

1 **Metabarcoding of unfractionated water samples relates phyto-, zoo-**
2 **and bacterioplankton dynamics and reveals a single-taxon bacterial**
3 **bloom**

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32 **Summary**

33 Most studies of aquatic plankton focus on either macroscopic or microbial
34 communities, and on either eukaryotes or prokaryotes. This separation is
35 primarily for methodological reasons, but can overlook potential interactions
36 among groups. We tested whether DNA-metabarcoding of unfractionated water
37 samples with universal primers could be used to qualitatively and
38 quantitatively study the temporal dynamics of the total plankton community in
39 a shallow temperate lake. We found significant changes in the relative
40 proportions of normalized sequence reads of eukaryotic and prokaryotic
41 plankton communities over a three-month period in spring. Patterns followed
42 the same trend as plankton estimates using traditional microscopic methods.
43 We characterized the bloom of a conditionally rare bacterial taxon belonging to
44 *Arcicella*, which rapidly came to dominate the whole lake ecosystem and would
45 have remained unnoticed without metabarcoding. Our data demonstrate the
46 potential of universal DNA-metabarcoding applied to unfractionated samples
47 for providing a more holistic view of plankton communities.

48

49 **Introduction**

50 Microbial communities are an integral component of total biodiversity
51 (Barberán *et al.*, 2014) and play key roles in all ecosystems. Understanding of
52 their composition and dynamics is a critical component of studying ecosystem
53 functions and services. Plankton communities in freshwater and marine
54 ecosystems are comprised of both microbial and macroscopic organisms from
55 all three domains of life (archaea, prokaryotes, and eukaryotes). Traditionally,
56 plankton is classified into functional groups such as phytoplankton,
57 zooplankton, and bacterioplankton; or into size classes such as picoplankton,

58 nanoplankton, and microplankton. This classification has resulted in the
59 emergence of independent fields of inquiry, particularly the separation of
60 prokaryotic and eukaryotic groups.

61

62 A consequence of this separation is that studies rarely survey all members of
63 the plankton community simultaneously, except for a few contemporary marine
64 surveys (Steele *et al.*, 2011, Lima-Mendez *et al.*, 2015). This is despite the
65 potential that integrated studies have for providing an interdisciplinary view of
66 plankton communities (Fuhrman *et al.*, 2015) by shedding light on the strength
67 of biotic interactions (Needham and Fuhrman 2016). Most plankton studies
68 employ size-selection steps (i.e. size fractionation by selective filtration) and
69 genetic markers targeting either bacteria, archaea, or eukaryotes. Our
70 literature review found that less than 0.5% of studies targeted all three (SI1).
71 This tradition impairs a full integration of microbial communities into ecological
72 concepts.

73

74 We used a universal 16S/18S primer pair to perform DNA-metabarcoding of
75 unfractionated water samples in a study of the entire plankton community of a
76 eutrophic, shallow, temperate lake (*Kleiner Gollinsee*) in northeastern Germany.
77 We extracted total DNA from direct-filtered (0.2 μm) lake water (0.5 to 1 L),
78 enabling us to screen all organisms from what is traditionally size-classified as
79 pico- to approximately mesoplankton (per definition 0.2 μm - 20 mm). Our aim
80 was to characterize the whole plankton community and its temporal dynamics
81 in relation to algal biomass over a three-month period in spring (April - June)
82 2010. This is the period with the largest changes in plankton abundance and a
83 high species turnover in most temperate eutrophic lakes. Our sampling (see SI1

84 for parameters and experimental procedures) was part of a larger, more
85 traditional whole-lake survey of bacteria, phytoplankton and zooplankton from
86 April 2010 to December 2011 (Brothers *et al.*, 2013, Hilt *et al.*, 2010) that we
87 used for comparison.

