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Interactions between the ice algae *Fragillariopsis cylindrus* and microplastics in sea ice

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ABSTRACT

High concentrations of microplastics have been found in sea ice but the mechanisms by which they get captured into the ice and which role ice algae might play in this process remain unknown. Similarly, we do not know how the presence of microplastics might impact the colonization of sea ice by ice algae. To estimate the ecological impact of microplastics for Polar ecosystems, it is essential to understand their behaviour during ice formation and possible interactions with organisms inhabiting sea ice.

In this study we tested the interaction between the ice algae *Fragillariopsis cylindrus* and microplastic beads with and without sea ice present and, in a third experiment, during the process of ice formation. With sea ice present, we found significantly less algae cells in the ice when incubated together with microplastics compared to the incubation without microplastics. However, during ice formation, the presence of microplastics did not impact the colonisation of the ice by *F. cylindrus* cells. Further, we observed a strong correlation between salinity and the relative amount of beads in the water and ice. With increasing salinity of the water, the relative amount of beads in the water decreased significantly. At the same time, the relative amount of beads in the ice increased significantly with increasing ice salinity. Both processes were not influenced by the presence of *F. cylindrus*. Also, we found indications that the presence of algae can affect the amount of microplastic beads sticking to the container walls. This could indicate that EPS produced by ice algae plays a significant role in surface binding properties of microplastics.

Overall, our results highlight that the interactions between algae and microplastics have an influence on the uptake of microplastics into sea ice with possible implications for the sea ice food web.

1. Introduction

Marine plastic pollution is an ongoing concern for the global oceans. In 2010, an estimated 4–10.6 million metric tons (MMT) of plastic waste have been released into the ocean just by the 20 most polluting countries alone (Jambeck et al., 2015). Worst case scenarios estimate that by 2025 about 250 MMT of plastic are accumulating in the ocean (Jambeck et al., 2015). Once entering the ocean, plastic can be dispersed by wind and currents and a recent study found that the poleward branch of the thermohaline circulation is a pathway for transport of floating debris from the North Atlantic into the Eurasian Arctic, which is considered to be a dead end for this plastic pollution (Cózar et al., 2017). Despite the Antarctic Circumpolar Current (ACC), which was considered to act as a barrier for any floating particles, a recent study could prove that plastic is also present around the entire Antarctic continent (Lacerda et al., 2019). Plastic waste includes large, visible particles but also small microplastics which are defined as particles < 5 mm. Microplastics can be directly produced and released as small particles (primary microplastics) e.g. from cosmetic products, sand-blasting media, or virgin pellets (Andrady, 2015). Secondary microplastics, on the other hand, originate from the breakdown of larger plastic particles by sunlight, temperature changes, mechanic abrasion, and wave action (Andrady, 2015). Even though microplastic pollution is not visible compared to macroplastic pollution, there is a growing concern about the effects on ecosystem and human health (Barboza et al., 2018; Botterell et al., 2019; Galloway, 2015; Lusher, 2015). Because of their small size, microplastics can be taken up by a wide range of organisms and cause problems such as abrasion and blockages of gut systems of small organisms (Wright et al., 2013 and references therein). In addition to these physical effects, microplastics leach plasticisers and can bind pollutants, including polycyclic aromatic hydrocarbons (PAHs). These pollutants are then potentially released following

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ingestion (Avio et al., 2017) and could have negative impacts on their health (Pittura et al., 2018) partly by affecting the organisms gut microbiome (Lu et al., 2019 and references therein). Overall, the effects of long-term exposure of microplastics on marine organisms and humans are still largely unknown.

Because of increased awareness and better detection methods, microplastics have been identified in numerous marine ecosystems globally (Kershaw and Rochman, 2016), including the Arctic (Lusher et al., 2015; Obbard et al., 2014) and the Southern Ocean (Isobe et al., 2017; Waller et al., 2017). Once entering the Arctic, microplastics have been found in various marine realms from sea ice to the deep sea sediments (Peeken et al., 2018a). Extremely high concentrations of microplastics have been found in sea ice (Peeken et al., 2018b), with concentrations in the same range of extremely polluted regions of South Korean waters (Song et al., 2015) or Lake Taihu in China (Su et al., 2016). Sea ice can act as a vector of both horizontal and vertical transport of microplastics (Peeken et al., 2018b) and elevated concentrations of microplastics on the sea floor underneath the marginal ice zone indicates that melting sea ice releases high concentrations of microplastics (Bergmann et al., 2017). A recent study further highlights the importance of an atmospheric input resulting in high microplastic concentrations in snow also found in the Arctic (Bergmann et al., 2019).

The processes how microplastics can be entrained in sea ice are not well understood but could include passive freezing or active transport into ice brine channels via organisms that have ingested microplastics or by sticking to the outside of organisms that inhabit sea ice. Especially pennate sea ice diatoms produce extracellular polymeric substances (EPS) that are important for adhesion and cell protection (among other things) and therefore play a vital role for the survival of these species in the harsh sea ice environment (Underwood et al., 2010). The high concentrations of these sticky EPS could potentially retain microplastics in the brine channels (Peeken et al., 2018b). *Fragillariopsis cylindrus* is well known as one of the most abundant sea ice alga in Arctic and Antarctic sea ice communities (Kang and Fryxell, 1992; Poulin et al., 2011; van Leeuwe et al., 2018) and can dominate the algae community in brine pockets and channels (Günther and Dieckmann, 2001; Lizotte, 2001). This species produces large amounts of EPS (Aslam et al., 2018) and could therefore be a significant transport mechanism of bringing microplastics bound to EPS deeper into sea ice. For some freshwater algae an increase in EPS production was observed when exposed to microplastics (Lagarde et al., 2016). Long et al. (2017) have reported the formation of hetero-aggregates between the diatom *Chaetoceros neogracile* and polystyrene microparticles during the stationary growth phase, but no such aggregates were observed for the prymnesiophyte and the dinoflagellate tested, indicating large species-specific differences in the interaction with microplastics.

We hypothesize that interactions between microplastics and the sea ice diatom *F. cylindrus* will impact the uptake of polystyrene beads into sea ice. To test this hypothesis, we performed three sets of incubation experiments looking at the interaction between *F. cylindrus* and polystyrene beads in seawater, in existing sea ice, and during the process of ice formation.

2. Methods

We performed three experiments to test the possible interaction of the ice algae *Fragillariopsis cylindrus* with microplastic beads in water (experiment 1), with pre-grown sea ice present (experiment 2) and during the process of ice formation (experiment 3).

2.1. Algae cultures and microplastic beads

The ice algae *F. cylindrus* was isolated from sea ice of the Weddell Sea (Antarctica) during the RV Polarstern expedition ANT XVI/3 (1999, for details see Mock and Valentin (2004)) and maintained in the AWI culture collection. The culture was kept under a 16:8 light cycle at 30 µE at 0 °C in f/2 medium based on 0.2 µm filtered Antarctic seawater collected during the Polarstern cruise ANT 27_2 (2011). The average cell size of this culture was 4–5 µm.

