north temperate lakes are generally net heterotrophic, as

bacterial respiration of allochthonous OC can proceed in the whole water column during the

entire year, while photosynthesis in most of the lakes is restricted to the uppermost water

390    layers (Jansson *et al.,* 2000). That is evident if a lake does not have extensive littoral

vegetation and the ratio of pelagic area to the area of littoral vegetation is very high.

However, northern areas have millions of lakes with mean depth less than 3 m and a littoral

zone that dominates the pelagic zone (Wetzel, 1990). In Finland, where ca. 10% of the

surface area is covered by lakes, over 95% of the 190 000 lakes are smaller than 1 km$^2$

395    (Raatikainen & Kuusisto, 1990) and a majority of these are humic with a high concentration

of organic matter (Kämäri *et al.,* 1991). Although our results are from only one lake, we can

assume that in small humic lakes, where littoral vegetation comprising floating moss and

macrophytes is a widespread characteristic, estimates of PP and lake metabolism based only

on measurements from the pelagic zone and ignoring epiphytic PP are likely to be seriously

400    erroneous.

Pelagic PP in Mekkojärvi was similar to that in previous studies of the lake (Salonen

*et al.,* 2005; Taipale, Kankaala & Jones*,* 2007): a clear spring peak was followed by a

decrease towards autumn with mean rates under 100 mg C m$^{-2}$ d$^{-1}$ and relatively high

variation. Salonen *et al.* (2005) measured the CR in Mekkojärvi *in situ* from a 1 m water

405    column in the epilimnion and found respiration rates 5.2 times higher than PP. Even so, they

concluded that total water column CR was likely to be considerably underestimated, as CR in

the anoxic hypolimnion has been shown to contribute significantly to whole-lake CR (Cole &

Pace, 1995). Our finding of a 77% contribution from the hypolimnetic fraction to total

pelagic CR, with a mean rate of almost 1 g C m$^{-2}$ d$^{-1}$ is consistent with this view. One of the

410    greatest uncertainties in measuring PP by $^{14}$C incorporation is whether it measures net or

gross primary production, or something in-between (Marra, 2009). We used a $^{14}$C

incorporation method with 24 h incubation, as has been widely used at other nearby humic

lakes (Arvola *et al.,* 1999), as a longer (24 h) incubation time has been considered appropriate

in unproductive lakes, and such an incubation time should give values approximating NPP.

415    Other uncertainty is involved in deriving GPP from NPP by adding CR, as this includes the respiration of organisms other than autotrophs and gives an overestimate of GPP. The true autotrophic respiration must be less than CR and therefore the true PP:R ratios of the lake will be higher than our estimates; hence the lake must actually be more autotrophic and our interpretations remain conservative.

420    Oxygen data from the pelagic (Table 1) at noon demonstrate undersaturation of $O_2$ in the epilimnion at the relevant temperatures, indicating apparent heterotrophy. Although we do not have DO data from the littoral in 2012, we measured DO from both habitats in late-May 2014 (Table 1). DO in the littoral was measured just above the moss from several sampling sites and from the epilimnion (0–0.5 m) in the pelagic. In the littoral zone oxygen saturation

425    varied between 105 and 120%, whereas in the pelagic it was under 70%. These results give support to our high PP rates and demonstrate marked spatial heterogeneity in oxygen in the lake. Hence the undersaturation of oxygen in the open water is not inconsistent with an overall metabolic balance for the lake.

    The extremely high littoral production we measured in Mekkojärvi is likely to support

430    a productive littoral food web. Van Duinen *et al.* (2013) found that periphyton constituted a significant food resource for aquatic macroinvertebrates in small and highly humic bog pools in southwest Estonia. Lau *et al.* (2014) also showed that autochthonous resources are the main driver of secondary production in five dystrophic lakes in Sweden. While they did not directly measure primary production, our results of high epiphytic production support their

435    findings. The most diverse and abundant macroinvertebrate community in Mekkojärvi lives within the littoral moss, and it is likely that periphyton is the basal food resource in this habitat. Moreover, the most widespread fish species in small humic lakes, European perch (*Perca fluviatilis*), relies mainly on littoral macroinvertebrates for food in such lakes (e.g.

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Nyberg *et al*., 2010). The contribution of periphyton to macroinvertebrate diets and the lake

440    food web remains unclear in Mekkojärvi and deserves further study.

Our findings demonstrate that littoral zones in humic lakes offer a highly productive

environment for lake biota, and are also as critical to whole-lake metabolism as the pelagic

processes that have been much better documented in the past. Littoral primary production can

dominate pelagic PP, and in doing so can offset the high heterotrophic pelagic respiration

445    even to the extent of producing a balanced or net autotrophic whole-lake metabolism. This

contradicts the most widely held current view (Del Giorgio, Cole & Cimbleris, 1997) and

demonstrates that there is much more to be learned regarding the role of littoral processes in

humic lake metabolism. However, our estimates cover only the ice-free period. Respiratory

processes, mainly due to decomposition of OC, continue in boreal Finnish lakes at a low

450    levels throughout the winter ice cover (Kortelainen *et al.,* 2006), which may still make these

lakes net heterotrophic on an annual basis.

We estimated that the ratio of pelagic to littoral zone by area in Mekkojärvi is 3.0,

which was derived from areal calculations of both habitats from satellite imagery. Littoral

area was determined as originating at the land-water interface and ending at the edge of the

455    vegetation.  Although crude, we believe this approach provides a reasonable estimate. The

number of lakes with the similar pelagic-littoral ratios approaches $10^5$ worldwide (Peters &

Lodge, 2010) with the majority other lakes having lower values. Yet, it is hard to give

quantitative estimates of the prevalence of humic lakes ringed by similar floating vegetation

with highly productive epiphyton as occurs in Mekkojärvi. Bryophyte mats and sedges are

460    characteristic of oligotrophic humic lakes (Rintanen, 1996; Heino & Toivonen, 2008) and

therefore there is a high probability that many lakes that are similar to Mekkojärvi in size and

region contain similar vegetation features. Aquatic moss stands in small, humic Finnish lakes

have expanded in recent decades showing a clear trend of filling the entire waterbody

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(Rintanen, 1996). A recent overview of the biodiversity and ecosystem functioning in natural

465    bog pools (Beadle *et al.,* 2015) highlighted the need for future studies, as knowledge of these

ecosystems is limited. While lakes like Mekkojärvi differ in some regards from bog pools,

there are relevant similarities in humic contents, vegetation and structure of the basins. Those

bog pools are assumed to be net heterotrophic, even though there is evidence that

invertebrates significantly rely on periphyton (Van Duinen *et al.,* 2013). We suggest that

470    littoral primary production and respiration should also be taken into account in the future

studies of humic systems, as our data demonstrate that they are likely to be more productive

and less heterotrophic then previously assumed.

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Figure 1. A) Location and bathymetry (numbers indicate depth contours in m) of the study lake Mekkojärvi. Dashed line illustrates the lake division curtain and squares the sampling points in the pelagic measurements. B) The mean (± S.E.) light attenuation in Mekkojärvi in 2012 (n = 13).

Figure 2. Pelagic (A) and littoral (B, C) net primary production (bars) and community respiration (lines) in Mekkojärvi in 2012. Pelagic NPP represents areal means (± S.E.) combining both basins of the lake. Some dates (19 July and 6 August for pelagic NPP and 6 August. for pelagic CR) have values only from one basin. Littoral NPP and CR represent mean (± S.E.) net primary production and community respiration of periphyton per gram dry-weighted substratum per hour (B) and per day (C). Differences in daily light regime account for inconsistencies in proportional scaling between hourly and daily rates on individual dates.

Figure 3. Pelagic (A) and periphyton (B) chl $a$ in Mekkojärvi in 2012. Pelagic chl $a$ represents mean (± S.E.) concentrations in three strata combining both basins. Periphyton chl $a$ is expressed as mean (± S.E.) chl $a$ content per gram dry-weighted substratum and combines both basins.

Figure 4. A) Estimates of the whole-lake littoral and pelagic net primary production and B) their proportions of whole-lake net primary production. C) and D) the same for the whole-lake community respiration.

Figure 5. Comparison of whole-lake GPP and CR from pelagic and littoral habitats, both separately and in combination. The values are plotted on a logarithmic scale. The line represents a GPP:CR ratio of 1.

Table 1. Mean (± SE) values for some chemical parameters in the epi-, meta- and

hypolimnion in Mekkojärvi during the open water period of 2012: chlorophyll $a$ (Chl $a$),

dissolved oxygen (DO), dissolved organic carbon (DOC), dissolved inorganic carbon (DIC),

particulate organic carbon (POC), total phosphorus (Tot-P), ammonium (NH$_4$-N) and

650 combined oxidized inorganic nitrogen (NO$_2$-N + NO$_3$-N). pH is expressed as median. N

refers to sampling occasions. Values from 2014 represent the mean (± SE) values from a

single sampling occasion in late-May.

| 2012 | N | 0–0.5 m | 0.5–1 m | 1–3 m |
|---|---|---|---|---|
| pH | 12 | 5.3* | 5.4* | 5.7* |
| Chl $a$ (µg L$^{-1}$) | 10 | 8.1 ± 0.0 | 27.2 ± 0.0 | 55.4 ± 0.0 |
| DO (mg O$_2$ L$^{-1}$) | 9 | 4.8 ± 0.4 | 1.9 ± 0.5 | 1.0 ± 0.0 |
| DOC (mg C L$^{-1}$) | 10 | 32.0 ± 1.5 | 33.0 ± 1.1 | 30.0 ± 0.5 |
| DIC (mg C L$^{-1}$) | 8 | 2.9 ± 0.2 | 4.5 ± 0.9 | 8.6 ± 1.7 |
| POC (mg C L$^{-1}$) | 10 | 0.5 ± 0.0 | 0.8 ± 0.1 | 1.0 ± 0.0 |
| Tot-P (µg P L$^{-1}$) | 10 | 12.0 ± 0.7 | 15.0 ± 2.0 | 58.0 ± 4.4 |
| NH$_4$ (µg L$^{-1}$) | 10 | 34.0 ± 4.0 | 55.0 ± 9.4 | 384.0 ± 33.5 |
| NO$_2$ + NO$_3$ (µg L$^{-1}$) | 10 | 36.0 ± 6.9 | 65.0 ± 8.9 | 74.0 ± 9.5 |
| 2014 | N | Pelagic | N | Littoral |
| DO (mg O$_2$ L$^{-1}$) | 2 | 7.3 ± 3.9 | 8 | 10.0 ± 0.5 |
| Oxygen saturation (%) | 2 | 68.0 ± 0.1 | 8 | 121.0 ± 4.1 |
| Temperature (ºC) | 2 | 11.5 ± 0.1 | 8 | 23.7 ± 0.3 |
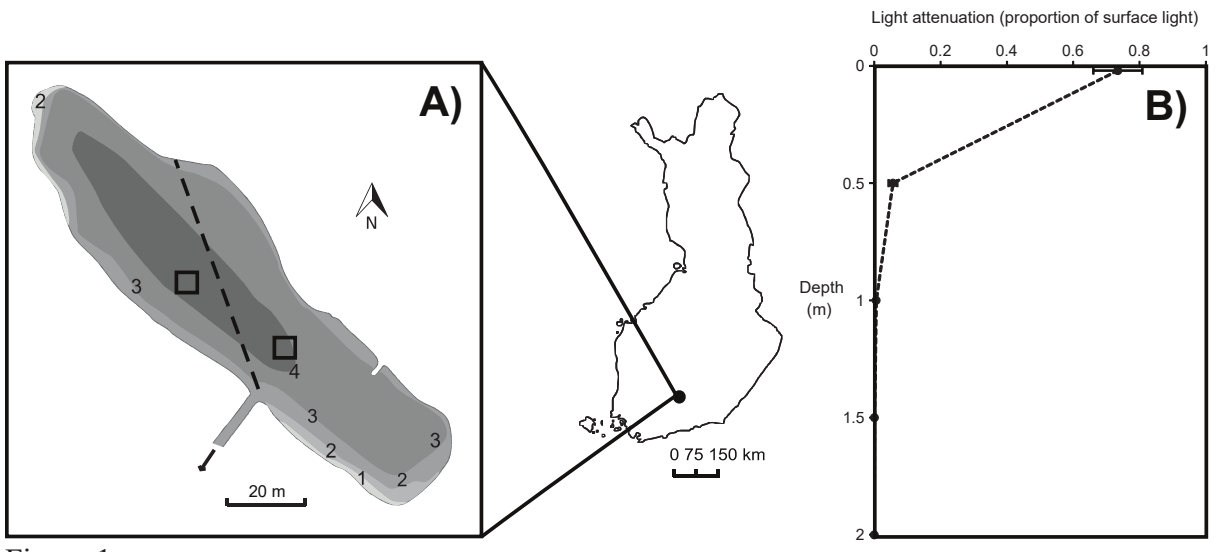
655    Figure 1.
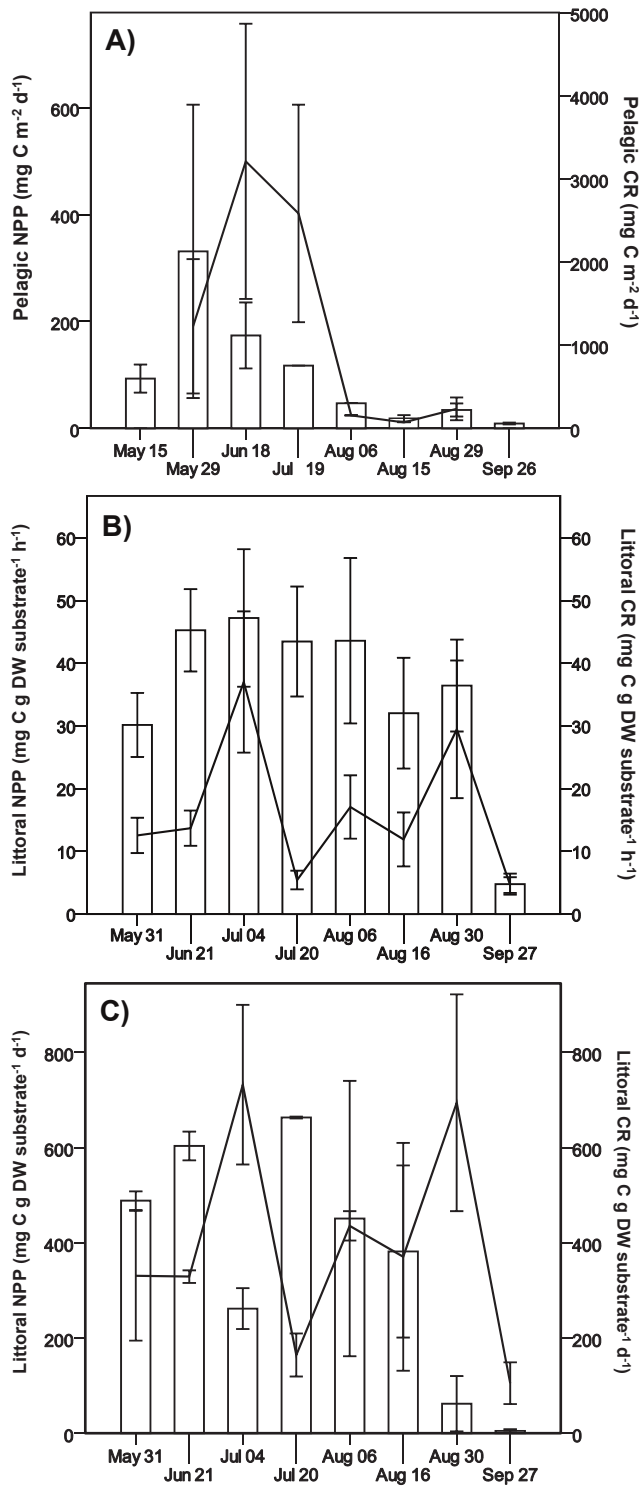
Figure 2.
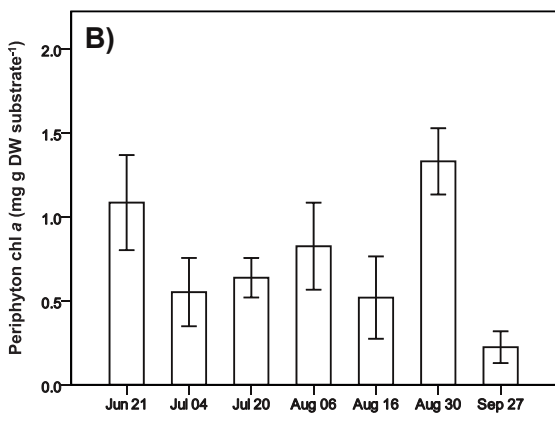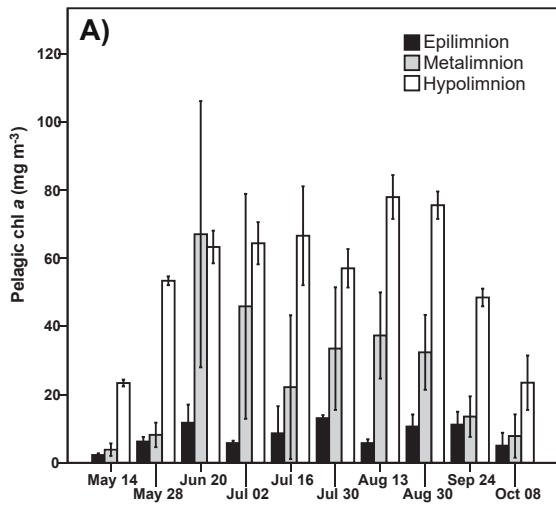
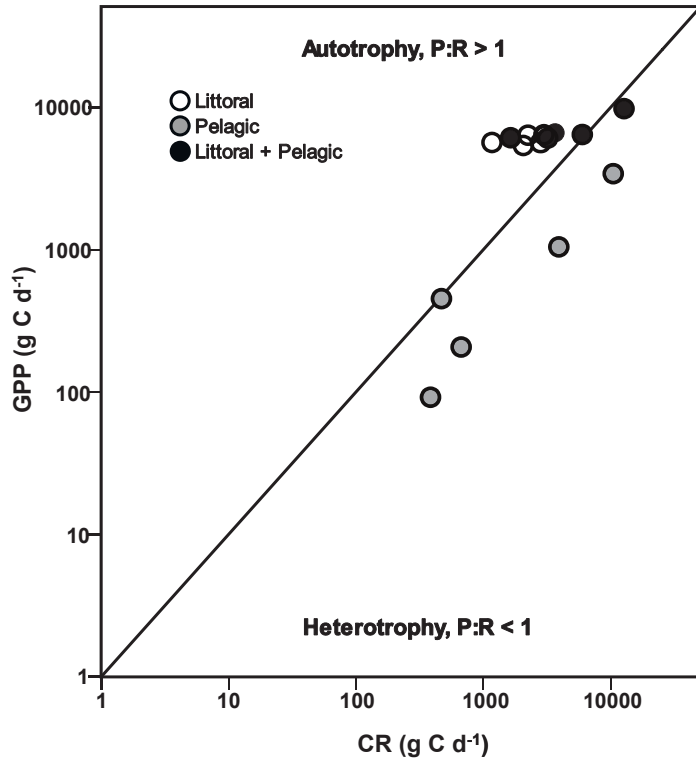660