88

89 **Results & Discussion**

90 *Prokaryotic- and eukaryotic population dynamics*

91 The DNA-metabarcoding of unfractionated water samples successfully
92 amplified organisms across all three domains of life, yielding a total of 1986
93 bacterial, 544 eukaryotic, and 315 archaeal operational taxonomic units (OTUs)
94 in the dataset. We recovered dominant organisms from nano- to mesoplankton
95 size classes (see SI2 for taxa lists) including typical freshwater bacteria (e.g.
96 *Polynucleobacter*, *Candidatus Aquirestis*), phytoplankton (e.g. *Cryptomonas*,
97 *Synechococcus*), and zooplankton (e.g., *Cryptocaryon*, Diaptomidae). One field
98 sample (June littoral zone sample) contained a small fish larva (inadvertantly
99 sampled) and this was detected by DNA-metabarcoding as 1% of sequencing
100 reads in that sample (classified as Cyprinidae, depicted as Teleostei in SI2).
101 Archaeal sequences were not abundant in our Lake Gollin samples. This was
102 not surprising because the lake was oxic during the sampling period and
103 Archaea are rarely found in oxygenated freshwaters (Pernthaler *et al.*, 1998,
104 Gies *et al.*, 2014). The low number of archaea was not likely caused by a primer
105 bias, because the primer pairs have been used successfully to detect a
106 dominance of *Archaea* in the anoxic zone of a meromictic lake (Gies *et al.*,
107 2014). We observed a pronounced shift in relative read abundance from a
108 dominance of eukaryotes in April to a dominance of prokaryotes in June for all
109 sampled water compartments (i.e. littoral, pelagic and sediment zones; Fig.

110 1a). This was accompanied by an increasing heterotrophs:phototrophs ratio
111 (SI3).

112

113 *Comparison with microscopical observations*

114 Abundance patterns based on DNA-metabarcoding data followed the trend of
115 the sum parameters of phyto-, zoo- and bacterioplankton obtained from
116 traditional microscopical counting data (e.g. ciliates in Fig. 1b, SI3). We
117 recovered all microscopically counted planktonic organisms, although there
118 were frequent mismatches between the classification depths in the two
119 methods (Table S2 in SI1). Counting and sequence data were not taken on the
120 same day and are therefore not directly comparable. Nonetheless, accounting
121 for this difference by averaging, we found a rank-based correlation between
122 phytoplankton reads and phytoplankton counts (Spearman's $\rho = 0.66$, $p <$
123 0.001). For zooplankton this relationship was not significant ($p > 0.05$),
124 although subsets exhibited a strong correlation (e.g. certain groupings of
125 ciliates). The strongest correlation between sequence and count datasets was
126 found with log-log transformed data for both phytoplankton (Pearson's $r = 0.45$,
127 $p < 0.001$) and zooplankton ($r = 0.37$, $p < 0.05$).

128

129 Our data also captured the temporal dynamics in reported species abundances
130 of the lake. A significant fish-kill event occurred in the winter prior to our study,
131 caused by a prolonged ice-cover that led to anoxia (Hilt *et al.*, 2015). This had
132 an important impact on the lake ecosystem, and a bloom of herbivorous ciliates
133 in April 2010 (Lischke *et al.*, 2016) was clearly visible in our sequence data
134 (approx. 30% of all reads, Fig. 1b). The ciliates would have exerted strong
135 grazing pressure on the small plankton ($< 5 \mu\text{m}$; Lischke *et al.*, 2016) but the

136 ciliate population crashed in May-June (Fig. 1b) and the role of grazer was then
137 filled by crustaceans (Hilt *et al.*, 2015). Similarly, our sequence data from the
138 pelagic zone indicated shifting crustacean:rotifer:ciliate read ratios, being
139 0:6:56 in April, 9:1:26 in May, and 38:6:5 in June (SI3). The replacement of
140 ciliates by crustaceans may have opened a niche for the observed bacterial
141 dominance in June, via reduced grazing pressure and increased substrate
142 supply via sloppy feeding of the copepods.

143

144 *Bloom-forming OTUs*

145 The bacterial dominance occurred in June and was attributed to OTUs classified
146 as *Arcicella* and *Variovorax* (Fig. 1c,d). June was also the period in which the
147 highest bacterial carbon production was measured (SI3, Brothers *et al.*, 2013;
148 Lischke *et al.*, 2016). A single *Arcicella* OTU was most abundant in the pelagic
149 open water and its greatest proportion of reads (40%) was observed at 1 m
150 sampling depth (Fig. 1c), suggesting it colonized the water surface. *Variovorax*
151 was more prevalent above the sediment, suggesting colonization from the
152 sediment (Fig. 1d). In contrast to *Variovorax*, which exhibited already stable
153 abundances at the other two sampling dates, *Arcicella* was present at very low
154 abundances in April and May (<0.2%) and can thus be classified as a
155 conditionally rare taxon (Lynch and Neufeld, 2015). There are few reports of
156 blooms of rare bacterial taxa correlated with algal blooms. Gilbert *et al.*, 2012
157 described a *Vibrio* sp. bloom in the English channel and Bizic-Ionescu *et al.*
158 (2014) described the genera *Flavobacterium* and *Undibacterium* associated
159 with a phytoplankton breakdown event in a lake. In order to test whether
160 *Arcicella* was a reoccurring taxon in Lake Gollin or if this was a unique
161 appearance related to the fish-kill disturbance, we screened additional samples