Polystyrene microplastic beads (Polysciences, Fluoresbrite yellow-green fluorescent, 0.5 µm diameter, density 1.05 g mL^{-1}) were used for all experiments. These beads have been used before in similar studies (Long et al., 2015; Long et al., 2017) in sea water. Due to their small size and density similar to water, settling effects are negligible in our setup. All experiments were performed in glass vessels to minimize binding of microplastic beads to the bottle walls (Long et al., 2017). Nevertheless, this process cannot be avoided and we thus always kept a bead only treatment during the course of the experiments to account for any wall effect over time.

For each experiment, the start cell concentration for *F. cylindrus* was 1 000 cells/mL both in the algae alone and the algae and beads treatments. The microplastic beads were added at a concentration of 90 000 beads/mL to the beads alone and the algae and beads treatments. The number of microplastic beads measured at the start of the experiment was set to be 100%. The number of microplastic beads that we measured in the water and/or ice during the incubation was named "free microplastic beads." This number was always between 2% and 41% lower compared to the initial concentration reflecting the beads bound to the container walls (wall bound beads). Cell numbers and number of microplastic beads in all experiments were measured using a Flow Cytometer (see below).

2.2. Experiment 1: Water experiment

We incubated 500 mL conical glass flasks, containing 300 mL of filtered f/2 medium based on Antarctic seawater at 0 °C and 30 µE for 24 days. Each of the three treatments (beads alone, algae alone, and beads and algae; see table 1) was run in triplicates. *F. cylindrus* microplastic beads were added at the concentrations mentioned above. The beads alone treatment was performed to test the binding of beads on the glass walls without algae present. The algae alone treatment was performed to test the growth rate of *F. cylindrus* without beads present. The combined algae and bead treatment tested possible effects of the presence of algae on the binding of beads onto the glass walls and the possible effect of beads on the growth rate of *F. cylindrus*.

Table 1
Overview of incubation conditions of the three experiments. Water and ice volume and salinity are values from the end of each experiment.

Experiment	treatments	Temp °C	Water salinity	Water volume (mL)	Ice salinity	Ice volume (mL)	Incubation period (days)	Number of replicates
1: Water	algae alone	0	35	300	–	–	24	3
	beads alone	0	35	300	–	–	24	3
	algae and beads	0	35	300	–	–	24	3
2: Ice colonization	algae alone	−4	51 ± 10	81 ± 25	13 ± 2	75 ± 23	6	9
	beads alone	−4	49 ± 9	88 ± 21	10 ± 2	61 ± 21	6	9
	algae and beads	−4	45 ± 5	95 ± 19	10 ± 1	61 ± 19	6	9
3: Ice formation	algae alone	−5	70 ± 3	28 ± 4	17 ± 0.4	24 ± 2	15	4
	beads alone	−5	68 ± 3	30 ± 2	17 ± 0.4	26 ± 2	15	4
	algae and beads	−5	67 ± 2	30 ± 1	17 ± 1	28 ± 3	15	4

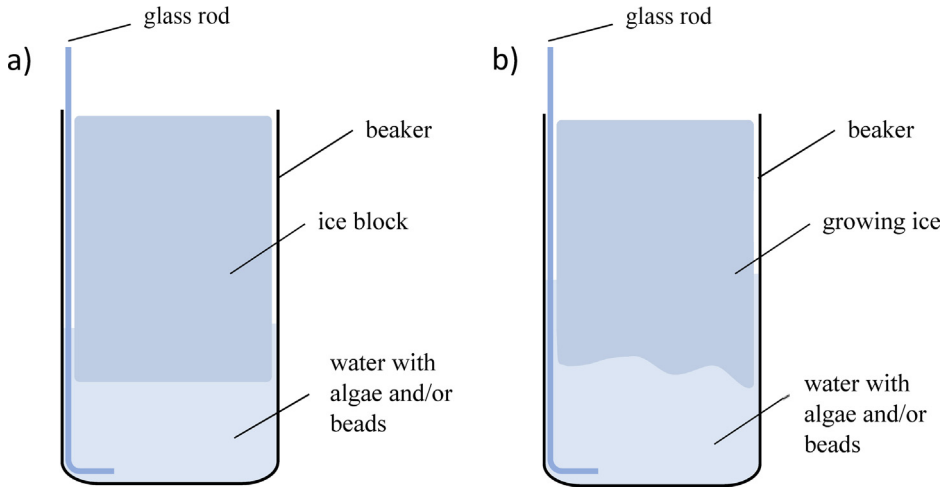

Fig. 1. Set up of the (a) experiment 2 (ice colonization) and (b) experiment 3 (ice formation). A glass rod was bent over a Bunsen burner to create a 90° hook at the end.

2.3. Experiment 2: Ice colonization experiment

27 glass beakers (150 mL, 9 replicates per treatment) were filled with 120 mL of f/2 medium based on 0.2 µm filtered Antarctic seawater and frozen into ice blocks at −20 °C. The ice blocks were carefully removed from the beakers by shortly putting the glass beaker into room temperature warm water until the surface was just melted enough to remove the ice block. We prepared three solutions of liquid f/2 medium containing algae and/or beads at the concentrations mentioned above. For each of the three treatments, we added 50 mL of the solution each to 9 of the beakers and carefully placed an ice block on top of the water using a bent glass rod (Fig. 1). We bent the glass rods over a Bunsen burner to create a 90° corner at the end of each rod. By using this glass rod, we were able to carefully lower the ice block and also remove it after the incubation. The beakers were placed in a cover of aluminium foil to allow light penetration only from the top of the incubated ice, mimicking natural growth conditions for the sea ice algae. The covered beakers with the ice blocks swimming on the water were incubated at −4°C for 6 days in a Rumed incubator (1301D). This temperature setting of the incubator was tested before to give stable conditions for a slight growth of the ice, despite the heat from the light bulbs. Irradiance in the beakers below the ice were measured with a Spherical Micro Quantum Sensor US-SQS/L (Waltz) and where in a range of 30 µmol m^{-2} s^{-1}. The light sources were Philips Master TL-D Reflex Eco set to a 16:8 h light:dark cycle. After the incubation, the ice blocks were removed and melted. We determined the volume and salinity (WTW LF 95, conductivity-measuring device) of each ice block and of the water remaining in the beaker and measured the number of *F. cylindrus* cells and microplastic beads in the water and the melted ice.

2.4. Experiment 3: Ice formation experiment

12 glass beakers (4 replicates per treatment) were filled with 60 mL f/2 medium based on 0.2 µm filtered Antarctic seawater containing algae and/or beads at the concentrations mentioned above. The beakers were incubated at −5 °C for two days a Rumed incubator, to get ice growing. Once the ice has established the experiment remained for 15 days in the same Rumed incubator with a 16:8 h light cycle. A bent glass rod was added to each beaker to enable us to remove the ice after the incubation as described above. Volume, salinity, and number of algae and beads in the water and ice was determined as described above.

2.5. Flow cytometry

F. cylindrus cell numbers and microplastic beads in all experiments were counted using an Accuri C6 (BD) Flow Cytometer. *F. cylindrus* samples were analysed for 1 to 4 min depending on cell density at a flow rate of 65 µl/min. Cell identification was triggered on chlorophyll fluorescence (FL3) and the threshold was set to 1000 on FL3. Microplastic beads were analysed for 1 min at a flow rate of 65 µl/min. Bead identification was triggered on green fluorescence (FL1) and the threshold was set to 1000 on FL1. For statistical analyses, Students t-tests were used. Differences found are reported as significant in the text if $p < 0.05$.

