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Effects of biofouling on the sinking behavior of microplastics

David Kaiser\textsuperscript{1,2}, Nicole Kowalski\textsuperscript{1} and Joanna J Waniek\textsuperscript{1}

\textsuperscript{1} Leibniz Institute for Baltic Sea Research, Seestrasse 15, 18119 Rostock, Germany
\textsuperscript{2} Author to whom any correspondence should be addressed.

E-mail: david.kaiser@io-warnemuende.de
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Abstract
Although plastic is ubiquitous in marine systems, our current knowledge of transport mechanisms is limited. Much of the plastic entering the ocean sinks; this is intuitively obvious for polymers such as polystyrene (PS), which have a greater density than seawater, but lower density polymers like polyethylene (PE) also occur in sediments. Biofouling can cause large plastic objects to sink, but this phenomenon has not been described for microplastics $<$5 mm. We incubated PS and PE microplastic particles in estuarine and coastal waters to determine how biofouling changes their sinking behavior. Sinking velocities of PS increased by 16\% in estuarine water (salinity 9.8) and 81\% in marine water (salinity 36) after 6 weeks of incubation. Thereafter sinking velocities decreased due to lower water temperatures and reduced light availability. Biofouling did not cause PE to sink during the 14 weeks of incubation in estuarine water, but PE started to sink after six weeks in coastal water when sufficiently colonized by blue mussels \textit{Mytilus edulis}, and its velocity continued to increase until the end of the incubation period. Sinking velocities of these PE pellets were similar irrespective of salinity (10 vs. 36). Biofilm composition differed between estuarine and coastal stations, presumably accounting for differences in sinking behavior. We demonstrate that biofouling enhances microplastic deposition to marine sediments, and our findings should improve microplastic transport models.

1. Introduction
Plastic is ubiquitous in the marine realm (Andrady 2011, Law 2017, Oberbeckmann \textit{et al} 2015, van Sebille \textit{et al} 2015), occurring from the equator to the poles (C\textit{\c{z}}ar \textit{et al} 2017), anthropogenic centers to remote areas, from beaches (Browne \textit{et al} 2011) to the deep sea (Van Cauwenbergh \textit{et al} 2013, Woodall \textit{et al} 2014). Nevertheless, there is a striking gap in our knowledge of the transport mechanisms that distribute plastics from sources to sinks around the globe. The gap is especially large for sub-surface transport, with recent studies focusing on surface drift of floating plastic (C\textit{\c{z}}ar \textit{et al} 2017, 2015, Lebreton \textit{et al} 2012, van Sebille \textit{et al} 2015, 2012). However, estimates of plastic in the surface ocean range in the tens to hundreds of thousands of metric tons (C\textit{\c{z}}ar \textit{et al} 2014, Law 2017, van Sebille \textit{et al} 2015), and only account for around 1\% of the estimated millions of metric tons of floating plastic input from land (C\textit{\c{z}}ar \textit{et al} 2014, Jambek \textit{et al} 2015, Lebreton \textit{et al} 2017). Much of the plastic introduced to surface waters may sink out through the water column, making the sea floor a suspected major sink (C\textit{\c{z}}ar \textit{et al} 2017, Van Cauwenbergh \textit{et al} 2013, Woodall \textit{et al} 2014). However, a large amount of produced plastic is buoyant (C\textit{\c{z}}ar \textit{et al} 2014) and should not sink on its own accord. Polyethylene (PE) alone, which has a specific density lower than water, constitutes over 1/3 of the global plastic production (Andrady 2011). Still, some low density plastic rests on the sea floor (Holmström 1975), corroborating the existence of mechanisms that facilitate sinking. One possible reason for sinking is biofouling that increases the specific density of the floating material it colonizes (Andrady 2011, Chubarenko \textit{et al} 2016, Fazey and Ryan 2016b, Morêt-Ferguson \textit{et al} 2010, Woodall \textit{et al} 2014, Ye and Andrady 1991). Biofouling on plastic is a successive buildup of organic matter and...
organisms. Stimulated by the hydrophobicity of plastic (Zettler et al 2013), the rapid adsorption of organic substances provides a ‘conditioning film’ (Artham et al 2009, Ye and Andrady 1991) and is swiftly followed by bacterial colonization and microalgal growth, which eventually shares space with colonizing invertebrates (Andrady 2011). The colonization speed and succession depend on polymer type, specific surface area (Ye and Andrady 1991), surface energy and roughness (Andrady 2011, Artham et al 2009, Kerr and Cowling 2003). It varies in space and time as ambient water conditions differ between geographic locations and between seasons (Andrady 2011, Artham et al 2009, Carson et al 2013, Eich et al 2015, Oberbeckmann et al 2015, 2014, Ye and Andrady 1991).