162 (two size-fractions in this case: 0.2-5 μm & > 5 μm , from 19 monthly taken
163 samples) that were available from Lake Gollin (Brothers *et al.*, 2013). *Arcicella*
164 occurred again in the following year (Fig. 2) and its periodical appearance may
165 be negatively related to chlorophyll-*a* concentrations and positively to
166 crustacean biomass (Fig. 2b; see Table S3 in SI1 for correlations). This bacterial
167 bloom therefore appears to be different from previously described blooms
168 which were positively linked to phytoplankton abundance (e.g. Gilbert *et al.*,
169 2012). We conclude that it was likely related to the fish-kill (see above), and
170 our data on microbial species turnover also suggested opening of a niche
171 allowing for bloom development of a single bacterial species. Conditionally rare
172 taxa can thus have a disproportional effect on community structure (Shade *et*
173 *al.*, 2014), although knowledge of their ecological and metabolic potential is
174 required better understand their ecosystem-wide consequences.

175

176 *What is Arcicella?*

177 We searched the freshwater and marine literature and found few reports of
178 *Arcicella* in environmental samples. *Arcicella* was the second-most abundant
179 OTU (6.8%) in a study of 5 large arctic rivers (Crump *et al.*, 2009), although the
180 taxon is not further discussed in their article. In some cases, *Arcicella* was
181 mentioned in a figure legend or in supplementary materials and we had to
182 request abundance data from the authors. One *Arcicella* OTU was among the
183 most dominant OTUs in the Danube river although it comprised only 2% (\pm 2%)
184 of relative read abundance (D. Savio pers. comm.; Savio *et al.*, 2015). A single
185 *Arcicella* OTU comprised 12 (\pm 4%) of the relative read abundance in a small
186 turbid glacial lake (Peter pers. comm.; Peter and Sommaruga, 2016). In spite of
187 these observations, their autecology has never been investigated.

188

189 *Study limitations and perspectives*

190 There was a large standard deviation among replicates for crustaceans and
191 rotifers (depicted as zooplankton in S3). These organisms belong to the
192 mesoplankton size class (0.2 to 20 mm) and it may be necessary to increase
193 the water volume of samples to better quantify them. Sequences belonging to
194 Metazoa could only be classified to phylum-class level (e.g. Maxillopoda,
195 Rotifera, Teleostei) using the automatic classifier. One reason for this is that the
196 SILVA reference taxonomy that we used has not implemented freshwater
197 zooplankton yet, but we partially resolved this problem by using the original
198 taxonomy provided by NCBI/EMBL. A second reason is that the SSU region
199 targeted here does not provide sufficiently variation for many Metazoan groups
200 (Tang *et al.*, 2012). We therefore estimated the resolution of the universal
201 marker for all of the planktonic organisms in Lake Gollin (see Table S2 in SI1).
202 Most Rotifera OTUs could be classified only to order, while most Maxillopoda
203 OTUs could be classified to family or genus level. Differing taxonomic resolution
204 among groups (in particular Metazoan taxa) is one limitation of using a
205 universal marker, and this should be considered in future studies of these
206 groups. The effect is most obvious when employing a fixed OTU cut-off such as
207 97%. One solution could be to combine universal metabarcoding with a
208 dynamic cutoff method focused on noise removal than on clustering (e.g., Eren
209 *et al.*, 2015). Such an approach may increase the phylogenetic resolution.
210 Moreover, biases in relative taxa abundance can be introduced through DNA
211 extraction, PCR (primer choice, amplification), library preparation and
212 sequencing steps (Gilbert *et al.*, 2012; Singer *et al.*, 2016). As a result,
213 quantitative estimates are likely to be semi-quantitative at best. Universal

214 metabarcoding has the same limitation but at least comes with the advantage,
215 that it provides a balance between all organisms, since most of the DNA
216 template will be derived from the target groups (only excluding viruses in this
217 case). To date, it has produced conclusive results for the general trends in
218 plankton communities (this study, see also Gies *et al.*, 2014, Parada *et al.*,
219 2016, Needham and Fuhrman, 2016).