3. Results

3.1. Experiment 1: Incubation without ice

The presence of microplastics had no impact on the growth of *F. cylindrus* (Fig. 2a, b). The growth rate of *F. cylindrus* was 0.28 for both treatments. However, the presence of algae did have a significant effect on the percentage of microplastic beads bound to the walls. Between 78 and 95% of the beads added at the beginning of the experiment stayed in solution when incubated together with the algae (Fig. 2a) compared to 59–75% when incubated without the algae (Fig. 2c). After day 10 of our experiment, significantly less of the microplastic beads were bound to the container walls when incubated together with algae compared to the incubations without algae (ANOVA, $p < 0.05$, Fig. 2d). Hetero-aggregates, which would indicate the active entrainment of microplastics into the algae, were not overserved during this study.

3.2. Experiment 2: Colonization of existing sea ice

In this experiment, we investigated the colonization of *F. cylindrus* cells and microplastic beads into an existing ice block. The presence of algae did not have a significant effect on the percentage of free beads in the water and ice (Fig. 3a). Without algae present, an average of 77% of the free microplastic beads were found in the water, while 23% were found in the ice (Fig. 3a). With algae present, an average of 80% of the free microplastic beads were found in the water, and 20% were found in the ice Fig. 3a). However, the percentage of wall bound beads was with an average of 31% significantly higher when incubated together with the algae compared to the incubations without algae (20%, Fig. 3b, *t*-test, $p = 0.0009$). This was the opposite trend compared to the experiment without ice (experiment 1, Fig. 2d).

While the presence of *F. cylindrus* cells had no significant effect on

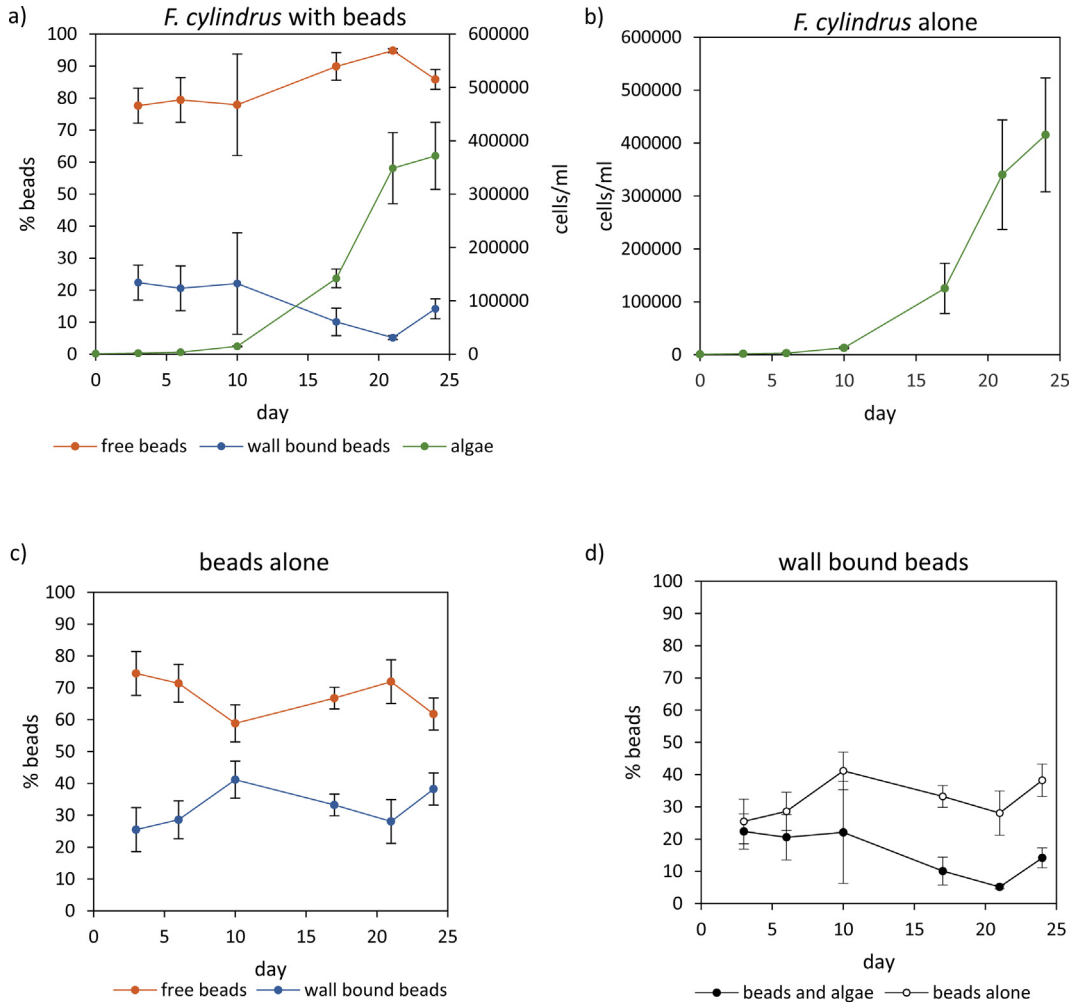

Fig. 2. Growth of *F. cylindrus* as well as percentage of beads in the experiment without ice. (a) Incubation of *F. cylindrus* and microplastic beads together, (b) growth of *F. cylindrus* without the presence of microplastic beads, (c) % of free and wall bound microplastics in the incubation without *F. cylindrus* present, and (d) % of wall bound beads in the incubations with and without *F. cylindrus*.

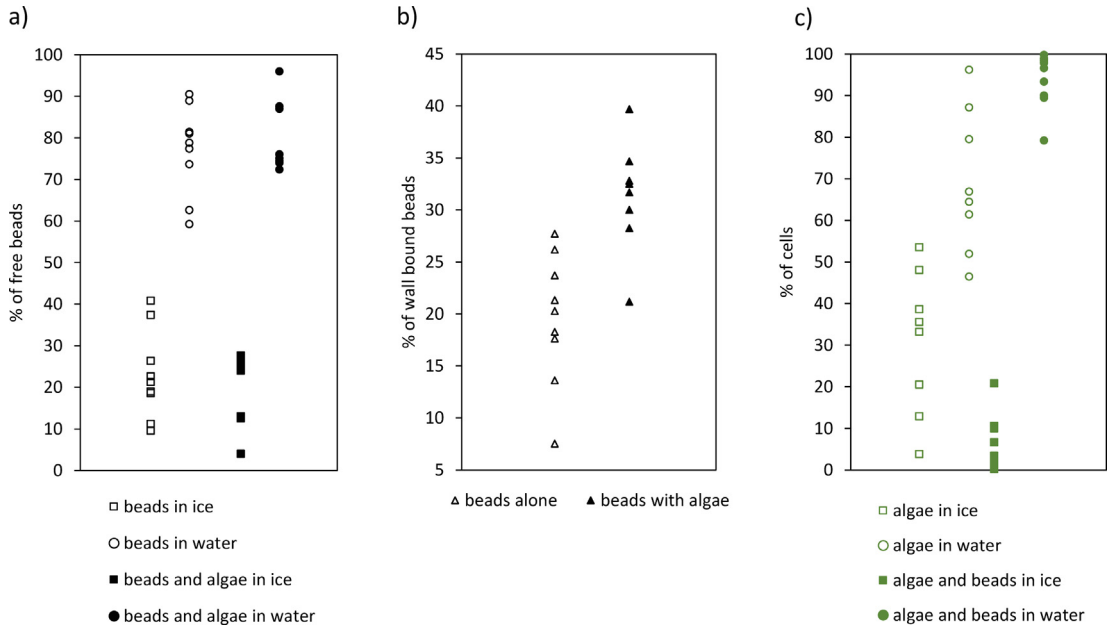

Fig. 3. Ice colonization experiment (a) Percentage of free microplastic beads in ice and water. (b) Percentage of wall bound beads in the incubation with and without algae. (c) Percentage of *F. cylindrus* cells in ice and water.