Biofouling can sink larger plastic objects, at least when colonization by macro-organisms occurs (Fazey and Ryan 2016a, Ye and Andrady 1991). Whether it sinks very small plastic items is still uncertain (Oberbeckmann et al 2015). By emerging consensus plastic \( \leq 5 \text{ mm} \) to micrometers in size are termed microplastic (Andrady 2011, Kowalski et al 2016, Law 2017, Oberbeckmann et al 2015, Tsang et al 2017, van Sebille et al 2015, Barnes et al 2009). Microplastic originates from cosmetics, synthetic fabric fibers (Browne et al 2011), and industrial raw resin (Andrady 2011, Van Cauwenbergh et al 2013), as well as the fragmentation of larger objects (Van Cauwenbergh et al 2013). The abundance of microplastic in surface waters is lower than expected from the rate of fragmentation of floating macroplastic (Eriksen et al 2014). There is evidence that the sea floor is a sink for high density microplastic (Van Cauwenbergh et al 2013). While some authors claim that low density microplastic have not been found at the sea floor (Chubarenko et al 2016), low density microplastic, including polyethylene, are abundant at least in near shore subtidal sediments (Thompson et al 2004, Vianello et al 2013). The transport and deposition mechanisms by which low density microplastic reaches the sediments is still poorly understood. Biofouling sufficient to sink buoyant microplastic has been described theoretically (Chubarenko et al 2016). While biofouling occurs on any kind and shape of plastic (Oberbeckmann et al 2015), and smaller objects with relatively higher surface area:volume ratio experience a more rapid relative change in specific density leading to their sinking (Chubarenko et al 2016, Fazey and Ryan 2016a, Ryan 2013), the small size of microplastic poses limits to the extent of biofouling.

In order to establish whether biofouling influences the transport of microplastic and enhances its removal from surface waters, we used field incubations to cause biofouling on microplastic particles and subsequently determined their sinking behavior. We hypothesize that (1) biofouling increases the sinking velocity of negatively buoyant microplastic particles and causes the sinking of positively buoyant microplastic particles, and that (2) the effects of biofouling on sinking behavior varies between locations.

2. Material and methods

2.1. Plastic particle incubation and sample preparation

Because geographic location and polymer type affect biofouling on plastic, we incubated two microplastic polymers at two locations. Polystyrene (PS, density \( 1050 \text{ kg m}^{-3} \), BASF 143 E) and high density polyethylene (PE, density 955 kg m\(^{-3}\), CS Plastik GmbH, Germany) were used in this study. Particles were produced at the IFP Dresden, Germany by extruding and cutting the resin to cylindrical shape with both height and diameter of about 1 mm; PS particles were marginally smaller than PE particles. Microplastic was incubated in the estuary of the Warnow river (54° 8’28.22″N, 12° 5’19.13″E) and at a coastal site near Heiligendamm (54° 8’46.11″N, 11°50’36.19″E), both in the Baltic Sea area of Germany. At both stations ten incubators, each filled with \( 10 \pm 0.1 \text{ g} \) of PE or PS, numbering around 4500 PE or 5300 PS particles, were suspended from jetties along ropes below the low tide water level at depths between 20–100 cm. Incubators were cylindrical (6.8 cm high, 7.2 cm internal diameter) with walls lined with 0.5 mm gauze to permit water passage. Incubation commenced on 6 July 2016 in the estuary and 19 July 2016 at the coastal station. One incubator of each polymer from both sites was sampled after 0, 2, 4, 6, 10, and 14 weeks of incubation and transported in station water to the laboratory. After sampling, 30 particles of each polymer were placed in pre-weighed tin cups for determination of their weight after drying at 40 °C for 24 hours. 20 particles each were photographed at 2.5–8 fold magnification under a SteREO Discovery.V8 stereo microscope (Carl Zeiss, Germany) equipped with an AxioCam Ic.c3 digital camera. From microscope images the shell length of attached mussels was determined as the longest distance from the umbo to the shell edge using ImageJ (imagej.net). Four particles of each polymer were fixed in 2% formaldehyde at 4 °C over night and then washed for 1 min in water filters. For imaging, particles were placed on a fume hood overnight. All reagents and equipment were sterilized by autoclaving or filtration over 0.2 μm filters. For imaging, particles were placed on pin stubs with adhesive carbon pads and sputter coated with chromium by argon plasma or carbon by high voltage. Images were taken with a MERLIN VP compact (Carl Zeiss) in vacuum at different magnifications, generally 40x (overview), 100x, 250x, 500x, 1000x, and 2500x.

