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

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1   **Epiphytic bacteria make an important contribution to bacterial production in a humic**

2   **boreal lake**

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4   RH: Littoral bacterial production

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15

16   Abstract

17   Bacterial production (BP) in lakes has generally been measured only in the pelagic zone

18   without accounting for littoral BP, and studies of BP at the whole-lake scale are very scarce. In

19   the dystrophic humic lakes which are common throughout the boreal region, low light

20   penetration through water has been assumed to seriously limit available habitats for littoral

21   organisms. However, many highly humic boreal lakes have extensive partly submerged

22   vegetation around the lake perimeter which can provide well-lit substrata for highly productive

23   epiphyton. We measured epiphytic BP on the littoral vegetation and pelagic BP in a small highly

24   humic boreal lake in Finland during an open water season and extrapolated the BP rates to the

25   whole-lake. Pelagic BP dominated the combined BP over the study period, but the epiphytic BP

26   contributed an average of 24% to overall BP over the sampling period and was almost equal to

27   pelagic BP in July. According to these results, a substantial component of BP has been previously

28   overlooked in the lake when BP has been measured only from the pelagic. Our study

29   demonstrates that the role of the littoral zone in bacterial production in highly humic lakes has

30   previously been understated, and needs to be taken into account in assessments of whole-lake

31   carbon cycling and metabolism.

32

33   Keywords: littoral, periphyton, pelagic, autotrophic, heterotrophic

34

Introduction

35

36   Pelagic and littoral habitats have generally been studied separately in lake ecosystem and

37 food web research, and only very few studies have examined productivity in both habitats

38 (Vadeboncoeur et al. 2002). Although pelagic and littoral production can be integrated by mobile

39 consumers like fish (Schindler & Scheuerell 2002) and even zooplankton (Van De Meutter et al.

40 2004) which utilize both pelagic and littoral resources, studies of the magnitudes of primary

41 production (PP) and particularly of heterotrophic bacterial production (BP) at the whole-lake

42 scale and including both pelagic and littoral habitats, are scarce. In highly humic lakes the

43 importance of littoral benthic production has been assumed to be minor due to the very low light

44 penetration into the water (e.g. Vadeboncoeur et al. 2002) together with very steep stratification,

45 which restricts illuminated and oxygenated benthic habitats. However, Vesterinen et al. (2016a)

46 showed that epiphyton on surrounding littoral vegetation dominated the whole-lake PP in highly

47 humic Lake Mekkojärvi in southern Finland, demonstrating that macrophytes and partly

48 submerged terrestrial vegetation can provide extensive well-lit substrata for epiphyton and make

49 the littoral an appreciable habitat for PP in humic lakes.

50   Algae and bacteria coexist in periphytic biofilms in an association that offers space and

51 resources to sustain production of both groups of organisms, and positive correlations between

52 periphyton PP and BP, as well as between algal and bacterial biomass, have been well

53 documented (e.g. Neely & Wetzel 1995, Rier & Stevenson 2002, Carr et al. 2005, Kuehn et al.

54 2014). This can be more pronounced if light is not limiting algal growth and biomass production,

55 when algae produce a substantial extracellular polysaccharide matrix that creates an isolated

56 microenvironment, where inorganic nutrients can be effectively recycled (Wetzel 1993). Highly

57 humic Lake Mekkojärvi has extensive littoral vegetation, which mostly lies just under the water

3

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

81   several times during an open water period in Mekkojärvi, extrapolated the results to the whole-

82   lake scale and compared the magnitude of BP in the two habitats.

83

84   Material and methods

85   *Study lake*

86   The study was conducted at Lake Mekkojärvi (61º13'N 25º3'E), a small (0.35 ha) and

87   highly humic headwater lake in the Evo forest region in southern Finland (Fig.1) with mean and

88   maximum depths of 2.0 and 4.3 m. The lake is sheltered by surrounding coniferous forest and

89   receives a high loading of terrestrial organic matter from its catchment causing high dissolved

90   organic carbon (DOC) concentrations (30–33 mg C L^{-1}), highly coloured water (300–800 mg Pt

91   l^{-1}) and low pH (5.3–5.7) (Devlin et al. 2015, Vesterinen et al. 2016a). This causes the lake to

