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NEWS AND VIEWS

Perspective

Who is where in the Plastisphere, and why does it matter?

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To fully understand how plastic is affecting the ocean, we need to understand how marine life interacts directly with it. Besides their ecological relevance, microbes can affect the distribution, degradation and transfer of plastics to the rest of the marine food web. From amplicon sequencing and scanning electron microscopy, we know that a diverse array of microorganisms rapidly associate with plastic marine debris in the form of biofouling and biofilms, also known as the "Plastisphere." However, observation of multiple microbial interactions in situ, at small spatial scales in the Plastisphere, has been a challenge. In this issue of *Molecular Ecology Resources*, Schlundt et al. apply the combination labelling and spectral imaging – fluorescence in situ hybridization to study microbial communities on plastic marine debris. The images demonstrate the colocalization of abundant bacterial groups on plastic marine debris at a relatively high taxonomic and spatial resolution while also visualizing biofouling of eukaryotes, such as diatoms and bryozoans. This modern imaging technology provides new possibilities to address questions regarding the ecology of marine microbes on plastic marine debris and describe more specific impacts of plastic pollution in the marine food webs.

KEYWORDS
biofilm, CLASI-FISH, plastic marine debris, plastisphere

Complex networks of microbial communities drive the flux of the biologically important elements in marine ecosystems. Hence, it is important to know how plastic litter in the oceans is affecting the ecology of microbes, starting by understanding what is there. Amplicon sequencing results from the Plastisphere—mainly of bacterial 16S rRNA genes–indicate that the surrounding environment largely influences the assemblage of plastic microbial communities (Oberbeckmann, Kreikemeyer, & Labrenz, 2018). However, high relative abundances of Bacteroidetes, Actinobacteria, Alphaproteobacteria and Cyanobacteria are often reported on microplastics (Reisser et al., 2014;Woodall et al., 2018). In this sense, this issue of *Molecular Ecology Resources*, Schlundt, Mark Welch, Knochel, Zettler, and Amaral-Zettler (2020) confirm in situ the relatively high abundance of some of these phyla with both combinatorial labelling and spectral imaging – fluorescence in situ hybridization (CLASI-FISH) and amplicon data, on plastic incubated at different locations of the Atlantic Ocean.

The results also reveal abundances at a lower taxonomic level, such as the case for Rhodobacteraceae (family). It is noteworthy that the use of (CARD [catalysed reporter deposition]) FISH to visualize bacteria at the genus level on plastics after environmental exposure has been published before (Harrison et al., 2014). In the case of CLASI-FISH, the images show the morphology of the cells and their location within the biofilm using confocal microscopy, spectral imaging and linear unmixing techniques. The method was first presented by Valm, Mark Welch, and Borisy (2012) and allows the use of several fluorophores at the same time (up to seven in this work, for different taxonomic levels) with relatively good separation of background noise. Therefore, the number of taxa analysed simultaneously is an improvement from other FISH methods. Finally, the observations by the authors in the samples from the Atlantic Ocean enable hypothesis-testing regarding colonization of plastic marine debris and the interpretation of amplicon sequencing results for bacteria. For example, is relative abundance of Bacteroidetes in amplicon data related to filament formation by this group on plastic marine debris in the oceans? Does cell organization impact the biomass per area or succession of bacterial groups? Also, how much do these results differ from natural particulate organic matter? In this regard, Dussud et al. (2018) demonstrated different communities (niche-specific) attached to plastic marine debris versus. organic particles in the Mediterranean, with an over-representation of Cyanobacteria on plastic marine debris.

The observation of bacteria directly in the biofilm is useful in the search of (noncultivable) pathogens in the Plastisphere. In the case of Vibrionaceae, a family containing important aquatic pathogens, the authors encountered difficulties in the selectivity of probes but still managed to show low but constant abundances of the group. The presence of this family was previously interpreted as a relevant feature of the Plastisphere after some sequencing and culturing efforts (Kirstein et al., 2016,). However, a substantial need to provide specific probes would be necessary for improving the search for bacterial pathogens. However, the procedure presented by the authors could allow other microbial interactions that are also of interest to animal and human health, such as horizontal gene exchange (e.g., of antibiotic resistance genes), to be demonstrated. Gene exchange has been analysed before within bacterial communities from microplastics by using fluorescently labelled gene markers (Arias-Andres, Klümper, Rojas-Jimenez, & Grossart, 2018), but the application of CLASI-FISH could demonstrate the exchange within the biofilm spatial structure.

Nevertheless, perhaps one of the most attractive results presented by Schlundt et al. (2020) is the visualization of bacteria and phytoplankton together and across time. Previous scanning electron microscopy on microplastics in the ocean has indicated the importance of diatoms in the early biofouling of the plastic marine debris, but with a low taxonomic resolution for the bacterial groups associated with them (Reisser et al., 2014). Meanwhile, the protocol presented by the authors allows the collaboration of bacterial groups and these autotrophic eukaryotes to be analysed in more detail. Eukaryote–Prokaryote interactions could be underlying the