Incubators were cleaned biweekly by rinsing and scrubbing with a horse-hair brush. During cleaning,
water temperature and salinity were measured using a WTW cond 1970i (Wuppertal, Germany), and water was sampled for filtration of 250–400–ml over combusted GF/F filters. Filters were analyzed for particulate organic carbon and nitrogen in the Elementar varioMICRO analyzer (determination limits: 1.1 μmol C and 0.3 μmol N; standard deviation of the method: 0.15 μmol C and 0.04 μmol N per sample). At the coastal station, chlorophyll a concentrations were determined fluorometrically from an ethanol (96%) extract of additional filters (repeated sample measurement precision ~15%) (Knap et al 1996). The filtrate was analyzed for dissolved inorganic nutrients using a FlowSys continuous flow analyzer (Alliance Instruments, Germany). Data of daily solar irradiance was obtained from the Climate Data Center of Deutscher Wetterdienst (ftp://ftp-cdc.dwd.de/pub/CDC/) for a meteorological station 16 and 4.3 km from the coastal and estuarine incubation stations, respectively.

### Table 1. Water characteristics during laboratory sinking experiments (means ± standard deviation).

| Polymer | Experimental Water | Temperature [°C] | Salinity | Water Density [kg m\(^{-3}\)] |
|---------|--------------------|------------------|----------|-----------------------------|
| PE      | coastal            | 20.6 ± 0.8       | 10.0 ± 0.3| 1005.6 ± 0.1                |
| PS      | coastal            | 20.7 ± 0.9       | 9.9 ± 0.0 | 1005.5 ± 0.2                |
| PE      | estuarine          | 20.7 ± 0.9       | 9.9 ± 0.0 | 1005.5 ± 0.2                |
| PS      | estuarine          | 20.6 ± 0.6       | 36.0 ± 0.0| 1025.3 ± 0.2                |
| PE      | Atlantic           | 20.6 ± 0.7       | 36.0 ± 0.0| 1025.3 ± 0.2                |
| PS      | Atlantic           | 20.6 ± 0.7       | 36.0 ± 0.0| 1025.3 ± 0.2                |

3. Results

#### 3.1. Environmental data

Over the course of the incubation, salinity, dissolved inorganic nutrients and particulate organic matter showed some degree of variability but no discernable trend. Water temperature remained stable over the summer months and deceased from mid-September on, i.e. after 8 and 10 weeks of incubation at the coastal and estuarine station, respectively. Similarly, solar irradiance time decreased in mid-September and was generally <5 h d\(^{-1}\) in October (figure 1). During the incubation period, mean salinity was slightly but significantly higher at the coast (11.7) than in the estuary (11.1). Particulate organic carbon and nitrogen as well as dissolved inorganic silicate and phosphorus were significantly lower at the coastal than the estuarine station, while there was no significant difference in nitrate concentrations between both stations (figure 1). Chlorophyll a concentrations at the coastal station during the incubation period ranged from 1.7–9.9 μg l\(^{-1}\), with a median of 3.0 μg l\(^{-1}\) and slightly higher values in October (data not shown).

#### 3.2. Biofilm development

The weight of incubated particles showed no trend over time and in most cases did not differ from that of virgin particles (figure S1 available at stacks.iop.org/ERL/12/124003/mmedia). Substantial weight increase was only observed for PE particles colonized by mussels at the coastal station.

