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Suspended micro-sized PVC particles impair the performance and decrease survival in the Asian green mussel *Perna viridis*

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ABSTRACT

Marine bivalves are known to ingest microplastics, but information on the consequences for their physiological performance is limited. To investigate a potential exposure pathway that has not yet been addressed, we mimicked the resuspension of microplastics from the sediment in a laboratory exposure experiment. For this, we exposed the Asian green mussel *Perna viridis* to 4 concentrations (0 mg/l, 21.6 mg/l, 216 mg/l, 2160 mg/l) of suspended polyvinylchloride (PVC) particles (1–50 μm) for two 2-hour-time-periods per day. After 44 days, mussel filtration and respiration rates as well as byssus production were found to be a negative function of particle concentration. Furthermore, within 91 days of exposure, mussel survival declined with increasing PVC abundance. These negative effects presumably go back to prolonged periods of valve closure as a reaction to particle presence. We suggest that microplastics constitute a new seston component that exerts a stress comparable to natural suspended solids.

1. Introduction

It was estimated that in 2010 a total of 4.8 to 12.7 million metric tons of plastics entered the oceans worldwide and the yearly input is predicted to increase by an order of magnitude until 2025 (Jambeck et al., 2015). Plastic particles are therefore accumulating in all parts of the oceans: at the sea surface, in the water column and in sediments (Moore et al., 2001; Thompson et al., 2004; Barnes, 2005). Their fragmentation at sea is the most important source of marine microplastics (i.e. plastic particles <5 mm) (Thompson et al., 2004; Claessens et al., 2011). In addition to this, micro-sized plastic particles enter the oceans as pre-production pellets, textile fibers and as scrubbers from cosmetics or from abrasives used in air-blasting machines (Gregory, 1996; Fendall and Sewell, 2009). Microplastics are widely distributed in the water column (Thompson et al., 2004; Lima et al., 2014; Song et al., 2014) as well as in sediments (Claessens et al., 2011; Vianello et al., 2013; Nor and Obbard, 2014) and have also reached pristine habitats like the deep sea (Van Cauwenberghe et al., 2013) and the Arctic ocean (Obbard et al., 2014).

Due to their small size, microplastics can be taken up by a wide range of animals. This includes large predators like fish (Davison and Asch, 2011), seabirds (Colabuono et al., 2009) and mammals (Besseling et al., 2015), but also benthic invertebrates (Thompson et al., 2004; Graham and Thompson, 2009, Goldstein and Goodwin, 2013, Watts et al., 2014) and even zooplankton (Cole et al., 2013; Besseling et al., 2014). However, since the majority of all plastic fragments will finally sink to the seafloor (Woodall et al., 2014), benthic invertebrates are presumably the group of organisms that is most exposed to microplastic pollution. This is particularly true for benthic filter feeders, such as mussels, which ingest organic material that is suspended in near-bottom waters. It originates either directly from vertical transport or from the wave- or current-induced resuspension of deposited material.

Several studies have documented the ingestion of microplastics by mussels and observed effects on their performance at the physiological, cellular and humoral level. Inflammatory responses followed by the accumulation of particles in the lysosomal system of the blue mussel *Mytilus edulis* occurred after the animals were exposed to polyethylene particles (1–80 μm) for a few hours (Von Moos et al., 2012). Furthermore, microplastics were found to be translocated to the cells of *M. edulis* 3 days after the ingestion of polystyrene beads (3 and 9.6 μm) (Browne et al., 2008) and reduced filtration rates were observed in individuals of *M. edulis* that were exposed to polystyrene particles (0.03 μm)

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for 8 h (Wegner et al., 2012). Many factors are assumed to influence the impact of microplastics on marine organisms, including particle size, shape, concentration, composition, weathering and erosional status as well as exposure time (Cole et al., 2011; Wright et al., 2013b), but the role of most of them remain to be investigated. Exposure time is of high ecological relevance, since marine invertebrates in many habitats most likely face microplastic pollution throughout their entire lifespan, which can reach from <1 year to >10 years, and this fact limits the interpretability of short-term tests. However, so far only one study on marine bivalves realized an exposure time that was longer than a few days: Sussarellu et al. (2016) exposed oysters to polystyrene microspheres (2 and 6 μm) for 2 months to cover a whole reproductive cycle and after this time they found that reproduction was impaired as they observed decreased sperm velocity, oocyte number and diameter as well as lower larval yield and growth. This was caused by a shift in energy allocation from reproduction to structural growth.

Although many of these experiments clearly documented negative effects of microplastics on animal performance, their interpretation is limited. This is because all but one were run for timespans that were by far shorter than the exposure times marine invertebrates very likely experience in polluted environments. However, exposure scenarios, which are realistic with regard to the length of exposure as well as to the concentration of particles, are central for assessing the ecological consequences of microplastic pollution. Therefore, more studies that cover the time scale of months to even years are currently needed to come to a comprehensive understanding of what the consequences of the enrichment of microplastics in marine systems can be.

