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Universal metabarcoding of pico- to mesoplankton reveals seasonal dynamics and a bacterial bloom

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Abstract

Most studies of biodiversity focus on either macroscopic or microbial communities, with little or no simultaneous study of eukaryotes and prokaryotes. We tested whether a universal metabarcoding approach could be used to study the total diversity and temporal dynamics of aquatic pico- to mesoplankton communities in a shallow temperate lake. The approach revealed significant changes in the relative abundance of eukaryotic and prokaryotic plankton communities over a period of three months. These patterns, based on sequencing reads, fit with counts using traditional methods. We also witnessed the bloom of a conditionally rare bacterial taxon belonging to *Arcicella*, a genus that has been largely overlooked in freshwaters. Our data demonstrate the potential of universal metabarcoding as a complement to traditional studies of plankton communities, and for long-term monitoring across a broad range of organisms.

Introduction

Microbial communities play key roles in ecosystems and knowledge on their composition and dynamics is critical for understanding ecosystem functions and services. Recently, Barberán et al. (2014) emphasized the importance of studying microbial diversity as an integral part of total biodiversity. In aquatic ecosystems, plankton communities are a mixture of prokaryotes and eukaryotes of a wide range of sizes, and the traditional separation into size classes and domains, with their largely independent research disciplines, impairs a full integration of microbial communities into ecological concepts. Simultaneous studies of diversity of all three domains are rare (< 1% of all studies, SI1) in environmental sequencing surveys (e.g., Steele *et al.*, 2011,

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Lima-Mendez *et al.,* 2015) and size pre-selection steps are common in most analyses. Nonetheless, bacterioplankton should exhibit temporal dynamics that are similar to those of macroorganisms (Shade et al. 2013), suggesting that the monitoring of temporal dynamics of all organisms has great potential for identifying ecological interactions (Fuhrman *et al.,* 2015). In addition, metadata on organismal ratios such as eukaryotes : prokaryotes or heterotrophs : phototrophs could be useful for monitoring ecosystem changes. Here we used a universal metabarcoding approach to simultaneously study the dynamics of both prokaryotic and eukaryotic plankton (pico- to mesoplankton) in a lake that exhibits pronounced seasonal plankton dynamics.

Methods

We applied universal metabarcoding to water samples of the eutrophic, shallow temperate Lake Gollin (Kleiner Gollinsee) in northwestern Germany. Lake Gollin was sampled monthly as part of a study of bacteria, phyto- and zooplankton from April 2010 to December 2011 (Brothers *et al.,* 2013; see SI1 for parameters and methods). Briefly, we took monthly samples in spring 2010 (April – June) from littoral water, pelagic water, and water above the pelagic sediment. DNA was extracted from filters (0.22 µm Sterivex; Millipore, Germany) using commercial kits and mechanical bead-beating. We used the V9 region of the ribosomal small subunit for taxa detection (Engelbrektson *et al.,* 2010). Methodological discussions on a related integrative single marker can be found in Parada *et al.* (2015). Amplicons were constructed and sequenced following the conditions described for pyrosequencing by Hölker *et al.* (2015) with the modification that we employed the AccuPrime High Fidelity Polymerase (Invitrogen, Carlsbad, USA). Sequences were processed in Mothur (version

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1.24.1; Schloss *et al.,* 2009) and classified with SINA aligner (version 1.2.11; Pruesse *et al.,* 2012) against the SILVA SSU reference database (115 Ref NR 99, www.arb-silva.de). After detection of a bacterial bloom (see below) we designed a PCR test for *Arcicella* bacteria for the monthly samples. PCR products were quantified by gel electrophoresis assigning them to four intensities.

Results & Discussion

There was a pronounced shift in dominance from mainly eukaryotes in April to predominantly prokaryotes in June (Fig. 1a; SI2 for taxa browsing). This was accompanied by an increased heterotrophs : phototrophs ratio (SI3). Archaea were not abundant in Lake Gollin, despite their high abundance in some meromictic lakes (Gies *et al.,* 2014). In the winter prior to our study, a significant fish-kill led to a bloom of herbivorous ciliates in April 2010 that was confirmed by our data (Fig. 1d). This likely exerted a very high grazing pressure upon the small plankton (<5 µm; Lischke *et al.,* 2016). Potentially, the abrupt disappearance of ciliates in May – June (Fig. 1d) following increasing crustacean abundances (Hilt *et al.,* 2015) reduced the abundance of algae and bacterial grazers. This may have opened a niche for the detected bacteria, in particular of the genera *Aricella* and *Variovorax* (see below). Our molecular data complement traditional counting data, following the trend of the sum parameters of phyto-, zoo- and bacterioplankton (SI3). The approach may therefore have the potential to follow dynamics of entire plankton communities in a single analysis.

