

Brief Report

Linking lifestyle and foraging strategies of marine bacteria: selfish behaviour of particle-attached bacteria in the northern Adriatic Sea

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Summary

Microbe-mediated enzymatic hydrolysis of organic matter entails the production of hydrolysate, the recovery of which may be more or less efficient. The selfish uptake mechanism, recently discovered, allows microbes to hydrolyze polysaccharides and take up large oligomers, which are then degraded in the periplasmic space. By minimizing the hydrolysate loss, selfish behaviour may be profitable for free-living cells dwelling in a patchy substrate landscape. However, selfish uptake seems to be tailored to algal-derived polysaccharides, abundant in organic particles, suggesting that particle-attached microbes may use this strategy. We tracked selfish polysaccharides uptake in surface microbial communities of the north-eastern Mediterranean Sea, linking the occurrence of this processing mode with microbial lifestyle. Additionally, we set up fluorescently labelled polysaccharides incubations supplying phytodetritus to investigate a ‘pioneer’ scenario for particle-attached microbes. Under both conditions, selfish behaviour was almost exclusively carried out by particle-

attached microbes, suggesting that this mechanism may represent an advantage in the race for particle exploitation. Our findings shed light on the selfish potential of particle-attached microbes, suggesting multifaceted foraging strategies exerted by particle colonizers.

Introduction

Heterotrophic microbial communities process much of the oceanic organic matter (OM) pool (Carlson *et al.*, 2007), transforming energy and matter into molecules essential for life (Falkowski *et al.*, 2008). The OM reservoir is shaped by a myriad of input and removal processes, resulting in a continuum of chemical and physical arrangements (Verdugo *et al.*, 2004; Dittmar *et al.*, 2021). Heterotrophic microbes preferentially direct their degradative efforts towards the high-molecular weight (HMW) end of this continuum, as it tends to be more bioavailable than the low-molecular weight (LMW) counterpart (Benner and Amon, 2015). Since the direct uptake of molecules through the prokaryotic cell wall is size constrained (i.e. <600 Da; Weiss *et al.*, 1991), the drawback of this food selectivity is the need to hydrolyze these HMW substrates before exploiting them. To this purpose, microbes use extracellular enzymes, which exist as cell-bound (i.e. attached to the outer cell membrane) and cell-free (i.e. dissolved into the surrounding environment; Chróst, 1990). By linking the enzyme-producing strategies with microbial lifestyle, a general microbial foraging framework can be outlined. Current hypotheses indicate that microbes dwelling as free-living (FL) would preferentially use cell-bound exoenzymes to cope with the patchy OM distribution in the ocean, whereas particle-attached (PA) cells, due to their proximity to the OM sources, may mainly rely on cell-free exoenzymes (Traving *et al.*, 2015; Baltar, 2018).

Whether cell-free or cell-bound, exoenzymes represent a considerable energetic investment (Arnosti, 2011). When producing exoenzymes, microbes operate, to a greater or lesser extent, at a loss, as a fraction of the

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LMW hydrolysis products will inevitably benefit cells that do not invest in exoenzyme production (Smith *et al.*, 1992; Folse and Allison, 2012). This scenario delineates two macro-categories of microbes: sharing and cheating (i.e. enzyme-producers and non-producers, respectively; Allison, 2005). In this framework, PA cells may be considered as the archetype of sharing microbes. Indeed, by degrading particulate organic matter (POM) through cell-free exoenzymes, PA microbes 'share' hydrolysis products with other members of the degrading consortium (Vetter *et al.*, 1998; Datta *et al.*, 2016). The discovery in the ocean of an alternative HMW-OM uptake mechanism, consisting in the transport of large, partially hydrolyzed, polysaccharide fragments into the periplasmic space of the cells, where further degradation occurs, challenged this two-player view (Reintjes *et al.*, 2017). Through this mechanism, HMW-OM can be directly taken up, minimizing the loss of LMW products derived from hydrolysis through cell-bound or cell-free exoenzymes (Reintjes *et al.*, 2017). These 'selfish' microbes represent a third player in microbial substrate utilization models, competing with those prokaryotes that preferentially use cell-bound or cell-free exoenzymes for HMW-OM degradation (Reintjes *et al.*, 2019).

Over the last few years, the selfish uptake of several polysaccharides has been shown to be widespread across marine systems, suggesting that this substrate processing mode is highly relevant for global biogeochemical dynamics (Arnosti *et al.*, 2021). Indeed, the ability to utilize biogeochemically relevant polysaccharides (i.e. pullulan, xylan, laminarin, chondroitin sulfate and arabinogalactan), both through external hydrolysis and selfish uptake, is widespread among marine bacteria (Arnosti, 2011; Reintjes *et al.*, 2019; Reintjes *et al.*, 2020a, 2020b). A substantial fraction of surface microbial communities can perform selfish polysaccharide uptake, including members of Bacteroidetes, Planctomycetes and Gammaproteobacteria (Reintjes *et al.*, 2019, 2020a,b). Representatives of these taxa are specifically involved in the degradation and remineralization of algal biomass (Grossart *et al.*, 2005; Teeling *et al.*, 2012) and are often found associated with particles in aquatic environments (Zoccarato and Grossart, 2019). Nonetheless, to date, investigations specifically looking for the presence of selfish bacteria on organic particles are missing. Since PA microbes process up to 70% of POM globally (Giering *et al.*, 2014), assessing how this carbon pool is processed along the selfish-sharing gradient is of prime importance to better resolve the microscale carbon fluxes that shape global biogeochemical dynamics.

To examine the occurrence of the selfish substrate processing mode among PA and FL microbes, we incubated surface microbial communities of the northeastern

Mediterranean Sea with fluorescently labelled polysaccharides (FLA-PS), covering an annual cycle (from January 2019 to January 2020) at monthly intervals. The selfish uptake mechanism allows the exploitation of HMW-OM without the production of hydrolysis by-products (Cuskin *et al.*, 2015), which, under the current microbial foraging theory, would be an advantageous scenario for FL microbes. Therefore, we hypothesized that selfish polysaccharide uptake would be more prominent in the FL fraction of the sampled microbial community.

In addition, we performed a second set of FLA-PS incubations, first removing larger particles and their climax assemblages, and then supplying fresh phytodetrital particles to the microbial community. With this setup, we aimed to achieve a 'new-colonization' scenario, to test whether PA microbes would selfishly exploit phytodetrital particles from the beginning of the particles' colonization process or would use this uptake mode to gain a competitive advantage in later colonization stages.