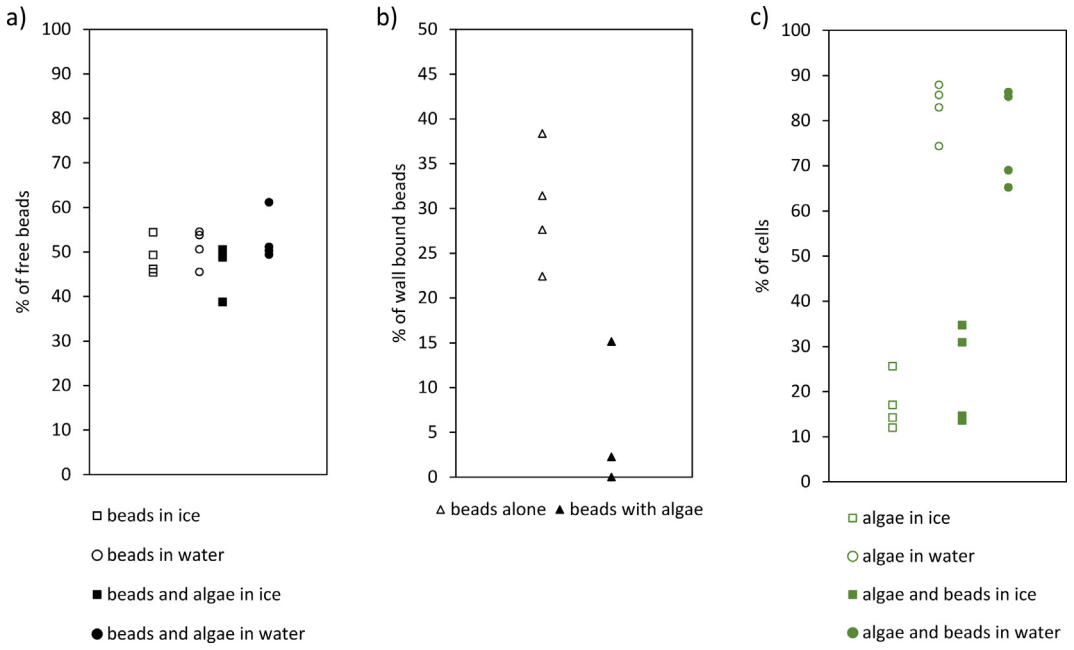

Fig. 4. Ice formation experiment (a) Percentage of free microplastics in ice and water. (b) Percentage of wall bound microplastics in the incubation with and without algae. c) Percentage of *F. cylindrus* cells in ice and water.

the relative amount of microplastic in the ice, the presence of microplastic did affect the colonisation of the added ice by *F. cylindrus* cells. Significantly more algae were found in the water underneath the ice when incubated together with microplastics (average of 94%) compared to the incubation without microplastics (average of 69%) and significantly less algae cells were found in the ice when incubated together with microplastics (average of 6%) compared to an average of 31% of the total cells when incubated without microplastics (Fig. 3c, *t*-test, p = 0.0014).

3.3. Experiment 3: Microplastic uptake during ice formation

In this experiment, we investigated the colonization of *F. cylindrus* cells and microplastic beads into sea ice during the process of ice formation. The presence of algae did not have a significant effect on the percentage of free microplastics in the water and the ice (Fig. 4a). Without algae present, an average of 51% of the free microplastic beads were found in the water, while 49% were found in the ice (Fig. 4a). With algae present, an average of 53% of the free microplastics were found in the water, and 47% were found in the ice (Fig. 4a). However, the percentage of wall bound microplastics was significantly higher (30%) when incubated alone compared to the incubations with algae (9%, Fig. 4b, *t*-test, p = 0.002). This was the same trend as observed in the experiment without ice (Fig. 2d). The presence of microplastics did not significantly affect the colonisation of the ice by *F. cylindrus* cells during ice formation. On average 83% of cells were found in the water and 17% in the ice when incubated without microplastics compared to 76% in water and 24% in the ice when incubated together with microplastic beads (Fig. 4c).

3.4. Microplastics and sea ice salinity

At the end of experiments 2 and 3 (ice colonisation and formation), we determined the salinity in the water underneath the ice and of the melted ice. Due to ice formation during experiment 3, the salinity of the water increased. We observed a strong correlation of salinity on the relative amount of beads in the water underneath the ice and the ice itself. With increasing salinity of the water from 34 to over 70, the relative amount of free beads in the water decreased from 96% to 50% (Fig. 5a). We did thereby not observe any significant difference between the incubations with and without *F. cylindrus* (ANCOVA, p = 0.30). At the same time, we observed that the relative amount of beads in the ice increased with increasing salinity. Here, we found the highest relative abundance of beads of about 50% in the ice with the highest salinity around 20 and the lowest relative abundance of beads of around 10% in the ice with the lowest salinity of around 10 (Fig. 5a). Again, the presence of the ice algae did not have a significant impact on this relationship (ANCOVA, p = 0.12). The salinity variation within each experiment arises from slightly different local temperature fields and thus different amount of ice melting and freezing. High salinity water indicative of more ice formation leads to a higher incorporation of beads into the ice and thus a lower proportion of beads in the water. Similarly, bulk ice salinity in our experiment is higher for enhanced ice growth, leading to a higher proportion of ice incorporated beads with increasing ice bulk salinity. Our observations thus show that microplastic beads of 0.5 μm diameter behave like a passive tracer and can easily be incorporated into the sea ice matrix. In particular microplastic beads are not rejected during ice formation such as salts dissolved in sea water.

At the same time, salinity of the water did not correlate with the relative amount of beads that were stuck to the walls of the incubation vessels (Fig. 5b). We did observe a somewhat similar correlation of salinity and the relative amount of *F. cylindrus* cells in the water or ice as with microplastic beads. However, this relationship is not statistically significant as the algal cells are not inert tracer particles, but are affected by a variety of factors (Fig. 5c).