Microscopic observations showed that particles incubated at the coast were colonized by individual
small mussels, *Mytilus edulis*, and filamentous red algae after the first 2 weeks. Few dark spots and streaks were also found on both polymers. Microfouling cover on PE did not apparently increase after 4 weeks, but patches of biomass occurred on PS. After 6 weeks those occurred also on PE and the size and number of mussels increased considerably. Cover increased similarly on PS. After 10 weeks, the first sampling after water temperature and sunlight hours started to decrease, unidentified biomass cover on PE did not increase perceptibly but the size of mussels further increased. PS was covered with less biomass and fewer attached mussels. At the end of the incubation period, after 14 weeks, most PE particles were aggregated by mussels, individual particles had lost most of the unidentified biomass patches but were covered in mussel byssus threads. Aggregation was less pronounced for PS, which was also covered with byssus threads. Occurrence of dark spots on PS was similar to week 10. The length of mussel shells increased from 0.3 mm after two weeks to 1.6 mm after 10 weeks (figure S2). In the estuary microscopic cover of PE and PS consisted of patchy and fluffy yellow-brown unidentified biomass growth. No mussels or other macro-organisms were observed. The area covered by biomass increased over time on both polymers, but starting from week 2, was larger on PE than PS.

SEM images showed that the microfouling composition was distinctly different between the stations (figures 2 and 3). At the coast, the biofilm consisted mostly of photosynthetic microorganisms, dominated by intact pennate diatoms, as well as chain-forming cyanobacteria and single-celled circular and rod-shaped bacteria. After 6 weeks, mussel byssus threads occurred and centric diatoms, as well as fragments of broken diatom frustules, became more frequent. Lithogenic particles were rarely present.Particles incubated in the estuary developed a distinguishable layer of organic matter covering the entire particle at the end of the incubation. Identified parts of the biofilm were dominated by fragments of broken diatoms of a larger variety than observed at the coast. Lithogenic particles were common throughout the incubation period, as were bacteria. Protozoans such as several ciliates, occurred frequently.

### 3.3. PS sinking velocity

Virgin PS particles had a sinking velocity of 0.015 m s$^{-1}$ in water from the Warnow estuary and the coastal Baltic Sea, and 0.009 m s$^{-1}$ in water from the Atlantic Ocean (figure 4).

After two weeks of incubation in the estuary, the sinking velocity of PS in estuarine and Atlantic water increased by 5% and 6%, respectively. Sinking velocity showed significant linear increase until the end of the incubation period ($p < 0.05$, $R^2 \geq 0.85$), to 0.017 m s$^{-1}$ in estuarine water and 0.012 m s$^{-1}$ in Atlantic water after 14 weeks (figure 4). At this time sinking velocity exceeded virgin particle velocity by 12% and 28% in estuarine and Atlantic water, respectively. Sinking velocity thus increased more strongly in Atlantic than estuarine water, with regression slopes of $2.5 \times 10^{-5}$ m s$^{-1}$ day$^{-1}$ and $2.9 \times 10^{-5}$ m s$^{-1}$ day$^{-1}$, respectively.

The change in sinking velocity was more complex for PS incubated at the coastal station. The velocity of PS in coastal water first significantly increased, by 16%, after 6 weeks (figure 4). Afterwards it decreased significantly to 6% over the velocity of virgin particles and retained a median velocity of 0.016 m s$^{-1}$ until the end of the incubation. Sinking in Atlantic water, PS velocity increased slightly but significantly after 2 weeks incubation at the coast. This combination showed highest overall velocity increase of 81% relative to virgin PS after 6 weeks. Consistent with PS sinking in coastal
water, velocity decreased between 6 and 10 weeks of incubation.

PS particles incubated at the coastal station exhibited higher variability in sinking velocity than those from the estuary, particularly when velocity was significantly increased. Highest variability coincided with highest median velocities (figure 4).

### 3.4. PE sinking velocity
As expected, PE did not initially sink. This did not change for PE incubated in the estuary, which floated in all tested cases, despite early patchy discoloration and microfouling.

Particles incubated at the coast first sank in both coastal and Atlantic water after 6 weeks of incubation (figure 5). Only particles colonized by macrozoobenthos, predominantly *Mytilus* sp. became negatively buoyant, and the percentage of sinking particles varied over time but did not continuously increase. From week 6 onwards the velocity of sinking particles increased significantly until the end of the incubation. Interestingly, there was no significant difference in velocity of PE

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**Figure 2.** SEM images of biofouling on polystyrene (PS) particles at a coastal and an estuarine station, after 2, 6, 10 and 14 weeks. Numbers show the size of bars in μm.
sinking in coastal or Atlantic water, despite considerable differences in fluid density.

