Author(s): Vesterinen, Jussi; Devlin, Shawn; Syväranta, Jari; Jones, Roger

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

Year: 2017

Version:

Please cite the original version:

Vesterinen, J., Devlin, S., Syväranta, J., & Jones, R. (2017). Epiphytic bacteria make an important contribution to heterotrophic bacterial production in a humic boreal lake. Aquatic Microbial Ecology, 79(3), 247-257. https://doi.org/10.3354/ame01835

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

RH: Littoral bacterial production

Jussi Vesterinen[1*], Shawn P. Devlin[1,2], Jari Syväranta[1,3], & Roger I. Jones[1]

1) University of Jyväskylä, Department of Biological and Environmental Science, P.O. Box 35, FI-40014 University of Jyväskylä, Finland
2) Flathead Lake Biological Station, University of Montana, Polson, MT, 59860.
3) University of Eastern Finland, Department of Environmental and Biological Sciences, P.O. Box 111, 80101 Joensuu, Finland

*Corresponding author: jussi.p.vesterinen@jyu.fi

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 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

58 surface in relatively well-lit conditions where it supports thick growths of epiphyton from spring
59 to early autumn (Vesterinen et al. 2016a). In view of the strong correlations found elsewhere
60 between PP and BP, we can expect that littoral epiphytic BP should be high and contribute
61 substantially to whole-lake BP in Mekkojärvi.
62 Heterotrophic bacteria are known to play a very important role in the carbon flux of
63 aquatic ecosystems, providing a link between autochthonous and allochthonous dissolved
64 organic matter (DOM) and bacterivores (Porter et al. 1988). In humic lakes, most of the DOM is
65 of allochthonous origin which is an important basal resource for both pelagic (Jones et al. 1992,
66 Pace et al. 2004, Jansson et al. 2007) and benthic (Premke et al. 2010, Karlsson et al. 2012) food
67 webs via microbial pathways. However, most studies of bacteria and their productivity in lakes
68 have concerned pelagic bacterioplankton alone without measuring productivity of bacteria
69 associated with profundal sediments or with periphyton in littoral benthic habitats, where
70 bacterial production (BP) can be of a similar magnitude to, or even higher than that in the pelagic
71 zone (Vadeboncoeur et al. 2002 and references therein). Benthic bacteria often outnumber
72 pelagic bacteria in lakes and rivers creating high spatial variability (Schallenberg & Kalff 1993,
73 Fischer & Pusch 2001), and the fraction of active bacterial cells in the total number of bacteria in
74 sediments and epiphytic biofilms can be much larger than in the pelagic (Haglund et al. 2002).
75 Therefore, measurements of BP in these different habitats are particularly needed in humic lakes,
76 where the importance of the littoral has been understated. Incorporation of littoral and pelagic as
77 integrated habitats into conceptual models of lake ecosystems will contribute to a more
78 comprehensive understanding of trophic dynamics (Vadeboncoeur et al. 2002) and of lake
79 metabolism, which is important in resolving organic carbon budgets in lakes (Hanson et al. 2015,
80 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: 0.0–0.2 m (surface), 0.2–0.5 m (epilimnion), 0.5–1.0 (metalimnion), 1.0–3.0 m (hypolimnion).

126 Pelagic bacterial production was measured five times between June and October in 2015
127 using a [^{14}C]-leucine uptake method (Kirchman et al. 1985) slightly modified according to
128 Tulonen (1993). From composite water samples collected from three stratum (epi- (0–0.5 m),
129 meta- and hypolimnion), triplicate subsamples of 5 mL were transferred to 20 mL pre-ignited
130 glass vials containing 30 nM of [^{14}C]-leucine (specific activity of 0.306 Ci mmol^{-1}, Amersham
131 Biosciences) and incubated for 60 min *in situ* in the strata from which they originated.
132 Glutaraldehyde-killed controls were run in parallel. After incubation, all the live samples were
133 killed with glutaraldehyde. In the laboratory, 0.5 mL of ice-cold 50% trichloroacetic acid (TCA)
134 was added into every sample to reach a final concentration of 5%. Samples were then cooled for
135 15 min followed by filtration onto 0.2 µm pore-size cellulose nitrate filters (Sartorius). The filters
136 were rinsed with 1 mL of ice-cold 5 % TCA and distilled water and then dissolved in 0.25 mL of
137 ethyleneglycolmonomethylether together with 9 mL of liquid scintillation cocktail (OptiPhase 3).
138 The total activity of the added [^{14}C]-leucine was counted from a subsample of 0.5 mL into which
139 0.5 mL of 1:7-ethanolamine/ethanol absorption liquid was added together with 9 mL of
140 scintillation cocktail. Samples were stored at room temperature for 24 h before their radioactivity
141 was counted with a Packard Tri-Carb® liquid scintillation counter (PerkinElmer, Waltham,
142 Massachusetts, USA).
143 Leucine incorporation rates ([dpm sample – dpm blank]/total activity of the added leucine) were
144 converted to biovolume by multiplying by 7.71 x 10^{15} (µm^{-3} mol^{-1}) and to carbon production by
145 multiplying by a carbon to biovolume ratio of 0.36 pg C µm^{-3}. Both factors are appropriate for
146 humic lakes according to their empirical determination in laboratory experiments (Tulonen
147 1993). Daily BP rates were calculated multiplying hourly rates by 24. Areal BP values were
148 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 (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 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 consists mainly of moss and partly submerged sedges in Mekkojärvi, these were the main representatives 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 [^{3}H]-leucine incorporation method described by Ask et al. (2009) based on the method originally developed by Smith & Azam (1992). [^{3}H]-leucine was used instead of [^{14}C]-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 [^{3}H]-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 [^{3}H]-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 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 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:

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

, 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:

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

195 , where 132.1 is the molecular weight of leucine, (%Leu) is the proportion of leucine in total
196 protein, assumed to be 0.073 (Simon & Azam 1989), (C:Protein) is the ratio of cellular C to
197 protein, assumed to be 0.86 (Simon & Azam 1989) and ID is the isotope dilution factor, which
198 was assumed to be 2 for samples from oligotrophic lakes (Simon & Azam 1989).
199 Substrata were dried in an oven at 60ºC for 24 h and dry-weight (DW) of substratum
200 [mean ± SE (g DW substratum) cm^{-1}] was recorded (0.00105 ± 0.0000876 g, n = 25). BP values
201 were then normalized to mg C g (DW substratum)$^{-1}$ h^{-1}. Daily rates were calculated by
202 multiplying hourly rates by 24. We examined how temperature changes during the day might
203 affect the BP rates by using temperature data from a miniDOT Logger (PME Inc. Vista, CA,
204 USA) which was placed in the surface water in the middle of a moss mat in the littoral in
205 Mekkojärvi for 2 months from July to August. Littoral BP values at noon over the sampling
206 period in 2015 plotted against the littoral surface temperature followed an exponential
207 relationship, and that function was used to estimate BP for every hour during the incubation
208 periods on 6 July and 5 August. These values were then summed and compared to the values
209 derived by multiplying noon rates by 24.
210 Whole-lake estimates for epiphytic BP were derived by first calculating the BP per m
211 lake shoreline using the average DW substratum^{-1} m^{-1} of lake shoreline (42.6 ± 3.4 g DW
212 substratum^{-1} m^{-1}), which was calculated by entirely removing the macrophyte and moss
213 vegetation along 40 cm of lakeshore from 24 sites around the lake (Vesterinen et al. 2016a). The
214 whole littoral epiphytic BP estimates were then calculated by multiplying BP per m lake
215 shoreline by the total shoreline length (320 m).
216
217 *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

Pelagic bacterial production

The mean O_2 concentrations over the study period were 4.1 ± 0.7 mg L^{-1} in the epilimnion, 1.2 ± 0.5 7 mg L^{-1} in the metalimnion and 0.8 ± 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 ± 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 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 ± 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).

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 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 ± 0.4 mg C [g DW substratum]$^{-1}$ d^{-1}). In August the similar

264 comparison was 2.0 versus 1.5 mg C (g DW substratum)$^{-1}$ d^{-1}, a difference of 15%. According to
265 these comparisons, multiplying noon BP h^{-1} by 24 gives slightly lower, and thus more
266 conservative, estimates of the daily epiphytic BP.
267
268 *Whole-lake pelagic and littoral bacterial production*
269 Whole-lake estimates for pelagic and littoral epiphytic BP revealed that the pelagic
270 dominated the combined BP over the open-water period, contributing over 80% to whole-lake
271 BP in early-June and in October (Fig. 6). Littoral epiphytic BP made the highest contribution to
272 overall BP during summer with the highest value (34.8 g C d^{-1}) and contribution (45%) in early-
273 July. The lowest littoral value (4.1 g C d^{-1}) and contribution (6%) occurred in October. The mean
274 values of pelagic and littoral epiphytic BP over the sampling period were 63.6 ± 15.6 and 20.5 ±
275 5.4 g C d^{-1}, respectively, and their respective mean proportions of the overall BP were 76 and
276 24%.
277
278 ## Discussion
279 Pelagic BP dominated the combined (pelagic + littoral epiphytic) BP in Mekkojärvi
280 during the study, but the littoral epiphytic BP contributed appreciably, particularly in summer.
281 According to our estimates of the mean whole-lake rates over the sampling period in 2015,
282 around one quarter of the combined (pelagic + littoral epiphytic) BP in Mekkojärvi has been
283 previously overlooked when the epiphyton has not been taken into account. However, the
284 complete whole-lake BP also include BP of sediment bacteria, which was not measured in this
285 study but can be assumed to make an appreciable contribution to the total BP of the lake, as their
286 production rates can be several times higher than in the overlying water (Sander & Kalff 1993,

287 Ask et a. 2009). In subarctic oligotrophic Swedish lakes, BP from allochthonous OC by sediment
288 bacteria was found to exceed the combined PP and BP in the pelagic (Ask et al. 2009). In our
289 study, littoral BP is represented as epiphytic BP, but it should be noted that the true littoral BP
290 also includes BP in the surrounding water in the littoral, which we did not measure. Considering
291 the higher surface water temperatures in the littoral than in the pelagic and the positive
292 relationship between temperature and BP together with potentially higher quantities of labile
293 organic compounds, such as periphytic algal exudates, and nutrients, BP in the in the littoral
294 water may be higher than that in the pelagic and can potentially increase the contribution of
295 littoral to whole-lake BP in Mekkojärvi. The strictly anaerobic green sulphur bacterium
296 *Chlorobium* is also abundant in the deeper layers in Mekkojärvi (Taipale et al. 2009, Karhunen et
297 al. 2013). As the BP samples in this study were exposed to O_2, the contribution of *Chlorobium* to
298 BP was probably underrepresented in our measurements. However, both the high production of
299 *Chlorobium* and potentially high production of sediment bacteria probably contribute the very
300 high community respiration rates reported from Mekkojärvi (Salonen et al. 2005, Vesterinen et
301 al. 2016). Despite these gaps, which do not allow us to report total whole-lake BP values, our
302 results clearly highlight how epiphytic BP can be a major part of the whole-lake BP in small
303 humic lakes.
304 The high BP measured in the epilimnion in spring weakened the correlation between
305 epilimnetic BP and the surface temperature, but indicated an association between BP and the
306 phytoplankton PP spring maximum, which has been documented in earlier studies in Mekkojärvi
307 (Salonen et al. 2005, Vesterinen et al. 2016a). These apparently related production maxima of
308 both groups of organisms may reflect exploitation by both groups of a pulse of nutrients from the
309 catchment with snow-melt in spring. Alternatively or additionally, it may be a result of bacterial