92   develop very steep temperature and oxygen gradients rapidly after ice-off in spring. Mekkojärvi

93   has ice cover usually from early November until the beginning of May. During the open water

94   period the thermocline lies between 0.5–1.0 m and anoxia occurs under that layer. Mekkojärvi

95   becomes totally anoxic during winter ice cover and therefore cannot sustain overwintering fish

96   populations, which has allowed development of very dense populations of the large-bodied

97   cladoceran *Daphnia longispina* in summer. Mekkojärvi has a depth ratio (DR = $\bar{z}/z_{max}$) of 0.47,

98   so the lake is relatively steep-sided and lacks illuminated benthic surfaces due to the highly

99   coloured water and very low light penetration (light-attenuation coefficient ranges from 4.5 to

100   7.5). Details of the lake's physical and chemical characteristics are presented elsewhere (e.g.

101   Vesterinen et al. 2016a). Mekkojärvi has been the subject of numerous studies, which have

102   revealed the importance of both allochthonous C and biogenic methane to productivity of the

103   pelagic system (e.g. Salonen & Hammar 1986, Jones et al. 1999, Salonen et al. 2005, Taipale et

5

104 al. 2008; 2011, Devlin et al. 2015). Bacterial densities are greater in the oxic-anoxic boundary

105 layer in the metalimnion and in the anoxic hypolimnion than in the oxic epilimnion (Arvola et al.

106 1992). The bacterial community in Mekkojärvi is mainly composed of heterotrophic,

107 chemoautotrophic and photoautotrophic bacteria, including photosynthetic green sulphur bacteria

108 (*Chlorobium* sp.) and methane-oxidizing bacteria (belonging to *Methylobacter* genus) which

109 contribute significantly to the bacterial biomass in the meta- and hypolimnion (Taipale et al.

110 2009). The littoral zone is not clearly defined in Mekkojärvi, but the lake has a surrounding

111 floating moss mat (consisting mainly of *Sphagnum* and *Warnstorfia* species) lining the lake

112 perimeter, with fallen terrestrial sedges (Carex sp.) and some macrophytes such as *Menyanthes*

113 *trifoliata*, *Phragmites australis* and *Utricularia* sp. associated with the moss mat. This

114 surrounding littoral vegetation does not extend further than ca. 1 m from the lake edge and not

115 deeper than ca. 0.5 m, but sustains highly productive periphyton assemblages, which have their

116 highest biomass in late-summer and can balance the whole-lake metabolism or even make the

117 lake net autotrophic (Vesterinen et al. 2016a).

118

119 *Pelagic bacterial production*

120   Pelagic sampling was carried out at the deepest point in the lake (Fig. 1). Temperature

121 and oxygen concentrations were measured at 0.5 m intervals from the surface to the bottom with

122 an oxygen and temperature sensor YSI 55 probe (YSI Inc., Yellow Springs, Ohio, USA) during

123 every sampling occasion in 2015. From these measurements the water column stratification was

124 defined as follows: 0.0–0.2 m (surface), 0.2–0.5 m (epilimnion), 0.5–1.0 (metalimnion), 1.0–3.0

125 m (hypolimnion).

126   Pelagic bacterial production was measured five times between June and October in 2015

127   using a [14C]-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 [14C]-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 [14C]-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

149 column and summing over depth. These were multiplied by the area of the lake to derive the

150 whole-lake BP values for the pelagic. To test the possible effect on anoxic hypolimnetic BP

151 samples of oxygen contamination from air in the incubation vessels, 5 parallel samples were

152 incubated in evacuated Labco Exetainers (Labco Limited, Lampeter, Ceredigion, UK)

153 simultaneously with other hypolimnetic samples in September.