Where possible the number of attached mussels was counted during the sinking of the particle. Up to six mussels were counted per PE particle. The dataset is small and mussels varied considerably in size (figure S2), but there appears to be a positive correlation between number of mussels and median sinking velocity ($R^2 = 0.94$, $p < 0.001$; figure S3). Not all PE with attached mussels sank, but some showed reduced upward floating velocity when submerged and released below the surface (not quantifiable). The variability in number and size of attached mussels causes the high observed variability in the velocity of sinking particles (figure 5).

### 4. Discussion

Biofouling does enhance microplastic sinking. The development of a biofilm increased the sinking velocity of negatively buoyant microplastic particles (figure 4), and marofouling caused positively buoyant microplastic particles to sink (figure 5). Despite differences in

Figure 3. SEM images of biofouling on polyethylene (PE) particles at a coastal and an estuarine station, after 2, 6, 10 and 14 weeks. Numbers show the size of bars in $\mu$m.
fluid density, the highest (after 6 weeks) sinking velocity of PS incubated at the coast was similar in coastal (0.018 m s\(^{-1}\)) and Atlantic water (0.016 m s\(^{-1}\)) (figure 4). The sinking velocity of PE was not significantly different between coastal and Atlantic water at any time of incubation (figure 5). The velocity of PS particles incubated in the estuary increased more strongly for particles sinking in higher salinity, thus converging toward the faster velocity of particles sinking in lower salinity. The sinking velocities of PE particles incubated at the coast were within a similar range to that of PS (0.01–0.04 m s\(^{-1}\)) despite fundamental differences in polymer density. These results show that biofouling reduces the effect of density differences between particle and fluid over time, and thus exerts major control over microplastic sinking.

The development of a microscopic biofilm alone did not increase the specific density of buoyant microplastic particles sufficiently to cause their sinking. The attachment of fouling macro-organisms appears necessary to transport buoyant microplastic through the water column. A strong effect of fouling macro-organisms on plastic sinking was also reported in other studies (Fazey and Ryan 2016a). With the exception of mussels, common plastic fouling macro invertebrates, such as barnacles (Andrady 2011, Fazey and Ryan 2016a, Ye and Andrady 1991), were absent from particles incubated at the coast, though they were present on the hard surface of the incubators from week 2. Organisms that need a surface to attach their whole body, unlike mussels that use threads, might be prevented from growing on microplastic due to the limiting available particle surface (Fazey and Ryan 2016a). This implies a lower limit to the size of particles that can be sunk by attached invertebrates. Much smaller microplastic particles than those used in this study occur in the ocean (e.g. Sutton et al 2016). Especially fibers may have surface areas that prevent the attachment of fouling macro-organisms. SEM pictures show that mussel byssus threads attach to microplastic particles over an area roughly 20 \(\mu\)m across (figure 3), which is similar to the thickness of PE microfibers (Kowalski et al 2016). Biofilm effects on the sinking rate of sub-mm microplastic should receive some scientific attention, especially because these particles are easily ingested by small aquatic organisms.
The distinct difference of the microscopic biofilm between particles incubated at the two stations is due to the different estuarine and coastal water conditions. No data for water transparency is available but at the coast the bottom at around 3 m was generally visible except during inclement weather, while in the estuary incubators suspended around 20 cm below the surface were not visible. The dominance of photosynthetic microalgae observed on particles incubated at the coast is common in biofilms on plastic in marine environments (Eriksen et al. 2014, Fazey and Ryan 2016a, Oberbeckmann et al. 2014, Zettler et al. 2013). However, the percentage of algal cover is a function of light availability (Ye and Andrady 1991). In the estuary, the low water transparency prevented the dominance of photosynthetic organisms. Moreover, the low transparency is a result of a high load of suspended matter and the observed brown water color indicates a negligible contribution of fresh phytoplankton. From this mostly non-living suspended matter lithogenic particles and dead phytoplankton fragments deposit onto the microplastic particles (figures 2 and 3). The high ambient organic matter concentration feeds the variety of heterotrophic organisms on the surface of the particle. Significant differences in the microbial biofilm composition on plastic at a similar spatial scale have also been shown by genetic methods (Oberbeckmann et al. 2014). The observed differences between stations in both biofouling composition and its effect on microplastic sinking, show that while fouling by photosynthetic organisms might be hampered when employing partly opaque incubators, spatial differences are well captured by this experiment.