310 stimulation by labile autochthonous OM released by phytoplankton, which couples BP with PP.
311 Such positive relationships between pelagic BP and PP and chlorophyll are well-documented
312 (e.g. White et al. 1991, Cole et al. 1988, Kritzberg et al. 2005). However, in DOC-rich
313 Mekkojärvi, the low concentrations of inorganic nutrients, and thus restricted resource
314 stoichiometry (i.e. high C:N:P ratio), for actively growing bacteria have been suggested to limit
315 the bacterial production on labile carbon substrates, such as algal exudates, in the pelagic
316 (Dorado-García et al. 2016). Therefore, higher nutrient availability, reflected also in enhanced
317 PP, appears the more likely explanation for high pelagic BP in spring. Generally the temperature
318 dependence of BP and growth is modulated by other environmental conditions, such as
319 availability of inorganic nutrients and quality and quantity of organic matter substrates (Apple et
320 al. 2006). The hypolimnetic fraction of the total pelagic BP was clearly higher than the
321 epilimnetic and metalimnetic fractions through the autumn, which is partly explained by higher
322 volume of water in the hypolimnion, but probably also reflects higher nutrient concentrations in
323 the hypolimnion as reported in previous studies (e.g. Vesterinen et al. 2016a). Temperature in the
324 hypolimnion remains around 4 ºC through the summer whereas surface temperature often rises
325 above 20 ºC. Nutrient concentrations, in turn, remain rather constant in the hypolimnion through
326 the stratification period (Vesterinen et al. 2016a). As only a small part of the total epiphyton
327 biomass in the littoral is grazed by littoral invertebrates during the summer (Vesterinen et al.
328 2016b), the remaining biomass is presumably decomposed in the water column and may
329 contribute to the relatively high hypolimnetic BP in the autumn.
330 Epiphytic BP in the littoral correlated positively with the surface temperature, and the
331 correlation was stronger than between pelagic BP and temperature in the epilimnion. As the
332 temperature logger data from the sampling occasions on July and August allowed us to calculate

333 the BP estimates for each hour on those days, which were within 10–15% of those calculated
334 multiplying by 24, the surface temperatures at noon were apparently close to the average daily
335 surface temperatures. However, the production by epiphytic heterotrophs is also light-mediated
336 and associated strongly with the epiphytic PP. Kuehn et al. (2014) found 60% higher production
337 rates in litter-associated bacteria which were exposed to light than those which were in dark. If
338 we assume a similar relationship between PP and BP and that a similar difference is applicable to
339 periphyton in Mekkojärvi, then the epiphytic BP rates would be 60% lower during the night.
340 However, day lengths in our study area range from 19.5 h in June to ca 10 h in October, so the
341 photoperiod is long during summer months and epiphyton is exposed to light for most of the day.
342 In autumn, in turn, day lengths are shorter but also the PP by epiphyton is low (Vesterinen et al.
343 2016a) and, like the epiphytic BP, shows a trend of decreasing towards autumn. How much this
344 light-dependent variation might truly affect epiphytic BP in Mekkojärvi, and in highly humic
345 lakes in general remains speculative.
346 The extent to which benthic bacteria in lakes use organic C of allochthonous or
347 autochthonous origin remains poorly known. In periphytic matrixes, the dissolved organic
348 carbon pool is a mixture of extracellular release from macrophytes, excretion of both attached
349 algae and bacteria, decomposition products following autolysis of epiphytes and dissolved
350 carbon compounds of both autochthonous and allochthonous origin (Allen 1971, Attermeyer et
351 al. 2014). The relative importance of these compounds likely varies between periphytic groups
352 colonizing different habitats, e.g. between epiphyton, epilithon and epipsammon. Ask et al.
353 (2009) showed that, although sediment bacteria in clear-water Swedish lakes were mainly fuelled
354 by benthic PP, allochthonous C made a substantial contribution to the benthic BP. In contrast,
355 Rodríguez et al. (2013) reported that benthic autochthonous OC supported pelagic BP in a small

clear-water lake. Allochthonous C has higher accessibility to sediment bacteria than to epiphytic bacteria due to high burial rates of allochthonous OM to 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. 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 ± 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 ± 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 the strong overall net heterotrophy and 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).

