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Ingestion and fragmentation of plastic carrier bags by the amphipod *Orchestia gammarellus*: effects of plastic type and fouling load

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Abstract: Inappropriate disposal of plastic debris has led to the contamination of marine habitats worldwide. This debris can be ingested by organisms; however, the extent to which chewing and gut transit modifies plastic debris is unclear. Detritivores, such as amphipods, ingest and shred natural organic matter and are fundamental to its breakdown. Here we examine ingestion and shredding of plastic carrier bags by *Orchestia gammarellus*. A laboratory experiment showed these amphipods shredded plastic carrier bags, generating numerous microplastic fragments (average diameter 488.59μm). The presence of a biofilm significantly increased the amount of shredding, but plastic type (conventional, degradable and biodegradable) had no effect. Subsequent field observations confirmed similar shredding occurred on the strandline. Rates of shredding will vary according to amphipod density; however, our data indicates that shredding by organisms could substantially accelerate the formation microplastics in the environment.

Keywords: Microplastic; Biofouling; Single-use carrier-bags; Polyethylene; Litter; Polymers.

1. Introduction

Plastic debris contaminates marine habitats worldwide (Barnes et al., 2009) and is now considered as a major environmental concern (Worm et al., 2017). Plastics are synthetic organic polymers mainly derived from oil or gas and typically incorporate a range of additive chemicals which increase functionality. Plastics are strong, durable, lightweight (For reviews see: Andrady and Neal, 2009; Thompson et al., 2009), and are inexpensive, making plastic an ideal material to produce a large array of products. However, these properties also lead to the accumulation and persistence of plastics in marine habitats creating considerable environmental challenges (Derraik, 2002; Cole et al., 2011).

Plastic debris is often divided into three size categories: Macro (>5mm), Micro (<5mm) (Barnes et al., 2009) and nano-sizes plastics (<100nm) (for reviews see Bergami et al., 2016; Gigault et al., 2016). There are numerous routes in which plastics can enter the marine environment originating from both marine and terrestrial sources (For review see: Auta et al., 2017). Microplastics can be directly emitted as small particles (primary sources), for example from industrial usage and from items such as cosmetics (e.g. Fendall and Sewell 2009; Napper et al., 2015). They can also originate from the fragmentation of larger plastic items (secondary sources) already present in the marine environment (Hidalgo-Ruz et al., 2012).

Because of their small size microplastics are potentially available via ingestion to a wider range of organisms than larger items of debris as they fall into the prey size range of many marine species (For review see: Galloway et al., 2017). Microplastics have been reported in a range of different habitats including the deep sea (Woodall et al., 2014) and arctic sea ice (Obbard et al., 2014) and their abundance is believed to be increasing (Law and Thompson, 2014). Their ingestion has been reported in 233 marine species (For review see: Law et al., 2017) by a diverse array of taxa including vertebrates (e.g. Simmonds, 2012) and invertebrates such as echinoderms (e.g. Graham and Thompson, 2009), arthropods, including the amphipods *O. gammarellus* and *Talitrus saltator* (e.g. Thompson et al., 2004; Ugolini et al., 2013), molluscs (e.g. Browne et al., 2008) and zooplankton (e.g. Cole et al., 2013; Desforges et al., 2015).

Ingested plastic has been reported to cause lethal and sub-lethal effects to marine organisms (Gall and Thompson, 2015). These may include direct physical effects, (Pierce et al., 2004; Kastelein and Lavaleije, 1992), compromised physiological performance (Wright et al., 2013; Cole et al., 2015) and indirect effects associated with the transfer of chemicals such as PCBs, DDT (Teuten et al., 2009; Rochman et al., 2013; Chua et al., 2014), decreased stomach volume due to the presence of plastic (Ryan, 1988) and reduced feeding rate (Welden and Cowie, 2016). As a result of plastic ingestion, an organism may also be more susceptible to stress and disease (Laist, 1987). Plastic debris can also impact at an assemblage level. Plastic carrier bags can create anoxic conditions within sediments reducing primary productivity,

organic matter and significantly reduce the abundance of infaunal invertebrates and the ecosystem services they provide (Green et al., 2015).

Over time plastic in the marine environment will become colonised by micro- and macro- marine organisms (Lobelle and Cunliffe, 2011); a process described as fouling, which can affect plastics in numerous ways. Firstly, a biofilm may 'shield' the plastic from UV light (O'Brine and Thompson, 2010) and since exposure to UV enhances degradation fouling will likely reduce degradation rates. Fouling can also make plastics negatively buoyant causing buoyant items to sink (Fazey and Ryan, 2016). It is also possible that the fouling of plastic could alter the palatability of plastics, thus increasing ingestion by marine organisms.