Figure 3.
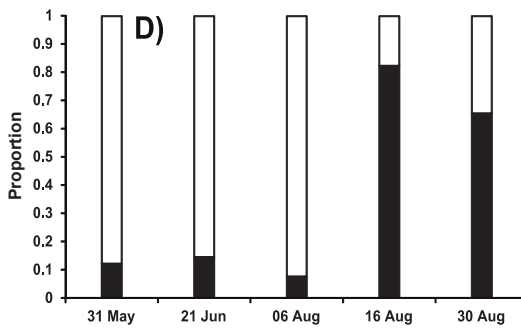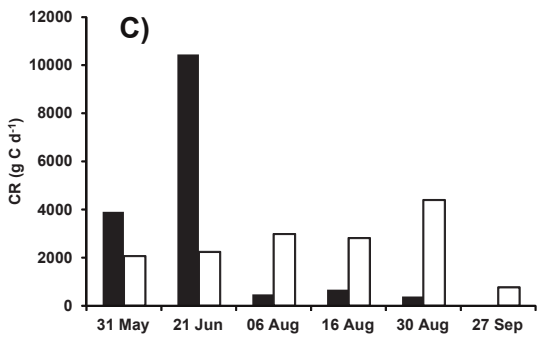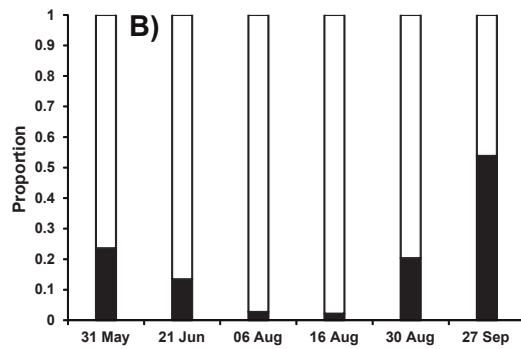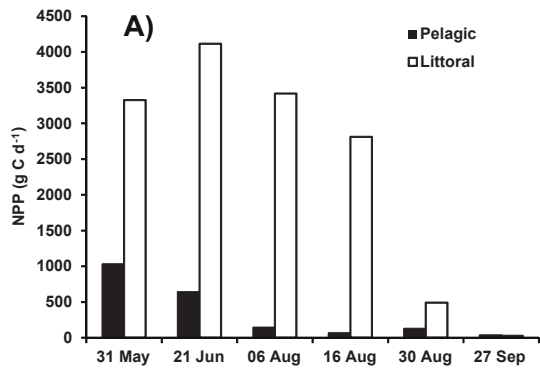


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Figure 4.

670    Figure 5.

# II

# PERIPHYTON SUPPORT FOR LITTORAL SECONDARY PRODUCTION IN A HIGHLY HUMIC BOREAL LAKE

by

Jussi Vesterinen, Jari Syväranta, Shawn P. Devlin, & Roger I. Jones 2016

# III

# EPIPHYTIC BACTERIA MAKE AN IMPORTANT CONTRIBUTION TO HETEROTROPHIC BACTERIAL PRODUCTION IN A HUMIC BOREAL LAKE

by

Jussi Vesterinen, Shawn P. Devlin, Jari Syväranta & Roger I. Jones 2017

Submitted manuscript

# Epiphytic bacteria make an important contribution to heterotrophic bacterial production in a humic boreal lake

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**Abstract** Bacterial production (BP) in lakes has generally been measured only in the pelagic zone without accounting for littoral BP, and studies of BP at the whole-lake scale are very scarce. In the dystrophic humic lakes which are common throughout the boreal region, low light penetration through water has been assumed to seriously limit available habitats for littoral organisms. However, many highly humic boreal lakes have extensive partly submerged vegetation around the lake perimeter which can provide well-lit substrata for highly productive epiphyton. We measured epiphytic BP on the littoral vegetation and pelagic BP in a small highly humic boreal lake in Finland during an open water season and extrapolated the BP rates to the whole-lake. Pelagic BP dominated the combined BP over the study period, but the epiphytic BP contributed an average of 24% to overall BP over the sampling period and and was almost equal to pelagic BP in July. According to these results, a substantial component of BP has been previously overlooked in the lake when BP has been measured only from the pelagic. Our study demonstrates that the role of the littoral zone in bacterial production in highly humic lakes has previously been understated, and needs to be taken into account in assessments of whole-lake carbon cycling and metabolism.
*Keywords: littoral, periphyton, pelagic, autotrophic, heterotrophic*

## Introduction

Pelagic and littoral habitats have generally been studied separately in lake ecosystem and food web research, and only very few studies have examined productivity in both habitats (Vadeboncoeur et al. 2002). Although pelagic and littoral production can be integrated by mobile consumers like fish (Schindler & Scheuerell 2002) and even zooplankton (Van De Meutter et al. 2004) which utilize both pelagic and littoral resources, studies of the magnitudes of primary production (PP) and particularly of heterotrophic bacterial production (BP) at the whole-lake scale and including both pelagic and littoral habitats, are scarce. In highly humic lakes the importance of littoral benthic production has been assumed to be minor due to the very low light penetration into the water (e.g. Vadeboncoeur et al. 2002) together with very steep stratification, which restricts illuminated and oxygenated benthic habitats. However, Vesterinen et al. (2016a) showed that epiphyton on surrounding littoral vegetation dominated the whole-lake PP in highly humic Lake Mekkojärvi in southern Finland,

demonstrating that macrophytes and partly submerged terrestrial vegetation can provide extensive well-lit substrata for epiphyton and make the littoral an appreciable habitat for PP in humic lakes.

Algae and bacteria coexist in periphytic biofilms in an association that offers space and resources to sustain production of both groups of organisms, and positive correlations between periphyton PP and BP, as well as between algal and bacterial biomass, have been well documented (e.g. Neely & Wetzel 1995, Rier & Stevenson 2002, Carr et al. 2005, Kuehn et al. 2014). This can be more pronounced if light is not limiting algal growth and biomass production, when algae produce a substantial extracellular polysaccharide matrix that creates an isolated microenvironment, where inorganic nutrients can be effectively recycled (Wetzel 1993). Highly humic Lake Mekkojärvi has extensive littoral vegetation, which mostly lies just under the water surface in relatively well-lit conditions where it supports thick growths of epiphyton from spring to early autumn (Vesterinen et al. 2016a). In view of the strong correlations found elsewhere between PP and BP, we can expect that littoral epiphytic BP should be high and contribute substantially to whole-lake BP in Mekkojärvi.

Heterotrophic bacteria are known to play a very important role in the carbon flux of aquatic ecosystems, providing a link between autochthonous and allochthonous dissolved organic matter (DOM) and bacterivores (Porter et al. 1988). In humic lakes, most of the DOM is of allochthonous origin which is an important basal resource for both pelagic (Jones et al. 1992, Pace et al. 2004, Jansson et al. 2007) and benthic (Premke et al. 2010, Karlsson et al. 2012) food webs via microbial pathways. However, most studies of bacteria and their productivity in lakes have concerned pelagic bacterioplankton alone and have not measured productivity of bacteria associated with profundal sediments or with periphyton in littoral benthic habitats, where bacterial production (BP) can be of a similar magnitude to, or even higher than that in the pelagic zone (Vadeboncoeur et al. 2002 and references therein). Benthic bacteria often outnumber pelagic bacteria in lakes and rivers creating high spatial variability (Schallenberg & Kalff 1993, Fischer & Pusch 2001), and the fraction of active bacterial cells in the total number of bacteria in sediments and epiphytic biofilms can be much larger than in the pelagic (Haglund et al. 2002). Therefore, measurements of BP in these different habitats are particularly needed in humic lakes, where the importance of the littoral has been understated. Incorporation of littoral and pelagic as integrated habitats into conceptual models of lake ecosystems will contribute to a more comprehensive understanding of trophic dynamics (Vadeboncoeur et al. 2002) and of lake metabolism, which is important in resolving organic carbon budgets in lakes (Hanson et al. 2015, Solomon et al. 2015).We measured BP in the littoral epiphyton and in the pelagic water column several times during an open water period in Mekkojärvi, extrapolated the results to the whole-lake scale and compared the magnitude of BP in the two habitats.

# Material and methods

## Study lake

The study was conducted at Lake Mekkojärvi (61º13'N 25º3'E), a small (0.35 ha) and highly humic headwater lake in the Evo forest region in southern Finland (Fig.1) with mean and maximum depths of 2.0 and 4.3 m. The lake is sheltered by surrounding coniferous forest and receives a high loading of terrestrial organic matter from its catchment causing high dissolved organic carbon (DOC) concentrations (30–33 mg C $L^{-1}$), highly coloured water (300–800 mg Pt $l^{-1}$) and low pH (5.3–5.7) (Devlin et al. 2015, Vesterinen et al. 2016a). This causes the lake to develop very steep temperature and oxygen gradients rapidly after ice-off in spring. Mekkojärvi has ice cover usually from early November until the beginning of May. During the open water period the thermocline lies between 0.5–1.0 m and anoxia occurs under that layer. Mekkojärvi becomes totally anoxic during winter ice cover and therefore cannot sustain overwintering fish populations, which has allowed development of very dense populations of the large-bodied cladoceran *Daphnia longispina* in summer. Mekkojärvi has a depth ratio (DR = $\bar{z}/z_{max}$) of 0.47, so the lake is relatively steep-sided and lacks illuminated benthic surfaces due to the highly coloured water and very low light penetration (light-attenuation coefficient ranges from 4.5 to 7.5). Details of the lake's physical and chemical characteristics are presented elsewhere (e.g. Vesterinen et al. 2016a). Mekkojärvi has been the subject of numerous studies, which have revealed the importance of both allochthonous C and biogenic methane to productivity of the pelagic system (e.g. Salonen & Hammar 1986, Jones et al. 1999, Salonen et al. 2005, Taipale et al. 2008; 2011, Devlin et al. 2015). Bacterial densities are greater in the oxic-anoxic boundary layer in the metalimnion and in the anoxic hypolimnion than in the oxic epilimnion (Arvola et al. 1992). The bacterial community in Mekkojärvi is mainly composed of heterotrophic, chemoautotrophic and photoautotrophic bacteria, including photosynthetic green sulphur bacteria (*Chlorobium* sp.) and methane-oxidizing bacteria (belonging to *Methylobacter* genus) which contribute significantly to the bacterial biomass in the meta- and hypolimnion (Taipale et al. 2009). The littoral zone is not clearly defined in Mekkojärvi, but the lake has a surrounding floating moss mat (consisting mainly of *Sphagnum* and *Warnstorfia* species) lining the lake perimeter, with fallen terrestrial sedges (Carex sp.) and some macrophytes such as *Menyanthes trifoliata*, *Phragmites australis* and *Utricularia* sp. associated with the moss mat. This surrounding littoral vegetation does not extend further than ca. 1 m from the lake edge and not deeper than ca. 0.5 m, but sustains highly productive periphyton assemblages, which have their highest biomass in late-summer and can balance the whole-lake metabolism or even make the lake net autotrophic (Vesterinen et al. 2016a).