401 The estimation of the variability in whole-lake extrapolation is challenging due to high spatial
402 heterogeneity and complex interactions (Pace 2001). Pelagic production rates can have high
403 spatial variability (Van de Bogert et al. 2007), and a particular challenge for estimating the error
404 for littoral epiphytic BP is associated with the variability of substrata around the lake.
405 Mekkojärvi, however, is a very small lake and has a relatively uniform basin morphometry, so
406 whole-lake extrapolations are likely to yield better constrained estimates than for larger lakes
407 with highly variable morphometry. As the quantification of available substrata for epiphyton
408 along the lake was done from 24 sites, which can be considered a rather high number of
409 replicates around this small lake (Vesterinen et al. 2016a), it can be expected to have yielded a
410 rather reliable estimate for the mean substratum weight per m lake shore (42.6 ± 3.2). Although
411 the BP samples were collected only from six randomly selected sites around the lake to keep the
412 workload reasonable, the spatial distribution of different plant species around the lake was well
413 represented in the samples, which consisted mainly of two dominant plant groups, sedges and
414 mosses. Some larger plant species (such as *Phragmites australis* and *Menyanthes trifoliate*),
415 which have a patchy appearance around the lake shore, were not sampled due to their large size
416 and the difficulties of incubating representative tissue samples with attached epíphyton in
417 Eppendorf tubes.
418 In conclusion, our study shows that littoral epiphytic bacteria can make a significant
419 contribution to whole-lake BP in humic lakes and, together with previous findings of highly
420 productive photosynthetic epiphyton in the littoral in Mekkojärvi (Vesterinen et al. 2016a),
421 demonstrates the importance of the littoral zone in the biomass production and C cycle in highly
422 humic lakes, at least in the small humic lakes like Mekkojärvi that are so abundant throughout
423 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.

Acknowledgements

We thank Lammi Biological Station for both financial support to JV and for accommodation and laboratory facilities. We also acknowledge financial support from the Maj and Tor Nessling Foundation, the Doctoral Programme in Biological and Environmental Science of the University of Jyväskylä, The Academy of Finland (projects 137671 to RJ and 296918 to JS) and the Marie Skłodowska-Curie actions (660655 to JS) under the EU Horizon 2020.

References

Allen HL (1971) Primary productivity, chemo-organotrophy, and nutritional interactions of epiphytic algae and bacteria on macrophytes in the littoral of a lake. Ecol Monogr 41:98–127.

Anesio AM, Hollas CB, Granéli W, Laybourn-Parry J (2004) Influence of humic substances on the bacterial and viral dynamics in freshwaters. Appl Environ Microbiol 78:4848–4854.

Apple JK, del Giorgio PA, Kemp WM (2006) Temperature regulation of bacterial production, respiration, and growth efficiency in a temperate salt-marsh estuary. Aquat Microb Ecol 43:243–254.

446 Arvola L, Salonen K, Kankaala P, Lehtovaara A (1992) Vertical distributions of bacteria and
447 algae in a steeply stratified humic lake under high grazing pressure from *Daphnia*
448 *longispina*. Hydrobiologia 229:253–269.

449 Ask J, Karlsson J, Persson L, Ask P, Byström P, Jansson M (2009) Whole-lake estimates of
450 carbon flux through algae and bacteria in benthic and pelagic habitats of clear-water lakes.
451 Ecology 90:1923–1932.

452 Attermeyer K, Hornick T, Kayler ZT, Bahr A, Zwirnmann E, Grossart H-P, Premke K (2014)
453 Enhanced bacterial decomposition with increasing addition of autochthonous to
454 allochthonous carbon without any effect on bacterial community composition.
455 Biogeosciences 11:1479–1489.

456 Azam F, Fenchel T, Field J, Gray J, Meyer-Reil L, Thingstad F (1983) The ecological role of
457 water-column microbes in the sea. Mar ecol prog ser 10:257–263.

458 Carr GM, Morin A, Chambers PA (2005) Bacteria and algae in stream periphyton along a
459 nutrient gradient. Freshwater Biol 50:1337–1350.

460 Cole JJ, Findlay S, Pace ML (1988) Bacterial production in fresh and saltwater ecosystems: a
461 cross-system overview. Mar ecol prog ser 43:1–10.

462 Cole JJ (2013) Burial efficiency and its possible controls. In: Kinne O (ed) Freshwater
463 ecosystems and the carbon cycle. International Ecology Institute, Oldendorf/Luhe,
464 Germany.

Danger M, Cornut J, Chauvet E, Chavez P, Elger A, Lecerf A (2013) Benthic algae stimulate leaf litter decomposition in detritus-based headwater streams: a case of aquatic priming effect. Ecology 94:1604–1613.

Devlin SP, Saarenheimo J, Syväranta J, Jones RI (2015) Top consumer abundance influences lake methane efflux. Nat Commun 6:8787.

Dorado-García I, Syväranta J, Devlin SP, Medina-Sánchez JM, Jones RI (2016) Experimental assessment of a possible microbial priming effect in a humic boreal lake. Aquat Sci 78:191–202.

Fischer H, Pusch M (2001) Comparison of bacterial production in sediments, epiphyton and the pelagic zone of a lowland river. Freshwater Biol 46:1335–1348.

Francoeur SN, Schaecher M, Neely RK, Kuehn KA (2006) Periphytic photosynthetic stimulation of extracellular enzyme activity in microbial communities associated with natural decaying wetland plant litter. Microb Ecol 52:662–669.

Haglund A, Törnblom E, Boström B, Tranvik L (2002) Large differences in the fraction of active bacteria in plankton, sediments, and biofilm. Microb Ecol 43:232–241.

Hanson PC, Pace ML, Carpenter SR, Cole JJ, Stanley EH (2015) Integrating landscape carbon cycling: research needs for resolving organic carbon budgets of lakes. Ecosystems 18:363–375.