The amphipod, *Orchestia gammarellus* (Pallas, 1766), is a detritivore that inhabits strandline habitats across Northwest Europe (Hayward and Ryland, 2017) and its diet includes plant detritus, decomposing organic matter, bacteria and diatoms (Créach et al., 1997). Amphipods play an important role in the breakdown of organic matter on the shoreline (Griffiths et al., 1983; Lastra et al., 2008) and in the organization of benthic marine communities in temperate regions worldwide (Duffy and Hay, 2000). Estimates suggest that up to 70% of plastic debris settles onto the benthos (Hammer et al., 2012) with significant accumulations in intertidal habitats worldwide (Barnes et al., 2009). Therefore, detritivores such as amphipods are likely to regularly come into contact with plastic debris. Hence there is clear ecological relevance to assessing ingestion of plastic debris by detritivores such as *O. gammarellus.* Early evidence of the interaction between amphipods and man-made materials was reported by Bate 1862; "*A lady's handkerchief which was dropped for a few minutes was perceived, upon being recovered, to be perforated by myriads of small holes, the work of these creatures… and in their turn these became food for birds, which devoured them greedily*" (Bate, 1862). Plastic ingestion could have deleterious effects on an organism health such as reduced growth rate and reproductive success (Au et al., 2015; Talley et al., 2015) however the role of ingestion in the formation of microplastics has yet to be examined.

Plastic bags are common items of marine debris, especially in intertidal and subtidal sediments (Green et al., 2015) and therefore have a high potential for interactions with a range of marine organisms. The aim of this investigation was to determine whether *O. gammarellus* can ingest and shred plastic carrier bags leading to the formation of microplastics and, if so, whether this was influenced by plastic type or fouling load.

2. Materials and Methods

2.1. Fouling of the plastics

Replicate 22 cm x 5 cm plastic samples were cut from carrier bags made of: 1) high density polyethylene (HDPE) produced from 15 % recycled material, 2) a plastic described as 'degradable' (d_2w) and 3) a plastic described as

‘biodegradable/compostable’. These samples were deployed at a depth of approximately 1.5m for 4 weeks during the summer of 2012 (July-August) at Queen Anne’s Battery’s, Plymouth, UK (50°36′58″N 4°12′63″W) in order to acquire a microbial biofilm. Once retrieved the samples were air-dried at 20 °C to simulate drying along the strandline in the intertidal.

2.2. Orchestia gammarellus plastic ingestion experiment.

Amphipods, *O. gammarellus*, were collected from the upper shore at Devil’s Point, Plymouth, UK (50°21′37″N 04°09′48″W). Single individuals were placed into 3.8 x 10 cm mesh bottomed tubes. The mesh allowed for faecal matter to fall through and collect beneath prior to analysis analysed. Amphipods of a similar size (average weight of 0.052g) were chosen throughout to ensure similarity across treatments.

Ten 1 cm^2 samples were cut from each of the three plastic types either with or without fouling (six treatments n= 10 for each). A razor blade was used to ensure straight edges allowing any signs of ingestion to be readily visualised and quantified. Each plastic sample was exposed to one amphipod in a tube for 7 days. Since the main objective of the laboratory experiment was to establish whether amphipods could shred the plastic and if so whether this was influenced by fouling or plastic type no other food source was made available during this period. All tubes were kept moist at 15 °C throughout. Control plastic samples (n= 60) were kept under identical conditions but were not exposed to *O. gammarellus.* After 7 days, the plastic samples were removed and dried. The dry weight of the amphipods was also determined.

Before and after exposure to *O. gammarellus,* the plastic samples were photographed using a light microscope and surface area was measured using ImageJ (plate 1 a - f). A scanning electron microscope was also used to provide detailed images of the edges of plastic that had been chewed and to allow comparison to controls (Plate 1, g).

2.3. Examination of plastics from faecal matter

The faecal material from amphipods that had ingested $\geq$ 1 mm^2 of plastic was examined for plastic fragments under an optical microscope. The fragments found in these samples were photographed (n= 522) and maximum length was measured using ImageJ. Average size data were used to estimate the number of fragments formed per individual per day. A scanning electron microscope was used to provide detailed images (Plate 1, h).

2.4. Data Analysis

The amount of plastic lost during exposure to the amphipods was calculated. Data were transformed when Levene’s test indicated variances were heterogeneous and analysed using a two-way ANOVA with plastic type and fouling both considered as fixed factors. Control plastic samples were analysed for loss using a Mann-Whitney U test for difference. Any differences in the mass of the amphipods across the

treatments were also assessed. The data were analysed using the statistical package MINITAB 16.