154

155 *Littoral epiphytic bacterial production*

156   Epiphytic BP was measured five times together with pelagic BP in 2015. Littoral

157 temperatures were measured with a YSI 55 probe (YSI Inc., Yellow Springs, Ohio, USA) during

158 every sampling occasion. Samples of littoral vegetation were collected randomly from 6 sites

159 around the lake into 2 L plastic buckets filled with lake water from each site. As the littoral

160 vegetation consists mainly of moss and partly submerged sedges in Mekkojärvi, these were the

161 main representatives in the samples. Some larger plants, such as *Menyanthes trifoliata*, were not

162 sampled, as they were difficult to process in the laboratory. Buckets were stored in a cool box

163 containing lake water and taken to the laboratory of Lammi Biological Station, about 30 km

164 south from Mekkojärvi. BP was measured from epiphytic biofilms using a modified version of

165 the [^{3}H]-leucine incorporation method described by Ask et al. (2009) based on the method

166 originally developed by Smith & Azam (1992). [^{3}H]-leucine was used instead of [^{14}C]-leucine,

167 since it was available at sufficiently higher concentrations. Six randomly selected 1 cm long

168 subsamples of plant substratum from each sampling site were clipped and put into 1.2 mL

169 Eppendorf tubes containing 30 µL of [^{3}H]-leucine (specific activity of 112 Ci mmol^{-1},

170 PerkinElmer, Inc.) and 70 µL of distilled water with the final concentration of 300 nM, and half

171 of the samples were immediately killed by addition of 130 µL of 50% TCA. To determine the

172 appropriate [³H]-leucine concentration and the maximum incorporation of leucine into protein in

173 epiphytic biofilms, a saturation experiment was conducted once in early-June in which samples

174 were incubated in 7 different concentrations ranging from 30 to 1000 nM. Eppendorf tubes were

175 incubated outside the laboratory in an open cool box containing lake water for 60 min. The

176 samples were submerged at the same depth from which they originated so that they experienced

177 similar light conditions. The temperature of the water was measured during the incubation and no

178 increase above the lake *in situ* temperature was observed. Incubation was then terminated by

179 adding 130 µL of 50% TCA into the live samples and vortexing them. Samples were centrifuged

180 at 12400 rpm for 10 min and the supernatant was gently removed using a thin pipette. No marked

181 loss of epiphyton from the substratum was visible (although was not confirmed by microscopy).

182 1.2 mL of 5 % TCA was then added and the samples were again vortexed and centrifuged at

183 12400 rpm for 10 min. The supernatant was then removed, 1.2 mL of 80% EtOH was added and

184 samples were centrifuged as above. Finally, the supernatant was removed, the sample was

185 aerated and 1.2 mL of scintillation cocktail (OptiPhase 3) was added. Sample radioactivity was

186 counted with a Packard Tri-Carb® liquid scintillation counter (PerkinElmer, Waltham,

187 Massachusetts, USA). Leucine uptake rate was calculated as:

188 $\text{mmols leucine (cm substratum}^{-1}) \text{ h}^{-1} = (4.5 \times 10^{-13}) \times (\text{dpm sample} - \text{dpm blank}) \times (\text{SA})^{-1} \times (\text{T})^{-1}$

189   Eq. 1

190 , where factor 4.5×10^{-13} is the number of curies dpm^{-1} (a constant), SA is the specific activity of

191 the leucine solution in curies mmol^{-1} and T is the incubation time in hours.

192 Bacterial production was calculated as:

193 $\text{mg C (cm substratum}^{-1}) \text{ h}^{-1} = (\text{Leucine uptake rate}) \times 132.1 \times (\%\text{Leu})^{-1} \times (\text{C:Protein}) \times \text{ID}$

194   Eq. 2

9

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*

218   Repeated measures of analysis of variance (RMA) was used to test the differences in

219   pelagic BP among the sampling occasions (dependent variable/within-subject variable) and

220   between the strata (grouping variable/between-subject factor). Normality and homoscedasticity

221   (Levene's test) of the data were tested before statistical analysis. RMA was also used to test the

222   differences in epiphytic BP among the sampling occasions (dependent variable/within-subject

223   variable). Independent t-test was used to test the possible difference in hypolimnetic BP in oxic

224   and anoxic vials. Regression analysis was used to test the relationships between surface

225   temperatures and epilimnetic and epiphytic BP. All the statistical tests were conducted with IBM

226   SPSS Statistics (version 20.0.0.2; IBM, Armonk, New York, USA). All the descriptive statistics

227   are means ± SE if not expressly noted.