Hessen DO (1985) The relation between bacterial carbon and dissolved humic compounds in oligotrophic lakes. FEMS Microbiol Ecol 1:215–223.

485 Hessen D, Andersen T, Lyehe A (1990) Carbon metabolism in a humic lake: Pool sires and
486 cycling through zooplankton. Limnol and Oceanogr 35:84–99.

487 Holgerson MA, Raymond PA (2016) Large contribution to inland water CO_2 and CH_4 emissions
488 from very small ponds. Nat Geosci 9:222–226.

489 Jansson M, Bergström A, Blomqvist P, Drakare S (2000) Allochthonous organic carbon and
490 phytoplankton/bacterioplankton production relationships in lakes. Ecology 81:3250–3255.

491 Jansson M, Persson L, De Roos AM, Jones RI, Tranvik L (2007) Terrestrial carbon and
492 intraspecific size-variation shape lake ecosystems. Trends Ecol Evol 22:316–322.

493 Jones RI, Salonen K (1985) The importance of bacterial utilization of released phytoplankton
494 photosynthate in two humic forest lakes in southern Finland. Holarc ecol 8:133–140.

495 Jones RI, Grey J, Sleep D, Arvola L (1999) Stable isotope analysis of zooplankton carbon
496 nutrition in humic lakes. Oikos 86:97–104.

497 Kairesalo T, Lehtovaara A, Saukkonen P (1992) Littoral-pelagial interchange and the
498 decomposition of dissolved organic matter in a polyhumic lake. Hydrobiologia 229:199−224.

499 Karhunen J, Arvola L, Peura S, Tiirola M (2013) Green sulphur bacteria as a component of the
500 photosynthetic plankton community in small dimictic humic lakes with an anoxic hypolimnion.
501 Aquat Microb Ecol 68:267–272.

502 Karlsson J, Berggren M, Ask J, Byström P, Jonsson A, Laudon H, Jansson M (2012) Terrestrial
503 organic matter support of lake food webs: Evidence from lake metabolism and stable
504 hydrogen isotopes of consumers. Limnol and Oceanogr 57:1042–1048.

505 Kirchman D, K'nees E, Hodson R (1985) Leucine incorporation and its potential as a measure of
506 protein synthesis by bacteria in natural aquatic systems. Appl Environ Microbiol 49:599–
507 607.

508 Kritzberg ES, Cole JJ, Pace MM, Granéli W (2005) Does autochthonous primary production
509 drive variability in bacterial metabolism and growth efficiency in lakes dominated by
510 terrestrial C inputs? Aquat Microb Ecol 38:103–111.

511 Kuehn KA, Francoeur SN, Findlay RH, Neely RK (2014) Priming the microbial landscape:
512 periphytic algal stimulation of litter-associated microbial decomposers. Ecology 95:749–
513 762.

514 McKnight DM, Aiken GR (1998) Sources and age of aquatic humus. In: Hessen D, Tranvik L
515 (ed) Aquatic humic substances. Springer Vertag, New York, New York, USA.

516 Neely RK, Wetzel RG (1995) Simultaneous use of ^{14}C and ^{3}H to determine autotrophic
517 production and bacterial protein production in periphyton. Microb Ecol 30:227–237.

518 Pace ML (2001) Getting It Right and Wrong, Extrapolations Across Experimental Scales. In:
519 Gardner RH, Kemp WM, Kennedy WS, Petersen J (ed) Scaling Relations In Experimental
520 Ecology. Columbia University Press, New York, USA.

521 Pace ML, Cole JJ, Carpenter SR, Kitchell JF, Hodgson JR, Van de Bogert MC, Bade DL,
522 Kritzberg ES, Bastviken D (2004) Whole-lake carbon-13 additions reveal terrestrial support
523 of aquatic food webs. Nature 427:240-243.

524 Paul A, Dziallas C, Zwirnmann E, Gjessing ET, Grossart HP (2012) UV irradiation of natural
525 organic matter (NOM); impact on organic carbon and bacteria. Aquat Sci 74:443–454.

526 Porter KG, Paerl R, Hodson M, Pace M, Priscu J, Riemann B, Scavia D, Stockner J (1988)
527 Microbial interactions in lake food webs. In: Carpenter SR (ed) Complex interactions in lake
528 communities. Springer, New York, USA.

529 Premke K, Karlsson J, Steger K, Gudasz C, von Wachenfeldt E, Tranvik LJ (2010) Stable
530 isotope analysis of benthic fauna and their food sources in boreal lakes. J N Am Benthol Soc
531 29:1339–1348.

532 Raymond PA, Hartmann J, Lauerwald R, Sobek S, McDonald C, Hoover M, Butman D, Striegl
533 R, Mayorga E, Humborg C, Kortelainen P, Durr H, Meybeck M, Ciais P, Guth P (2013)
534 Global carbon dioxide emissions from inland waters. Nature 503:355–359.

535 Rodríguez P, Ask J, Hein CL, Jansson M, Karlsson J (2013) Benthic organic carbon release
536 stimulates bacterioplankton production in a clear-water subarctic lake. Freshwater Sci
537 32:176–182.

538 Salonen K, Hammar T (1986) On the importance of dissolved organic matter in the nutrition of
539 zooplankton in some lake waters. Oecologia 68:246–253.

540 Salonen K, Hammar T, Kuuppo P, Smolander U, Ojala A (2005) Robust parameters confirm
541 predominance of heterotrophic processes in the plankton of a highly humic pond.
542 Hydrobiologia 543:181–189.