Results and discussion

Selfish polysaccharide uptake – natural samples

To evaluate the distribution of selfish polysaccharide uptake mechanisms among the PA and FL fraction of microbial communities, surface (~1 m depth) seawater was sampled monthly from January 2019 to January 2020 at a coastal station in the northern Adriatic Sea. Subsamples were taken in 15 ml vials, spiked with one of 5 FLA-PS (pullulan, xylan, laminarin, chondroitin sulfate and arabinogalactan) and incubated at *in situ* temperature for 2 h. Triplicate 5 ml aliquots, from each substrate incubation, were prepared for epifluorescence microscopy analysis (as detailed in the study by Celussi *et al.*, 2017). Total and FLA-PS positive cells were identified and enumerated as FL or PA at 1000× magnification (Olympus BX60F5) under a UV (BP 330–385 nm, BA 420 nm) and blue (450–480 nm, BA 515 nm) filter set for 4,6-diamidino-2-phenylindole (DAPI, Sigma Aldrich) and FLA-PS fluorescence, respectively, in at least 15 randomly selected fields. In DAPI fields, at least 100 and 200 cells were counted for PA and FL lifestyles, respectively; under blue light, between 5 and 50 cells were counted for each replicate to measure the abundance of FLA-PS-stained cells. Cells were identified as substrate-stained if they showed overlapping signals in both DAPI and FLA-PS fields. No FLA-PS-stained cells were observed in the killed controls, prepared as described in the Supporting Information S1. Detailed experimental procedures and representative micrographs of FLA-PS-stained cells (Supporting Information Fig. S1) are available in the Supporting Information S1.

Through the study period (January 2019 to January 2020), microbial communities from surface water of the northern Adriatic Sea showed selfish uptake of four out of the five tested FLA-PS (Fig. 1). Selfish uptake of pullulan and xylan was measurable throughout the study period, whereas laminarin- and chondroitin sulfate-positive cells were observed starting from March and April 2019, respectively. Although some arabinogalactan-positive cells were observed, their abundance was not high enough to statistically validate the epifluorescence counts. No substrate-stained cells were observed in any of the killed controls.

PA and FL fractions showed rather similar temporal dynamics (Fig. 1). Selfish activity maxima were measured in June 2019 for all substrate incubations (Fig. 1); winter minima characterized pullulan and xylan incubations, whereas laminarin- and chondroitin sulfate-positive cells showed the lowest abundance between September and November 2019 (Fig. 1). In laminarin incubations, the summer peak was mostly evident for the PA fraction, whereas FL cells showed a less pronounced seasonal variability (Fig. 1). Chondroitin sulfate-positive cells followed an opposite pattern, with FL cells showing a higher degree of seasonal variability when compared with PA ones (Fig. 1). This temporal pattern appeared to be linked to the overall Chlorophyll *a* and particulate organic carbon (POC) temporal dynamics, both peaking between May and June (Fig. 2, determined as detailed in the study by Manna *et al.*, 2021).

The set of FLA-PS used in the present study includes some of the most abundant or readily hydrolysed polysaccharides in the ocean (Arnosti, 2011; Becker *et al.*, 2020), which are likely derived from primary

producers (Mühlenbruch *et al.*, 2018; Bäumgen *et al.*, 2021). The study area is heavily influenced by riverine inputs, representing the major source of inorganic nutrients (Cozzi *et al.*, 2020), driving seasonal and inter-annual changes in both autochthonous and allochthonous OM standing stocks and, consequently, in microbial OM utilization patterns (Celussi and Del Negro, 2012; Manna *et al.*, 2021).

Xylan is a structural polysaccharide contained in land plants as well as in macroalgae and phytoplankton cell walls (Beg *et al.*, 2001), and its fundamental units (i.e. xylose molecules, Bastawde, 1992) are broadly present in the HMW-OM pool in the study area, peaking between May and June (Sist and Urbani, 2017). The extensive presence of these molecules, likely due to the influence of terrestrial runoff at our coastal sampling station, matches with maxima observed in FLA-xylan amended incubations (Fig. 1), establishing a link between HMW-OM composition and selfish polysaccharide degradation.

In the investigated system, *Synechococcus* spp. dominates as the main photoautotrophic plankton over the nutrient-depleted summer (Fonda Umani *et al.*, 2012). Among the substrates tested, prolonged summer maxima were only observed in chondroitin sulfate incubations (June–August, Fig. 1). *Synechococcus* strains naturally produce chondroitin sulfate (Sarnaik *et al.*, 2019), suggesting that these organisms may have represented a natural source of chondroitin sulfate, promoting the selfish uptake of this substrate. We found indeed a significant correlation between total FLA-chondroitin sulfate-positive cells and *Synechococcus* abundance (Spearman's $\rho = 0.94$, $P < 0.001$, $n = 10$), linking the observed

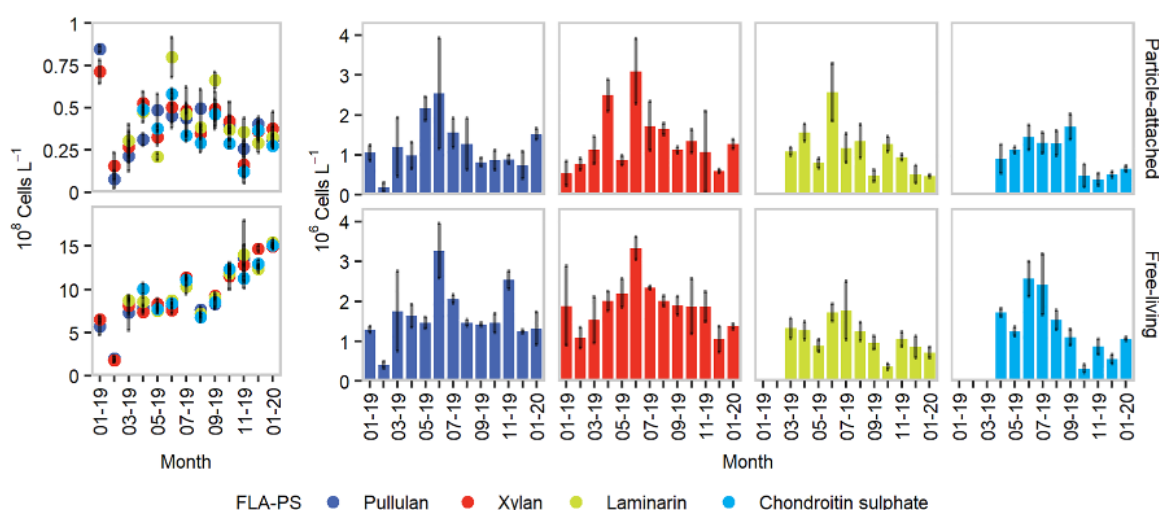


Fig. 1. Temporal trends of total DAPI-stained (left panel) and selfish (right panel) cell counts in unamended (i.e. without phytodetritus) samples incubated with different FLA-PS. Error bars depict standard deviations calculated from three experimental replicates. For visualization purposes, X-axis labels are displayed every other month.