228

229   Results

230   *Pelagic bacterial production*

231   The mean O_2 concentrations over the study period were 4.1 ± 0.7 mg L^{-1} in the

232   epilimnion, 1.2 ± 0.5 7 mg L^{-1} in the metalimnion and 0.8 ± 0.1 7 mg L^{-1} in the hypolimnion.

233   Total pelagic BP was highest in early summer, and decreased steadily towards autumn (Fig. 2).

234   After the early summer peak, BP remained under 20.0 mg C m^{-2} d^{-1} (Fig. 2). Epilimnetic and

235   metalimnetic BP together constituted 85 % of the total pelagic BP in early-June. In July the rates

236   were similar in all the three strata. Hypolimnetic BP increased slightly towards autumn and

237   constituted the largest fraction of total pelagic BP in late-summer and autumn (56–63%). The

238   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

239   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

240   time was significant (RMA, $F_{4, 24} = 6.0$, $p < 0.01$), as were the interactions between time and

241   strata (RMA, $F_{8,24} = 4.0$, $p < 0.01$). Tukey's HSD test revealed significant differences in BP

242   between meta- and hypolimnion. Epilimnetic BP appeared to be generally related to the surface

243   temperature (Fig. 3A), but a high value in early summer prevented a significant correlation

244   (exponential regression, $F_{1,3} = 6.320$, $R^2 = 0.678$, $p = 0.087$). No statistically significant

245   difference (*t*-test, $t_6 = -0.606$, $p = 0.606$) was found between values of hypolimnetic BP

246   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

247   mg C m^{-3} d^{-1} in the anoxic).

248

249   *Littoral epiphytic bacterial production*

250   Based on the test conducted in early June, saturation of leucine incorporation into protein

251   appeared at 300 nM concentration (Fig. 4) and this concentration was therefore applied in the

252   production measurements. Variability among replicates may be the result of patchy occurrence of

253   periphyton on the substrata or variability of chlorophyll *a* (chl *a*) along the substrata, assuming

254   that there was a positive relationship between the periphyton chl *a* and BP. Epiphytic BP was

255   highest in summer (June and July) and decreased towards autumn (August, September, October;

256   Fig. 5). The change in BP over time was significant (RMA, $F_{4,68} = 17.6$, $p < 0.01$), and Tukey

257   HSD revealed significant differences between summer and autumn. Epiphytic BP correlated

258   significantly with littoral surface temperature (Fig. 3B; exponential regression, $F_{1,3} = 21.7$, $R^2 =$

259   0.878, $p = 0.019$).

260   Daily epiphytic BP in July calculated from the exponential function of BP and

261   temperature (Fig. 3B) and hourly temperatures from the *in situ* data logger was 2.8 mg C (g DW

262   substratum)$^{-1}$ d^{-1}, which is only ca. 10% higher than the value estimated multiplying hourly

263   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 $\pm$ 15.6 and 20.5 $\pm$

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

14

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 °C through the summer whereas surface temperature often rises

325 above 20 °C. 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

15

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

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356   clear-water lake. Allochthonous C has higher accessibility to sediment bacteria than to epiphytic

357   bacteria due to high burial rates of allochthonous OM to lake sediments particularly in smaller

358   lakes (Cole 2013). In small humic lakes, where non-illuminated sediments lack benthic

359   autotrophic production, all potential autochthonous C for sediment bacteria comes from the

360   upper water layers and will have been at least partly decomposed by pelagic bacteria. Therefore,

361   allochthonous C is presumably more important for sediment bacteria in small humic lakes.

362   Wetzel & Søndergaard (1998) described how macrophytes provide an extensive and diverse

363   three-dimensional habitat for microbial colonization, which results in a shift from dominance of

364   the macrophytes to the very high productivity of the attached microbiota. Theil-Nielsen &

365   Søndergaard (1999) described epiphytic biofilms as "hotspots" for BP, exploiting exudation of

366   DOC from macrophytes and epiphyton. Photolysis of recalcitrant allochthonous DOM can

367   produce labile organic molecules that are more available for heterotrophic bacteria (Wetzel et al.

