

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 only been measured 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 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 BP in highly humic lakes has previously been understated, and needs to be taken into account in assessments of whole-lake carbon cycling and metabolism.

KEY WORDS: Littoral · Periphyton · Pelagic · Autotrophic · Heterotrophic

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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.

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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 effect can be more pronounced if light is not limiting algal growth and biomass production; under these conditions 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 and is an important basal resource for both pelagic (Jones 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 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 2 habitats.

MATERIALS AND METHODS

Study lake

The study was conducted at Lake Mekkojärvi (61° 13' N, 25° 3' 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, respectively. 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 to 33 mg C l⁻¹), highly coloured water (300 to 800 mg Pt l⁻¹) and low pH (5.3 to 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 and 1.0 m depth 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

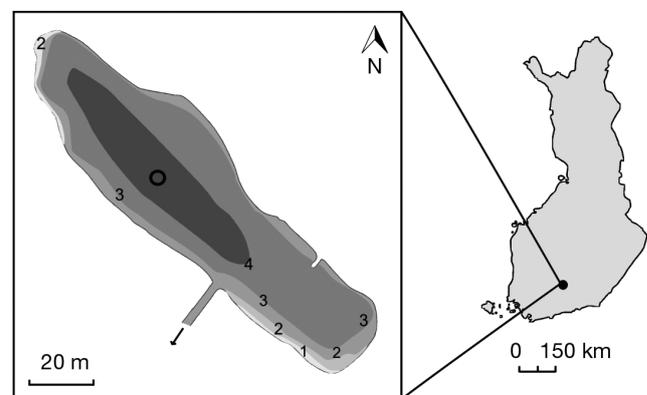


Fig. 1. Location and bathymetry of Lake Mekkojärvi, southern Finland, site for the study of open-water season bacterial production (BP) in pelagic and littoral zones. Open circle denotes the sampling point for the pelagic measurements. Numbers show depths in metres

depth ratio (DR = mean depth:maximum depth) 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* spp.) 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 is 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 during every sampling occasion in 2015. From these measurements the water column stratification was defined as follows: epilimnion, 0 to 0.5 m; metalimnion, 0.5 to 1.0 m; and hypolimnion, 1.0 to 3.0 m.

Pelagic bacterial production was measured 5 times between June and October in 2015 using a [¹⁴C]-leucine uptake method according to Kirchman et al. (1985), slightly modified according to Tulonen (1993).

From composite water samples collected from each of the 3 strata in the water column (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 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 ethanolamine:ethanol (1:7, v/v) 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).

Leucine incorporation rates (calculated as [dpm sample – dpm blank]/total activity of the added leucine, where dpm is disintegrations per minute) were converted to biovolume by multiplying by 7.71 × 10¹⁵ (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 vessels, 5 parallel samples were incubated in evacuated Labco Exetainers simultaneously with other hypolimnetic samples in September.

Littoral epiphytic bacterial production

Epiphytic BP was measured 5 times together with pelagic BP in 2015. Littoral water temperatures were measured with a YSI 55 probe during every sampling occasion. Samples of littoral vegetation were col-

lected 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 constituents of 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 112 Ci mmol⁻¹, PerkinElmer) 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 at 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 (42976 × g) 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). Following addition of 1.2 ml of 5% TCA, the samples were again vortexed and centrifuged at 12400 rpm (42976 × g) 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). Leucine uptake rate was calculated as:

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

where factor 4.5×10^{-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.

BP was calculated as:

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

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°C for 24 h and dry weight (DW) of substratum was recorded as $(1.05 \times 10^{-3}) \pm (8.76 \times 10^{-5})$ g DW substratum cm⁻¹, mean ± SE (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) which was placed in the surface water in the middle of a moss mat in the littoral in Mekkojärvi for 2 mo 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°C for 48 h and DW of substrata were recorded as described for BP samples. The whole littoral epi-

phytic BP estimates were then calculated by multiplying BP per metre of 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. All the descriptive statistics are mean \pm SE if not expressly noted.