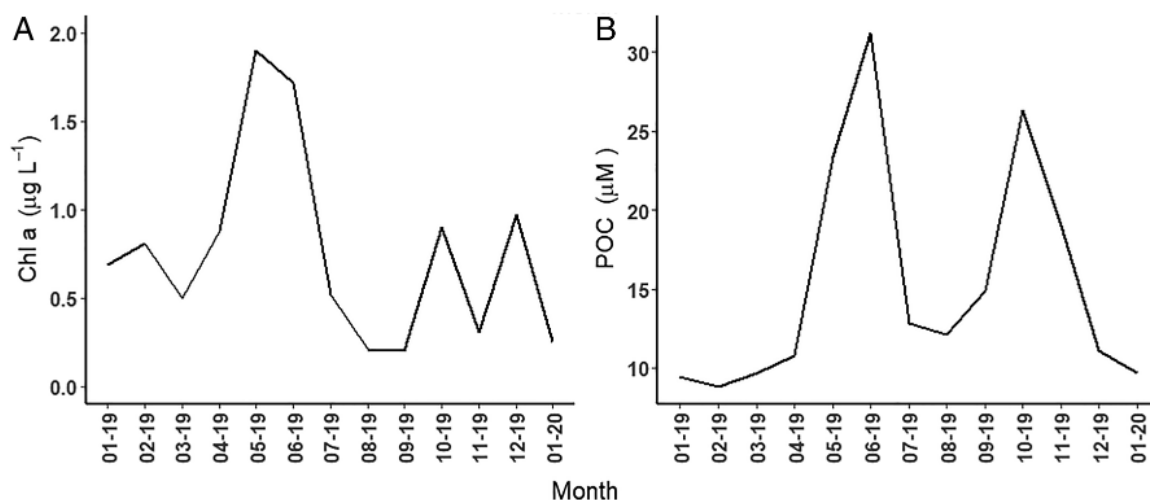


Fig. 2. Trends of (A) Chl a and (B) POC measured *in situ* from January 2019 to January 2020. Sampling dates are indicated with the format mm-yy.

selfish uptake pattern with *Synechococcus* dynamics, an important nutritional source for heterotrophic microbes in the study area (Manna *et al.*, 2021).

We noted that during the investigation period, the selfish uptake of arabinogalactan was not observed. Arabinogalactans are branched polysaccharides composed of arabinose and galactose monomers (Showalter, 2001), also present in brown macroalgae (Hervé *et al.*, 2016). Along the easternmost coast of the northern Adriatic Sea, where our sampling site is located, brown macroalgae are broadly present (Orlando-Bonaca *et al.*, 2013), suggesting that arabinogalactan may be an abundant polysaccharide in this area. Arabinose and galactose monosaccharides, the fundamental units of the arabinogalactan molecule, represent a consistent fraction of the dissolved OM pool at the sampling station (7%–11% and 17%–27%, respectively; Sist and Urbani, 2017). Since the signature of external hydrolysis is the production of LMW products (i.e. mono- or disaccharides, Vetter *et al.*, 1998), we speculate that the bulk of arabinogalactan in the study area is carried out through external hydrolysis (i.e. through cell-bound or cell-free exoenzymes), thus reducing the availability of the whole arabinogalactan molecule for selfish microbes. However, this hypothesis remains to be tested by direct measurement of arabinogalactan hydrolysis rates.

In terms of absolute cell abundance, PA and FL substrate-stained cells showed in general similar dynamics (Fig. 1). However, when compared to the total DAPI-stained cell counts, the uptake of fluorescent polysaccharides emerged as more widespread among PA microbes (Fig. 3).

Substrate-stained cells represented between $0.72\% \pm 0.35\%$ and $6.25\% \pm 1.68\%$ of the total PA cells in unamended incubations. The highest values were

observed in pullulan and xylan incubations ($5.67\% \pm 1.40\%$ and $6.25\% \pm 1.68\%$, respectively; Fig. 3), whereas the contribution of laminarin and chondroitin sulfate-stained cells to the total PA abundance was lower (laminarin: $0.72\% \pm 0.18\%$ – $3.77\% \pm 0.69\%$; chondroitin sulfate: $1.39\% \pm 0.26\%$ – $4.42\% \pm 0.47\%$; Fig. 2). Gene groups implicated in polysaccharides utilization (e.g. TBDTs and carbohydrate-active enzymes) are generally more abundant in the PA fraction of a given microbial community (Smith *et al.*, 2013; Simon *et al.*, 2014; Bunse *et al.*, 2021), suggesting that they have the potential to express the metabolic machinery for selfish polysaccharide uptake. Free-living substrate-stained cells represented a remarkably lower fraction of the total, generally below 0.5% (Fig. 3). This result was unexpected since the selfish mechanism allows the exploitation of HMW-OM minimizing the production of hydrolysis by-products, which, under the current microbial foraging theory, would be an advantageous scenario for FL microbes (Baltar, 2018).

However, the origin of the polysaccharides used in our incubations suggests that microbes closely associated with living and/or detrital POM are likely equipped to exploit these carbon sources, either exclusively in a selfish way (e.g. fucoidan; Reintjes *et al.*, 2019) or concurrently with external hydrolysis (e.g. laminarin; Reintjes *et al.*, 2020b). These polysaccharides indeed, can be directly produced by phytoplankton, as storage or structural compounds (laminarin and xylan, respectively; Hsieh and Harris, 2019; Becker *et al.*, 2020), by fungi as an extracellular coating agent (pullulan, Singh *et al.*, 2008), by macroalgae and seagrasses as cell wall components (Hervé *et al.*, 2016; Pfeifer *et al.*, 2020) or resemble algal-derived polysaccharides (e.g. chondroitin sulfate; Bhaskar *et al.*, 2005; Zhang *et al.*, 2008).