368   1995, Paul et al. 2012). Since we only measured production of the epiphytic bacteria in

369   Mekkojärvi, we cannot distinguish between autochthonous and allochthonous C sources

370   supporting BP. As the littoral ambient water is brown with high quantities of allochthonous DOM

371   (Kairesalo et al. 1992) bacteria may utilise that directly and after photolysis. However,

372   considering the probable substantial release of labile autochthonous C from the highly productive

373   epiphytic biofilms in the littoral (Vesterinen et al. 2016a), the bacteria are likely to rely heavily

374   on autochthonous C. A light-mediated biotic decomposition process via algal stimulation of

375   litter-associated microbial heterotrophs has recently been recognized (e.g. Francoeur et al. 2006,

376   Danger et al. 2013). Kuehn et al. (2014) studied this process and concluded that periphytic algae

377   function as a photosynthetic conduit for labile carbon supply to microbial heterotrophs (bacteria

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378   and fungi) over very short time intervals, demonstrating the important role of bacteria and fungi

379   in this light-mediated carbon cycling process.

380   Vesterinen et al. (2016a) demonstrated how the littoral in Mekkojärvi was strongly net

381   autotrophic and reported 364 ± 66 mg C (g DW substratum)$^{-1}$ d^{-1} as the mean PP by epiphyton in

382   Mekkojärvi in 2012. Comparison to the mean daily epiphytic BP of 1.52 ± 1.36 mg C (g DW

383   substratum)$^{-1}$ d^{-1} in this study reveals the strong dominance of autotrophic production in the

384   biofilms in the littoral and large quantities of autochthonous C potentially available for secondary

385   production. A similar comparison between pelagic PP (in 2012) and BP (in 2015) reveals that the

386   PP during the phytoplankton spring maximum can be 10 times higher than BP but the rates later

387   in summer and autumn can be very even. Since the strong overall net heterotrophy and very high

388   bacterial respiration has been demonstrated in the pelagic in Mekkojärvi (Salonen et al. 2005,

389   Vesterinen et al. 2016a), it is likely that both anaerobic bacteria (e.g. green sulphur bacteria) and

390   sediment bacteria contribute strongly to the whole-lake metabolism. However, comparison of PP

391   and BP measured in different years can only be considered indicative. We did not measure

392   epiphytic PP or chl *a* in this study, but comparison between the PP rates and chl *a* in the

393   epiphyton in Mekkojärvi in 2012 (Vesterinen et al. 2016a) and BP in this study reveals a similar

394   trend of increase from spring to late summer and then decrease towards autumn. This also

395   indicates the possible positive relationship between the epiphytic BP and the autochthonous C

396   produced by epiphyton. However, the relative concentrations of OC originating from internal net

397   primary production (NPP) versus allochthonous OM loading to lake metabolism remains

398   unresolved (Hanson et al. 2015).

399   There are various sources of error and uncertainty included in any studies which attempt

400   to upscale rate estimates made in bottle incubations to the whole-lake scale (Hanson et al. 2015).

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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,

424 Holgerson & Raymond 2016). Although it is reasonable to suppose that in Mekkojärvi labile

425 autochthonous C produced by epiphytic algae is an important source for closely associated

426 bacteria, our study does not provide direct evidence of this phenomenon. This question merits

427 future study in which more sophisticated whole-lake scale approaches, which account for both

428 pelagic and littoral habitats, can address the role of the littoral zone in humic lakes.