RESULTS

Pelagic bacterial production

The mean O₂ concentrations over the study period were 4.1 ± 0.7 mg l⁻¹ in the epilimnion, 1.2 ± 0.5 mg l⁻¹ in the metalimnion and 0.8 ± 0.1 mg l⁻¹ in the hypolimnion. Total pelagic BP was highest in early summer, and decreased 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 3 strata. Hypolimnetic BP increased slightly towards autumn and constituted the largest fraction of total pelagic BP in late-summer and autumn (56 to 63%). The mean BP over the sampling period was 11.6 ± 2.0 mg C m⁻² d⁻¹ in the epilimnion, 5.7 ± 2.0 mg C m⁻² d⁻¹ in the metalimnion and 6.0 ± 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 be-

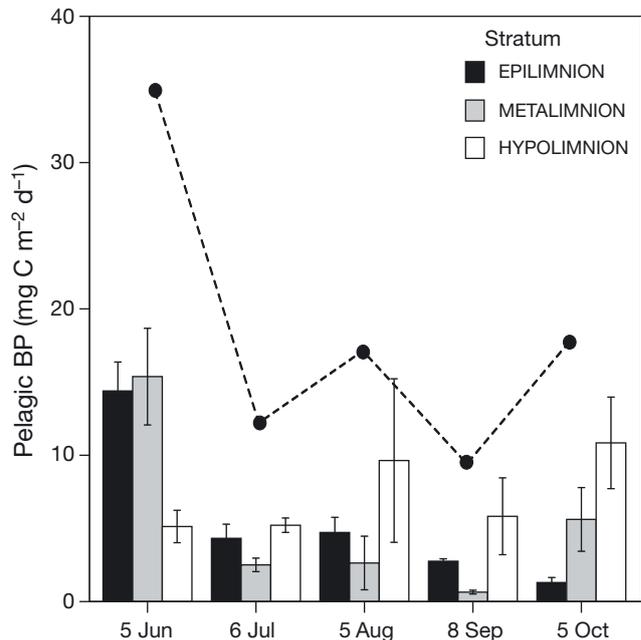


Fig. 2. Pelagic bacterial production (BP) per unit area (mean \pm SE) in 3 different strata in Lake Mekkajärvi, southern Finland, during the open-water season of 2015. The dashed line expresses the areal BP in the whole water column as the sum of values from the 3 strata

tween 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 ± 1.3 mg C m⁻³ d⁻¹ in the oxic and 2.0 ± 1.0 mg C m⁻³ d⁻¹ in the anoxic).

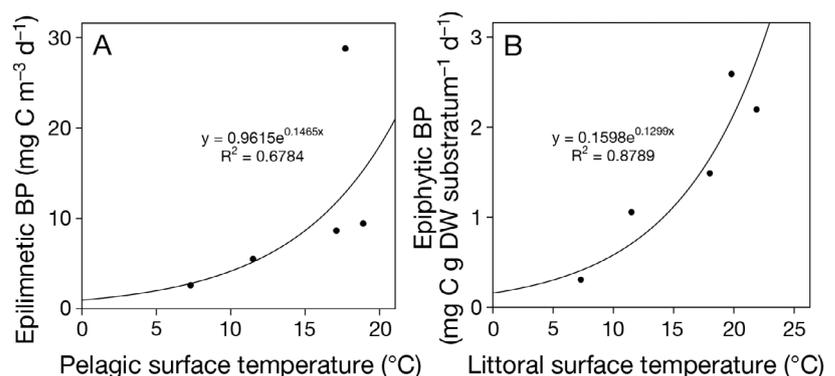


Fig. 3. Exponential relationships between (A) epilimnetic (pelagic) and (B) littoral epiphytic BP and surface temperature in Lake Mekkajärvi, southern Finland

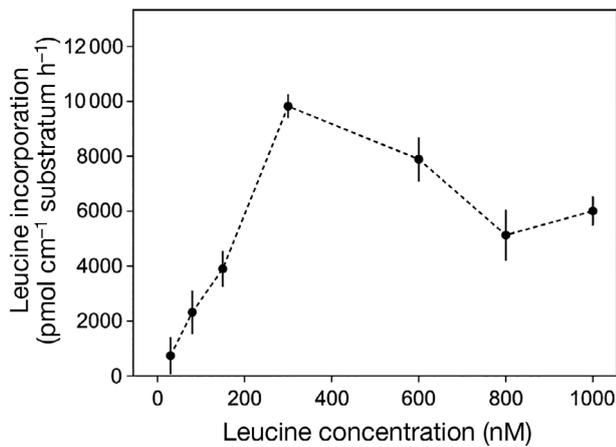


Fig. 4. Mean \pm SE uptake of leucine for epiphytic bacteria in 7 different leucine concentrations in Lake Mekkojärvi, southern Finland