When comparing the relative amount of microplastic beads in the ice between the two ice experiments, we observed that the process of ice formation traps significantly more microplastic beads in the ice compared to the first experiment where we added the already frozen ice (Fig. 6a). On average 23 and 18% of the microplastic beads were trapped in the ice in experiment 2 (ice colonization) when incubated alone or with algae cells, respectively. During experiment 3 (ice formation), on average 49 and 47% of the free microplastic beads were trapped in the ice when incubated alone or with algae cells, respectively. This difference was significant both for the incubations with and without *F. cylindrus* (*t*-test, p = 0.0007 and 0.0002). This is consistent with the hypothesis, that microplastic particles mainly get incorporated

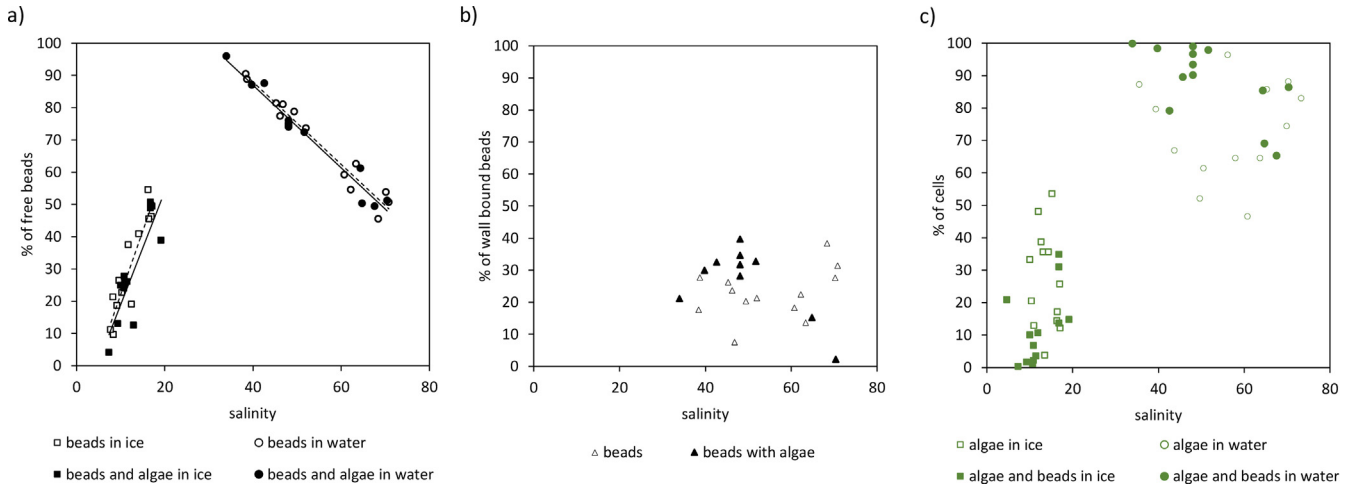

Fig. 5. (a) Percentage of free beads in the water and ice as a function of water salinity in both ice experiments. (b) Percentage of wall bound beads as a function of water salinity in both ice experiments. (c) Percentage of *F. cylindrus cells* in the water and the ice as a function of water salinity in both ice experiments.

into sea-ice during the growth phase.

We further observed a significant impact of the process of ice formation (experiment 3) on the relative amount of *F. cylindrus* cells in the ice (Fig. 6b). When incubated without microplastics, only 17% of cells colonised the ice during experiment 3 (ice formation) compared to 31% during experiment 2 (ice colonisation (*t*-test, p = 0.044). When incubated together with microplastic beads, 24% of cells were found in the ice during experiment 3 (ice formation) compared to 6% during experiment 2 (ice colonisation; *t*-test, p = 0.043).

4. Discussion

4.1. Interactions between Fragillariopsis cylindrus and microplastics

There is contradicting information in the current literature about the impact of microplastic presence on marine phytoplankton growth. While some studies, including ours, have found no effect (Davarpanah and Guilhermino, 2015; Long et al., 2017; Prata et al., 2018) other studies have reported a significant reduction of algae growth rate when exposed to microplastics (Bergami et al., 2017; Sjollema et al., 2016; Zhang et al., 2017). The main reason for the contradicting results is possibly due to large differences in the different plastic material used (PS, PE, PVC), bead size (0.04–10 μm), microplastic concentration

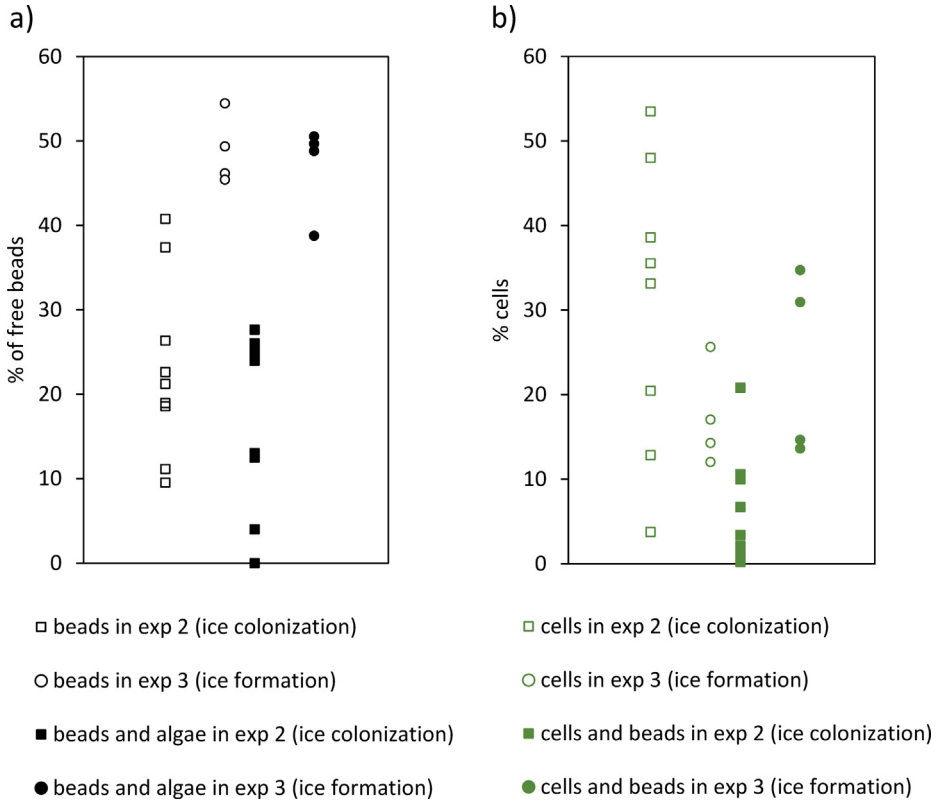

Fig. 6. (a) Percentage of microplastic beads in ice in both ice experiments. (b) Percentage of *F. cylindrus* cells in ice in both ice experiments.

(0–7500 mg L^{-1}), and algae species used (Prata et al., 2019). Also, surface chemistry of microplastic particles influences adsorption and therefore can have an influence on toxicity (Bergami et al., 2017). In general it seems that smaller sizes and positive surface charges have more negative effects on microalgae (Prata et al., 2019 and references therein). Several studies have reported the formation of hetero-aggregates between microplastics and freshwater algae (Bhattacharya et al., 2010; Cunha et al., 2019; Lagarde et al., 2016) as well as marine algae (Cunha et al., 2019; Long et al., 2017; Zhang et al., 2017). However, there seems to be a large difference in the formation of hetero-aggregates between different algae species due to differences in their individual EPS production (Cunha et al., 2019; Long et al., 2017). Further, the methods for heteroaggregates detection differ between the studies (microscopy (Cunha et al., 2019; Lagarde et al., 2016), spectrophotometry (Bhattacharya et al., 2010), Flow Cytometry (Long et al., 2017)). It is possible that Flow Cytometry does not detect weaker bound hetero-aggregates between algae and microplastic beads as these might break when the sample is forced into a fine stream prior to detection in the instrument. Therefore, even though we did not observe hetero-aggregates with Flow Cytometry in our study it is still possible that the microplastic beads stuck to the algae cells.