429

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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
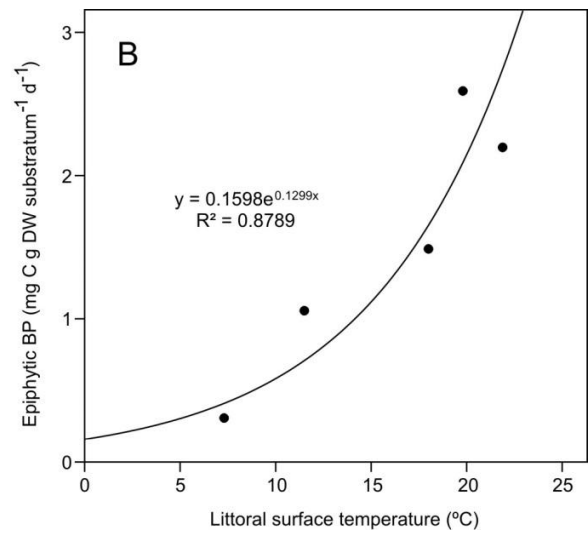
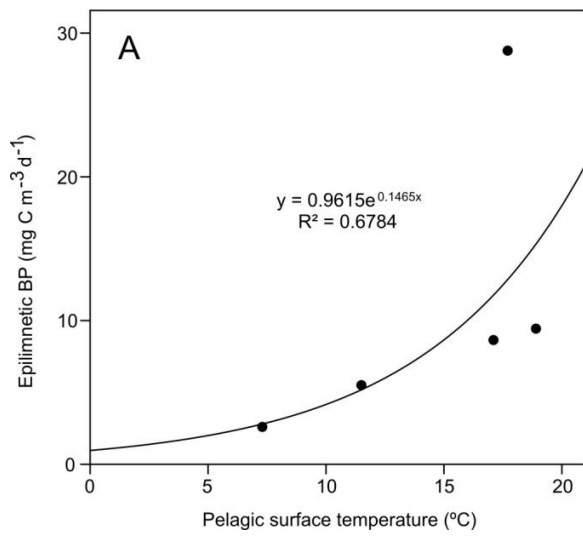623   bacteria and B) their relative proportions.
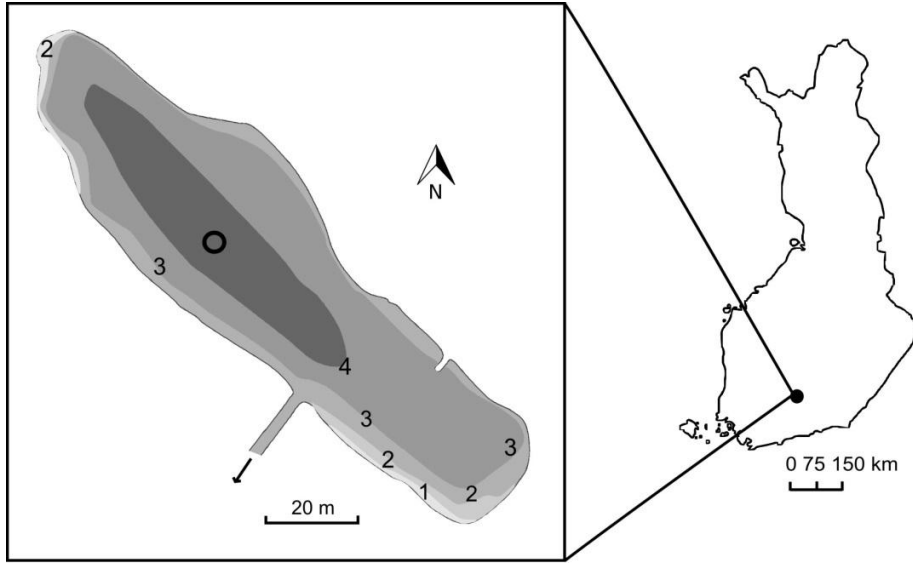