## Pelagic bacterial production

Pelagic sampling was carried out at the deepest point in the lake (Fig. 1). Temperature and oxygen concentrations were measured at 0.5 m intervals from the surface to the bottom with an oxygen and temperature sensor YSI 55 probe (YSI Inc., Yellow Springs, Ohio, USA) during every sampling occasion in 2015. From these measurements the water column stratification was defined as follows: 0–0.5 m (epilimnion), 0.5–1.0 (metalimnion), 1.0–3.0 m (hypolimnion).
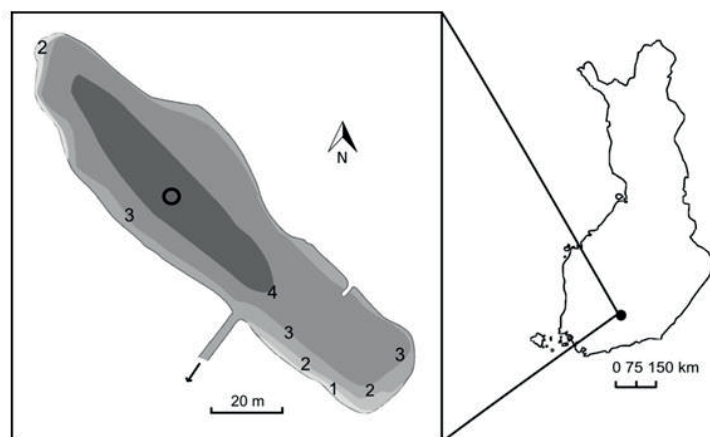
**Figure 1.** Location and bathymetry of Lake Mekkojärvi in southern Finland. Open circle denotes the sampling point for the pelagic measurements. Numbers refer to depth in meters.

Pelagic bacterial production was measured five times between June and October in 2015 using a [14C]-leucine uptake method (Kirchman et al. 1985) slightly modified according to Tulonen (1993). From composite water samples collected from each of the epi- meta- and hypolimnion), triplicate subsamples of 5 mL were transferred to 20 mL pre-ignited glass vials containing 30 nM of [14C]-leucine (specific activity of 0.306 Ci mmol$^{-1}$, Amersham Biosciences) and incubated for 60 min *in situ* in the strata from which they originated. Glutaraldehyde-killed controls were run in parallel. After incubation, all the live samples were killed with glutaraldehyde. In the laboratory, 0.5 mL of ice-cold 50% trichloroacetic acid (TCA) was added into every sample to reach a final concentration of 5%. Samples were then cooled for 15 min followed by filtration onto 0.2 µm pore-size cellulose nitrate filters (Sartorius). The filters were rinsed with 1 mL of ice-cold 5 % TCA and distilled water and then dissolved in 0.25 mL of ethyleneglycolmonomethylether together with 9 mL of liquid scintillation cocktail (OptiPhase 3). The total activity of the added [14C]-leucine was counted from a subsample of 0.5 mL into which 0.5 mL of 1:7-ethanolamine/ethanol absorption liquid was added together with 9 mL of scintillation cocktail. Samples were stored at room temperature for 24 h before their radioactivity was counted with a Packard Tri-Carb® liquid scintillation counter (PerkinElmer, Waltham, Massachusetts, USA).

Leucine incorporation rates ([dpm sample − dpm blank]/total activity of the added leucine) were converted to biovolume by multiplying by 7.71 x 10$^{15}$ (µm$^{-3}$ mol$^{-1}$) and to carbon production by multiplying by a carbon to biovolume ratio of 0.36 pg C µm$^{-3}$. Both factors are appropriate for humic lakes according to their empirical determination in laboratory experiments (Tulonen 1993). Daily BP rates were calculated multiplying hourly rates by 24. Areal BP values were calculated by multiplying the volumetric values by the fraction of each stratum of the water column and summing over depth. These were multiplied by the area of the lake to derive the whole-lake BP values for the pelagic. To test the possible effect on anoxic hypolimnetic BP samples of oxygen contamination from air in the incubation

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vessels, 5 parallel samples were incubated in evacuated Labco Exetainers (Labco Limited, Lampeter, Ceredigion, UK) simultaneously with other hypolimnetic samples in September.

*Littoral epiphytic bacterial production*

Epiphytic BP was measured five times together with pelagic BP in 2015. Littoral water temperatures were measured with a YSI 55 probe (YSI Inc., Yellow Springs, Ohio, USA) during every sampling occasion. Samples of littoral vegetation were collected randomly from 6 sites around the lake into 2 L plastic buckets filled with lake water from each site. As the littoral vegetation in Mekkojärvi consists mainly of moss and partly submerged sedges, these were the main representatives in the samples. Both aged and fresh plant tissues were included in the samples. Some larger plants, such as *Menyanthes trifoliata*, were not sampled, as they were difficult to process in the laboratory. Buckets were stored in a cool box containing lake water and taken to the laboratory of Lammi Biological Station, about 30 km south from Mekkojärvi. BP was measured from epiphytic biofilms using a modified version of the [3H]-leucine incorporation method described by Ask et al. (2009) based on the method originally developed by Smith & Azam (1992). [3H]-leucine was used instead of [14C]-leucine, since it was available at sufficiently higher concentrations. Six randomly selected 1 cm long subsamples of plant substratum from each sampling site were clipped and put into 1.2 mL Eppendorf tubes containing 30 µL of [3H]-leucine (specific activity of 112 Ci mmol$^{-1}$, PerkinElmer, Inc.) and 70 µL of distilled water with the final concentration of 300 nM, and half of the samples were immediately killed by addition of 130 µL of 50% TCA. To determine the appropriate [3H]-leucine concentration and the

maximum incorporation of leucine into protein in epiphytic biofilms, a saturation experiment was conducted once in early-June in which samples were incubated in 7 different concentrations ranging from 30 to 1000 nM. Eppendorf tubes were incubated outside the laboratory in an open cool box containing lake water for 60 min. The samples were submerged at the same depth from which they originated so that they experienced similar light conditions. The temperature of the water was measured during the incubation and no increase above the lake *in situ* temperature was observed. Incubation was then terminated by adding 130 µL of 50% TCA into the live samples and vortexing them. Samples were centrifuged at 12400 rpm for 10 min and the supernatant was gently removed using a thin pipette. No marked loss of epiphyton from the substratum was visible (although this was not confirmed by microscopy). 1.2 mL of 5 % TCA was then added and the samples were again vortexed and centrifuged at 12400 rpm for 10 min. The supernatant was then removed, 1.2 mL of 80% EtOH was added and samples were centrifuged as above. Finally, the supernatant was removed, the sample (plant substratum with associated epiphyton) was aerated and 1.2 mL of scintillation cocktail (OptiPhase 3) was added. Sample radioactivity was counted with a Packard Tri-Carb® liquid scintillation counter (PerkinElmer, Waltham, Massachusetts, USA). Leucine uptake rate was calculated as:

$$\text{mmols leucine (cm substratum}^{-1})\ h^{-1} = (4.5 \times 10^{-13}) \times (\text{dpm sample} - \text{dpm blank}) \times (SA)^{-1} \times (T)^{-1} \quad \text{Eq. 1}$$

, where factor 4.5 x 10$^{-13}$ is the number of curies dpm$^{-1}$ (a constant), SA is the specific activity of the leucine solution in curies mmol$^{-1}$ and T is the incubation time in hours. Bacterial production was calculated as:

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$$mg\,C\,(cm\,substratum^{-1})\,h^{-1} =$$
$$(\text{Leucine uptake rate}) \times 132.1 \times (\%Leu)^{-1}$$
$$\times\,(C\text{:}Protein) \times ID \quad Eq.\,2$$

, where 132.1 is the molecular weight of leucine, (%Leu) is the proportion of leucine in total protein, assumed to be 0.073 (Simon & Azam 1989), (C:Protein) is the ratio of cellular C to protein, assumed to be 0.86 (Simon & Azam 1989) and ID is the isotope dilution factor, which was assumed to be 2 for samples from oligotrophic lakes (Simon & Azam 1989).

Epiphyton was completely removed from 1 cm long randomly clipped substrata (separately from the BP samples) by scraping with a spatula and the substrata were dried in an oven at 60 ºC for 24 h and dry-weight (DW) of substratum [mean ± SE (g DW substratum) cm$^{-1}$] was recorded (0.00105 ± 0.0000876 g, n = 25). BP values were then normalized to mg C g (DW substratum)$^{-1}$ h$^{-1}$. Daily rates were calculated by multiplying hourly rates by 24. We examined how temperature changes during the day might affect the BP rates by using temperature data from a miniDOT Logger (PME Inc. Vista, CA, USA) which was placed in the surface water in the middle of a moss mat in the littoral in Mekkojärvi for 2 months from July to August. Littoral BP values at noon over the sampling period in 2015 plotted against the littoral surface temperature followed an exponential relationship, and that function was used to estimate BP for every hour during the incubation periods on 6 July and 5 August. These values were then summed and compared to the values derived by multiplying noon rates by 24.

Whole-lake estimates for epiphytic BP were derived by first calculating the BP per m lake shoreline using the average DW substratum$^{-1}$ m$^{-1}$ of lake shoreline (42.6 ± 3.4 g DW substratum$^{-1}$ m$^{-1}$), which was calculated by entirely removing the macrophyte and moss vegetation along 40 cm of lakeshore from 24 sites around the lake and dividing the weights by 2 to conservatively estimate the illuminated sides of the flat and horizontally aggregated substrata (Vesterinen et al. 2016a). Substrata were dried in an oven at 60 ºC for 48 h and dry-weight (DW) of substrata were recorded as described for BP samples. The whole littoral epiphytic BP estimates were then calculated by multiplying BP per m lake shoreline by the total shoreline length (320 m).

### Statistical analyses

Repeated measures of analysis of variance (RMA) was used to test the differences in pelagic BP among the sampling occasions (dependent variable/within-subject variable) and between the strata (grouping variable/between-subject factor). Normality and homoscedasticity (Levene's test) of the data were tested before statistical analysis. RMA was also used to test the differences in epiphytic BP among the sampling occasions (dependent variable/within-subject variable). Independent *t*-test was used to test the possible difference in hypolimnetic BP in oxic and anoxic vials. Regression analysis was used to test the relationships between surface temperatures and epilimnetic and epiphytic BP. All the statistical tests were conducted with IBM SPSS Statistics (version 20.0.0.2; IBM, Armonk, New York, USA). All the descriptive statistics are means ± SE if not expressly noted.

## Results

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*Pelagic bacterial production*

The mean $O_2$ concentrations over the study period were $4.1 \pm 0.7$ mg $L^{-1}$ in the epilimnion, $1.2 \pm 0.5$ 7 mg $L^{-1}$ in the metalimnion and $0.8 \pm 0.1$ 7 mg $L^{-1}$ in the hypolimnion. Total pelagic BP was highest in early summer, and decreased steadily towards autumn (Fig. 2). After the early summer peak, BP remained under 20.0 mg C $m^{-2}$ $d^{-1}$ (Fig. 2). Epilimnetic and metalimnetic BP together constituted 85 % of the total pelagic BP in early-June. In July the rates were similar in all the three strata. Hypolimnetic BP increased slightly towards autumn and constituted the largest fraction of total pelagic BP in late-summer and autumn (56–63%). The mean BP over the sampling period was $11.6 \pm 2.0$ mg C $m^{-2}$ $d^{-1}$ in the epilimnion, $5.7 \pm 2.0$ mg C $m^{-2}$ $d^{-1}$ in the metalimnion and $6.0 \pm 1.8$ mg C $m^{-2}$ $d^{-1}$ in the hypolimnion. The change in BP over time was significant (RMA, $F_{4, 24} = 6.0$, $p < 0.01$), as were the interactions between time and strata (RMA, $F_{8, 24} = 4.0$, $p < 0.01$). Tukey's HSD test revealed significant differences in BP between meta- and hypolimnion. Epilimnetic BP appeared to be generally related to the surface temperature (Fig. 3A), but a high value in early summer prevented a significant correlation (exponential regression, $F_{1,3} = 6.320$, $R^2 = 0.678$, $p = 0.087$). No statistically significant difference ($t$-test, $t_6 = -0.606$, $p = 0.606$) was found between values of hypolimnetic BP measured in oxic or anoxic vials (mean values $2.9 \pm 1.3$ mg C $m^{-3}$ $d^{-1}$ in the oxic and $2.0 \pm 1.0$ mg C $m^{-3}$ $d^{-1}$ in the anoxic).

