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Aging of microplastics promotes their ingestion by marine zooplankton☆

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ABSTRACT

Microplastics (<5 mm) are ubiquitous in the marine environment and are ingested by zooplankton with possible negative effects on survival, feeding, and fecundity. The majority of laboratory studies has used new and pristine microplastics to test their impacts, while aging processes such as weathering and biofouling alter the characteristics of plastic particles in the marine environment. We investigated zooplankton ingestion of polystyrene beads (15 and 30 μm) and fragments (≤30 μm), and tested the hypothesis that microplastics previously exposed to marine conditions (aged) are ingested at higher rates than pristine microplastics. Polystyrene beads were aged by soaking in natural local seawater for three weeks. Three zooplankton taxa ingested microplastics, excluding the copepod *Pseudocalanus* spp., but the proportions of individuals ingesting plastic and the number of particles ingested were taxon and life stage specific and dependent on plastic size. All stages of *Calanus finmarchicus* ingested polystyrene fragments. Aged microbeads were preferred over pristine ones by females of *Acartia longiremis* as well as juvenile copepodites CV and adults of *Calanus finmarchicus*. The preference for aged microplastics may be attributed to the formation of a biofilm. Such a coating, made up of natural microbes, may contain similar prey as the copepods feed on in the water column and secrete chemical exudates that aid chemo-detection and thus increase the attractiveness of the particles as food items. Much of the ingested plastic was, however, egested within a short time period (2–4 h) and the survival of adult *Calanus* females was not affected in an 11-day exposure. Negative effects of microplastics ingestion were thus limited. Our findings emphasize, however, that aging plays an important role in the transformation of microplastics at sea and ingestion by grazers, and should thus be considered in future microplastics ingestion studies and estimates of microplastics transfer into the marine food web.

1. Introduction

Microplastics, <5 mm in size, are omnipresent in marine environments (Andrady, 2011) and due to their minuteness, can be readily bioavailable to a range of aquatic organisms, and marine zooplankton in particular (Desforges et al., 2015). In situ, microplastics have been found inside filter-feeders such as copepods and euphausiids (Desforges et al., 2015), and planktivorous fishes (Boerger et al., 2010; Lusher et al., 2013; Bråte et al., 2016), demonstrating the potential for both direct feeding on microplastics as well as transfer up the food chain and possible biomagnification at the base of the marine food web. As grazers on primary producers (mainly microalgae), zooplankton represent an important link in energy transfer to higher trophic levels, including commercially important fish species. As zooplankton target prey in a size range similar to that of microplastics, it is important to study their ingestion of microplastics and potential adverse effects on the resilience of zooplankton communities. In the laboratory, ingestion of microplastics by copepods had been initially confirmed in early feeding studies of the 1970s and 80s (Ayukai, 1987; Wilson, 1973) and has recently been revisited in the context of plastic pollution for a variety of zooplankton taxa (Cole et al., 2013; Setälä et al., 2014). Whether zooplankton ingest microplastics or not depends on the size and feeding strategy of the organism and its preferred prey size range versus the microplastics size and shape encountered (Cole et al., 2013; Setälä et al., 2014). In the present study, we