Bacterial OTUs of the genera *Arcicella* and *Variovorax* were major representatives of the bacterial "bloom" that dominated the lake in June. *Arcicella* was more abundant in the upper water layers and potentially

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colonized the water from the neuston, whereas *Variovorax* was more abundant above the sediment suggesting colonization from the sediment (Fig. 1b, c). Interestingly, *Arcicella* was rare in April and May (<0.2%). There are few reports of blooms of rare bacterial taxa (e.g., Gilbert et al., 2012) and causes for single taxa bacterial blooms are often connected to phytoplankton events (Bizic-Ionescu et al. 2014). *Arcicella* re-occurred the following year (Fig. 2) and we were able to relate its appearance to the phyto- and zooplankton dynamics (SI1). In contrast to the *Vibrio* sp. bloom described by Gilbert *et al*. (2012), our *Arcicella* OTU can be classified as conditional/recurrent rare taxa (Lynch and Neufeld, 2015). Searching through existing freshwater data sets, we found *Arcicella* to be a prevalent freshwater lineage on a global scale, especially in lotic ecosystems. *Arcicella* was reported as the second most abundant OTU (6.8%) in all large circumpolar streams (Crump *et al.,* 2009). In other studies, Arcicella was detected but not explicitly reported. For example, a single *Arcicella* OTU was among the dominant taxa in the Danube River, comprising 2% ± 2% (Savio pers. comm.; Savio *et al.*, 2015) and a single *Arcicella* OTU reached 12 ± 4% in a small turbid glacial lake (Peter pers. comm.; Peter and Sommaruga, 2016). Despite those previous appearances, their autecology has never been discussed, suggesting that we have probably revealed a thus far unrecognized major freshwater lineage.

Using universal metabarcoding we could monitor major changes in freshwater pico- to mesoplankton with a single water sample analysis. Changes in relative abundance of OTUs matched the seasonal dynamics that have previously been reported for this lake. The discovery of a conditionally rare taxon supports the observed plankton dynamics for the bacterioplankton, since such taxa are of a

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disproportional significance for the overall community dynamics (Shade *et al.,* 2014). Integrative analysis may help to answer broader ecological questions related to the role of keystone species, ecosystem resilience and resistance, and cross-domain interactions of species (Lima-Mendez *et al.,* 2015). Universal metabarcoding represents a valuable means of studying plankton dynamics in aquatic systems and shows promise for long-term monitoring.

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Conflict of interest

The authors declare no conflict of interest.

Supplementary information is available

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Figure Legends

Figure 1. Spatial and temporal changes of microorganisms in Lake Gollin from three samplings (April, May, June) and locations (littoral, pelagic, and above sediment) displaying abundances (mean ± standard deviation) based on a rarefied OTU matrix (1744 reads/sample) of (a) all three domains with a total of 1307 bacteria, 388 eukaryote, and 190 archaea OTUs in the dataset; (b) the dominant *Arcicella* OTU; (c) the dominant *Variovorax* OTU and (d) the sum of all ciliate OTUs (70 OTUs).

Figure 2. Seasonal appearance of *Arcicella* assessed via a PCR assay and evaluated based on the band intensity in four categories: 0 = no product, 1 = very weak product, 2 = weak product, 3 = medium product, 4 = strong product. *Arcicella* exhibited pronounced maxima and minima over the course of the 2 years and appeared in the particle-attached (> 5 µm) and free-living fraction (0.2 - 5 µm).