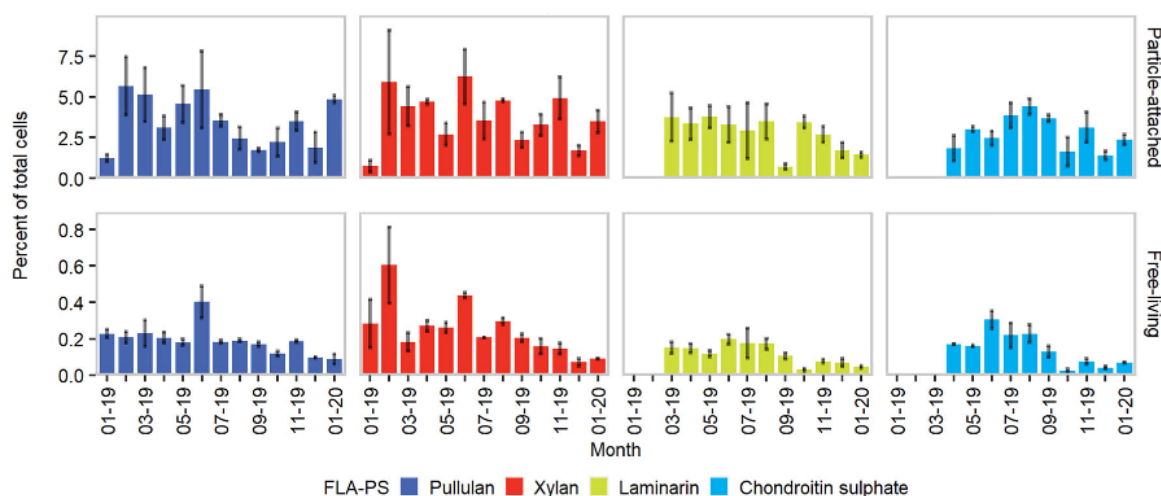


Fig. 3. Temporal trends of selfish cells (percentage over total DAPI-stained cells) in unamended (i.e. without phytodetritus) samples incubated with different FLA-PS. Error bars depict standard deviations calculated from three experimental replicates. For visualization purposes, X-axis labels are displayed every other month.

Furthermore, the selfish uptake strategy appears to be widespread across marine bacterial taxa known to be associated with organic particles (e.g. Flavobacteriaceae, Planctomycetes and Gammaproteobacteria representatives; Reintjes *et al.*, 2019; Supporting Information Fig. S2). In the study area, POM concentration is a major forcing of heterotrophic microbial metabolism (Manna *et al.*, 2021), indicating that particles represent an important driver in planktonic microbial foraging dynamics. From this perspective, the selfish uptake of specific polysaccharides may represent a substantial advantage in the race for POM exploitation, likely explaining its prevalence among PA microbes (Fig. 3).

Short-term dynamics of selfish polysaccharides uptake

Organic particles represent dynamic environments, where the competition for resource access is likely to be strong (Long and Azam, 2001; Bižić-Ionescu *et al.*, 2015). During the particles' colonization and degradation processes, different microbial assemblages develop over time, delineating a successional transition from primary particle degraders to secondary consumers (Datta *et al.*, 2016; Bunse *et al.*, 2021). The latter are often represented by cheating microbes, benefitting from hydrolysis by-products released by primary degraders (Datta *et al.*, 2016; Koch *et al.*, 2019; Baltar *et al.*, 2021; Bunse *et al.*, 2021). Selfish polysaccharides uptake may represent an effective strategy that primary particle degraders implement to avoid being outcompeted by secondary consumers. However, this behaviour may be difficult to observe in naturally occurring large particles, since they are colonized by climax communities in which individual organisms occupy a defined degradation niche

(Datta *et al.*, 2016; Bunse *et al.*, 2021). To test whether selfish polysaccharides uptake would be a prominent strategy in the early phase of particle colonization, we supplied model phytodetrital particles (prepared as described in the Supporting Information S1) to natural communities, after the removal of large (i.e. >5 µm) particles. In this 'new colonization' scenario, we observed that selfish polysaccharide uptake was more common among PA cells, and very few FL cells showed selfish activity at either time point (i.e. 2 h and 24 h; Fig. 4 and Supporting Information Fig. S3), confirming the results observed for unamended natural samples.

Phytodetritus enrichments resulted in a general increase of substrate-stained cells when compared with unamended incubations (Figs. 3 and 4, respectively). However, selfish cells still represented a minor fraction of the total (i.e. DAPI stained) cells, with higher relative proportions observed in the PA fraction (ranges: PA 2 h, $0.28 \pm 0.06\%$ – $6.53 \pm 2.83\%$; FL 2 h, $0.04 \pm 0.01\%$ – $0.51 \pm 0.11\%$; PA 24 h, $0.05 \pm 0.06\%$ – $5.90 \pm 0.76\%$; FL 24 h, $0.01 \pm 0\%$ – $0.54 \pm 0.09\%$; Supporting Information Fig. S3). A paired T-test (Student, 1908) was applied to detect significant changes in (i) total DAPI-stained cell and (ii) FLA-PS-stained cell abundance after 2 h and 24 h of incubation with detrital particles (details are present in the Supporting Information S1). As in previous FLA-PS incubation experiments, we expected to observe an increase in selfish cell abundance over the 24 h incubation period (e.g. Reintjes *et al.*, 2019). We found instead a significant decrease of selfish cells between 2 h and 24 h, except for pullulan and FL cells in xylan incubations (Table 1). DAPI-stainable cells significantly increased over the 24 h substrate incubations, except for PA cells amended with FLA-xylan (Table 1). These