543 Sander BC, Kalff J. 1993. Factors controlling bacterial production in marine and freshwater
544 sediments. Microb Ecol 26:79–99.

545 Schallenberg M, Kalff J (1993) The ecology of sediment bacteria in lakes and comparisons with
546 other aquatic ecosystems. Ecology 74:919–934.

547 Schindler D, Scheuerell M (2002) Habitat coupling in lake ecosystems. Oikos 98:177–189.

548 Seekell DA, Lapierre J, Ask J, Bergström A, Deininger A, Rodríguez P, Karlsson J (2015) The
549 influence of dissolved organic carbon on primary production in northern lakes. Limnol and
550 Oceanogr 60:1276–1285.

551 Simon M, Azam F (1989) Protein content and protein synthesis rates of planktonic marine
552 bacteria. Mar Ecol Prog Ser 51:201–213.

553 Smith DC, Azam F (1992) A simple, economical method for measuring bacterial protein
554 synthesis rates in seawater using ^{3}H-leucine. Mar Microb Food Webs 6:107–114.

555 Solomon CT, Jones SE, Weidel BC, Buffam I, Fork ML, Karlsson J, Larsen S, Lennon JT, Read
556 JS, Sadro S (2015) Ecosystem consequences of changing inputs of terrestrial dissolved
557 organic matter to lakes: current knowledge and future challenges. Ecosystems 18:376–389.

558 Sundh I, Bell RT (1992) Extracellular dissolved organic carbon released from phytoplankton as a
559 source of carbon for heterotrophic bacteria in lakes of different humic content. In: Kairesalo
560 T, Jones R, Salonen K (ed) Dissolved Organic Matter in Lacustrine Ecosystems: Energy
561 Source and System Regulator. Springer Science+Business Media, B.V.

562 Taipale S, Kankaala P, Tiirola M, Jones RI (2008) Whole-lake dissolved inorganic ^{13}C additions
563 reveal seasonal shifts in zooplankton diet. Ecology 89:463–474.

564 Taipale S, Kankaala P, Hämäläinen H. Jones RI (2009) Seasonal shifts in the diet of lake
565 zooplankton revealed by phospholipid fatty acid analysis. Freshwater Biol 54:90–104.

566 Taipale S, Kankaala P, Hahn MW, Jones RI, Tiirola M (2011) Methane-oxidizing and
567 photoautotrophic bacteria are major producers in a humic lake with a large anoxic
568 hypolimnion. Aquat Microb Ecol 64:81–95.

569 Theil-Nielsen J, Søndergaard M (1999) Production of epiphytic bacteria and bacterioplankton in
570 three shallow lakes. Oikos 86:283–292.

571 Tranvik LJ (1988) Availability of dissolved organic carbon for planktonic bacteria in
572 oligotrophic lakes of differing humic content. Microb Ecol 16:311–322.

573 Tranvik LJ (1989) Bacterioplankton growth, grazing mortality and quantitative relationship to
574 primary production in a humic and a clearwater lake. J Plankton Res 11:985–1000.

575 Tranvik LJ (1992) Allochthonous dissolved organic matter as an energy source for pelagic
576 bacteria and the concept of the microbial loop. In: Kairesalo T, Jones R, Salonen K (ed)
577 Dissolved Organic Matter in Lacustrine Ecosystems: Energy Source and System Regulator.
578 Springer Science+Business Media, B.V.

579 Tulonen T (1993) Bacterial production in a mesohumic lake estimated from [^{14}C] leucine
580 incorporation rate. Microb Ecol 26:201–217.

581 Vadeboncoeur Y, Peterson G, Vander Zanden MJ, Kalff J (2008) Benthic algal production across
582 lake size gradients: interactions among morphometry, nutrients, and light. Ecology 89:2542–
583 2552.

584 Vadeboncoeur Y, Vander Zanden MJ, Lodge DM (2002) Putting the lake back together:
585 reintegrating benthic pathways into lake food web models. Bioscience 52:44–54.

586 Van de Bogert MC, Carpenter SR, Cole JJ, Pace ML (2007) Assessing pelagic and benthic
587 metabolism using free water measurements. Limnology and Oceanography: Methods 5:145–
588 155.

589 Van de Meutter F, Stoks R, De Meester L (2004) Behavioral linkage of pelagic prey and littoral
590 predators: microhabitat selection by *Daphnia* induced by damselfly larvae. Oikos 107:265–
591 272.

592 Vesterinen J, Devlin SP, Syväranta J, Jones RI (2016a) Accounting for littoral primary
593 production by periphyton shifts a highly humic boreal lake towards net autotrophy.
594 Freshwater Biol 61:265–276.

595 Vesterinen J, Syväranta J, Devlin SP, Jones RI (2016b) Periphyton support for littoral secondary
596 production in a highly humic boreal lake. Freshwater Sci. In press, DOI: 10.1086/689032

597 Wetzel RG (1993) Microcommunities and microgradients: linking nutrient regeneration,
598 microbial mutualism, and high sustained aquatic primary production. Neth J Aquat Ecol
599 27:3–9.

600 Wetzel RG, Hatcher PG, Bianchi TS (1995) Natural photolysis by ultraviolet irradiance of
601 recalcitrant dissolved organic matter to simple substrates for rapid bacterial metabolism.
602 Limnol and Oceanogr 40:1369–1380.

