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Interactions between polystyrene microplastics and marine phytoplankton lead to species-specific hetero-aggregation☆

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ABSTRACT

To understand the fate and impacts of microplastics (MP) in the marine ecosystems, it is essential to investigate their interactions with phytoplankton as these may affect MP bioavailability to marine organisms as well as their fate in the water column. However, the behaviour of MP with marine phytoplanktonic cells remains little studied and thus unpredictable. The present study assessed the potential for phytoplankton cells to form hetero-aggregates with small micro-polystyrene (micro-PS) particles depending on microalgal species and physiological status. A prymnesiophycea, *Tisochrysis lutea,* a dinoflagellate, *Heterocapsa triquetra,* and a diatom, *Chaetoceros neogracile,* were exposed to micro-PS (2 μm diameter; 3.96 μg L^{-1}) during their growth culture cycles. Micro-PS were quantified using an innovative flow-cytometry approach, which allowed the monitoring of the micro-PS repartition in microalgal cultures and the distinction between free suspended micro-PS and hetero-aggregates of micro-PS and microalgae. Hetero-aggregation was observed for *C. neogracile* during the stationary growth phase. The highest levels of micro-PS were "lost" from solution, sticking to flasks, with *T. lutea* and *H. triquetra* cultures. This loss of micro-PS sticking to the flask walls increased with the age of the culture for both species. No effects of micro-PS were observed on microalgal physiology in terms of growth and chlorophyll fluorescence. Overall, these results highlight the potential for single phytoplankton cells and residual organic matter to interact with microplastics, and thus potentially influence their distribution and bioavailability in experimental systems and the water column.

1. Introduction

Microplastics (MP), defined as plastic particles below 5 mm diameter (Arthur et al., 2008), constitute an emerging threat in marine ecosystems due to the overall quantity of plastic debris entering the oceans every year. Indeed, Jambeck et al. (2015) estimated that 4.8 to 12.7 million metric tons of plastic waste entered the world's oceans in 2010, with a steady increase expected in the following years. In addition, the ubiquitous nature of these microparticles has been shown to lead to their accumulation in oceans over the last decades (Eriksen et al., 2014; Woodall et al., 2014). According to Van Sebille et al. (2015), up to 51.2 × 10^{12} MP particles are presently floating in marine environments worldwide.

Ingestion of MP by marine organisms, via direct uptake of free MP (Cole et al., 2011; Rochman et al., 2016; Sussarellu et al., 2016) or through consumption of contaminated preys (Farrell and Nelson, 2013), has been demonstrated in laboratory studies. Numerous field studies have also shown the presence of MP in fishes (Boerger et al., 2010; Lusher et al., 2013; Rummel et al., 2016; Sanchez et al., 2014), crustaceans (Murray and Cowie, 2011) and a wide range of filter feeders including bivalves (Van Cauwenberghe and Janssen, 2014), polychaetes (Van Cauwenberghe et al., 2015) and whales (Besseling et al., 2015). Such findings are of great concern, as physical and toxicological impacts have been observed both in cases of MP ingestion (for review see Rochman et al. (2016); Wright et al. (2013); for recent studies see Green (2016); Jemec et al. (2016); Paul-Pont et al. (2016); Sussarellu et al. (2016)) and food chain transfer arising from predator/prey interactions (Farrell and

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Nelson, 2013; Setälä et al., 2014). Nevertheless, it is still not clear how MP enter the marine food web. While free MP can be taken up directly by organisms (e.g. from mussels to crabs (Farrell and Nelson, 2013) or from beach hoppers to ray-finned fish (Tosetto et al., 2017)), they could also be ingested via vectors. For instance (Ward and Kach, 2009), experimentally demonstrated a facilitated transfer of nanoplastics (NP) to filter feeders through marine aggregates.

In the marine environment, MP can drift alone in the water column, but are also likely to interact with the surrounding marine plankton (Cole et al., 2016, 2013; Lagarde et al., 2016; Long et al., 2015; Setälä et al., 2014). For instance MP and NP can be efficiently incorporated in marine phytoplankton aggregates, which modify MP settlement rates in the water column (Long et al., 2015) and favour ingestion of NP by suspension-feeding bivalves (Ward and Kach, 2009). However, the behaviour of MP with phytoplanktonic aggregates and single cells has been little studied. The interactions of MP with phytoplankton have only been reported at very high concentrations, ranging from 25 mg to 2 g L^{-1}. Studies on freshwater microalgae demonstrated significant interactions and rapid formation of hetero-aggregates when microalgae were exposed to 20 nm nano-polystyrene at 80–800 mg L^{-1} (Bhattacharya et al., 2010) and 400–1000 μm MP (polypropylene and high-density polyethylene) at 1 g L^{-1} (Lagarde et al., 2016). The only report of aggregation with a marine species was observed between the diatom *Skeletonema costatum* and micro-polyvinyl chloride (micro-PVC at 50–2000 mg L^{-1}) (Zhang et al., 2017). Significant impact on growth rate was reported for the marine flagellate *Dunaliella tertiolecta* exposed to 250 mg L^{-1} of 0.05 μm micro-polystyrene (micro-PS) (Sjollema et al., 2016), but no hetero-aggregation was observed.