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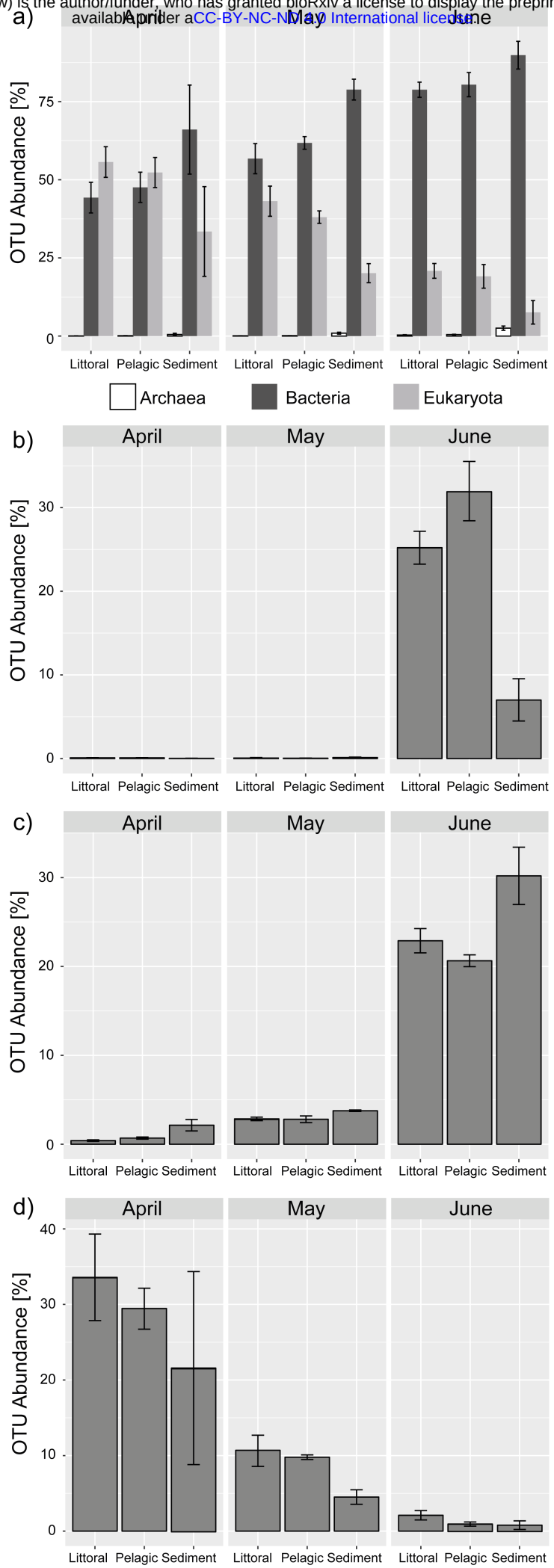

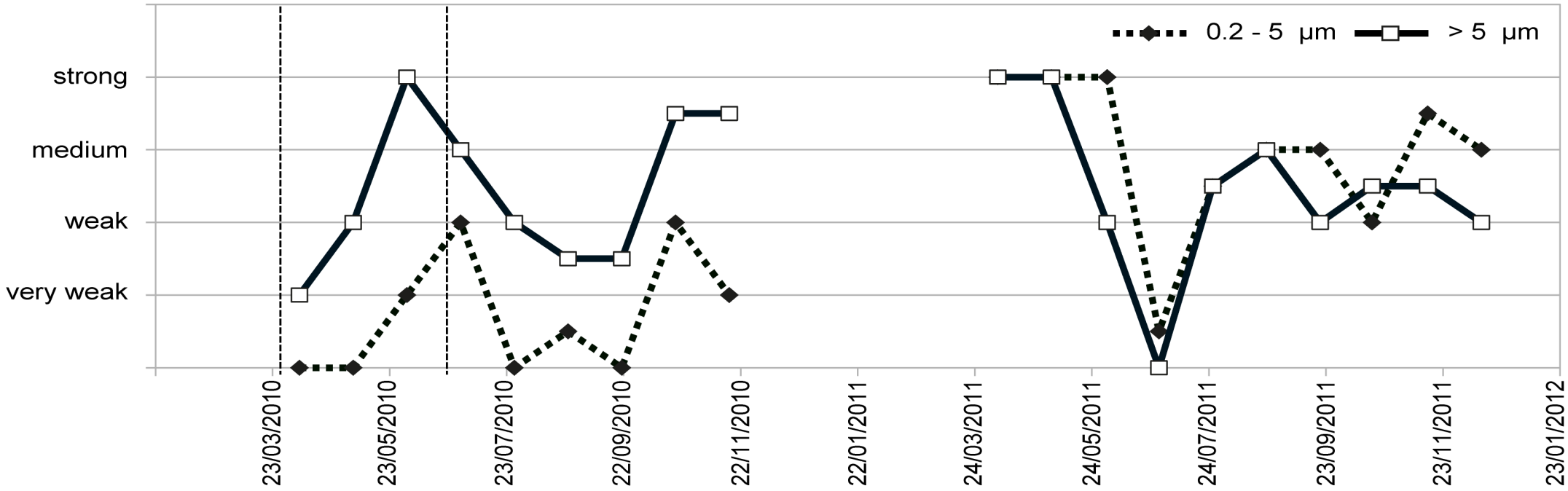
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