*Littoral epiphytic bacterial production*

Based on the test conducted in early June, saturation of leucine incorporation into protein appeared at 300 nM concentration (Fig. 4) and this concentration was therefore applied in the production measurements. Variability among replicates may be the

result of patchy occurrence of periphyton on the substrata or variability of chlorophyll *a*
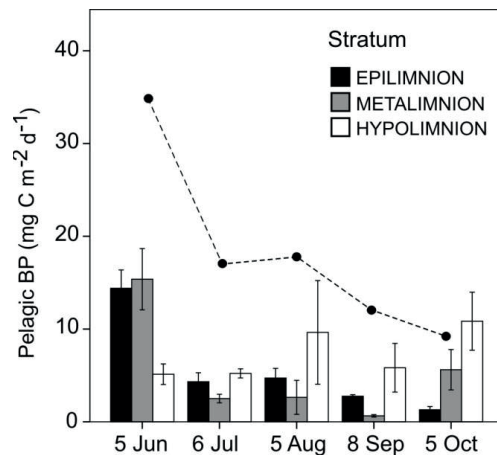


**Figure 2.** Pelagic bacterial production (BP) per unit area (mean ± SE) in three different strata in 2015. The dotted line expresses the areal BP in the whole water column as the sum of values from three strata.

(chl *a*) along the substrata, assuming that there was a positive relationship between the periphyton chl *a* and BP. Epiphytic BP was highest in summer (June and July) and decreased towards autumn (August, September, October; Fig. 5). The change in BP over time was significant (RMA, $F_{4, 68} = 17.6$, $p < 0.01$), and Tukey HSD revealed significant differences between summer and autumn. Epiphytic BP correlated significantly with littoral surface temperature (Fig. 3B; exponential regression, $F_{1,3} = 21.7$, $R^2 = 0.878$, $p = 0.019$).

Daily epiphytic BP in July calculated from the exponential function of BP and temperature (Fig. 3B) and hourly temperatures from the *in situ* data logger was 2.8 mg C (g DW substratum)$^{-1}$ $d^{-1}$, which is only ca. 10% higher than the value estimated multiplying hourly leucine incorporation rates by 24 ($2.6 \pm 0.4$ mg C [g DW substratum]$^{-1}$ $d^{-1}$). In August the similar

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comparison was 2.0 versus 1.5 mg C (g DW substratum)$^{-1}$ d$^{-1}$, a difference of 15%. According to these comparisons, multiplying noon BP h$^{-1}$ by 24 gives slightly lower, and thus more conservative, estimates of the daily epiphytic BP.

### *Whole-lake pelagic and littoral bacterial production*

Whole-lake estimates for pelagic and littoral epiphytic BP revealed that the pelagic dominated the combined BP over the open-water period, contributing over 80% to whole-lake BP in early-June and in October (Fig. 6). Littoral epiphytic BP made the highest contribution to overall BP during summer with the highest value (34.8 g C d$^{-1}$) and contribution (45%) in early-July. The lowest littoral value (4.1 g C d$^{-1}$) and contribution (6%) occurred in October. The mean values of pelagic and littoral epiphytic BP over the sampling period were 63.6 ± 15.6 and 20.5 ± 5.4 g C d$^{-1}$, respectively, and their respective mean proportions of the overall BP were 76 and 24%.
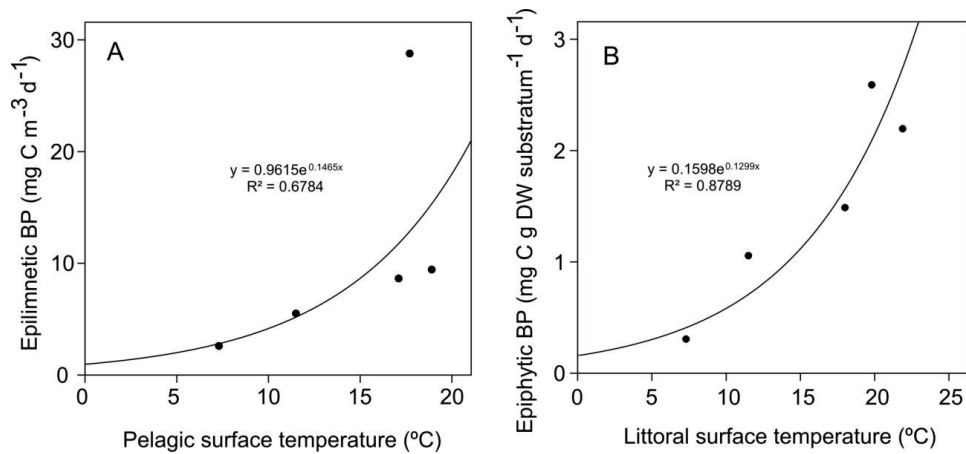


**Figure 3.** Exponential relationships between (A) epilimnetic (pelagic) and (B) littoral epiphytic BP and surface temperature.
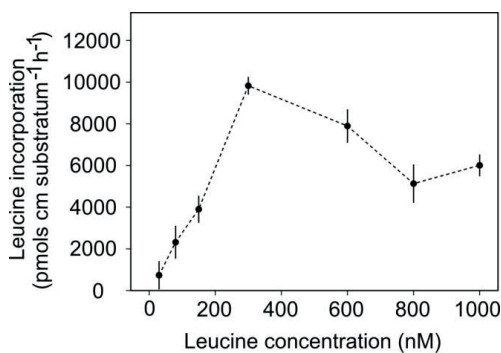


**Figure 4.** The mean ± SE uptake of leucine for epiphytic bacteria in 7 different leucine concentrations.
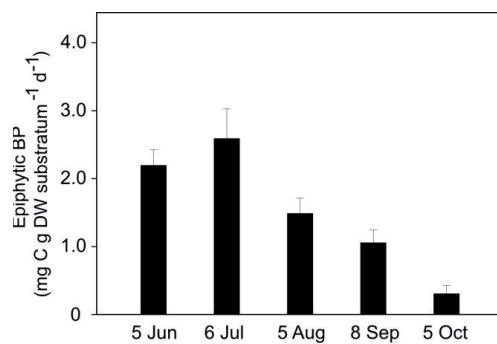
**Figure 5.** Littoral daily (mean ± SE) epiphytic BP in Mekkojärvi, derived from the noon rates by multiplying by 24 and then normalized to g dry-weight of substratum.

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# Discussion

Pelagic BP dominated the combined (pelagic + littoral epiphytic) BP in Mekkojärvi during the study, but the littoral epiphytic BP contributed appreciably, particularly in summer. According to our estimates of the mean whole-lake rates over the sampling period in 2015, around one quarter of the combined (pelagic + littoral epiphytic) BP in Mekkojärvi has been previously overlooked when the epiphyton has not been taken into account. However, the complete whole-lake BP also includes BP of sediment bacteria, which was not measured in this study but can be assumed to make an appreciable contribution to the total BP of the lake, as their production rates can be several times

higher than in the overlying water (Sander & Kalff 1993, Ask et al. 2009). In subarctic oligotrophic Swedish lakes, BP from allochthonous OC by sediment bacteria was found to exceed the combined PP and BP in the pelagic (Ask et al. 2009). However, the chemical and physical conditions in oligotrophic lakes are rather different from those in anoxic dystrophic lakes and presumably have a strong influence on the sediment BP rates. Low $O_2$ concentrations together with high allochthonous inputs have been suggested as the major reason for high burial rates of organic C in northern lakes (Cole 2013).
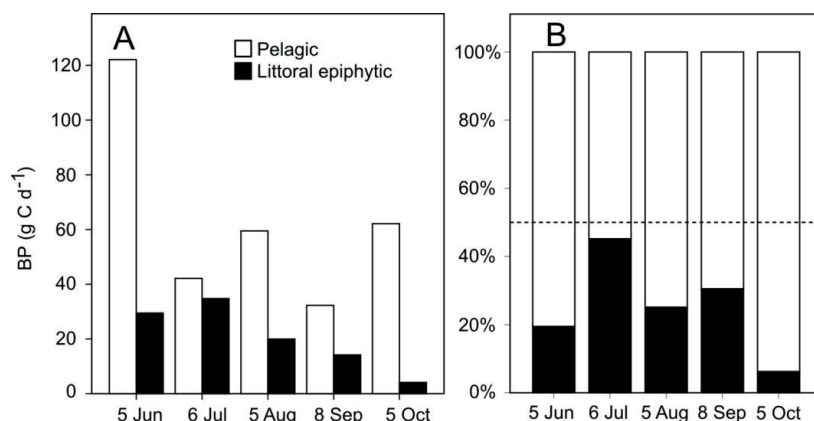


**Figure 6.** A) Whole-lake estimates for BP of pelagic bacterioplankton and littoral epiphytic bacteria and B) their relative proportions.

In our study, littoral BP is represented as epiphytic BP, but it should be noted that the true littoral BP also includes BP in the surrounding water in the littoral, which we did not measure. Considering the higher surface water temperatures in the littoral than in the pelagic and the positive relationship between temperature and BP, together with potentially higher quantities of labile organic compounds, such as

periphytic algal exudates, and nutrients, BP in the littoral water may be higher than that in the pelagic and can potentially increase the contribution of littoral to whole-lake BP in Mekkojärvi. The strictly anaerobic green sulphur bacterium *Chlorobium* is also abundant in the deeper layers in Mekkojärvi (Taipale et al. 2009, Karhunen et al. 2013). As the BP samples in this study were exposed to $O_2$, the contribution of

*Chlorobium* to BP was probably underrepresented in our measurements. However, both the high production of *Chlorobium* and potentially high production of sediment bacteria probably contribute to the very high community respiration rates reported from Mekkojärvi (Salonen et al. 2005, Vesterinen et al. 2016). Despite these gaps, which do not allow us to report total whole-lake BP values, our results clearly highlight how epiphytic BP can be a major part of the whole-lake BP in small humic lakes.

Generally the temperature dependence of BP and growth is modulated by other environmental conditions, such as availability of inorganic nutrients and quality and quantity of organic matter substrates (Apple et al. 2006). The hypolimnetic fraction of the total pelagic BP was clearly higher than the epilimnetic and metalimnetic fractions through the autumn, which is partly explained by the higher volume of water in the hypolimnion, but probably also reflects higher nutrient concentrations in the hypolimnion as reported in previous studies (e.g. Vesterinen et al. 2016a). Temperature in the hypolimnion remains around 4 ºC through the summer whereas surface temperature often rises above 20 ºC. Nutrient concentrations, in turn, remain rather constant in the hypolimnion through the stratification period (Vesterinen et al. 2016a). The study year 2015 can be considered as representative in terms of weather and physical and chemical conditions of the lake, as the summer was not particularly warm or rainy.

The high BP measured in the epilimnion in spring weakened the correlation between epilimnetic BP and the surface temperature, but indicated an association between BP and the phytoplankton PP spring maximum, which has been documented in earlier studies in Mekkojärvi (Salonen et al. 2005, Vesterinen et al. 2016a). These apparently related production maxima of both groups of organisms may reflect exploitation by both groups of a pulse of nutrients from the catchment with snow-melt in spring. Alternatively or additionally, it may be a result of bacterial stimulation by labile autochthonous OM released by phytoplankton, which couples BP with PP. Such positive relationships between pelagic BP and PP and chlorophyll are well-documented (e.g. White et al. 1991, Cole et al. 1988, Kritzberg et al. 2005). However, in DOC-rich Mekkojärvi, the low concentrations of inorganic nutrients, and thus restricted resource stoichiometry (i.e. high C:N:P ratio), for actively growing bacteria have been suggested to limit the bacterial production on labile carbon substrates, such as algal exudates, in the pelagic (Dorado-García et al. 2016). Therefore, higher nutrient availability, reflected also in enhanced PP, appears the more likely explanation for high pelagic BP in spring. Epiphytic BP in the littoral correlated clearly and positively with the surface temperature, and the correlation was stronger than between pelagic BP and temperature in the epilimnion. As the temperature logger data from the sampling occasions on July and August allowed us to calculate the BP estimates for each hour on those days, which were within 10–15% of those calculated multiplying by 24, the surface temperatures at noon were apparently close to the average daily surface temperatures. However, the production by epiphytic heterotrophs is also light-mediated and associated strongly with the epiphytic PP. Kuehn et al. (2014) found 60% higher production rates in litter-associated bacteria which were exposed to light than those which were in dark. If we assume a similar relationship between PP and BP and that a similar difference is applicable to periphyton

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in Mekkojärvi, then the epiphytic BP rates would be 60% lower during the night. However, day lengths in our study area range from 19.5 h in June to ca 10 h in October, so the photoperiod is long during summer months and epiphyton is exposed to light for most of the day. In autumn, in turn, day lengths are shorter but also the PP by epiphyton is low (Vesterinen et al. 2016a) and, like the epiphytic BP, shows a trend of decreasing towards autumn. How much this light-dependent variation might truly affect epiphytic BP in Mekkojärvi, and in highly humic lakes in general remains speculative.

The extent to which benthic bacteria in lakes use organic C of allochthonous or autochthonous origin remains poorly known. In periphytic matrixes, the dissolved organic carbon pool is a mixture of extracellular release from macrophytes, excretion of both attached algae and bacteria, decomposition products following autolysis of epiphytes and dissolved carbon compounds of both autochthonous and allochthonous origin (Allen 1971, Attermeyer et al. 2014). The relative importance of these compounds likely varies between periphytic groups colonizing different habitats, e.g. between epiphyton, epilithon and epipsammon. Ask et al. (2009) showed that, although sediment bacteria in clear-water Swedish lakes were mainly fuelled by benthic PP, allochthonous C made a substantial contribution to the benthic BP. In contrast, Rodríguez et al. (2013) reported that benthic autochthonous OC supported pelagic BP in a small clear-water lake. Allochthonous C is more accessible to sediment bacteria than to epiphytic bacteria due to high burial rates of allochthonous OM in lake sediments particularly in smaller lakes (Cole 2013). In small humic lakes, where non-illuminated sediments lack benthic autotrophic production, all potential autochthonous C for sediment bacteria comes from the upper water layers and will be at least partly decomposed by pelagic bacteria. Therefore, allochthonous C is presumably more important for sediment bacteria in small humic lakes. As only a small part of the total epiphyton biomass in the littoral is grazed by littoral invertebrates during the summer (Vesterinen et al. 2016b), the remaining biomass is presumably decomposed in the water column and may contribute to the relatively high hypolimnetic BP in the autumn.