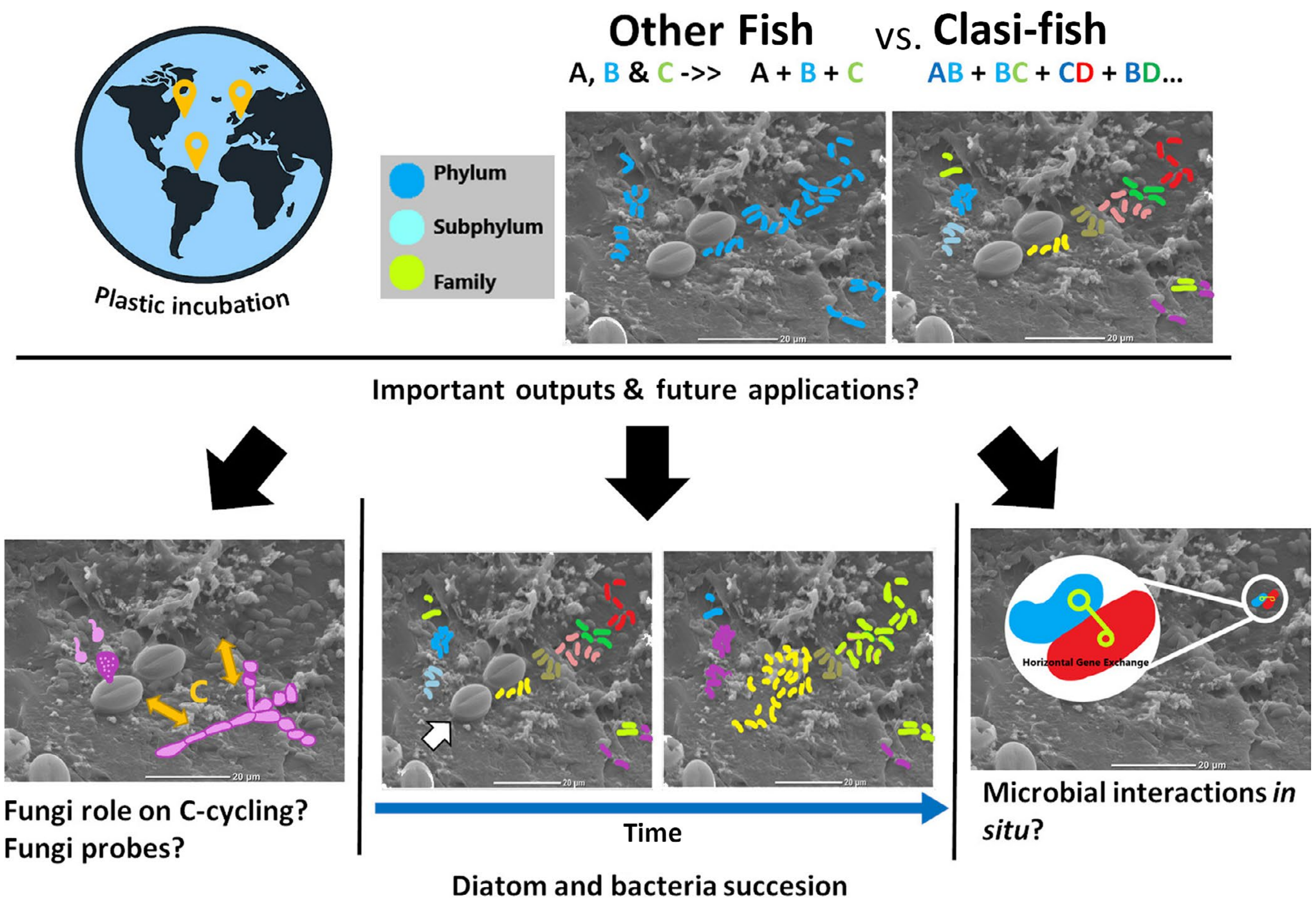

FIGURE 1 CLASI-FISH and microbial ecology applications in the Plastisphere demonstrated with samples from the Atlantic Ocean by Schlundt et al. (2020). The application of spectral imaging in FISH improves the number of bacterial groups that can be visualized on plastic marine debris simultaneously, compared with other FISH methods (e.g., CARD-FISH), and including different levels of taxonomic resolution (above; bacteria represented in colours). This provides the opportunity to propose hypotheses with regard to functional and structural aspects of the biofilm communities. Relevant topics are, for example: the role of filamentous or zoosporic fungi in degrading and producing different carbon substrates within the biofilm (below left), the role of eukaryotes on shaping prokaryotic communities on plastic debris and vice versa (below middle; white arrow indicates diatoms), and bacterial interactions of interest to public health such as the horizontal gene transfer of antibiotic resistance genes by plasmids (below right)

differences in diatom coverage and subsequent succession of bacterial groups observed among the three locations analysed by the authors. Of course, the number of samples that can be processed by confocal microscopy is small compared to amplicon sequencing. However, as the authors show, image analysis is an important tool for observation of specific succession patterns in the biofilms and for testing hypotheses based on sequence data.

Although not mentioned in Schlundt et al. (2020), the future use of probes for fungi could give more insight into carbon processing in the Plastisphere. Indeed, there is evidence of a diverse fungal community on plastic (Kettner, Rojas-Jimenez, Oberbeckmann, Labrenz, & Grossart, 2017). Moreover, the group seems of relevance in sequences from larger size fractions (>3 µm) from seawater where the attached lifestyle is relevant (Comeau, Vincent, Bernier, & Lovejoy, 2016) and its importance in aquatic ecosystem food webs is receiving increased attention by ecologists (Grossart et al., 2019). Therefore, fungi are likely to be key to carbon processing within the new plastic habitat as well, and we look forward to more work using probes designed to target them. Meanwhile, the use of dyes or fluorophores that interact with chitin could perhaps be used to distinguish groups broadly.

Going “back to the microscope” offers new and interesting possibilities after the last 5 years of amplicon sequencing (much limited to bacteria) on plastic biofilms (Figure 1). Perhaps the recent advancements in machine learning could further increase the resolution and improve image analysis using this technology (McRae, Oleksyn, Miller, & Gao, 2019). Overall, the spatial approach implemented by Schlundt et al. (2020) should be of interest to those working in microbial interactions on biofilms in marine substrates.

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