603 Wetzel RG, Søndergaard M (1998) Role of submerged macrophytes for the microbial
604 community and dynamics of dissolved organic carbon in aquatic ecosystems. In: Jeppesen
605 E, Søndergaard M, Søndergaard M, Christoffersen K (ed) The structuring role of submerged
606 macrophytes in lakes. Springer-Verlag, New York, New York, USA.

607 White PA, Kalff J, Rasmussen JB, Gasol JM (1991) The effect of temperature and algal biomass
608 on bacterial production and specific growth rate in freshwater and marine habitats. Microb
609 Ecol 21:99–118.

610

611 Figure 1. Location and bathymetry of Lake Mekkojärvi in southern Finland. Open circle denotes
612 the sampling point for the pelagic measurements. Numbers refer to depth in meters.

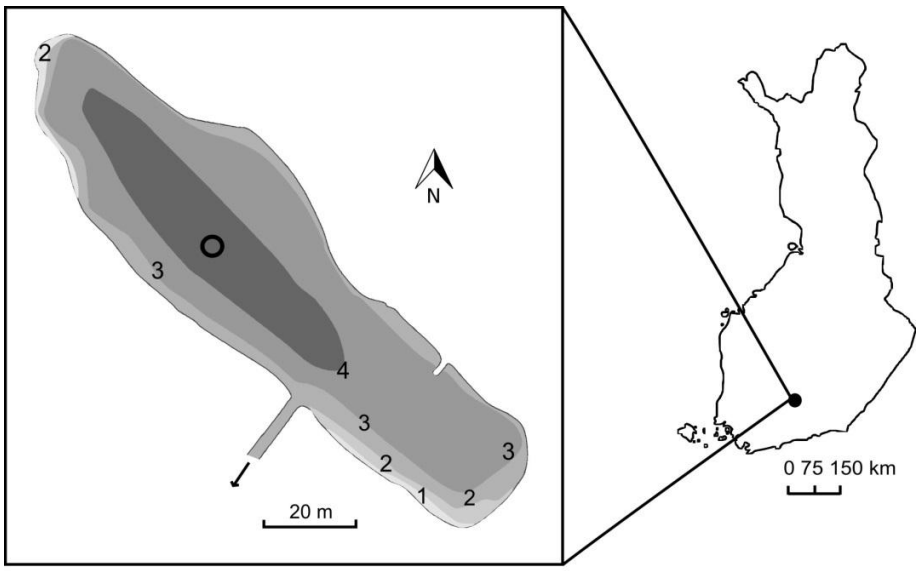
613 Figure 2. Pelagic bacterial production (BP) per unit area (mean ± SE) in three different strata in
614 2015. The dotted line expresses the areal BP in the whole water column as the sum of values
615 from three strata.

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

618 Figure 4. The mean ± SE uptake of leucine for epiphytic bacteria in 7 different leucine
619 concentrations.

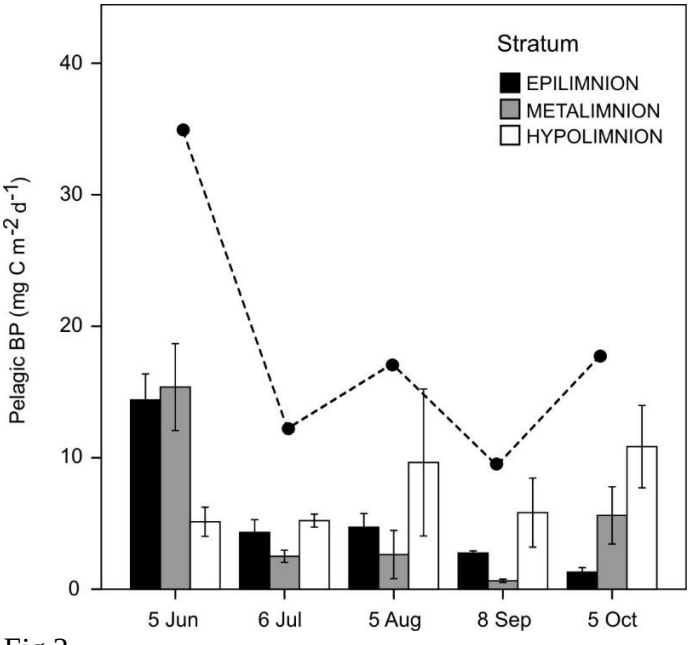
620 Figure 5. Littoral daily (mean ± SE) epiphytic BP in Mekkojärvi, derived from the noon rates by
621 multiplying by 24 and then normalized to g dry-weight of substratum.