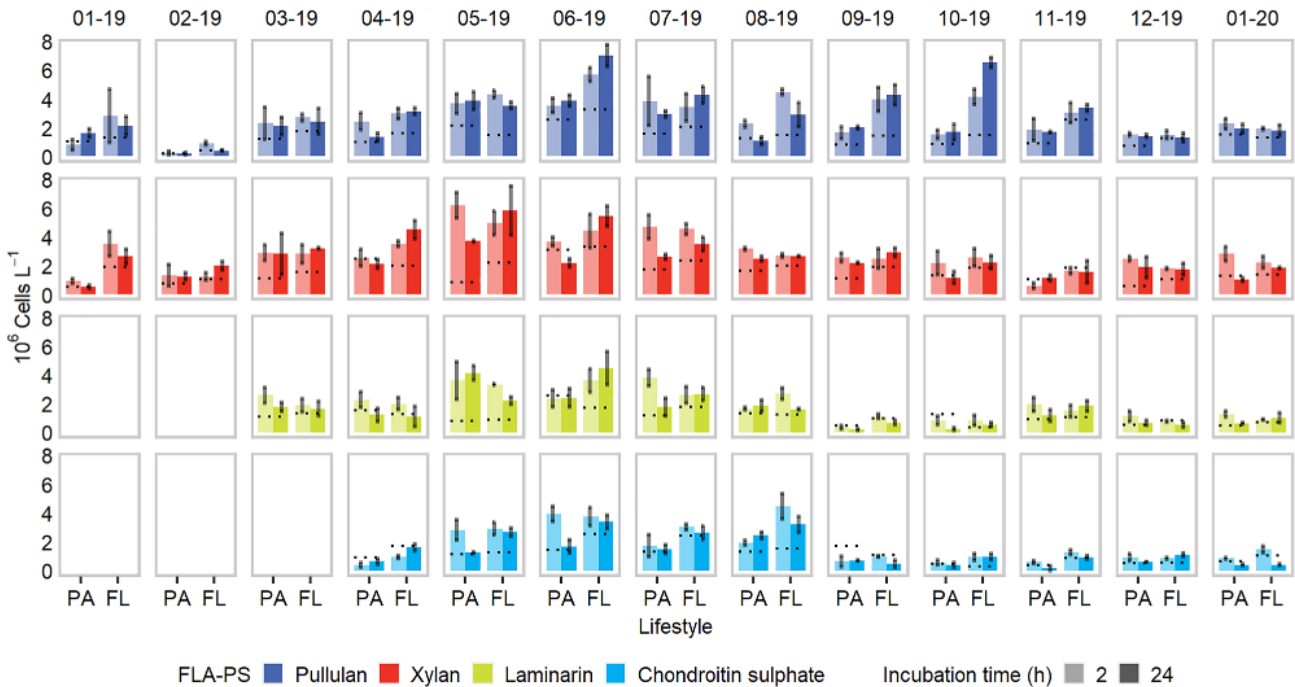


Fig. 4. Temporal trends of selfish cell counts in phytodetritus-amended samples incubated with different FLA-PS. Error bars depict standard deviations calculated from three experimental replicates. Superimposed dashed lines report values of FLA-PS positive cells as in Fig. 2 to aid comparison. On the top of each subpanel, the sampling date (mm-yy format) is present. PA = particle-attached; FL = free-living.

Table 1. Summary table of paired T-test statistics carried out on log-transformed cell counts in phytodetritus amended incubations.

		DAPI stained		T-test statistics		
		Mean $\pm$ SD		df	t	P
		2 h	24 h			
Pullulan	PA	18.51 $\pm$ 0.83	18.69 $\pm$ 0.74	37	3.27	<0.01
	FL	21.03 $\pm$ 0.52	21.25 $\pm$ 0.60	37	5.41	<0.001
Xylan	PA	18.55 $\pm$ 0.66	18.62 $\pm$ 0.76	37	1.37	ns
	FL	21.02 $\pm$ 0.55	21.25 $\pm$ 0.59	37	6.21	<0.001
Laminarin	PA	18.73 $\pm$ 0.38	19.01 $\pm$ 0.42	32	5.48	<0.001
	FL	21.18 $\pm$ 0.25	21.47 $\pm$ 0.33	32	5.78	<0.001
Chondroitin sulfate	PA	18.82 $\pm$ 0.27	19.07 $\pm$ 0.36	29	4.64	<0.001
	FL	21.23 $\pm$ 0.24	21.48 $\pm$ 0.23	29	7.07	<0.001
		FLA-PS-stained		T-test statistics		
		Mean $\pm$ SD		df	t	P
		2 h	24 h			
Pullulan	PA	14.38 $\pm$ 0.78	14.30 $\pm$ 0.76	37	-1.01	ns
	FL	14.87 $\pm$ 0.52	14.80 $\pm$ 0.76	37	-1.06	ns
Xylan	PA	14.67 $\pm$ 0.66	14.37 $\pm$ 0.52	37	-3.88	<0.001
	FL	14.80 $\pm$ 0.43	14.84 $\pm$ 0.46	37	0.656	ns
Laminarin	PA	14.29 $\pm$ 0.71	13.81 $\pm$ 0.97	32	-4.69	<0.001
	FL	14.31 $\pm$ 0.58	14.08 $\pm$ 0.72	32	-2.86	<0.01
Chondroitin sulfate	PA	13.85 $\pm$ 0.81	13.51 $\pm$ 0.78	29	-2.96	<0.01
	FL	14.33 $\pm$ 0.63	14.10 $\pm$ 0.78	29	-2.33	<0.05

Mean and standard deviations (SD) are calculated on log-transformed DAPI- and FLA-PS-stained cell counts (ln cells L⁻¹). PA, particle attached; FL, free living; ns, not significant; df, degrees of freedom.

results contrast with previous FLA-PS incubation experiments (Reintjes *et al.*, 2019).

With the addition of phytodetrital particles, we aimed to mimic the late stage of a phytoplankton bloom. In similar environmental conditions (i.e. the demise of a phytoplankton bloom, Reintjes *et al.*, 2020b), it has been observed that polysaccharide utilization through the selfish mechanism is reduced, while the spectrum and rates of external hydrolysis increase. In this scenario, fast-growing external hydrolysers may become prevalent in a given microbial community, limiting the flux of HMW molecules processed in a selfish manner (Reintjes, *et al.*, 2020b; Arnosti *et al.*, 2021). Given this framework, the concurrent decrease of FLA-PS-positive cells and the increase of total DAPI-stained cells observed during our incubation with phytodetrital particles would suggest that, despite a relatively short time frame (i.e. 24 h), selfish cells may have been outcompeted by external degraders, in line with previous, although longer, experiments (Reintjes *et al.*, 2020b). However, since the selfish uptake is considered to be particularly effective in presence of highly complex substrates, it is possible that increasing the incubation time, the selfish behaviour among PA-bacteria would become prevalent in the effort of degrading the refractory OM accumulating over time.

Since the detrital pool used for phytodetritus-amended incubation was diatom-based, we expected to observe a high prevalence of laminarin-stained selfish cells, as laminarin is central in diatoms metabolism (Biersmith and Benner, 1998). Despite this premise, we did not observe a major increase of laminarin selfish uptake when compared to the other substrates tested in the phytodetritus-amended incubations (Fig. 4 and Supporting Information Fig. S3). Laminarin is an abundant polysaccharide in the marine environment (Becker *et al.*, 2020) and thus this substrate is likely targeted not only by selfish uptake but also processed through external hydrolysis, especially when it is more bioavailable, as during the late stages of a diatom bloom (Reintjes *et al.*, 2020b).