The presence of algae in our experiments did have an impact on the surface binding properties of the microplastic and significantly affected the amount of wall bound beads in our incubations. The presence of algae resulted in significantly lower amounts of polystyrene beads bound to the container walls when incubated without ice (Fig. 2d) and during ice formation (Fig. 4b). On the other hand, the presence of algae did significantly increase the amount of wall bound beads during ice colonization (Fig. 3b). On average, the range of wall bound beads in our experiment was well below what is reported for similar experiments with polystyrene beads and algae. Long et al. (2017) found that over 50% of polystyrene beads were bound to the glass walls in their control incubation without algae present. Different algae species significantly changed the surface binding properties of the particles in their incubations with only 14% ± 3 of wall bound beads when incubated with the diatom *Chaetoceros neogracile* and up to 85% ± 2 when incubated with *Heterocapsa triquetra* (Long et al., 2017). It is possible that the production of EPS by the algae is responsible for this process. Ice algae in particular are known to have a high EPS production as an adaption strategy to survive in the harsh environmental conditions present in sea ice (Aslam et al., 2018, and references therein) which could explain our relative high interactions of beads and algae. Chen et al. (2011) report that the presence of PS microbeads had a significant impact on the assemblage of EPS into microgels in the marine algae *Amphora* sp., while only minor effects were found for *Ankistrodesmus angustus* and *Phaeodactylum tricornutum*. These effects could also impact the binding of microplastics to marine aggregates, which has been shown to be a major sink for microplastics (Long et al., 2015).

The lack of this relationship in pre-grown ice (experiment 2) might be due to relative lower surface to volume ratio in this experiment. Since here, the beads had not the opportunity to attach to the entire beaker, due to the overlaying ice, the effect here is less pronounced compared to the other two experiments. Overall, the large loss of microplastics to incubation container walls and especially the significant impact that different phytoplankton species can have on this process, highlights the importance to monitor the free microplastic concentration during incubation experiments in order to quantify the difference between treatments. Especially when working with different microplastics concentrations, it cannot just be assumed that the number of microplastics added stays constant during incubation.

4.2. Microplastic and ice colonisation

Microplastics did have a significantly negative effect on the amount of *F. cylindrus* cells colonizing already established ice (experiment 2). With microplastics present, a significantly lower relative number of algae cells was found in the ice (Fig. 3c), suggesting some interaction between the beads and the algae.

This preferential entrainment of beads in the ice, could hamper the recolonization of sea ice, which is an important process to transfer sea ice species from multiyear ice to first-year ice (Olsen et al., 2017). Ice algae are an important food source for higher trophic levels (Soreide et al., 2010) and thus the increasing presence of microplastics in the Arctic might be an additional stressor for this sensitive ecosystem, with implications for higher trophic levels including fish. Ice algae communities face rapid changes of their environment through climate change related stressors such as ocean warming and ocean acidification which are likely to affect productivity and community composition (McMinn, 2017; McMinn et al., 2014; Tedesco et al., 2019). Any additional stressor such as microplastics could have wide-reaching implications for the whole ecosystem. Interestingly, as opposed to experiment 2, no interaction between algae and microplastic beads was observed during ice formation (experiment 3, Fig. 4c). This, together with the observed relationship between microplastics and salinity in our experiments rather suggests a passive entrainment process of microplastic beads into sea ice during ice growth driven by changes in seawater salinity (density) that affects the floating of microplastics. The strong relationship between salinity and microplastic concentration could also impact the general distribution of microplastics via brine convection as already suggested by Pittura et al. (2018). We had initially hypothesised that the presence of ice algae would have an impact on the amount of microplastic particles being transported into existing sea ice but our experiments have not shown such a relationship. It remains to be studied, if organisms that ingest microplastics and colonise brine channels could play a significant role in incorporating and distributing microplastics in sea ice under more natural conditions.

4.3. Microplastic and ice properties

Passive uptake of particles in general during ice formation is a well-known process that incorporates particles such as sediments in sea ice (Nürnberg et al., 1994). This would especially affect those particles with relatively low densities and therefore lower sinking rates. Our study did not find a relative concentration of microplastics in the ice during the process of ice formation (Fig. 4a), however, we did observe that the process of ice formation trapped significantly more microplastic beads compared to experiment 2 where ice was already present (Fig. 6a), which coincides with the assumption that this process is responsible for incorporation of (organic) particles into sea ice (Peeken et al., 2018b).

In a recent study, Geilfus et al. (2019) studied the effect of microplastics on sea ice formation in an outdoor pool without algae added. In their study, a mixture of polypropylene (PP, density 0.855 g cm^{-3}), polyvinyl chloride (PVC, density 1.38 g cm^{-3}) and polyethylene terephthalate (PET, density 1.37 g cm^{-3}) particles, with irregular shapes ranging from µm to mm size, did not have an impact on sea ice growth (Geilfus et al., 2019). This is in agreement with our results where the presence of microplastics and/or algae did not affect the ice volume in both ice experiments (Table 1). Geilfus et al. (2019) further report the highest number of microplastic beads in the surface of their ice cores. We did not see a difference in microplastic concentrations between the bottom and the surface of the ice in our incubations and have therefore combined the two parts of the ice in the analysis of our data. This discrepancy between our study and the one from Geilfus et al. (2019) is likely due to differences in the experimental set up. While we worked with small scale indoor incubations where ice growth, the resulting properties and texture is not strictly representative of natural conditions, Geilfus et al. (2019) used outdoor mesocosms of 1000 L each, using artificial seawater, much lower temperatures (< 10 °C), and pumped the water at a speed of 12 mL min^{-1} during their experiments to ensure water circulation. Also, the PP particles had a much lower density (0.855) than the PS particles used in our study (1.05) and the

water circulation in the Geilfus et al. (2019) study might have mixed up the denser PVC and PET particles. This could explain the differences in our studies. For future experiments investigating the impact of microplastics on ice formation and the interaction with ice algae it would be good to use growth tanks with larger volumes under controlled conditions and using a variety of polymer types, sizes, and concentrations to better mimic natural conditions.

Interestingly, Geilfus et al. (2019) also observed a correlation between the amount of microplastics added to their experiments and the salinity in their ice core, which is similar to our findings. The treatments with the highest amount of microplastics added had ice bulk salinities of up close to 20 in the surface of the ice core while the control and the treatments with low microplastic concentrations added had surface ice salinities of < 10. While we did not observe higher sea ice salinities in our treatments with microplastics added, we did find a strong correlation between the % of free beads and salinity both in the water and the ice of our experiments. Similar to Geilfus et al. (2019), our highest sea ice salinities of around 20 showed the highest relative concentrations of microplastics (Fig. 5a). Sea ice salinity is directly related to both, the salinity of the water when it is formed from and in particular the growth rate during ice formation. Fast forming ice incorporates more seawater inclusions leading to a higher salinity and in this case also a higher microplastic concentration. However, it remains unclear, to which extent the presence of microplastics could play a significant role in the physical properties of sea ice, and thereby have indirect implications for sea ice inhabiting organisms and the polar food chain.