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focus on the marine copepods *Calanus finmarchicus* and *Acartia longiremis*: planktonic filter feeders between 1 and 4 mm in length, depending on species and stage. Copepods, abundant zooplankton in all world oceans, can ingest microplastics in the micro size range (<1 mm) (Cole et al., 2013; Setälä et al., 2014; Lee et al., 2013), which in turn may impact their health and/or that of higher trophic levels in their food chain. High concentrations of microplastics (several thousand per mL) negatively affected feeding rates of the copepod *Centropages typicus* (Cole et al., 2013) and survival and fecundity of *Tigriopus japonicus* (Lee et al., 2013). At a lower concentration (75 particles mL^{-1}), Cole et al. (2015) found energetic depletion and reduced reproductive output in the copepod *Calanus helgolandicus* feeding on 20 μm polystyrene (PS) beads. They concluded that microplastics competed with food items for ingestion and that the copepods did not select against non-nutritious particles. Food selectivity is, however, a trait that has been described for zooplankton and for calanoid copepods in particular (Leising et al., 2005). The level of selectivity appears to be dependent on both the physiology of the grazer ('hunger') and the abundance and properties of prey items, such as toxicity (Cowles et al., 1988; Kleppel, 1993). Surface characteristics play an essential role in particle detection, as many species are able to discriminate between inert and edible particles (DeMott, 1988a). Most laboratory studies on microplastics ingestion used laboratory grade microbeads, which are in a "pristine" state. This implies a smooth, sterile surface, and suspension in an anti-microbial agent. Microplastics in the marine environment, on the other hand, are exposed to seawater for extended periods of time. Due to this exposure, breakdown of plastic items takes place, creating irregularly shaped microplastics fragments. Consequently, fibres and fragments are found more frequently in the world's oceans than beads (Moore et al., 2001; Lusher et al., 2014; Thompson et al., 2004). Shape may influence microplastic ingestion as well as effects (Lambert et al., 2017): in the amphipod *Gammarus fossarum* fibres significantly reduced assimilation efficiency, whereas this effect was not found using beads (Blarer and Burkhardt-holm, 2016). On top of this, sea water exposure results in microplastic aging, altering particle surface characteristics from smooth and regular to brittle with cracks and rifts (Andrady, 2011). Furthermore, as plastic surfaces are hydrophobic, organic molecules will adsorb (Lobelle and Cunliffe, 2011). Together with the brittle, uneven surface, this creates a matrix to which microbes can attach, a process known as biofouling, or biofilm formation (Zettler et al., 2013). Adsorption of organic molecules occurs rapidly, within hours after microplastics enter the seawater, followed by colonization of microbes (Oberbeckmann et al., 2015). The first discovery of microbe-colonized microplastics in the marine environment dates back to samples collected in the 1970's in the Sargasso Sea (Carpenter and Smith, 1972). More recent findings report biofouled microplastics in a range of marine environments, such as the North Pacific Gyre and the North Atlantic ocean (Zettler et al., 2013; Oberbeckmann et al., 2015; Carson et al., 2013). The biofilms on these plastics contain a variety of organism groups, including diatoms and dinoflagellates (Oberbeckmann et al., 2015) and form diverse communities, which represent a nutritious coating around the - otherwise indigestible – microplastics. Microplastics may thus become attractive food items to grazing zooplankton, such as copepods. Consequently, biofilms may alter the interactions between microplastics and planktonic grazers, increasing ingestion rates of microplastics (Harrison et al., 2011). Many planktonic taxa, including calanoid copepods, possess chemo- and mechanoreceptors for prey detection, which assist in the decision to ingest or reject a given particle (Kleppel, 1993). Suggested mechanisms behind this are chemical cues emitted by particles (Poulet and Marsot, 1978) as well as their surface electrical charge and wettability (Gerritsen and Porter, 1982). The influence of surface characteristics on microplastics ingestion has been studied for specific artificial coatings, for instance using microbeads inoculated with a marine bacterium (Powell and Berry, 1990), or soaked in algae (DeMott, 1988b). However, whether aging in natural sea water has any influence on microplastics ingestion has not been investigated to date. Lastly, the fate of microplastics post-ingestion remains unclear. While in some species microplastics can be retained in the gut for days (Cole et al., 2013) or translocate within the body if small enough (Browne et al., 2008), in others they are egested within hours (Cole et al., 2013; Kaposi et al., 2014). Data on egestion and accumulation of microplastics in prey organisms are crucial to assess the risks of bioaccumulation and trophic transfer through biomagnification.

Accordingly, we postulate that several factors pertaining to microplastics ingestion require more detailed investigation: 1. the role of particle size and shape, 2. the importance of microplastics aging (due to weathering and biofilm formation), 3. potential differences in the disposition to ingest microplastics between life stages of a given grazer species, and 4. differences in gut passage times between beads and fragments. We exposed zooplankton to PS particles of two different sizes (15 and 30 μm diameter) and shapes (beads and fragments) and studied their ingestion, egestion, accumulation and impact on grazer survival. We further investigated the role of plastic aging for ingestion in *Calanus finmarchicus* and *Acartia longiremis*, two abundant copepod species in the northern Atlantic Ocean, including differences between males, females and juvenile copepodite stages CV.

2. Materials and methods

2.1. Zooplankton collection

Zooplankton samples were taken on board of RV Hyas on May 19th and June 23rd, 2015 in the Norwegian Sea at Håkøybotn, Norway (69° 40′N, 18° 46′E). Vertical hauls were conducted with a plankton net (mesh size 180 μm) at a speed of 0.2 m s^{-1} from a depth of 35–45 m and contained mostly *Acartia longiremis*, *Pseudocalanus* spp. and decapod larvae (indet.). An additional sample was taken on June 26th in a deep water location (>100 m) in Balsfjord, Norway (69° 18′N 19° 12′E) to target *Calanus finmarchicus*. Plankton was kept in the laboratory at ambient temperature in 20 L plastic buckets provided with gentle aeration for up to a week until sorting with a dissecting stereoscope. Individuals of the same species or taxon were placed in new aerated buckets containing filtered seawater (60 μm) for up to three weeks prior to experiments. The plastic material of the buckets used is considered inert, and consumption of microplastics from bucket material due to abrasion is unlikely. No plastic particles other than fluorescently labelled beads used for experimental exposures (see below) have been observed in the experiments. Zooplankton was fed once a week with algae (Reed Mariculture, Shellfish Diet, 1800®) at 5000 cells mL^{-1}. The water in buckets was replaced regularly and dead zooplankton was removed. Only healthy-looking (intact and active) individuals were selected for use in experiments.

2.2. Seawater preparation

Seawater used prior to and during bioassays was supplied by the facility's tap system. Water was pumped from the fjord adjacent to the lab, mixing water from a layer at 60 m depth with surface water. This was subsequently filtered over a 60 μm filter and a 1 μm filter, to remove larger plankton and debris. Filtered seawater used in experiments is 1 μm filtered unless stated otherwise. Temperature of the incoming water was monitored daily and was on average 6.4 °C during our study.