The outcomes of phytodetrital amendments would suggest that early particles colonizers tend to maximize polysaccharides exploitation via selfish uptake. We found indeed higher numbers of PA selfish cells in early colonization stages, decreasing as the particles, and their associated communities, age. However, the cumulative evidence gathered on the selfish substrate processing mode suggests that there is a gradient of 'selfishness' (see Arnosti *et al.*, 2021 for a review). Processing polysaccharides through a selfish mechanism may grant exclusive access to specific HMW-OM components, providing a competitive advantage. Furthermore, selfish processing of certain substrates might still release oligomers (i.e. in the case of 'sensing enzymes', *sensu* Thomas *et al.*, 2012), which in turn may induce the

external hydrolysis of the target compounds (Arnosti, 2004), further enhancing the production of LMW hydrolysate, benefitting the entire community (Smith *et al.* 1992). Therefore, it appears that the selfish uptake mechanism is not solely useful in competing for resource access but plays a key role also in cooperative interactions.

Conclusions

As the first evidence of selfish polysaccharide uptake in the ocean resulted in an adjustment of the sharing-cheating model (Allison, 2005; Reintjes *et al.*, 2019), the finding of a higher proportion of PA cells performing selfish FLA-PS uptake challenges the idea that PA microbes behave as sharing entities, releasing external hydrolysis by-products (i.e. small oligomers and monomers) into the surrounding environment (Smith *et al.*, 1992; Simon *et al.*, 2002). With this work, we provide evidence that, in temperate coastal waters and for the selected set of polysaccharides, selfish uptake is predominantly carried out by the PA fraction of the heterotrophic microbial community. Furthermore, the incubations with detrital particles highlighted that the selfish strategy is preferentially adopted in the early phase of particles colonization, decreasing in importance as the particles age. However, these results refer to a limited set of molecules, which, although including some of the most abundant or readily degraded polymers in marine systems (Arnosti, 2011; Becker *et al.*, 2020), are far from describing OM chemical diversity in the ocean (Riedel and Dittmar, 2014). It is therefore not possible to exclude that in the presence of other polysaccharides, lipids or proteins and/or in other environmental conditions, PA microbes can behave as 'sharing' individuals (e.g. Rakoff-Nahoum *et al.*, 2016), or even adopt a combined degradation strategy, coupling external hydrolysis with selfish behaviour (Reintjes *et al.*, 2020b). This work underlines the need to extend such investigations, broadening the spectrum of target molecules as well as the breadth of surveyed environments. Coupling this information with insights on microbial lifestyle will be fundamental in future experimental and modelling studies, as microbe-mediated OM fluxes at the microscale inherently shape the biogeochemistry of the global ocean.

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References

- Allison, S.D. (2005) Cheaters, diffusion and nutrients constrain decomposition by microbial enzymes in spatially structured environments. *Ecol Lett* **8**: 626–635. <https://doi.org/10.1111/j.1461-0248.2005.00756.x>.
- Amosti, C. (2004) Speed bumps and barricades in the carbon cycle: substrate structural effects on carbon cycling. *Mar Chem* **92**: 263–273. <https://doi.org/10.1016/j.marchem.2004.06.030>.
- Amosti, C. (2011) Microbial extracellular enzymes and the marine carbon cycle. *Ann Rev Mar Sci* **3**: 401–425. <https://doi.org/10.1146/annurev-marine-120709-142731>.
- Amosti, C., Wietz, M., Brinkhoff, T., Hehemann, J.-H., Probandt, D., Zeugner, L., and Amann, R. (2021) The biogeochemistry of marine polysaccharides: sources, inventories, and bacterial drivers of the carbohydrate cycle. *Ann Rev Mar Sci* **13**: 81–108. <https://doi.org/10.1146/annurev-marine-032020-012810>.
- Baltar, F. (2018) Watch out for the “living dead”: cell-free enzymes and their fate. *Front Microbiol* **8**: 2438. <https://doi.org/10.3389/fmicb.2017.02438>.
- Baltar, F., Alvarez-Salgado, X.A., Arístegui, J., Benner, R., Hansell, D.A., Herndl, G.J., and Lønborg, C. (2021) What is refractory organic matter in the ocean? *Front Mar Sci* **8**: 642637. <https://doi.org/10.3389/fmars.2021.642637>.
- Bastawde, K.B. (1992) Xylan structure, microbial xylanases, and their mode of action. *World J Microbiol Biotechnol* **8**: 353–368. <https://doi.org/10.1007/BF01198746>.
- Bäumgen, M., Dutschei, T., and Bornscheuer, U.T. (2021) Marine polysaccharides: occurrence, enzymatic degradation and utilization. *Chembiochem* **22**: 2247–2256. <https://doi.org/10.1002/cbic.202100078>.
- Becker, S., Tebben, J., Coffinet, S., Wiltshire, K., Iversen, M. H., Harder, T., et al. (2020) Laminarin is a major molecule in the marine carbon cycle. *Proc Natl Acad Sci U S A* **117**: 6599–6607. <https://doi.org/10.1073/pnas.1917001117>.
- Beg, Q., Kapoor, M., Mahajan, L., and Hoondal, G. (2001) Microbial xylanases and their industrial applications: a review. *Appl Microbiol Biotechnol* **56**: 326–338. <https://doi.org/10.1007/s002530100704>.
- Benner, R., and Amon, R.M.W. (2015) The size-reactivity continuum of major bioelements in the ocean. *Ann Rev Mar Sci* **7**: 185–205. <https://doi.org/10.1146/annurev-marine-010213-135126>.
- Bhaskar, P.V., Grossart, H.-P., Bhosle, N.B., and Simon, M. (2005) Production of macroaggregates from dissolved exopolymeric substances (EPS) of bacterial and diatom origin. *FEMS Microbiol Ecol* **53**: 255–264. <https://doi.org/10.1016/j.femsec.2004.12.013>.
- Biersmith, A., and Benner, R. (1998) Carbohydrates in phytoplankton and freshly produced dissolved organic matter. *Mar Chem* **63**: 131–144. [https://doi.org/10.1016/S0304-4203\(98\)00057-7](https://doi.org/10.1016/S0304-4203(98)00057-7).
- Bizjić-Ionescu, M., Zeder, M., Ionescu, D., Orlić, S., Fuchs, B. M., Grossart, H.-P., and Amann, R. (2015) Comparison of bacterial communities on limnic versus coastal marine particles reveals profound differences in colonization: marine and limnic particle-associated bacteria. *Environ Microbiol* **17**: 3500–3514. <https://doi.org/10.3389/fmicb.2018.02569>.
- Bunse, C., Koch, H., Breider, S., Simon, M., & Wietz, M. (2021). Sweet spheres: succession and CAZyme expression of marine bacterial communities colonizing a mix of alginate and pectin particles. *Environ Microbiol* **23**: 3130–3148. <https://doi.org/10.1111/1462-2920.15536>.
- Carlson, C.A., del Giorgio, P.A., and Herndl, G.J. (2007) Microbes and the dissipation of energy and respiration: from cells to ecosystems. *Oceanography* **20**: 89–100. <https://doi.org/10.5670/oceanog.2007.52>.
- Celussi, M., and Del Negro, P. (2012) Microbial degradation at a shallow coastal site: long-term spectra and rates of exoenzymatic activities in the NE Adriatic Sea. *Estuar Coast Shelf Sci* **115**: 75–86. <https://doi.org/10.1016/j.ecss.2012.02.002>.
- Celussi, M., Malfatti, F., Annalisa, F., Gazeau, F., Giannakourou, A., Pitta, P., et al. (2017) Ocean acidification effect on prokaryotic metabolism tested in two diverse trophic regimes in the Mediterranean Sea. *Estuar Coast Shelf Sci* **186**: 125–138. <https://doi.org/10.1016/j.ecss.2015.08.015>.
- Chróst, R.J. (1990) Microbial ectoenzymes in aquatic environments. In *Aquatic Microbial Ecology*. Brock, T.D., Overbeck, J., and Chróst, R.J. (eds). Springer: New York, NY, pp. 47–78. https://doi.org/10.1007/978-1-4612-3382-4_3.
- Cozzi, S., Cabrini, M., Kralj, M., De Vittor, C., Celio, M., and Giani, M. (2020) Climatic and anthropogenic impacts on environmental conditions and phytoplankton community in the Gulf of Trieste (northern Adriatic Sea). *Water* **12**: 2652. <https://doi.org/10.3390/w12092652>.
- Cuskin, F., Lowe, E.C., Temple, M.J., Zhu, Y., Cameron, E. A., Pudlo, N.A., et al. (2015) Human gut Bacteroidetes can utilize yeast mannan through a selfish mechanism. *Nature* **517**: 165–169. <https://doi.org/10.1038/nature13995>.
- Datta, M.S., Sliwerska, E., Gore, J., Polz, M.F., and Cordero, O.X. (2016) Microbial interactions lead to rapid micro-scale successions on model marine particles. *Nat Commun* **7**: 11965. <https://doi.org/10.1038/ncomms11965>.
- Dittmar, T., Lennartz, S.T., Buck-Wiese, H., Hansell, D.A., Santinelli, C., Vanni, C., et al. (2021) Enigmatic persistence of dissolved organic matter in the ocean. *Nat Rev Earth Environ* **2**: 570–583. <https://doi.org/10.1038/s43017-021-00183-7>.
- Falkowski, P.G., Fenchel, T., and Delong, E.F. (2008) The microbial engines that drive earth's biogeochemical cycles. *Science* **320**: 1034–1039. <https://doi.org/10.1126/science.1153213>.
- Folse, H.J., and Allison, S.D. (2012) Cooperation, competition, and coalitions in enzyme-producing microbes: social