Wetzel & Søndergaard (1998) described how macrophytes provide an extensive and diverse three-dimensional habitat for microbial colonization, which results in a shift from dominance of the macrophytes to the very high productivity of the attached microbiota. Theil-Nielsen & Søndergaard (1999) described epiphytic biofilms as "hotspots" for BP, exploiting exudation of DOC from macrophytes and epiphyton. Photolysis of recalcitrant allochthonous DOM can produce labile organic molecules that are more available for heterotrophic bacteria (Wetzel et al. 1995, Paul et al. 2012). Since we only measured production of the epiphytic bacteria in Mekkojärvi, we cannot distinguish between autochthonous and allochthonous C sources supporting BP. As the littoral ambient water is brown with high quantities of allochthonous DOM (Kairesalo et al. 1992) bacteria may utilise that directly and after photolysis. However, considering the probable substantial release of labile autochthonous C from the highly productive epiphytic biofilms in the littoral (Vesterinen et al. 2016a), the bacteria are likely to rely heavily on autochthonous C. A light-mediated biotic decomposition process via algal stimulation of litter-associated microbial heterotrophs has recently been recognized (e.g. Francoeur et al. 2006, Danger et al. 2013). Kuehn et al. (2014) studied this process and concluded that periphytic algae function as a photosynthetic

conduit for labile carbon supply to microbial heterotrophs (bacteria and fungi) over very short time intervals, demonstrating the important role of bacteria and fungi in this light-mediated carbon cycling process.

Vesterinen et al. (2016a) demonstrated how the littoral in Mekkojärvi was strongly net autotrophic and reported $364 \pm 66$ mg C (g DW substratum)$^{-1}$ d$^{-1}$ as the mean PP by epiphyton in Mekkojärvi in 2012. Comparison to the mean daily epiphytic BP of $1.52 \pm 1.36$ mg C (g DW substratum)$^{-1}$ d$^{-1}$ in this study reveals the strong dominance of autotrophic production in the biofilms in the littoral and large quantities of autochthonous C potentially available for secondary production. A similar comparison between pelagic PP (in 2012) and BP (in 2015) reveals that the PP during the phytoplankton spring maximum can be 10 times higher than BP but the rates later in summer and autumn can be very even. Since strong overall net heterotrophy associated with very high bacterial respiration has been demonstrated in the pelagic in Mekkojärvi (Salonen et al. 2005, Vesterinen et al. 2016a), it is likely that both anaerobic bacteria (e.g. green sulphur bacteria) and sediment bacteria contribute strongly to the whole-lake metabolism. However, comparison of PP and BP measured in different years can only be considered indicative. We did not measure epiphytic PP or chl *a* in this study, but comparison between the PP rates and chl *a* in the epiphyton in Mekkojärvi in 2012 (Vesterinen et al. 2016a) and BP in this study reveals a similar trend of increase from spring to late summer and then decrease towards autumn. This also indicates the possible positive relationship between the epiphytic BP and the autochthonous C produced by epiphyton. However, the relative concentrations of OC originating from internal net primary production (NPP) versus allochthonous OM

loading to lake metabolism remains unresolved (Hanson et al. 2015).

There are various sources of error and uncertainty included in any studies which attempt to upscale rate estimates made in bottle incubations to the whole-lake scale (Hanson et al. 2015). Estimation of the variability in whole-lake extrapolation is challenging due to high spatial heterogeneity and complex interactions (Pace 2001). Even pelagic production rates can have high spatial variability (Van de Bogert et al. 2007), but a particular challenge for estimating the error for littoral epiphytic BP is associated with the variability of substrata around the lake. Mekkojärvi, however, is a very small lake and has a relatively uniform basin morphometry, so whole-lake extrapolations are likely to yield better constrained estimates than for larger lakes with highly variable morphometry. As the quantification of available substrata for epiphyton along the lake was done from 24 sites, which can be considered a rather high number of replicates around this small lake (Vesterinen et al. 2016a), it can be expected to have yielded a rather reliable estimate for the mean substratum weight per metre of lake shore. Since the substrata mostly consisted of flat and horizontally aggregated plant pieces, the weight was divided by two to estimate the illuminated side of substrata. Furthermore, over half of the collected substrata had substantial development of epiphyton, so this approach likely yielded a conservative estimate of available substrata. Although the BP samples were collected from only six randomly selected sites around the lake to keep the workload reasonable, the spatial distribution of different plant species around the lake was well represented in the samples, which consisted mainly of two dominant plant groups, sedges and mosses. Some larger plant species (such as *Phragmites australis* and *Menyanthes trifoliate*), which have a patchy appearance

around the lake shore, were not sampled due to their large size and the difficulties of incubating representative tissue samples with attached epíphyton in Eppendorf tubes. Altogether, these uncertainties demonstrate the very real challenges to obtaining reliable quantitative estimates from highly complex littoral environments, which have contributed to the underrepresentation of littoral zones in lake studies (Vadeboncoeur et al. 2002). Whole-lake extrapolation approaches need to be treated with caution as small changes in the factors used can potentially change the littoral contribution from "important" to "dominant". As our approach is likely to have yielded a conservative estimate, the essential message of high but often overlooked littoral BP is not altered. However, these issues are important to recognize for development of more reliable approaches to study whole-lake processes.

In conclusion, our study shows that littoral epiphytic bacteria can make a significant contribution to whole-lake BP in humic lakes and, together with previous findings of highly productive photosynthetic epiphyton in the littoral in Mekkojärvi (Vesterinen et al. 2016a), demonstrates the importance of the littoral zone in the biomass production and C cycle in highly humic lakes, at least in the small humic lakes like Mekkojärvi that are so abundant throughout the boreal region and contribute substantially to greenhouse gas emissions (Raymond et al. 2013, Holgerson & Raymond 2016). Although it is reasonable to suppose that in Mekkojärvi labile autochthonous C produced by epiphytic algae is an important source for closely associated bacteria, our study does not provide direct evidence of this phenomenon. This question merits future study in which more sophisticated whole-lake scale approaches, which account for both pelagic and littoral habitats, can address the role of the littoral zone in humic lakes.

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# IV

# INFLUENCE OF LITTORAL PERIPHYTON ON WHOLE-LAKE METABOLISM RELATES TO LITTORAL VEGETATION IN HUMIC LAKES.

by

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# Influence of littoral periphyton on whole-lake metabolism relates to littoral vegetation in humic lakes

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**Abstract** The role of littoral habitats in lake metabolism has been underrated, especially in humic lakes, based on an assumption of low benthic primary production (PP) due to low light penetration into water. This assumption has been challenged by recent recognition of littoral epiphyton dominance of whole-lake PP in a small highly humic lake and of epiphyton as an important basal food source for humic lake biota. However, as these studies have mostly concerned single lakes, there is a need to test their wider generality. We studied the whole-lake PP and community respiration (CR) in eight small humic lakes in southern Finland during July 2015 using $^{14}C$ incorporation to measure pelagic PP and the changes in dissolved inorganic carbon in light and dark in situ incubations to measure CR and littoral PP by epiphyton. Changes in $O_2$ concentration in both pelagic and littoral surface water were measured periodically from each lake and, additionally, continuously with a data logger from one lake during the study period. The results revealed that the littoral dominated whole-lake net primary production (NPP) in five of the eight lakes, which was consistent with observed $O_2$ supersaturation in the littoral surface water in most of the lakes. Calculated pelagic:littoral ratios by area correlated negatively with both littoral NPP and littoral contribution to whole-lake NPP. Moreover, there was a significant positive relationship between littoral NPP and the fraction of lake surface area covered by littoral vegetation. This demonstrates that increased surrounding littoral vegetation cover increases the overall importance of the littoral to whole-lake PP in highly humic lakes. Littoral NPP also correlated strongly with littoral $O_2$ saturation, and the continuously measured $O_2$ revealed substantial temporal variation in $O_2$ saturation, particularly in the littoral zone. Whole-lake gross primary production:community respiration (GPP:CR) ratios revealed that accounting for littoral metabolism produced a marked shift towards lake metabolic balance, although seven of the eight lakes remained net heterotrophic. This study emphasises that littoral metabolism needs to be accounted for when estimating whole-lake C fluxes in all lakes, even in highly coloured humic waters.
*Keywords:* Epiphyton, lake metabolism, whole-lake primary production, community respiration, boreal lakes, lake browning

# Introduction

Most studies of production in lakes have concerned only the pelagic habitat without measuring littoral autotrophic production and metabolism (Vadeboncoeur et al. 2002, Cantonati and Lowe 2014). Of the few studies covering both habitats most have focused either on eutrophic or clear oligotrophic lakes (Vadeboncoeur et al. 2003, Anderson and Brunberg 2006, Ask et al. 2009a, Devlin et al. 2015), where littoral autotrophy can make a substantial contribution to whole-lake primary production (PP). If lakes are steep-sided, general models of benthic algal production, based on variables such as light, basin morphometry and nutrients, yield a negligible benthic contribution to whole-lake PP (Vadeboncoeur et al. 2008). However, these models do not account for the surrounding aquatic and submerged terrestrial vegetation which varies greatly between lakes and is important for many interactions in lake communities (Jeppesen et al. 2002).

In highly humic lakes the role of the littoral zone in whole-lake PP has been particularly underrated based on an assumption of low benthic PP due to the very low light penetration into water. Many boreal lakes have high concentrations of allochthonous dissolved organic matter (DOM) rich in humic substances, which restrict light penetration to the uppermost water layers and absorb heat very efficiently, resulting in steep vertical temperature and $O_2$ gradients. While allochthonous DOM directly controls light and temperature conditions, it also affects PP, metabolic rates and several other fundamental functions in lake ecosystems (Solomon et al. 2016). Besides constraining intrinsic PP (Karlsson et al. 2009), incorporation of allochthonous organic carbon into lake food webs increases community respiration (CR), which is often reported to exceed gross primary production (GPP) and make lakes net heterotrophic (Del Giorgio and Peters 1994, Duarte and Agusti 1998). Small boreal lakes are predominately shallow due to flat topography, and allochthonous inputs are diluted in a small volume of water, resulting in higher water colour than in deeper lakes (Kortelainen et al. 1999). Many surface waters of glaciated landscapes across North America and Europe have experienced an increase in dissolved organic carbon (DOC) concentration, which is expected to continue in the future (e.g. Monteith et al. 2007, Solomon et al. 2016). As this lake "browning" further limits light availability in lakes, it presumably has a negative effect on benthic PP.

Small humic boreal lakes often have surrounding moss stands and submerged terrestrial vegetation among other emergent, floating and submerged aquatic macrophytes, and this surrounding vegetation has been reported to have expanded over recent decades in Finnish lakes (Rintanen 1996). These vegetation stands can provide extensive substrata for epiphyton growth in relatively well-illuminated conditions just under the water surface. Vesterinen et al. (2016a) recently reported that PP of epiphyton on the littoral vegetation dominated the summer whole-lake PP in a small humic lake. Concurrent measurements of CR revealed that the littoral was strongly net autotrophic, to the extent of being able to balance the whole-lake metabolism and even make the lake net autotrophic occasionally during the open water period. Lakes of similar size are very common throughout the boreal zone (Downing et al. 2006, Verpoorter et al. 2014, Messager et al. 2016), while globally the estimated total number of lakes with surface area from 0.1 to 1 ha is $2.77 \times 10^8$ according to Downing et al. (2006) or slightly lower according to Verpoorter et al. (2014). In the long term, expansion of littoral vegetation cover (Rintanen 1996) together with postglacial land uplift (Ekman 1991)

may offset the negative effect of browning on benthic PP in boreal lakes by providing more extensive well-lit substrata for attached algae.

Hence the widely held view of a very low contribution of littoral production to whole-lake PP is being challenged by recent studies. However, as the evidence derives largely from studies of individual lakes there is a clear need to test the wider generality of the findings. In this study we investigated the prevalence of highly productive littoral habitats in several small humic lakes in southern Finland. We studied the magnitudes of autotrophic production and respiration in both the pelagic and littoral habitats the lakes during July 2015, extrapolated to the whole-lake scale and related the values to different environmental variables. We predicted that the littoral epiphyton would make a substantial contribution to whole-lake PP and strongly influence the apparent whole-lake metabolic balance, but that the littoral contribution amongst the lakes would decrease as a function of increasing ratio of pelagic to littoral area.

## Methods

### *Study lakes*

This study was conducted between 8 and 22 July 2015 and encompassed eight lakes located in the Evo forest area in southern Finland (an area of ca. 18 km$^2$ between 61° 10' and 61° 13' N and 25° 5' and 25° 12' E; Fig. 1). This area contains mainly small humic headwater lakes inter-connected by brooks and forming a network of lakes in the landscape (Järvinen et al. 2002, Vehkaoja et al. 2015). Most of these lakes have one or more inflows and one outflow and are predominantly fed by surface runoff, while a smaller fraction of the lakes are seepage lakes (Arvola et al. 2010).
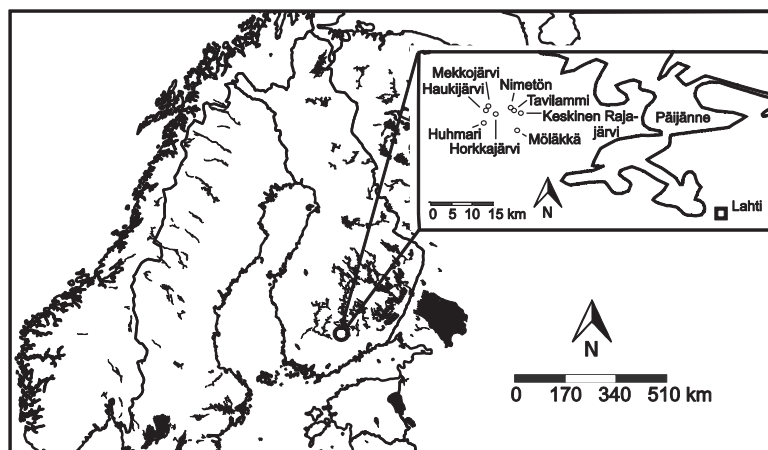


**Figure 1.** The location of the eight study lakes in southern Finland (map derived following Shorthouse 2010).

The study lakes are small (mean ± SE surface area 1.0 ± 0.2 ha) and highly humic (mean ± SE water color 280 ± 57 mg Pt L$^{-1}$). The lakes are surrounded by coniferous forest with patches of deciduous trees. There is no agricultural activity remaining in the study area, and the development of the forests in Evo is strongly influenced by commercial forestry. The lakes have ice cover usually from early November until the beginning of May. Being highly colored, light penetration is restricted to the uppermost water layers, and steep temperature and $O_2$ gradients form very rapidly in the water

column after ice-off in spring, with anoxia developing in the hypolimnion in every study lake.