A further aspect of microplastic pollution is the interaction of these materials with organic pollutants. In the marine environment plastic particles accumulate and transport persistent organic pollutants (POPs) like polychlorinates biphenyls (PCBs) or polycyclic aromatic hydrocarbons (PAHs) (Rios et al., 2007) and it is widely assumed that by this they can serve as a vector for pollutants to organisms (Teuten et al., 2007). The microplastics-mediated transfer and subsequent accumulation of POPs in tissues of *Arenicola marina* have already been documented for 19 different polychlorinated biphenyls (PCBs) (Besseling et al., 2013). Furthermore, increased genotoxic effects were observed in *Mytilus galloprovincialis* as a response to a 7-day exposure to pyrene-contaminated polyethylene and polystyrene (<100 μm) in comparison to virgin plastic. Although the authors found an accumulation of pyrene in the tissue, the majority of the observed biological responses (immunological, lysosomal, cholinesterasic and antioxidant effects) were particle-induced and not influenced by the pollutant (Avio et al., 2015). These studies show that a microplastics-mediated transfer of POPs is possible, but it is still controversially debated whether this actually represent an ecologically relevant pathway for organic pollutants to marine organisms (Koelmans et al., 2013; Ziccardi et al., 2016).

In a laboratory study, we simulated the resuspension of microplastics from coastal sediments and implemented different pollution levels. The particle loads covered several orders of magnitude to determine the threshold concentration that leads to a measurable impairment in physiological performance. Respiration and clearance rates were measured because they have widely been recognized as sensitive and reliable measures of stress in mussels (Chandurvelan et al., 2013; Zhao et al., 2014). Furthermore, we recorded byssus production as a response variable to assess physiological activity and to detect the impact of unfavorable conditions that may trigger stress responses that, in turn, consume energy which is then not available for the formation of byssus threads (Paul, 1980; Wang et al., 2012). Since microplastics can constitute a stress for mussels, we hypothesized the physiological performance to decrease with increasing amounts of microplastics. We used PVC particles (1–50 μm) that were contaminated with an organic pollutant to mimic the situation in the field. The influence of microplastics on the performance of the Asian green mussel *Perna viridis* was then assessed over the course of 91 days.

2. Methods

2.1. Study site and mussel collection

Individuals of the Asian green mussel *Perna viridis* used in this study were collected in Jakarta Bay, which is under the influence of various anthropogenic impacts. The waters of the bay are eutrophic and polluted with heavy metals and organic pollutants (Damar, 2003; Arifin, 2004). Concentrations of PAHs in Jakarta Bay sediments were found to range between 257 and 1511 ng/g due to petrogenic pollutants that presumably originate from the use of fossil fuels (Rinawati et al., 2012), while a constant import of suspended particulate matter into the bay leads to enhanced sedimentation rates (Arifin, 2004). Furthermore, water turbidity is high due to the complete mixing of the water column during each tidal change as Jakarta Bay is a shallow sea area with a mean depth of 8.6 m (Damar, 2003).

We collected mussel individuals with a shell length of 3.5–4.0 cm from bamboo constructions near Muara Kamal (6° 4′ S, 106° 43′ E). The racks were deployed by fishermen as a settlement substratum for the bivalves, which represent an important protein source for the coastal population of the region (Arifin, 2004). During the 2 h transport to the marine laboratory in Bogor, we kept the mussels in cooled insulation boxes without seawater. After arrival, we randomly formed 6 groups of 30 mussels each, which all had the same shell length. Each group was transferred into a glass aquarium with 20 l of seawater and provided constant aeration. For the experiment, 2000 l of clean seawater were transported from the coast to the laboratory in Bogor, where they were distributed to two separate water cycles, of which both contained a storage tank and a filter unit. Mussel acclimatization to laboratory conditions lasted for 2 weeks, during which half of the water in the aquaria was exchanged daily and the mussels were fed with one million cells of an *Isochrysis galbana* culture per aquarium twice per day.

Prior to the exposure experiment, we tested whether *P. viridis* takes up the PVC particles we chose for this study (see below). Ingestion of particles was confirmed by microscopic inspection of the faeces. Animals that were used in this pilot study were not included in the main experiment.

2.2. Exposure to microplastic particles

2.2.1. Plastic material

We chose PVC particles due to their negative buoyancy in seawater, which makes them available for benthic invertebrates even if they are devoid of fouling. Furthermore, PVC makes up 19% of the global plastic production and is therefore a common component of marine microplastic waste (Andrady, 2011; Browne et al., 2010; Nor and Obbard, 2014). We purchased the material in a size range of 1–50 μm from PyroPowders (www.pyropowders.de).

Prior to the experiment, the PVC material was submersed in fluoranthene-contaminated seawater for 24 days. This was done to mimic the conditions in the Bay of Jakarta, where plastic material presumably gets quickly contaminated due to the omnipresence of PAHs of which fluoranthene is a common component (Rinawati et al., 2012). Fluoranthene is a PAH that is released through the combustion of fossil fuels and has already been detected on microplastic particles in the ocean (Rios et al., 2007). For contaminating the particles in the laboratory, 100 g of PVC were mixed into 500 ml of seawater that contained 2 μg fluoranthene per l. We chose this concentration as a rather high but still ecologically relevant level since similar amounts of PAHs can occur in seawater close to oil spills and petroleum installations (Jensen et al., 2012; Neff et al., 2006; Reddy and Quinn, 1999). As fluoranthene is a hydrophobic solid, it was first dissolved in acetone and then aliquots of a stock solution with 100 μg fluoranthene per 1 ml of acetone were mixed into the seawater. This water came from Jakarta Bay and therefore surely contained organic material, including bacteria and pollutants, which are likely to have interacted with the PVC particles as