- evolution and nutrient depolymerization rates. *Front Microbiol* **3**: 338. <https://doi.org/10.3389/fmicb.2012.00338>.
- Fonda Umani, S., Malfatti, F., and Del Negro, P. (2012) Carbon fluxes in the pelagic ecosystem of the Gulf of Trieste (northern Adriatic Sea). *Estuar Coast Shelf Sci* **115**: 170–185. <https://doi.org/10.1016/j.ecss.2012.04.006>.
- Giering, S.L.C., Sanders, R., Lampitt, R.S., Anderson, T.R., Tamburini, C., Boutrif, M., et al. (2014) Reconciliation of the carbon budget in the ocean's twilight zone. *Nature* **507**: 480–483. <https://doi.org/10.1038/nature13123>.
- Grossart, H.-P., Levold, F., Allgaier, M., Simon, M., and Brinkhoff, T. (2005) Marine diatom species harbour distinct bacterial communities. *Environ Microbiol* **7**: 860–873. <https://doi.org/10.1111/j.1462-2920.2005.00759.x>.
- Hervé, C., Siméon, A., Jam, M., Cassin, A., Johnson, K.L., Salmeán, A.A., et al. (2016) Arabinogalactan proteins have deep roots in eukaryotes: identification of genes and epitopes in brown algae and their role in *Fucus serratus* embryo development. *New Phytol* **209**: 1428–1441. <https://doi.org/10.1111/nph.13786>.
- Hsieh, Y.S.Y., and Harris, P.J. (2019) Xylans of red and green algae: what is known about their structures and how they are synthesised? *Polymers (Basel)* **11**: 354. <https://doi.org/10.3390/polym11020354>.
- Koch, H., Dürwald, A., Schweder, T., Noriega-Ortega, B., Vidal-Melgosa, S., Hehemann, J.-H., et al. (2019) Biphasic cellular adaptations and ecological implications of *Alteromonas macleodii* degrading a mixture of algal polysaccharides. *ISME J* **13**: 92–103. <https://doi.org/10.1038/s41396-018-0252-4>.
- Long, R.A., and Azam, F. (2001) Antagonistic interactions among marine pelagic bacteria. *Appl Environ Microbiol* **67**: 4975–4983. <https://doi.org/10.1128/AEM.67.11.4975-4983.2001>.
- Manna, V., De Vittor, C., Giani, M., Del Negro, P., and Celussi, M. (2021) Long-term patterns and drivers of microbial organic matter utilization in the northernmost basin of the Mediterranean Sea. *Mar Environ Res* **164**: 105245. <https://doi.org/10.1016/j.marenvres.2020.105245>.
- Mühlenbruch, M., Grossart, H.P., Eigemann, F., and Voss, M. (2018) Mini-review: phytoplankton-derived polysaccharides in the marine environment and their interactions with heterotrophic bacteria. *Environ Microbiol* **20**: 2671–2685. <https://doi.org/10.1111/1462-2920.14302>.
- Orlando-Bonaca, M., Mannoni, P.-A., Poloniato, D., and Falace, A. (2013) Assessment of *Fucus virsoides* distribution in the Gulf of Trieste (Adriatic Sea) and its relation to environmental variables. *Bot Mar* **56**: 451–459. <https://doi.org/10.1515/bot-2013-0027>.
- Pfeifer, L., Shafee, T., Johnson, K.L., Bacic, A., and Classen, B. (2020) Arabinogalactan-proteins of *Zostera marina* L. contain unique glycan structures and provide insight into adaptation processes to saline environments. *Sci Rep* **10**: 8232. <https://doi.org/10.1038/s41598-020-65135-5>.
- Rakoff-Nahoum, S., Foster, K.R., and Comstock, L.E. (2016) The evolution of cooperation within the gut microbiota. *Nature* **533**: 255–259. <https://doi.org/10.1038/nature17626>.
- Reintjes, G., Arnosti, C., Fuchs, B., and Amann, R. (2019) Selfish, sharing and scavenging bacteria in the Atlantic Ocean: a biogeographical study of bacterial substrate utilisation. *ISME J* **13**: 1119–1132. <https://doi.org/10.1038/s41396-018-0326-3>.
- Reintjes, G., Arnosti, C., Fuchs, B.M., and Amann, R. (2017) An alternative polysaccharide uptake mechanism of marine bacteria. *ISME J* **11**: 1640–1650. <https://doi.org/10.1038/ismej.2017.26>.
- Reintjes, G., Fuchs, B.M., Amann, R., and Arnosti, C. (2020b) Extensive microbial processing of polysaccharides in the south pacific gyre via selfish uptake and extracellular hydrolysis. *Front Microbiol* **11**: 583158. doi: 10.3389/fmicb.2020.583158.
- Reintjes, G., Fuchs, B.M., Scharfe, M., Wiltshire, K.H., Amann, R., and Arnosti, C. (2020a) Short-term changes in polysaccharide utilization mechanisms of marine bacterioplankton during a spring phytoplankton bloom. *Environ Microbiol* **22**: 1884–1900. <https://doi.org/10.1111/1462-2920.14971>.
- Riedel, T., and Dittmar, T. (2014) A method detection limit for the analysis of natural organic matter via Fourier transform ion cyclotron resonance mass spectrometry. *Anal Chem* **86**: 8376–8382. doi: 10.1021/ac501946m.
- Sarnaik, A., Abernathy, M.H., Han, X., Ouyang, Y., Xia, K., Chen, Y., et al. (2019) Metabolic engineering of cyanobacteria for photoautotrophic production of heparosan, a pharmaceutical precursor of heparin. *Algal Res* **37**, 57–63. doi: 10.1016/j.algal.2018.11.010.
- Showalter, A.M. (2001) Arabinogalactan-proteins: structure, expression and function. *Cell Mol Life Sci* **58**: 1399–1417. doi: 10.1007/PL00000784.
- Simon, H.M., Smith, M.W., and Herfort, L. (2014) Metagenomic insights into particles and their associated microbiota in a coastal margin ecosystem. *Front Microbiol* **5**: 3389. doi: 10.3389/fmicb.2014.00466.
- Simon, M., Grossart, H.-P., and Schweitzer, B. (2002) Microbial ecology of organic aggregates in aquatic ecosystems. *Aquat Microb Ecol* **28**: 175–211. doi: 10.3354/ame028175.
- Singh, R.S., Saini, G.K., and Kennedy, J.F. (2008) Pullulan: microbial sources, production and applications. *Carbohydr Polym* **73**: 515–531. doi: 10.1016/j.carbpol.2008.01.003.
- Sist, P., and Urbani, R. (2017) Polysaccharide characterisation of marine organic matter in a coastal station of the Gulf of Trieste (northern Adriatic Sea, Italy). *Thalass An Int J Mar Sci* **33**: 139–150. doi: 10.1007/s41208-017-0031-4.
- Smith, D.C., Simon, M., Alldredge, A.L., and Azam, F. (1992) Intense hydrolytic enzyme activity on marine aggregates and implications for rapid particle dissolution. *Nature* **359**: 139–142. doi: 10.1038/359139a0.
- Smith, M., Zeigler Allen, L., Allen, A., Herfort, L., and Simon, H. (2013) Contrasting genomic properties of free-living and particle-attached microbial assemblages within a coastal ecosystem. *Front Microbiol* **4**: 120. doi: 10.3389/fmicb.2013.00120.
- Student. (1908) The probable error of a mean. *Biometrika* **6**: 1–25. doi: 10.2307/2331554.
- Teeling, H., Fuchs, B.M., Becher, D., Klockow, C., Gardebrecht, A., Bennke, C.M., et al. (2012) Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. *Science* **336**: 608–611. doi: 10.1126/science.1218344.