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

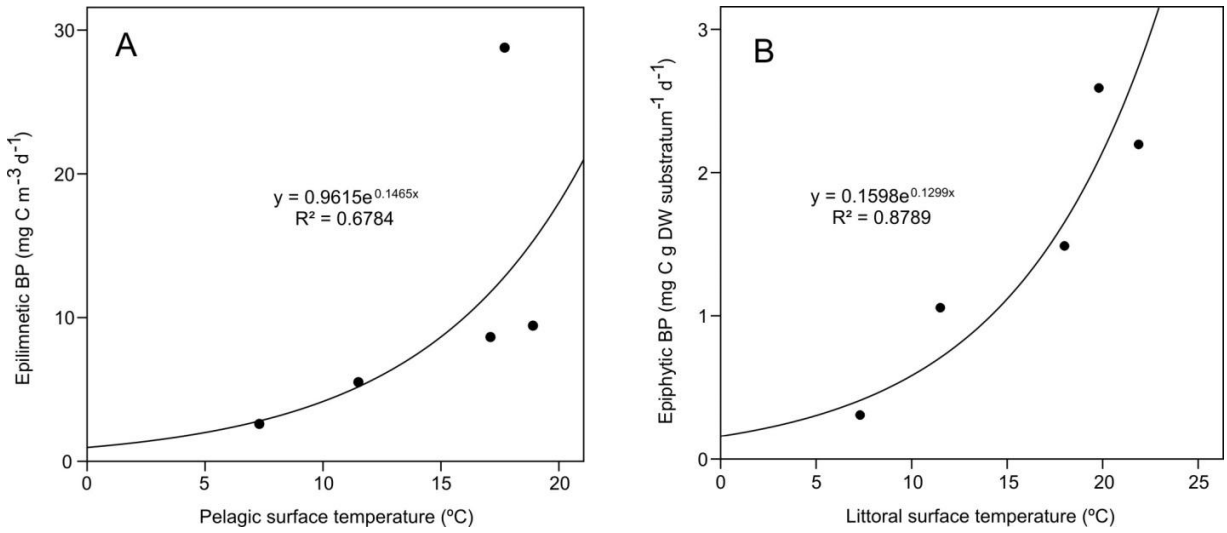


624
625 Fig 1.

626
627
628



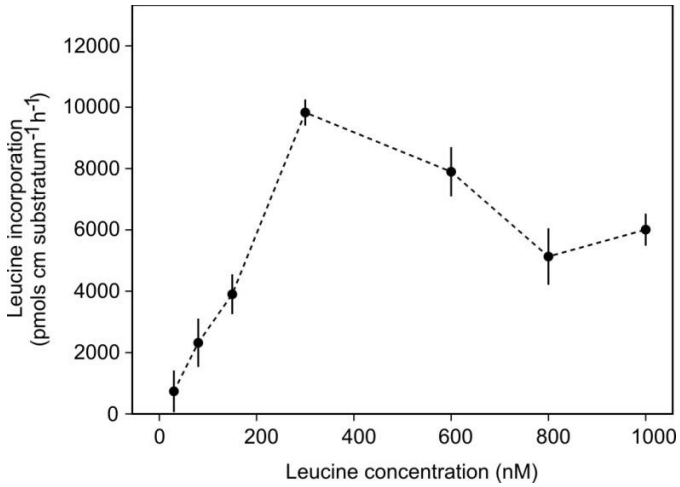
629
630 Fig 2.
631



632

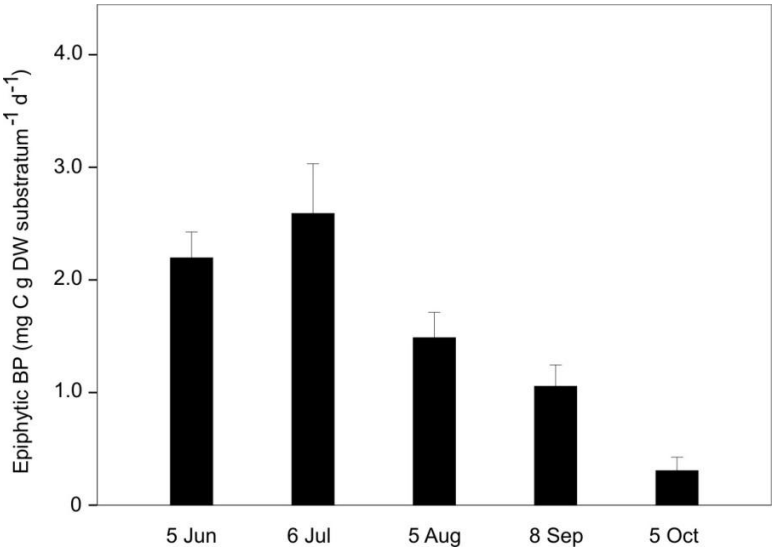
633 Fig 3.

634



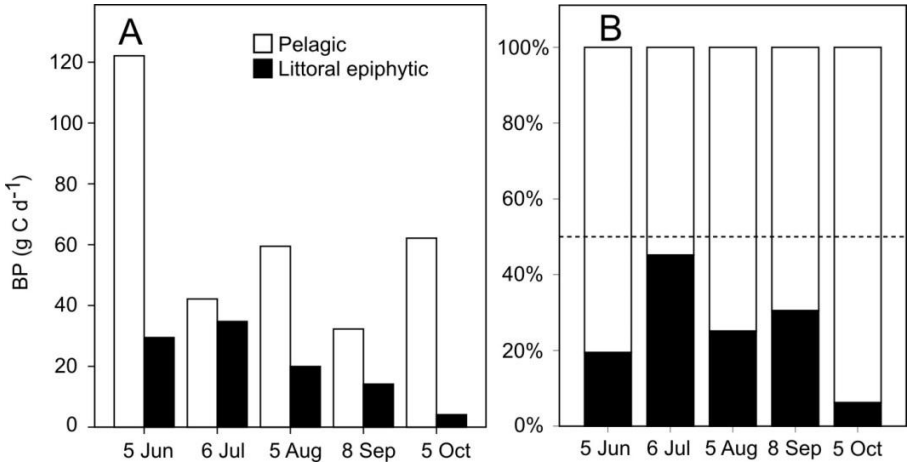
635

636 Fig 4.



637
638
639 Fig 5.

640



641
642 Fig 6.