220

221 **Conclusions**

222 Using unfractionated water samples with universal DNA metabarcoding allowed
223 us to document major changes in almost the entire size- and functional
224 spectrum of freshwater plankton with a single water sample analysis. Changes
225 in the relative abundance of OTUs closely matched the seasonal dynamics of
226 phyto-, zoo-, and bacterioplankton reported for this lake based on microscopy,
227 indicating that relative abundance data based on read counts are ecologically
228 meaningful. The discovery of a bloom of a largely overlooked freshwater
229 bacteria genus was remarkable, with potential implications for the whole lake
230 ecosystem. Our results highlight the potential of simultaneously studying both
231 microbial and macrobial communities for an improved understanding of whole
232 ecosystem changes. Integrative and interdisciplinary analyses may help to
233 answer broad ecological questions in freshwater systems related to the role of
234 keystone species; ecosystem resilience and resistance; and cross-domain
235 interactions of species. Unfractionated sampling coupled with metabarcoding
236 using a universal primer provides a powerful approach for studying plankton
237 dynamics in aquatic systems and shows promise for long-term whole
238 community monitoring.

239

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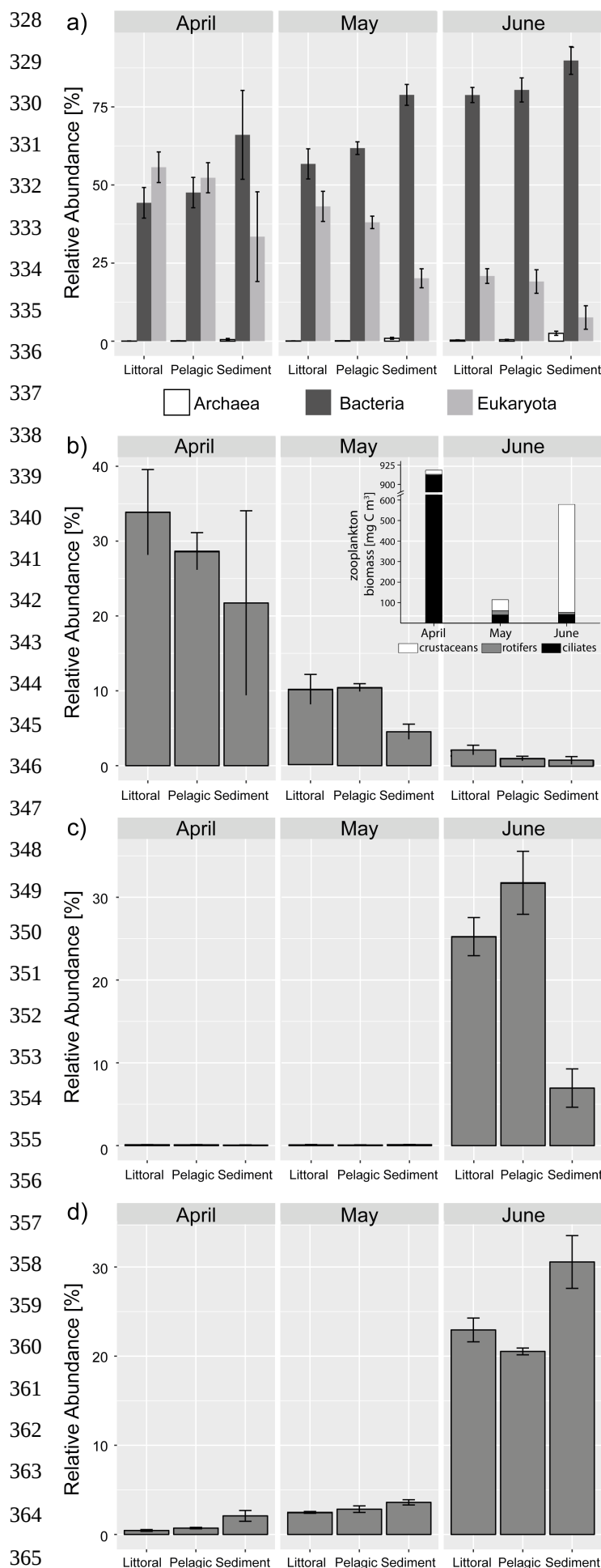
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366 **Figure 2.** Seasonal appearance of (a) *Arcicella* exhibited pronounced maxima and
367 minima over the course of the 2 years and appeared in the particle-attached ($> 5 \mu\text{m}$)
368 and free-living fraction ($0.2 - 5 \mu\text{m}$). *Arcicella* was detected using a PCR assay (see
369 experimental procedures S1) and evaluated based on gel electrophoresis band
370 intensity where 0 = no PCR product, 1 = very weak product, 2 = weak product, 3 =
371 medium product, 4 = strong product. For this data we applied rank based correlation
372 tests based on local similarities for time-series analysis (see Table S3 in SI1).
373 Correlations that were significant correlated with the band intensity of *Arcicella*
374 products derived from both size fractions were Chlorophyll a and crustacean biomass,
375 both of which were plotted in panel (b).