Most of these lakes are steep-sided and have very little illuminated benthic area, but some do have some shallower areas close to the lake edges, where light can penetrate to the bottom. Littoral areas consist mostly of surrounding aquatic vegetation and associated epiphytes, which vary among the lakes. A peripheral floating moss mat (mostly of *Warnstorfia* and *Sphagnum* species) is a predominant characteristic in Lakes Mekkojärvi, Möläkkä and Nimetön. Littoral areas in these lakes also comprise sedges (*Carex* sp.), which are partly fallen into the lake, and some other floating, emergent and submerged macrophytes, such as *Menyanthes trifoliata*, *Phragmites*

*australis* and *Utricularia* sp. Lakes Horkkajärvi and Tavilammi have the most bare rocky shore and the least vegetation with some patchy appearance of emergent and floating macrophytes. Lakes Haukijärvi, Huhmari and Keskinen Rajajärvi can be considered as intermediate in terms of vegetation cover, which consists mostly of some floating, emergent and submerged plants, but without such an extensive moss bed as in Mekkojärvi and Nimetön. All the study lakes, except for Keskinen Rajajärvi, have been monitored ca. twice per year (in spring and in autumn) by staff from nearby Lammi Biological Station so that some additional chemical and physical parameters not measured in this study were available. More characteristics of the study lakes are presented in Table 1.

**Table 1.** Some characteristics of the eight study lakes.

| Lake | Sampling date | SA (ha) | CA (ha) | Max. depth (m) | Mean depth (m) | P:L ratio | pH [1] | Color (mg Pt L⁻¹)[1] | Tot-N (µg L⁻¹)[1] | Tot-P (µg L⁻¹)[1] | DOC (mg L⁻¹)[2] | Chl *a* (mg m⁻³)[3] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mekkojärvi | 8.7.2015 | 0.4 | 24.8 | 4.3 | 2.0 | 3.0 | 5.9 | 429.0 | 635.0 | 16.0 | 24.0 | 3.0 |
| Horkkajärvi | 9.7.2015 | 1.1 | 62.1 | 12 | 7.7 | 19.8 | 5.9 | 402.0 | 745.0 | 21.0 | 27.0 | 5.0 |
| Huhmari | 13.7.2015 | 1.1 | 102.0 | 8 | 4.2 | 5.5 | 6.4 | 34.0 | 265.0 | 8.0 | 6.0 | 7.0 |
| Möläkkä | 14.7.2015 | 0.7 | 4.4 | 15 | 6.0 | 5.4 | 4.6 | 233.0 | 593.0 | 23.0 | 17.0 | 5.0 |
| Nimetön | 15.7.2015 | 0.4 | 32.3 | 11 | 8.6 | 2.9 | 4.6 | 449.0 | 758.0 | 18.0 | 30.0 | 2.0 |
| Tavilammi | 16.7.2015 | 0.8 | 18.1 | 7 | 5.2 | 8.9 | 4.6 | 199.0 | 680.0 | 15.0 | 14.0 | 11.0 |
| Keskinen Rajajärvi | 21.7.2015 | 1.5 | 109.9 | 12 | 6.6 | 4.0 | n/a | n/a | n/a | n/a | n/a | 15.0 |
| Haukijärvi | 22.7.2015 | 2.3 | 661.3 | 8.5 | 3.8 | 8.4 | 6.5 | 216.0 | 524.0 | 13.0 | 22.0 | 7.0 |

SA = Surface area, CA = Catchment area, P:L ratio = Pelagic:Littoral ratio by area, [1] = Sampled on 3.11.2015,

[2] = Sampled on 27.-28.11.2014, [3] = measured from epilimnion

### *Physical parameters*

Light attenuation was measured at 0.5 m intervals from the surface to the bottom in the pelagic area of every study lake using a single planar LI-COR (Li-192) Underwater Quantum light sensor (LI-COR Inc., Lincoln, Nebraska, USA). Temperature and $O_2$ concentrations were measured at 0.5 m intervals from the pelagic areas and

at six randomly selected sites from the littoral areas with a YSI 55 $O_2$ and temperature sensor (YSI Inc., Yellow Springs, Ohio, USA). In Mekkojärvi, two miniDO$_2$T data loggers (PME Inc., Vista, California, USA) were used to record littoral and pelagic surface temperatures and $O_2$ concentrations continuously during July. One data logger was placed above the moss mat (but under the water surface) in the littoral and the other was in the pelagic

ca. 1 m from the edge of the littoral vegetation. Daily irradiance values (measured with a Kipp & Zonen B.V., model CM11; Delft, The Netherlands) and day lengths were obtained from the Finnish Meteorological Institute measurement station in Jokioinen, ca. 110 km west from our study area in Evo. The area of littoral vegetation mat in each study lake was calculated from a satellite image using an area calculator tool (in Retkikartta.fi, © Metsähallitus, MML, CGI, 2016) and this was used for estimating pelagic:littoral ratios (P:L ratios) by area for each lake.

### *Pelagic chlorophyll a, phytoplankton primary production and community respiration*

Chlorophyll *a* (chl *a*) was measured from 0.2–0.5 m depth (representing the epilimnion) from each lake. Samples were filtered onto Whatman GF/C glass fiber filters and chl *a* was measured using a Shimadzu UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) according to a standard spectrophotometric method SFS 5772 (Finnish Standards Association, Helsinki, Finland).

Pelagic PP was measured according to the $^{14}C$ incorporation method described by Schindler et al. (1972). Composite water samples were taken with an acrylic tube sampler Limnos (Limnos Ltd., Komorów, Poland) by combining three pulls from each of four strata corresponding to surface, epi-, meta- and hypolimnion in each lake, which were determined according to $O_2$ measurements. Subsamples were decanted into 20 mL glass vials containing $NaH^{14}CO_3$ with four replicates (one dark sample in each depth) and were then incubated *in situ* in each stratum for 24 h. The $^{14}C$ activity was measured with a Packard Tri-Carb® liquid scintillation counter (PerkinElmer, Waltham, Massachusetts, USA). 24 h incubations were expected to yield PP values which approximate most closely to

net primary production (NPP) (Salonen and Holopainen 1979, Marra 2009). PP of the water column was converted from volumetric basis to areal basis by multiplying the values from each stratum by the total depth of the stratum and then summing over the whole water column. Whole-lake daily PP was estimated by multiplying the daily areal values by the lake surface area.

Pelagic CR was measured concurrently with PP as the increase of dissolved inorganic carbon (DIC) concentration during dark incubations. Subsamples (two initials and two samples for dark incubations) were taken from the composite water samples from each stratum (surface, epi-, meta- and hypolimnion) into 50 mL glass BOD bottles. Half of the bottles were wrapped in aluminium foil, put into light-impermeable tubes (filled with water) and incubated *in situ* in each stratum for 24 h. The other half ($t_{0h}$) were placed on ice and taken immediately to the laboratory, where DIC was measured with a DIC-analyser (Salonen 1981), and this was repeated for $t_{24h}$ samples the next day. The areal values for CR were calculated by multiplying the average volumetric values by the mean depth, and the whole-lake estimates were calculated as for PP. Pelagic GPP was calculated as the sum of NPP and CR in the photic zone, assuming that most of CR in the photic zone was attributable to primary producers.

### *Littoral primary production, community respiration and periphyton biomass*

Periphyton PP and CR in littoral habitats were measured concurrently with the pelagic measurements from the difference of DIC during *in situ* incubations of epiphyton samples in both dark and transparent 20 mL glass vials, where consumption or production of DIC over time indicates either production or respiration. Following the methods described by Vesterinen et al. (2016a), we

mostly sampled sedges and other plant substrata which had started decaying, as these were effectively metabolically inert and easier to process compared to the more complex moss substratum. The samples were collected from six randomly selected sites around the lake shoreline by clipping randomly chosen pieces of plant substratum (four replicates per site) into 20 mL glass vials and filling with surrounding littoral water. Trial incubations of bare littoral water had not revealed substantive changes in DIC concentrations over time (Vesterinen et al. 2016a). Surrounding littoral water was sampled from each site to obtain initial DIC concentrations. Periphyton samples were incubated *in situ* for 2 h around noon, half of the samples in the dark conditions and the other half in the surface water under the same light condition as those from which the periphyton originated (barely submerged under the water surface). After the incubations, the samples were placed on ice in a cool box and taken to the laboratory where DIC concentrations were measured immediately with a DIC-analyzer (Salonen 1981). Periphyton was then entirely removed from the substrata of six randomly selected samples by scraping with a spatula and the samples were filtered onto glass fiber filters (Whatman GF-F). Chl *a* contents in these were measured as described above for phytoplankton, and the periphyton biomass was estimated as the total chl *a* per dry weight (DW) of substratum in a sample. Substrata were dried at 60 ºC for 24 h and dry mass was recorded.

Gross primary production (GPP) of periphyton was calculated from the difference in DIC between light and dark samples (GPP = $DIC_{dark}$ − $DIC_{light}$ / incubation time) and the CR from the differences between dark and initial samples ($DIC_{dark}$ − $DIC_{initial}$ / incubation time). NPP was then calculated as NPP = GPP − CR, assuming that most of the CR is attributable to primary producers. The values of each sample were normalized to

mg C g DW substratum$^{-1}$. To estimate the whole-lake littoral PP, the availability of littoral substrata at each lake was quantified according to Vesterinen et al. (2016a). The littoral substratum along 50 cm of lakeshore from six sites around the lake was entirely removed, carefully collected and dried at 60 ºC for 48 h. Light penetration into each lake was used to determine the depth to which submerged and emergent macrophytes were removed (the depth reached by 1% of incident photosynthetically active radiation). The average DW of littoral substratum per meter of lake shore was calculated for each lake and, as the substratum consists mainly of flat and horizontally aggregated macrophytes and moss leaves, the weight was divided by two in order to estimate the mass of the illuminated side of the substratum. This method was evaluated by Vesterinen et al. (2016a) for Lake Mekkojärvi and concluded to be conservative. Daily PP was calculated according to Vesterinen et al. (2016a) using Eqn. 1, which is a modified version of the calculation described by Vadeboncoeur et al. (2008). For whole-lake estimates, PP per metre of lake shore was calculated and multiplied by the total shoreline length of each lake. Daily estimates for periphyton respiration were calculated by multiplying the mean hourly values around noon by 24.

$$\text{Periphyton PP}^{-day} = \Delta t \sum_{sunrise}^{sunset}[PP_{max} \quad \tanh(I_{zt}/I_k)] \quad \text{(Eqn 1)}$$

where $\Delta t$ is the time increment (h), $PP_{max}$ is the light-saturated primary production right under the water surface at noon (mg C g dry-weighted substratum$^{-1}$ h$^{-1}$), tanh is the hyperbolic tangent function, $I_{zt}$ is the light at depth z ($\mu$mol m$^{-2}$ s$^{-1}$) and time t (h) and $I_k$ is the light intensity for onset of saturation, which was set to 300 $\mu$mol m$^{-2}$ s$^{-1}$ according to Hill (1996). The irradiance data and day lengths were obtained from the Finnish Meteorological Institute in Jokioinen. To define $I_z$, the photosynthetically active radiation (PAR)

on the surface was multiplied by the fraction of light at the incubation depth in each lake.

*Statistical analyses*

Welch's *t*-test was used to test for differences in pelagic and littoral NPP and CR and periphyton chl *a* between the lakes, when sample sizes were unequal. Log-transformation was used for data with unequal variances, and the Tukey post-hoc test was used to explore which means were significantly different from each other. Regression analysis was used to test the relationships between epilimnetic NPP and chl *a*, periphyton NPP and periphyton chl *a*, littoral NPP and P:L ratio, littoral proportion of whole-lake NPP and P:L ratio, and littoral $O_2$ saturation and littoral NPP. All the statistical tests were conducted with IBM SPSS Statistics (version 20.0.0.2; IBM, Armonk, New York, USA). The daily estimates of periphyton PP (Eqn 1) were performed using R project for Statistical Computing version 3.3.2 (R Core Team, 2016). All the descriptive statistics are reported as means ± SE if not expressly noted.

# Results

*Physical parameters in pelagic and littoral areas*

Light attenuation was rapid in all the lakes (Fig. 2A), with the depth of 1% of surface irradiance being between 0.5 and 1.0 m. Surface water temperatures in the pelagic areas were similar in all eight lakes during the study period (mean 18.1 ± 0.31 ºC) and temperature gradients were steep in all the lakes (mean temperature at 2 m depth 9.0 ± 0.91 ºC). Littoral surface temperatures were similar in all the lakes with a mean of 20.6 ± 0.30 ºC), and on average 2.5 degrees higher than in the pelagic.
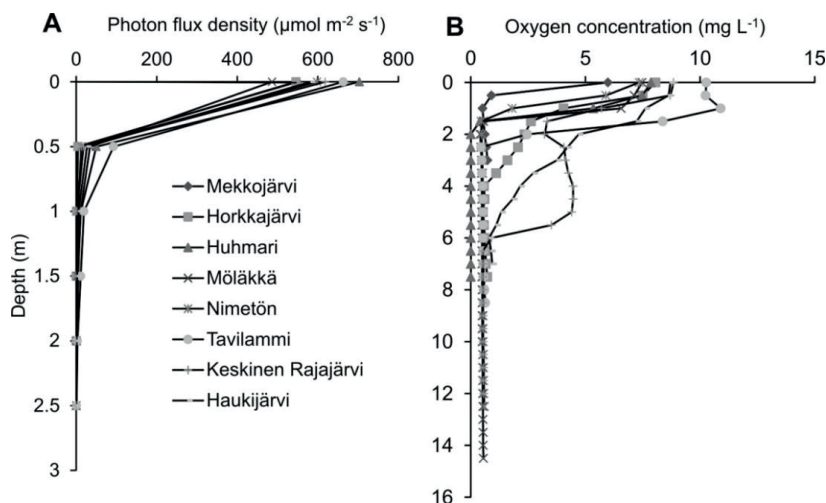


**Figure 2.** A) Light attenuation in the study lakes in July 2015 when incident light at the lake surface was set to 772 µmol m$^{-2}$ s$^{-1}$. B) Oxygen concentrations in the study lakes in July 2015.

Oxygen concentration gradients were also similarly steep in all the lakes (Fig. 2B). However, Tavilammi, Keskinen Rajajärvi and Haukijärvi had the highest $O_2$ saturation in the surface water (over 90%), and Tavilammi was the only lake in which $O_2$ supersaturation was recorded in the pelagic (Fig. 3A). In contrast, clear $O_2$ supersaturation was observed in the littoral areas of all the lakes except Horkkajärvi

and Haukijärvi. Logger data for $O_2$ saturation in Mekkojärvi during July revealed that the littoral was often strongly supersaturated at noon but $O_2$ decreased to almost zero during night (Fig. 3B). The pelagic logger, which was only ca. 1 m from the edge of the littoral moss mat, showed a similar diel pattern but with clearly lower maximum and higher minimum values, as the $O_2$ saturation usually remained under 80% at noon and above 30% at night.