From recent literature (Maes et al., 2017; Sgier et al., 2016; Shim et al., 2016), tools based on fluorescence analysis, such as Flow cytometry (FCM), appear to be a relevant approach for rapid and robust detection and analysis of microplastics. FCM is a routine method that allows the analysis of various particle types within a flux. This technique enables the quantification and the characterization of particle parameters through measurements of light scattering and fluorescence (natural or following staining) after excitation by a laser beam. FCM has already been widely used for the study of microorganisms, including marine phytoplankton (Estrada et al., 2004; Lelong et al., 2011b; Pomati et al., 2011). FCM has the advantage of quickly and simultaneously analysing several parameters on large quantities of suspended cells. Moreover, FCM can be combined with several extensions such as camera or cell sorter, which allows the detection of microplastics (Sgier et al., 2016).

The aim of the present study is to investigate the potential for marine phytoplankton cells to aggregate with micro-PS depending on phytoplankton species and physiological state under experimental conditions. The present study focused on small MP (2-μm yellow-green fluorescent polystyrene microspheres; micro-PS). Polystyrene is one of the three most commonly used plastic polymers worldwide (alongside polyethylene and polypropylene), and is frequently found among microplastics sampled at sea (Barnes et al., 2009; Browne et al., 2010; Hidalgo-ruz et al., 2012). Three different marine phytoplankton species from different taxonomic groups were tested. The species were selected based on their predominance in marine phytoplankton communities and because of their common inclusion in bivalve diets (Dalsgaard et al., 2003; Malviya et al., 2016; Robert et al., 2004). The diatom *Chaetoceros neogracile* is a non-motile cell (width = 4 μm, length = 7 μm) encased in siliceous valves known as frustules covered with an organic coating (Hecky et al., 1973). The prymnesiophyceae *Tisochrysis lutea* is a small motile cell (width = 5 μm, length = 6 μm) covered by a dense layer of thin organic scales (Bendif et al., 2013). The dinoflagellate *Heterocapsa triquetra* is a motile cell (diameter = 16 μm, length = 23 μm) encased in a theca covered by an external plasmic membrane (Dodge and Crawford, 1970). The partitioning (aggregation and attachment to algae) of micro-PS was evaluated over an entire culture cycle, from seeding to stationary growth phase for each algal species. The potential toxic effects of MP on microalgal physiology was investigated through the analysis of growth rates and chlorophyll auto-fluorescence, which can be used as a proxy of photosynthetic efficiency (Lelong et al., 2011a). All analyses (cell and particle counts, formation of hetero-aggregates, algal viability and photosynthetic activity) were performed by flow cytometry.

2. Materials and methods

2.1. Algal culture

Two species of algae, *Chaetoceros neogracile* (strain CCAP 1010-3) and *Tisochrysis lutea* (strain CCAP 927/14 - Bendif et al., 2013) were obtained from the Scottish Marine Institute. The dinoflagellate *Heterocapsa triquetra* (strain HT99PZ - Ehrenberg, 1840) was isolated in the Penzé river (bay of Morlaix, France). Non-axenic cultures were grown in autoclaved F/2 medium (Guillard, 1975) made with filtered (0.22 μm) natural seawater. F/2 medium was enriched with silica (1.07 10^{-4} M) for the diatom *C. neogracile*. Cultures were performed in 250 mL glassware balloon flasks filled with 100 mL of medium maintained at 16 °C under a 12/12 h photoperiod with 92 ± 13 μmol photons m^{-2} s^{-1}.

2.2. Microplastic exposure

Pristine 2-μm polystyrene beads (micro-PS) (yellow-green fluorescent, density of 1.05 g mL^{-1}, smooth and uncharged, solution in deionised water containing 0.1% Tween 20) obtained from Polysciences Inc. were used in this experiment. Exposures were performed in glass flasks to minimize losses caused by plastic attraction to the flask walls. Micro-PS were added to the medium at the beginning of the experiment just after microalgal inoculation (9 10^5 micro-PS mL^{-1}, corresponding to 3.96 μg L^{-1}). It is noteworthy that the same experiment was also performed using a concentration of micro-PS ten times higher (9 10^6 micro-PS mL^{-1}, corresponding to 39.6 μg L^{-1}) than the concentration presented here. Data from this second experiment are not discussed in the paper as they give identical results, but they are available in supplementary tables 1 to 7. A good dispersion of the micro-PS in the culture media was confirmed in a preliminary experiment. Two sets of controls were set up: non-exposed microalgae cultured without addition of micro-PS, and micro-PS maintained in clean F/2 medium without any algae. Cultures and control flasks were set up in triplicate and were sampled 6 to 8 times from early exponential phase to stationary growth phase for flow cytometric and microscopic analyses. Micro-PS and microalgae were kept in suspension and homogenized by gently stirring the flasks every day and prior to sampling. Micro-PS detection and count were performed on fresh samples, while algal cell concentrations and chlorophyll fluorescence intensity were measured on fixed samples (glutaraldehyde, 0.3% final concentration), frozen in liquid nitrogen and stored at −80 °C. Growth rates (day^{-1}) were estimated according to the following equation:

$$\mu = \frac{\mathrm{Ln}(C_2/C_1)}{T_2 - T_1}$$