The spatial difference in biofilm composition determines the change of sinking velocities over time at the two stations. Increasing sinking velocity of PS incubated at the coast during the warm water period and the rapid decrease coinciding with decreasing temperature and sunshine hours show that the effect of a fresh, mostly living biofilm follows a growing season. The decrease is due to reduced biofouling, which declines as growth conditions deteriorate (Artham et al. 2009). However, this change in sinking velocity did not correlate with the development of chlorophyll a in the water, suggesting different governing factors for free living phytoplankton and microplastic-attached algae. Other factors also determine the degree of macro-fouling, as the effect of attached mussels on PE sinking continues to increase until the end of the incubation period (figure 5). Similar to the microscopic biofilm, fouling by macro invertebrates is reversible. In the case of PE, and other low density plastic, defouling will return particles to the surface, where fouling might increase again, causing the plastic to oscillate through the water column over time until persistently colonized by macro organisms (Andrady 2011, Ye and Andrady 1991). It is also conceivable that in areas of high sediment deposition or bioturbation rates buoyant microplastic might be buried permanently even when defouling happens. In contrast to coastal waters, estuarine biofouling continues to increase microplastic sinking velocity after ambient water temperature and sunshine hours decrease (figures 1 and 4). The high proportion of non-living matter makes biofouling in the turbid environment less dependent on growth conditions for microorganisms. This spatio-temporal variability in biofouling poses a challenge for the transfer of results to other aquatic systems with different environmental conditions. In addition, the experimental setup used in this study likely caused conditions for fouling to differ from those occurring naturally. Fouling by photosynthetic organisms might have been affected by reduced light availability inside the incubators. It is however difficult to estimate the magnitude of this effect; originally sinking PS can experience low light conditions also in sediment or at the sediment.
surface, while fouling of originally floating PE can be reduced at the water-atmosphere-interface (see Fazey and Ryan 2016a). The high concentration of particles inside the incubators might have increased physical weathering through abrasion and might have scoured larger organisms off the biofilm or prevented their establishment after settlement. Free particles likely also experience changing fouling conditions as they are transported between locations, which would change the composition of biofouling. Unfortunately, the progressive fouling of free microplastic particles would be impossible to track. Despite these restrictions, the experiment could show the effect of progressive biofouling in different locations on the sinking behavior of microplastic.

The results of this study offer the first empirical values to incorporate the effect of biofouling on microplastic sinking velocities. Theoretically, sinking velocities can be calculated adequately for virgin particles with regular shape to within a few percent (e.g. Khatmullina and Isachenko 2017). The effect of substantial biofouling can be modeled by adding a factor to that theoretical velocity. The value of that factor would depend on fouling location, time and duration. These conditions would also affect particle changes due to weathering by physical abrasion and UV radiation. Additional factors would be the numerous characteristics of plastic released to the aquatic environment (Law 2017).

In nature, the effect of biofouling on sinking velocity will be different for positively and negatively buoyant microplastic. High density microplastic that sinks on its own accord, will probably reach the seafloor before any acceleration by fouling can take place. For instance at a sinking velocity of 0.01 m s$^{-1}$ virgin PS particles used in this study would need 5.8 d to sink through 5000 m of water in the Atlantic. Velocity increase over that time, according to our results, would be minimal, certainly <5% (figure 4). Even if such particles became resuspended from the sediment, the responsible turbulence would apparently exert the dominant effect on vertical transport. In smaller systems like coastal seas (e.g. the Baltic Sea) floating microplastic might be transported to shore in a shorter time than is necessary for biofouling to cause it to sink (Chubarenko et al 2016). However, the presence of low density microplastic in nearshore subtidal sediments (e.g. Thompson et al 2004, Vianello et al 2013) and of low density plastic sheets at the sea bottom (Holmström 1975) strongly suggest that some portion of floating microplastic remains at sea for a time sufficient to cause its sinking by biofouling.

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ORCID iDs

David Kaiser  https://orcid.org/0000-0003-3629-6336

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