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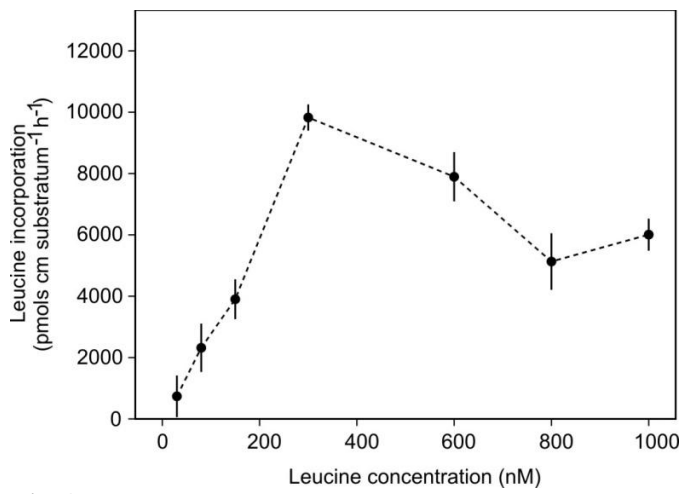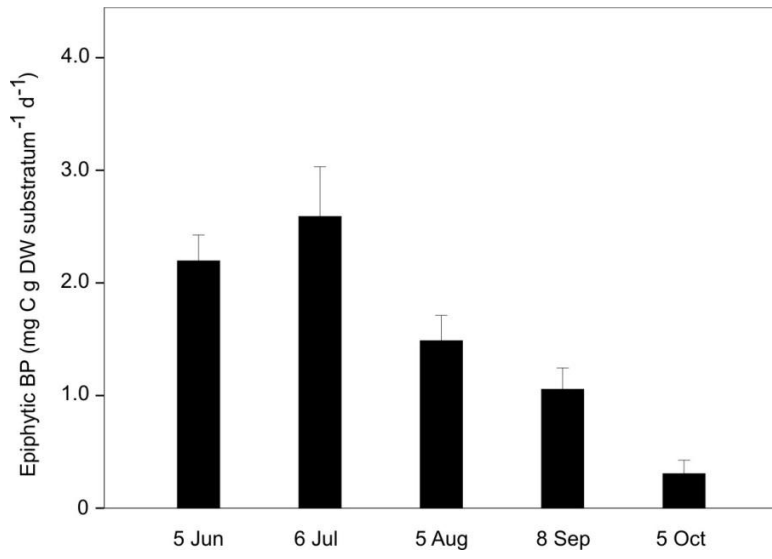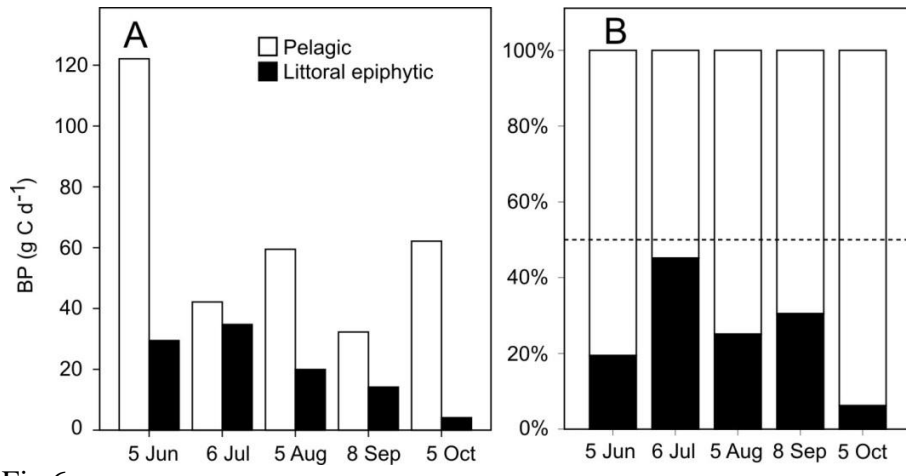626
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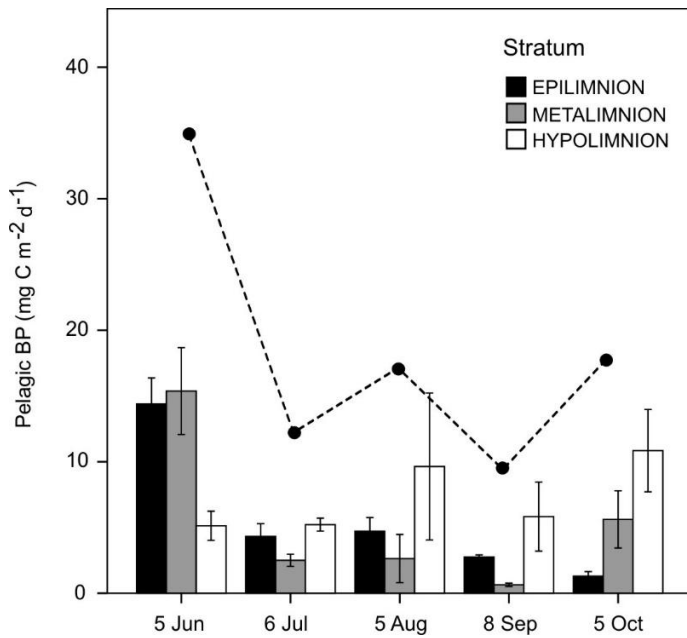
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