5. Conclusion

While we did not find any evidence for an increased transport of microplastics into sea ice by sticking to *F. cylindrus* cells as initially hypothesized, we did find indications for an interaction between ice algae and microplastic beads. With sea ice present, significantly less algae cells were found in the ice when incubated together with microplastics compared to the incubation without microplastics. However, during ice formation, the presence of algae did not have a significant effect on the percentage of free microplastics in the water and the ice and the presence of microplastics did not significantly affect the colonisation of the ice by *F. cylindrus* cells. Further, we found indications that the presence of algae can affect the amount of microplastic beads sticking to surfaces such as the container walls both depending on the experimental setup. This could indicate that EPS produced by ice algae plays a significant role in surface binding properties of microplastics.

Another interesting result of our study is the observation how strongly the amount of microplastics correlates with salinity in sea ice. The highest relative amount of microplastic beads was found in sea ice with the highest salinity. As ice salinity differs depending on many factors such as air temperature and water salinity conditions during ice formation, this could help understand how microplastics are being entrained into sea ice. In order to better understand the processes that determine the uptake of microplastics into sea ice we therefore need to understand both the physical and biological factors that influence microplastic uptake and distribution.

6. Author statement

L.H. and I. P. have planned and performed the experiment. E. A. and S. L. E. have helped with maintaining cultures and sampling. L.H., I. P., and C. K. analysed the data. L. H. wrote the manuscript with input from I. P. All authors provided feedback and helped edit the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Andrady, A. L. 2015. Persistence of plastic litter in the oceans, p. 57-72. In M. Bergmann, G. L and M. Klages [eds.], Marine Anthropogenic Litter. Springer.
Aslam, S.N., Strauss, J., Thomas, D.N., Mock, T., Underwood, G.J.C., 2018. Identifying metabolic pathways for production of extracellular polymeric substances by the diatom Fragilariopsis cylindrus inhabiting sea ice. ISME J 12, 1237–1251.
Avio, C.G., Gorbi, S., Regoli, F., 2017. Plastics and microplastics in the oceans: from emerging pollutants to emerged threat. Mar. Environ. Res. 128, 2–11.
Barboza, L.G.A., Dick Vethaak, A., Lavorante, B.R.B.O., Lundebye, A.-K., Guilhermino, L., 2018. Marine microplastic debris: An emerging issue for food security, food safety and human health. Mar. Pollut. Bull. 133, 336–348.
Bergami, E., Pugnalini, S., Vannuccini, M.L., Manfra, L., Faleri, C., Savorelli, F., Dawson, K.A., Corsi, I., 2017. Long-term toxicity of surface-charged polystyrene nanoplastics to marine planktonic species *Dunaliella tertiolecta* and *Artemia franciscana*. Aquat. Toxicol. 189, 159–169.
Bergmann, M., Mützel, S., Primpke, S., Tekman, M.B., Trachsel, J., Gerdts, G., 2019. White and wonderful? Microplastics prevail in snow from the alps to the arctic. Sci. Adv. 5 eaax1157.
Bergmann, M., Wirzberger, V., Krumpen, T., Lorenz, C., Primpke, S., Tekman, M.B., Gerdts, G., 2017. High quantities of microplastic in arctic deep-sea sediments from the HAUSGARTEN observatory. Environ. Sci. Technol. 51, 11000–11010.
Bhattacharya, P., Lin, S., Turner, J.P., Ke, P.C., 2010. Physical adsorption of charged plastic nanoparticles affects algal photosynthesis. J. Phys. Chem. C 114, 16556–16561.
Botterell, Z.L.R., Beaumont, N., Dorrington, T., Steinke, M., Thompson, R.C., Lindeque, P.K., 2019. Bioavailability and effects of microplastics on marine zooplankton: a review. Environ. Pollut. 245, 98–110.
Chen, C.-S., Anaya, J.M., Zhang, S., Spurgin, J., Chuang, C.-Y., Xu, C., Miao, A.-J., Chen, E.Y.T., Schwehr, K.A., Jiang, Y., Quigg, A., Santschi, P.H., Chin, W.-C., 2011. Effects of engineered nanoparticles on the assembly of exopolymeric substances from phytoplankton. PLoS ONE 6, e21865.
Cózar, A., Martí, E., Duarte, C.M., García-de-Lomas, J., van Sebille, E., Ballatore, T.J., Eguíluz, V.M., González-Gordillo, J.I., Pedrotti, M.L., Echevarría, F., Troublè, R., Irigoien, X., 2017. The Arctic Ocean as a dead end for floating plastics in the North Atlantic branch of the Thermohaline Circulation. Sci. Adv. 3, e1600582.
Cunha, C., Faria, M., Nogueira, N., Ferreira, A., Cordeiro, N., 2019. Marine vs freshwater microalgae exopolymers as biosolutions to microplastics pollution. Environ. Pollut. 249, 372–380.
Davarpanah, E., Guilhermino, L., 2015. Single and combined effects of microplastics and copper on the population growth of the marine microalgae Tetraselmis chuii. Estuar. Coast. Shelf Sci. 167, 269–275.
Galloway, T. S. 2015. Micro- and nano-plastics and human health, p. 343-366. In M. Bergmann, L. Gutow and M. Klages [eds.], Marine Anthropogenic Litter. Springer.
Geilfus, N.X., Munson, K.M., Sousa, J., Germanov, Y., Bhugaloo, S., Babb, D., Wang, F., 2019. Distribution and impacts of microplastic incorporation within sea ice. Mar. Pollut. Bull. 145, 463–473.
Günther, S., Dieckmann, G.S., 2001. Vertical zonation and community transition of sea-ice diatoms in fast ice and platelet layer, Weddell Sea, Antarctica. Ann. Glaciol. 33, 287–296.
Isobe, A., Uchiyama-Matsumoto, K., Uchida, K., Tokai, T., 2017. Microplastics in the Southern Ocean. Mar. Pollut. Bull. 114, 623–626.
Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science 347, 768–771.
Kang, S.-H., Fryxell, G.A., 1992. *Fragilariopsis cylindrus* (Grunow) Krieger: the most abundant diatom in water column assemblages of Antarctic marginal ice-edge zones. Polar Biol. 12, 609–627.
Kershaw, P., Rochman, C.M., 2016. Sources, fate and effects of microplastics in the marine environment: part two of a global assessment. Rep. Stud. GESAMP 93, 220.
Lacerda, A. L. d. F., L. d. S. Rodrigues, E. van Sebille, F. L. Rodrigues, L. Ribeiro, E. R. Secchi, F. Kessler, and M. C. Proietti. 2019. Plastics in sea surface waters around the Antarctic Peninsula. Sci. Rep. 9: 3977.
Lagarde, F., Olivier, O., Zanella, M., Daniel, P., Hiard, S., Caruso, A., 2016. Microplastic interactions with freshwater microalgae: hetero-aggregation and changes in plastic density appear strongly dependent on polymer type. Environ. Pollut. 215, 331–339.
Lizotte, M.P., 2001. The contributions of Sea Ice Algae to Antarctic marine primary production. Integr. Comp. Biol. 41, 57–73.
Long, M., Moriceau, B., Gallinari, M., Lambert, C., Huvet, A., Raffray, J., Soudant, P., 2015. Interactions between microplastics and phytoplankton aggregates: Impact on their respective fates. Mar. Chem. 175, 39–46.
Long, M., Paul-Pont, I., Hégaret, H., Moriceau, B., Lambert, C., Huvet, A., Soudant, P., 2017. Interactions between polystyrene microplastics and marine phytoplankton lead to species-specific hetero-aggregation. Environ. Pollut. 228, 454–463.
Lu, L., Luo, T., Zhao, Y., Cai, C., Fu, Z., Jin, Y., 2019. Interaction between microplastics