The calculated P:L ratios for each lake are presented in Table 1. The mean P:L ratio was 7.2 ± 1.9. The lowest P:L ratios, and, thus the most extensive potential aquatic vegetation cover, were found from Nimetön and Mekkojärvi, and the highest P:L ratio and the least extensive potential vegetation cover from Horkkajärvi.
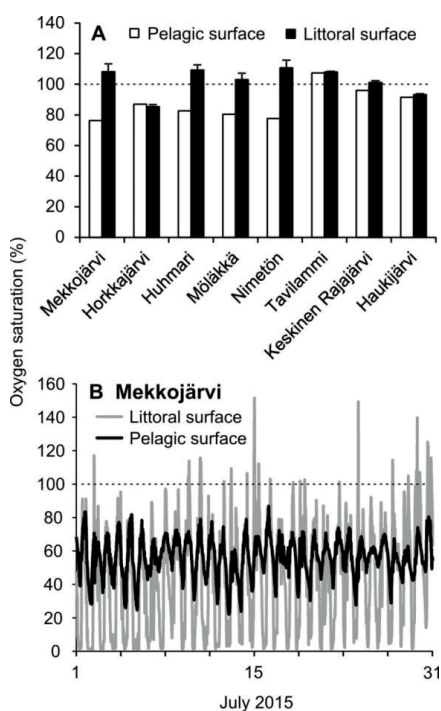


**Figure 3.** A) Oxygen % saturation in the pelagic and littoral areas (mean ± SE, $n$ = 6) in the study lakes in July 2015. B) Logger data of oxygen % saturation in the littoral and pelagic areas in Mekkojärvi during July 2015.

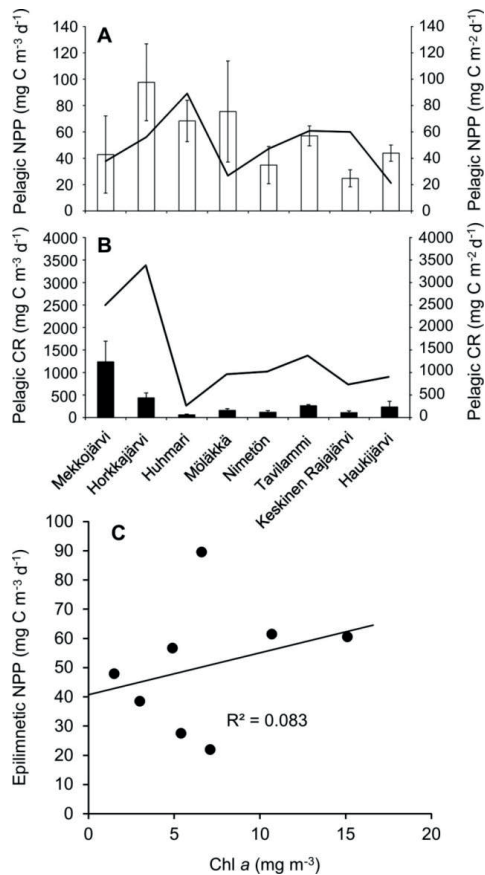## Pelagic primary production, community respiration and chlorophyll a

Pelagic NPP $m^{-3}$ was low in all the lakes (Fig. 4A; mean 55.6 ± 8.5 mg C $m^{-3}$ $d^{-1}$), and there were no statistically significant differences between the lakes (Welch's $t$-test, $t_{7,\ 20.8}$ = 2.2, $p$ = 0.079). The mean areal NPP was 50.5 ± 7.6 mg C $m^{-2}$ $d^{-1}$.

Pelagic CR $m^{-3}$ was substantially higher than NPP (Fig. 4B; mean 363 ± 137 mg C $m^{-3}$ $d^{-1}$), and there were statistically significant differences between the lakes (Welch's $t$-test, $t_{7.,\ 22.0}$ = 4.4, $p$ < 0.01) which, according to the Tukey post hoc test, were between Mekkojärvi and Huhmari, Mekkojärvi and Nimetön and Mekkojärvi and Keskinen-Rajajärvi. The mean areal CR value was 1375 ± 364 mg C $m^{-2}$ $d^{-1}$, and the highest values were in Horkkajärvi and Mekkojärvi (Fig. 4B).

Epilimnetic chl $a$ in the lakes (Table 1) ranged from 1.5 mg $m^{-3}$ in Nimetön to 15.1 mg $m^{-3}$ in Keskinen Rajajärvi (mean 6.8 ± 1.5 mg $m^{-3}$). No significant relationship was observed between epilimnetic NPP and chl $a$ (Fig. 4C; linear regression, $R^2$ = 0.083, $F_{1,6}$ = 0.54, $p$ = 0.489). However, most of the study lakes contain appreciable quantities of bacteriochlorophyll (BChl), which overlaps with chl $a$ in spectrophotometric determination (Karhunen et al. 2013) and may obscure any potential correlation between the NPP and chl $a$.

## Littoral primary production, community respiration and periphyton biomass

Periphyton NPP in the littoral at noon (Fig. 5A) varied substantially between the lakes (mean 22.9 ± 4.4 mg C (g DW substratum)$^{-1}$ $h^{-1}$). The highest values were observed in Mekkojärvi and Nimetön and the lowest value in Horkkajärvi. There was a statistically significant difference in noon values between the lakes (Welch's $t$-test, $t_{7,\ 23.2}$ = 9.810, $p$ < 0.001). Tukey's post hoc test revealed that Mekkojärvi differed from Tavilammi, Keskinen Rajajärvi and Haukijärvi, whereas Horkkajärvi differed

**Figure 4.** Pelagic A) net primary production (NPP) and B) community respiration (CR) per unit volume (bars) and per unit area (lines) in the study lakes in July 2015. C) The relationship between pelagic NPP and chlorophyll *a*.

from all the lakes except Tavilammi, Keskinen Rajajärvi and Haukijärvi. Huhmari and Möläkkä differed only from Horkkajärvi, whereas Nimetön differed from Horkkajärvi, Tavilammi, Keskinen Rajajärvi and Haukijärvi. Tavilammi differed from Nimetön and Mekkojärvi, and both Keskinen-Rajajärvi and Haukijärvi differed from Mekkojärvi and Nimetön, Daily NPP values (g DW substratum)$^{-1}$ (Fig. 5A) were also highest in Nimetön and Mekkojärvi and lowest in Horkkajärvi (mean daily NPP 251 ± 99 mg C (g DW substratum)$^{-1}$ d$^{-1}$).

There was also substantial variation in periphyton CR between the lakes (Fig.

5A; mean ± SE noon value 14.5 ± 2.1 mg C (g DW substratum)$^{-1}$ h$^{-1}$) with the highest values in Möläkkä, Mekkojärvi and Nimetön. There were statistically significant differences between the lakes (Welch's *t*-test, $t_{7, 33.2}$ = 8.862, $p$ < 0.001). Tukey's post hoc test revealed that Mekkojärvi differed from Horkkajärvi, Tavilammi and Keskinen Rajajärvi and Horkkajärvi differed from Mekkojärvi, Möläkkä and Nimetön. Möläkkä differed from Horkkajärvi and Tavilammi, whereas Nimetön differed from Horkkajärvi, Tavilammi and Keskinen Rajajärvi. Tavilammi differed from Mekkojärvi, Möläkkä and Nimetön, whereas Keskinen-Rajajärvi differed from Mekkojärvi and Nimetön.

Periphyton chl *a* varied substantially between the lakes (mean 0.55 ± 0.11 mg (g DW substratum)$^{-1}$), and there were statistically significant differences between lakes (Welch's *t*-test, $t_{7, 15.5}$ = 15.525, $p$ < 0.001). The highest chl *a* content was recorded from Nimetön (1.8 ± 0.6 mg (g DW substratum)$^{-1}$) and the lowest from Horkkajärvi (0.04 ± 0.01 mg (g DW substratum)$^{-1}$). Tukey's post hoc test revealed that Mekkojärvi differed from Horkkajärvi, Horkkäjärvi from Mekkojärvi and Nimetön, and Nimetön differed from Horkkajärvi and Tavilammi. There was a significant positive correlation between the periphyton NPP and chl *a* (Fig. 5B; linear regression, $R^2$ = 0.7451, $F_{1, 7}$ = 17.7, $p$ < 0.01).

*Whole-lake primary production, respiration and production:respiration (GPP:CR) ratios*

Whole-lake extrapolation revealed that the littoral NPP exceeded pelagic PP in five of the eight lakes (Fig. 6A). Nimetön and Mekkojärvi had over 90% and Möläkkä over 80% littoral contribution to whole-lake NPP, while in Horkkajärvi and Tavilammi pelagic was also clearly dominating with over 80% contribution (Fig. 6B). On average, the littoral contributed 58% to whole-lake NPP in the
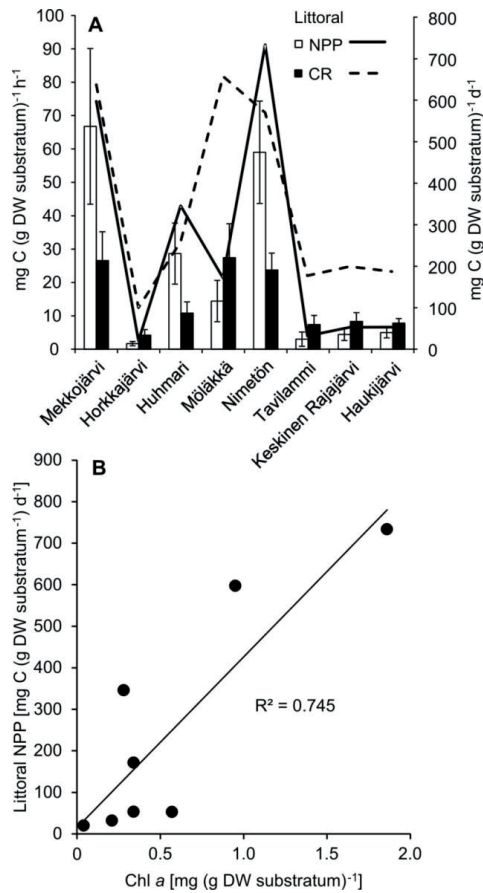
**Figure 5.** A) Mean (± SE) littoral net primary production (NPP) and community respiration (CR) per hour (boxes) and per day (lines) in the study lakes. B) The relationship between littoral NPP and and chlorophyll a.

study lakes. The pelagic clearly dominated the whole-lake CR in every lake, except in Möläkkä where the littoral contribution of 45% almost equalled that of the pelagic (Fig. 6C, 6D). On average, the pelagic contributed 79% to whole-lake CR in the study lakes.

GPP:CR ratios demonstrate predominant net heterotrophy in the pelagic areas and net autotrophy in the littoral areas (Fig. 7). The mean GPP:CR ratio in the study lakes was $0.35 \pm 0.10$ in the pelagic and $1.59 \pm 0.17$ in the littoral. Huhmari had the least heterotrophic pelagic area with a GPP:CR ratio of 0.83. The highest littoral GPP:CR ratios were in

Huhmari (2.33), Nimetön (2.28) and Mekkojärvi (1.93). When pelagic and littoral were combined, the mean whole-lake GPP:CR ratio was $0.63 \pm 0.13$. Huhmari, with the a GPP:CR ratio of 1.29, was the only net autotrophic lake; Horkkajärvi and Tavilammi, with GPP:CR of 0.12 and 0.29, were the most heterotrophic. Other lakes were closer to metabolic balance, ranging between 0.53 (in Mekkojärvi) and 0.82 (in Keskinen Rajajärvi).

A significant negative relationship between whole-lake littoral NPP and P:L ratio was observed (Fig. 8A; inverse linear regression, $R^2 = 0.700$, $F_{1,\ 6} = 14.01$, $p = 0.01$, and logarithmic regression, $R^2 = 0.686$, $F_{1,\ 6} = 13.10$, $p = 0.011$), demonstrating higher littoral NPP with increasing relative potential vegetation cover. A strong negative logarithmic relationship was also observed between the littoral proportion of whole-lake NPP and P:L ratio (Fig. 8B; logarithmic regression, $R^2 = 0.754$, $F_{1,\ 6} = 18.416$. $p < 0.01$), which demonstrates higher littoral contribution to whole-lake PP with increasing relative potential vegetation cover. This was also supported by the strong positive linear relationship between whole-lake littoral NPP and the fraction of littoral vegetation cover of the lake surface area (Fig, 8C; linear regression, $R^2 = 0.706$, $F_{1,\ 6} = 14.433$. $p < 0.01$). There was a strong and statistically significant positive linear relationship between the littoral $O_2$ saturation and littoral NPP (kg C $d^{-1}$) for data from seven of the lakes with Tavilammi excluded (Fig. 8D solid line; linear regression, $R^2 = 0.875$, $F_{1,\ 5} = 34.885$, $p < 0.001$); with Tavilammi included the relationship was weaker and non-significant (Fig. 8D dashed line; linear regression, $R^2 = 0.400$, $F_{1,\ 6} = 4.001$, $p = 0.092$). Periphyton NPP was very low in Tavilammi but the littoral surface water was supersaturated with $O_2$ as was the pelagic, presumably due to relatively high phytoplankton production in both habitats.

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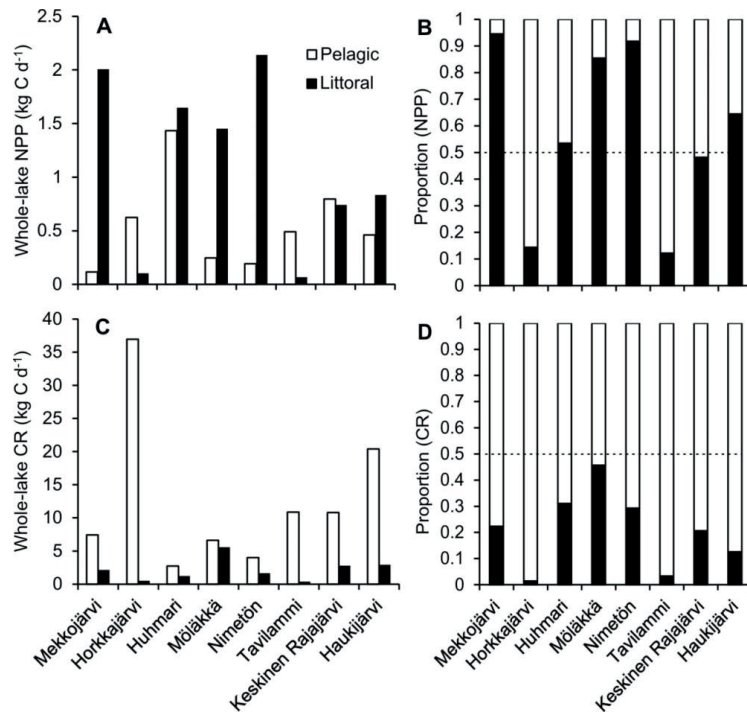
**Figure 6.** A) Whole-lake net primary production (NPP) in the pelagic and littoral areas in the study lakes and, B) their proportions of the whole-lake PP. C) Whole-lake community respiration (CR) in the pelagic and littoral areas in the study lakes and, D) their proportions of the whole-lake CR.