- Thomas, F., Barbeyron, T., Tonon, T., Génicot, S., Czjzek, M., and Michel, G. (2012) Characterization of the first alginolytic operons in a marine bacterium: from their emergence in marine Flavobacteriia to their independent transfers to marine Proteobacteria and human gut Bacteroides. *Environ Microbiol* **14**: 2379–2394, doi: 10.1111/j.1462-2920.2012.02751.x.
- Traving, S.J., Thygesen, U.H., Riemann, L., and Stedmon, C.A. (2015) A model of extracellular enzymes in free-living microbes: which strategy pays off? *Appl Environ Microbiol* **81**: 7385–7393, doi: 10.1128/aem.02070-15.
- Verdugo, P., Alldredge, A.L., Azam, F., Kirchman, D.L., Passow, U., and Santschi, P.H. (2004) The oceanic gel phase: a bridge in the DOM-POM continuum. *Mar Chem* **92**: 67–85, doi: 10.1016/j.marchem.2004.06.017.
- Vetter, Y.A., Deming, J.W., Jumars, P.A., and Krieger-Brockett, B.B. (1998) A predictive model of bacterial foraging by means of freely released extracellular enzymes. *Microb Ecol* **36**: 75–92, doi: 10.1007/s002489900095.
- Weiss, M.S., Abele, U., Weckesser, J., Welte, W., Schiltz, E., and Schulz, G.E. (1991) Molecular architecture and electrostatic properties of a bacterial porin. *Science* **254**: 1627–1630, doi: 10.1126/science.1721242.
- Zhang, S., Xu, C., and Santschi, P.H. (2008) Chemical composition and ²³⁴Th (IV) binding of extracellular polymeric substances (EPS) produced by the marine diatom *Amphora* sp. *Mar Chem* **112**: 81–92, doi: 10.1016/j.marchem.2008.05.009.
- Zoccarato, L., and Grossart, H.P. (2019) Relationship between lifestyle and structure of bacterial communities and their functionality in aquatic systems. In *The Structure and Function of Aquatic Microbial Communities. Advances in Environmental Microbiology*, Vol. 7. Hurst, C.J. (ed). Cham: Springer, pp. 13–52. https://doi.org/10.1007/978-3-030-16775-2_2.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1: Supporting information