and microorganism as well as gut microbiota: A consideration on environmental animal and human health. Sci. Total Environ. 667, 94–100.
Lusher, A. L. 2015. Microplastics in the marine environment: distribution, interactions and effects, p. 245-307. In M. Bergmann, L. Gutow and M. Klages [eds.], Marine Anthropogenic Litter. Springer.
Lusher, A.L., Tirelli, V., O'Connor, I., Officer, R., 2015. Microplastics in Arctic polar waters: the first reported values of particles in surface and sub-surface samples. Sci. Rep. 5, 14947.
McMinn, A., 2017. Reviews and syntheses: Ice acidification, the effects of ocean acidification on sea ice microbial communities. Biogeosciences 14, 3927–3935.
McMinn, A., Müller, M.N., Martin, A., Ryan, K.G., 2014. The response of Antarctic sea ice algae to changes in pH and CO_2. PLoS ONE 9, e86984.
Mock, T., Valentin, K., 2004. Photosynthesis and cold acclimation: Molecular evidence from a polar diatom. J. Phycol. 40, 732–741.
Nürnberg, D., Wollenburg, I., Dethleff, D., Eicken, H., Kassens, H., Letzig, T., Reimnitz, E., Thiede, J., 1994. Sediments in Arctic sea ice: Implications for entrainment, transport and release. Mar. Geol. 119, 185–214.
Obbard, R.W., Sadri, S., Wong, Y.Q., Khitun, A.A., Baker, I., Thompson, R.C., 2014. Global warming releases microplastic legacy frozen in Arctic Sea ice. Earth's Future 2, 315–320.
Olsen, L.M., Laney, S.R., Duarte, P., Kauko, H.M., Fernandez-Mendez, M., Mundy, C.J., Rosel, A., Meyer, A., Itkin, P., Cohen, L., Peeken, I., Tatarek, A., Rozanska-Pluta, M., Wiktor, J., Taskjelle, T., Pavlov, A.K., Hudson, S.R., Granskog, M.A., Hop, H., Assmy, P., 2017. The seeding of ice algal blooms in Arctic pack ice: the multiyear ice seed repository hypothesis. J. Geophys. Res.-Biogeo. 122, 1529–1548.
Peeken, I., M. Bergmann, G. Gerdts, C. Katlein, T. Krumpen, S. Primpke, and M. B. Tekman. 2018a. Microplastics in the Marine Realms of the Arctic with Special Emphasis on Sea Ice Arctic Report Card 2018.
Peeken, I., Primpke, S., Beyer, B., Gütermann, J., Katlein, C., Krumpen, T., Bergmann, M., Hehemann, L., Gerdts, G., 2018. Arctic sea ice is an important temporal sink and means of transport for microplastic. Nat. Commun. 9, 1505.
Pittura, L., C. G. Avio, M. E. Giuliani, G. d'Errico, S. H. Keiter, B. Cormier, S. Gorbi, and F. Regoli. 2018. Microplastics as vehicles of environmental PAHs to marine organisms: Combined chemical and physical hazards to the mediterranean mussels, Mytilus galloprovincialis. Front. Marine Sci. 5.
Poulin, M., Daugbjerg, N., Gradinger, R., Ilyash, L., Ratkova, T., von Quillfeldt, C., 2011. The pan-Arctic biodiversity of marine pelagic and sea-ice unicellular eukaryotes: a first-attempt assessment. Mar. Biodivers. 41, 13–28.
Prata, J.C., da Costa, J.P., Lopes, I., Duarte, A.C., Rocha-Santos, T., 2019. Effects of microplastics on microalgae populations: a critical review. Sci. Total Environ. 665, 400–405.
Prata, J. C., B. R. B. O. Lavorante, M. d. C. B.S.M. Montenegro, and L. Guilhermino. 2018. Influence of microplastics on the toxicity of the pharmaceuticals procainamide and doxycycline on the marine microalgae Tetraselmis chuii. Aquat. Toxicol. 197: 143-152.
Sjollema, S.B., Redondo-Hasselerharm, P., Leslie, H.A., Kraak, M.H.S., Vethaak, A.D., 2016. Do plastic particles affect microalgal photosynthesis and growth? Aquat. Toxicol. 170, 259–261.
Song, Y.K., Hong, S.H., Jang, M., Han, G.M., Rani, M., Lee, J., Shim, W.J., 2015. A comparison of microscopic and spectroscopic identification methods for analysis of microplastics in environmental samples. Mar. Pollut. Bull. 93, 202–209.
Soreide, J.E., Leu, E., Berge, J., Graeve, M., Falk-Petersen, S., 2010. Timing of blooms, algal food quality and Calanus glacialis reproduction and growth in a changing Arctic. Glob. Change Biol. 16, 3154–3163.
Su, L., Xue, Y., Li, L., Yang, D., Kolandhasamy, P., Li, D., Shi, H., 2016. Microplastics in Taihu Lake. China. Environ. Pollut. 216, 711–719.
Tedesco, L., Vichi, M., Scoccimarro, E., 2019. Sea-ice algal phenology in a warmer Arctic. Sci. Adv. 5, eaav4830.
Underwood, G.J.C., Fietz, S., Papadimitriou, S., Thomas, D.N., Dieckmann, G.S., 2010. Distribution and composition of dissolved extracellular polymeric substances (EPS) in Antarctic sea ice. Mar. Ecol. Prog. Ser. 404, 1–19.
van Leeuwe, M.A., Tedesco, L., Arrigo, K.R., Assmy, P., Campbell, K., Meiners, K.M., Rintala, J.-M., Selz, V., Thomas, D.N., Stefels, J., 2018. Microalgal community structure and primary production in Arctic and Antarctic sea ice: a synthesis. Elem. Sci. Anth. 6, 2–25.
Waller, C.L., Griffiths, H.J., Waluda, C.M., Thorpe, S.E., Loaiza, I., Moreno, B., Pacherres, C.O., Hughes, K.A., 2017. Microplastics in the Antarctic marine system: An emerging area of research. Sci. Total Environ. 598, 220–227.
Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: a review. Mar. Pollut. Bull. 133, 336–348.
Zhang, C., Chen, X., Wang, J., Tan, L., 2017. Toxic effects of microplastic on marine microalgae Skeletonema costatum: Interactions between microplastic and algae. Environ. Pollut. 220, 1282–1288.