## Discussion

Our study addressed the often neglected but important question of the relative contributions of pelagic phytoplankton and littoral periphyton to whole-lake PP in lake ecosystems (Cantonati and Lowe 2014), focusing on small and highly humic boreal lakes. Our results test the generality of the recent demonstration of littoral periphyton dominance of whole-lake PP in Mekkojärvi (Vesterinen et al. 2016a) by surveying whole-lake PP in eight small humic lakes in southern Finland. Our demonstration of littoral dominance of whole-lake PP in five of the eight lakes in July 2015 suggests that this is probably a widespread characteristic of such lakes. The recorded $O_2$ supersaturation in the littoral areas in most of the lakes during the study provides an additional indication of high littoral PP, which also correlated positively with periphyton NPP in the littoral. The significant negative relationship between littoral NPP and P:L ratio by area demonstrates how a change in lake morphometry with an associated potential increase in littoral vegetation cover and the resulting increase in physical substrata available for periphyton can increase littoral NPP and its contribution to whole-lake PP in highly humic lakes. Accounting for littoral PP by periphyton can even balance the whole-lake metabolism of these lakes, which have previously been considered strongly net heterotrophic according to pelagic measurements alone. Furthermore, as macrophytes themselves may appreciably increase $CO_2$ uptake (Brothers et al. 2013, Peixoto et al. 2016), their carbon fixation together with that of associated periphyton may shift these small humic lakes to strongly net autotrophic in summer. Thus

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increased DOC concentration, which has been reported to increase net heterotrophy in lakes through the respiration of allochthonous C and through the decrease in both pelagic and benthic PP due to restricted light penetration (Ask et al. 2012, Godwin et al. 2014), is not always a good predictor of whole-lake production and metabolism. Littoral vegetation cover and the associated periphyton need to be taken into account, since the periphyton can form highly productive biofilms under shallow well-lit conditions even in highly humic lakes.
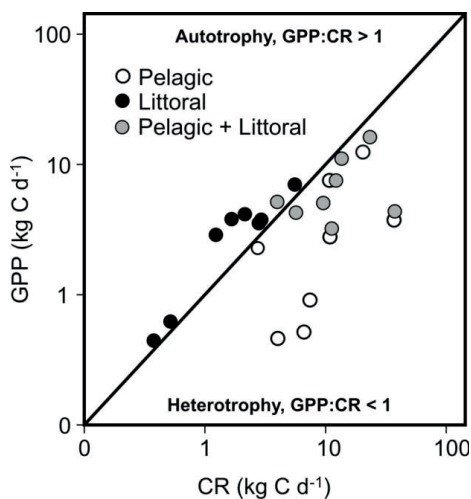


**Figure 7.** The relationship between gross primary production (GPP) and community respiration (CR) in the pelagic, littoral and whole lake (both habitats combined) in the study lakes. The line represents a GPP:CR ratio of 1 (i.e. metabolic balance).

As the area of our study lakes and their P:L ratios by area are both small, the littoral in these lakes is a relatively large proportion of the lake surface area and contributes strongly to whole-lake metabolism. When P:L ratio by area increases together with lake depth, the overall net heterotrophy increases and the relative importance of the littoral in whole-lake metabolism decreases. In this study we defined the littoral area as the area of vegetation cover, and greater vegetation cover was associated with higher littoral

NPP by epiphyton. As a few of the study lakes have some illuminated sediments/rocks, and, thus likely also some associated benthic algae, our definition of the littoral is incomplete. Therefore, our estimates of whole-lake littoral PP are likely underestimates, at least for some of the lakes. However, any contribution to whole-lake PP from benthic algae other than those associated with the shallow littoral vegetation is probably very small due to the low light penetration into the water in every lake. Although seven of the eight lakes in this study remained net heterotrophic when the whole-lake GPP:CR ratios were calculated, incorporation of the net autotrophic littoral into the calculation shifted them towards metabolic balance. As our study only encompassed PP and CR on a single summer day in each lake, it does not account for temporal variation of the PP and CR rates, which was demonstrated in Mekkojärvi in 2012 by Vesterinen et al. (2016a). However, the logger data from Mekkojärvi during July shows pronounced weather-associated temporal variation in $O_2$ saturation, which indicates high variation in PP rates and metabolism during summer. Comparison of metabolism of different lakes can be misleading when lakes are not sampled simultaneously and the weather changes during the sampling period. The days when Mekkojärvi and Horkkajärvi were sampled in this study were cloudier than the rest of the sampling days, which were all sunny and almost cloudless. This weather associated variation in light particularly affects the relationship between PP and CR by reducing PP. For instance, Mekkojärvi was found to be frequently net autotrophic during summer 2012 (Vesterinen et al. 2016a), but was net heterotrophic in this study. This could be result of lower light and reduced PP together with higher respiration in the lake. Indeed, pelagic CR was particularly high in Mekkojärvi relative to its smaller lake volume than in the other study lakes. This

illustrates the temporal variation in metabolism which occurs both within and between years.

Comparison of pelagic and littoral loggers indicates substantial spatial heterogeneity in PP rates in the lake, which was also shown by Van de Bogert et al. (2007). The remarkable differences in $O_2$ saturation between the pelagic and littoral loggers only ca 1 m apart, demonstrate very low horizontal fluxes between the pelagic and littoral in the small and sheltered Mekkojärvi. Remarkable variation of littoral $O_2$ from supersaturation at noon to near anoxia during night indicates extreme conditions for invertebrates living in the littoral vegetation of Mekkojärvi, Despite this the littoral of the lake sustains relatively rich invertebrate communities compared to the pelagic, and many of the species are tightly connected to the surrounding moss (Vesterinen et al. 2016b). Whether those species are well adapted to low $O_2$ conditions or whether they move location in response to changing $O_2$ conditions remains unresolved. Probably part of the species are well adapted, but as lake has always been sampled during the day possible behavioral differences have not been observed. However, how the littoral invertebrates cope with such extreme conditions certainly merits future study.

The upscaling of rate estimates made in bottle incubations to the ecosystem level inevitably has marked uncertainties (Hanson et al. 2015). Considering this, our whole-lake littoral PP and CR estimates from the structurally more complex littoral zones undoubtedly involve higher uncertainty than those from the more homogenous pelagic. However, as the rates were so high, the associated uncertainty cannot alter the essential message of highly important littoral autotrophic production in most of the lakes. Moreover, as the littoral NPP values were calculated by subtracting CR from GPP and the true autotrophic respiration must be less than the measured CR, the littoral NPP values must actually be underestimates. There is also debate about whether the ^{14}C incorporation technique most closely estimates NPP or GPP (Marra 2009). This technique has been widely used in studies of humic lakes, with an incubation time of 24 h considered to approximate NPP. Remaining uncertainty is associated with summing up the pelagic NPP and photic zone CR to estimate pelagic GPP assuming that most of the photic zone respiration is attributable to primary producers. As this overestimates GPP, it to some extent yields higher GPP:CR ratios for the pelagic. The P:L ratios by area were obtained using areal calculations from satellite imagery, which undoubtedly gives only coarse estimates due to rather low resolution. However, P:L ratios correlated significantly with the littoral NPP estimates, demonstrating the validity of our approach, which nevertheless might be improved with higher resolution satellite imagery.

Changing inputs of terrestrial organic matter to lakes and predicted browning as a consequence in complex lake ecosystems is a challenging and still poorly understood topic, which needs further study (Solomon et al. 2016). While browning of lakes is expected to negatively affect lake benthic PP due to decreased light availability, there are other widely occurring environmental changes, such as the expansion of surrounding macrophyte cover in shallow Finnish lakes (Rintanen 1996) and postglacial land uplift (Ekman 1991), which may compensate the negative effect of browning on benthic and whole-lake PP by providing more well-lit substrata for epiphyton. Increase in macrophyte cover allows development of the periphytic community which is better adapted to low light conditions (Rodríguez et al. 2012). Nevertheless, it is difficult to predict whether these environmental changes will increase the overall productivity of these systems and what might be their consequences for lake
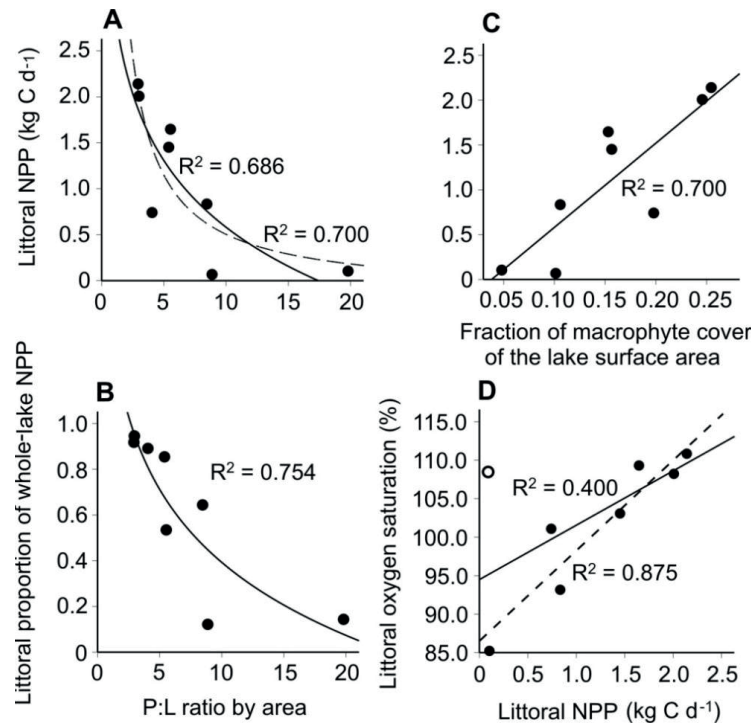
**Figure 8.** A) Inverse linear (dashed line) and logarithmic (solid line) relationships between whole-lake littoral NPP and pelagic:littoral ratio by area (P:L ratio). B) Logarithmic relationship between littoral proportion of whole-lake NPP and P:L ratio by area. C) Linear relationship between whole-lake littoral NPP and the fraction of the lake surface area occupied by littoral vegetation. D) Linear relationship between littoral oxygen saturation and littoral NPP, where the dashed line has been fitted to data from only 7 lakes (omitting Lake Tavilammi shown by the open circle).

biota. Vesterinen et al. (2016b) demonstrated the importance of periphyton as a basal food resource for invertebrates in Mekkojärvi, as also suggested by studies in other humic ecosystems (van Duinen et al. 2013, Lau et al. 2014). Despite their remarkable diel fluctuations in $O_2$ concentration, these littoral habitats appear to sustain relatively rich invertebrate communities supported strongly by periphyton (Vesterinen et al. 2016b), so the expansion of these habitats may increase their overall species richness and secondary production.

The relative contribution of littoral and pelagic habitats to whole-lake PP is a fundamental ecosystem attribute that remains poorly explored and is in urgent need of further study (Vadeboncoeur et al. 2002, Cantonati and Lowe 2014). Our

study addressed this using a novel approach to demonstrate the importance of littoral PP in humic lakes. However, all of our study lakes are small and shallow, and most of them have relatively large fractions of the lake surface area covered by littoral vegetation; therefore whether the reported relationship between littoral epiphytic NPP and P:L ratio (defined as the extent of littoral vegetation cover) is also applicable to larger humic lakes remains to be tested. Depth ratio (DR = $\bar{z}/z_{max}$) together with light attenuation have been used for predicting benthic algal contribution to whole-lake PP, which in deeper oligotrophic and mesotrophic lakes is predicted as moderate, ranging between 10 and 50% (Vadeboncoeur et al. 2008). DR alone tells us little about the distribution of illuminated benthic habitat.

If the mean depth is well below the depth 1% of light, the majority of benthic habitat is not illuminated, and the benthic algae contributes only little to whole-lake PP regardless of DR (Vadeboncoeur et al. 2008). Whether the P:L ratio by area increases along with increasing lake size depends on light availability and lake morphometry. Allochthonous DOM is a major factor controlling light availability in oligotrophic lakes (Ask et al. 2009b), and DOC concentration is very strongly connected to the light penetration into water particularly in small boreal lakes (Jones and Arvola 1984). Larger lakes tend to be less humic than the small ones due to relatively much smaller allochthonous DOM loads and faster net sedimentation rate of humic matter (Eloranta 1999). Thus, the increase in lake size increases available habitats for benthic algae and the total area of littoral zone. Lake morphometry also affects the distribution of macrophytes in lakes. Shallow humic lakes with clearly larger surface area than our study lakes can have very high macrophyte cover providing extensive substrata for epiphyton under well-lit conditions, as has been demonstrated in some humic lakes in South American wetlands (Rodriguez et al. 2012). Lakes with similar characteristics can be found also in the boreal zone, although P:L ratios

presumably tend to increase in larger humic boreal lakes. However, the extent of macrophyte cover together with illuminated sediments should be quantified in order to estimate the P:L ratios and whole-lake PP also in larger humic lakes. The model fit to our data suggests <10% littoral contribution to whole-lake PP in lakes with P:L ratio over 20, but empirical studies are needed to test this for higher number of lakes including larger ones. Improved satellite imagery can be an effective tool for such studies, which are technically challenging, yet necessary.

As littoral zones are very abundant and are among the most productive ecosystems in the world (Wetzel 1990), greater focus on their trophic structure, metabolism and biogeochemistry is very important. The number of boreal lakes similar to those we studied is very high globally (Downing et al. 2006). Thus, their role in regional C budgets is large and they are considered "hotspots" of C sequestration, receiving high allochthonous inputs, which shift these systems towards heterotophy (Cole 2013). However, we suggest that many of these lakes are actually more productive and not as heterotrophic as is previously assumed, when the potentially high production of periphyton on aquatic vegetation is accounted for.

*Acknowledgements*

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