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* (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 $2.8 \text{ mg C (g DW substratum)}^{-1} \text{ d}^{-1}$, which is only ca. 10% higher than the value estimated multiplying hourly leucine incorporation rates by 24 ($2.6 \pm 0.4 \text{ mg C [g DW substratum]}^{-1} \text{ d}^{-1}$). In August the similar comparison was 2.0 versus $1.5 \text{ mg C (g DW substratum)}^{-1} \text{ d}^{-1}$, a difference of 15%. According to these comparisons, multiplying noon BP by 24 gives

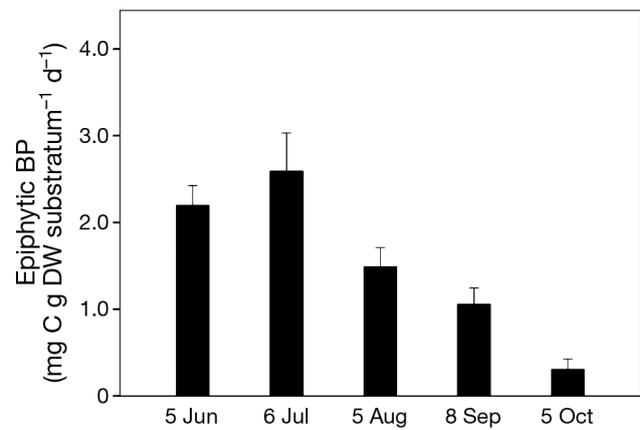


Fig. 5. Littoral daily (mean \pm SE) epiphytic BP in Lake Mekkojärvi, southern Finland, derived from the noon rates by multiplying by 24 and then normalized to gram dry-weight of substratum

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

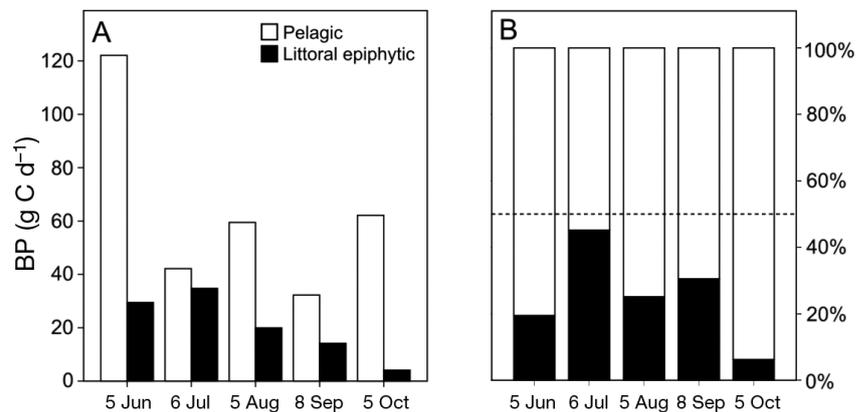


Fig. 6. (A) Whole-lake estimates for BP of pelagic bacterioplankton and littoral epiphytic bacteria in Lake Mekkojärvi, southern Finland, and (B) their relative proportions

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 %.

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 those in the overlying water (Sander & Kalff 1993, Ask et al. 2009). In subarctic oligotrophic Swedish lakes, BP from allochthonous organic carbon (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 O₂ 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).

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 O₂, 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. 2016a). 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°C through the summer whereas surface temperature often rises above 20°C. 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 organic matter (OM) released by phytoplankton, which couples BP with PP. Such positive relationships between pelagic BP and PP and chlorophyll are well documented (e.g. Cole et al. 1988, White et al. 1991, 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 BP 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 correla-

tion 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 to 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 the dark. If we assume a similar relationship between PP and BP and that a similar difference is applicable to periphyton 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 DOC 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 have been 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 3-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 $364 \pm 66 \text{ mg C (g DW substratum)}^{-1} \text{ d}^{-1}$ as the mean PP by epiphyton in Mekkojärvi in 2012. Comparison to the mean daily epiphytic BP of $1.52 \pm 1.36 \text{ mg C (g DW substratum)}^{-1} \text{ d}^{-1}$ 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 similar. 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 2 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 6 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 2 dominant plant groups, sedges and mosses. Some larger plant species (such as *Phragmites australis* and *Menyanthes trifoliata*), which have a patchy appearance around the lake shore, were not sampled due to their size and the difficulties of incubating representative tissue samples with attached epiphyton 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, it is important to recognize these issues in order to develop more reliable approaches for studying 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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