2.5 Field Trial

Field observations were conducted to confirm whether the shredding observed in the laboratory also occurred under field conditions where natural food sources were available to the amphipods. Biodegradable and non-degradable carrier bags attached to frames were deployed at Coxside Marina, Plymouth, UK to acquire a bio-fouling layer. After 7 weeks' immersion in seawater, ten 2 x 5cm^2 samples of the fouled biodegradable and non-degradable plastic carrier bags were exposed to amphipods on the supralittoral strandline at Mount Batten Beach, Mount Batten, Plymouth, SW England (50º21'24. 86" N, 4º07'33.19" W). *Orchestia mediterranea* and *O. gammarellus* are abundant at this shore level (densities up to 12 individuals/100 cm^2). The plastics were positioned under and attached to rocks with cotton twine to prevent them being relocated. Samples were recovered from the shore after 24 days and examined for bite-marks

3. Results

The laboratory control samples, i.e. without amphipods, showed no plastic loss. Therefore, any plastic lost from samples in the other treatments was assumed to result from ingestion and shredding. There were no significant differences in the amphipod dry weights across the treatments, therefore it can be deduced that amphipod size did not affect the amount of plastic ingested across the treatments.

Two-way ANOVA showed the presence of a fouling load significantly increased the amount of plastic lost from the samples ($F_{1,54}$ = 21.00; p = <0.001; Figure 1; plate 1, a - f). However, plastic type had no significant effect on amount of plastic eaten ($F_{2,54}$ = 0.76; p = 0.42; Figure 1; plate 1 a - f). There was no significant interaction between fouling load and plastic type. An average 8.23 fragments were generated / amphipod/ day across all fouled treatments compared to 2.04 fragments / amphipod/ day across all the un-fouled treatments.

Plastic pieces were not found within any the solid amphipod faecal matter; therefore, the liquid in which the faeces were present at the bottom of the containers was also examined. These contained substantial quantities of plastic fragments with an average diameter of 488.59 µm (range 86 - 1351 µm, Figure 2). Scanning electron microscope images showed the fragments had signs of stretching, which may have been caused by shredding and/ or ingestion (Plate 1, h). The plastic samples from the field trial also showed signs of ingestion with "bite marks" of similar shape and size to that observed on the plastics in the laboratory trial.

4. Discussion

Shredding of plastic bags by *O. gammarellus* was present across all treatments in the

laboratory trial. Significantly greater amounts of fouled plastic were shredded compared to the non-fouled plastic by approximately four-fold, resulting in an average of 8.23 fragments per amphipod per day. This would appear to indicate a greater preference for fouled plastics. Evidently, a variety of factors lead to the formation of microplastics in the marine environment including chemical, biological and mechanical actions (Phuong et al., 2016). However, within a hypothetical scenario of shredding alone, our data suggests that an entire plastic carrier bag could generate approximately 1.75 million microplastic pieces as a consequence of shredding by *O. gammarellus*.

Scanning electron microscope images showed the presence of 'bite-marks' created by *O. gammarellus*. These images revealed long scars across the surface of the samples, potentially created by the organisms' mouthparts while feeding on micro-epiphytes (Plate 1, g). It appears that the action of the amphipods mouthparts caused the plastic to stretch and tear away from the edges of the samples. The microplastics generated also showed signs of stretching and distortion (Plate 1, h). Greater quantities of fouled plastic were ingested, indicating that *O. gammarellus* may be attracted by the presence of a biofilm which could be acting as a feeding cue. Similar findings have recently been shown for seabirds (Savoca et al., 2016). Other marine organisms may be predisposed to ingest plastic debris that has become fouled therefore we suggest that future research should examine feeding cues associated with plastic ingestion. Ingestion in the non-fouled treatments may have resulted because no other substrate was available.

Microplastic fragments were found with the faecal matter, although a limited quantity of complete faecal pellets was found. It is possible that the fragments were released during degradation of the faecal pellets into the surrounding container before examination. However, since the fragments were not exclusively found in the faecal material itself it is not clear whether the plastics were chewed and rejected or chewed and ingested. Earlier work by Thompson et al., (2004) demonstrated plastic can be ingested by *O. gammarellus* in laboratory conditions (Thompson et al., 2004) and we suspect here that a combination of shredding followed by ingestion and shredding followed by rejection is most likely. Further work would be needed to clarify this.

Evidence of similar shredding was observed in the field trial confirming fragmentation of plastic bags by amphipods or similar detritivores can occur in the natural environment even where natural food sources are also available. Densities of up to 12 *O. gammarellus* individuals/ 100 cm^2 were estimated at our field study site, which is similar to densities reported at other locations. Future studies could investigate plastic ingestion by other strandline fauna to establish a wider picture of the fate of plastics on the shore.

O. gammarellus is a detritivore which dominates supralittoral strandlines across Northwest Europe (Hayward and Ryland, 2017). The fragmentation of plastics by detritivores could, therefore, have a substantial effect in increasing the breakdown of

plastic debris in the marine environment, increasing the number of fragments present and the speed at which macro-plastics breakdown into smaller pieces. This fragmentation therefore increases the potential for a wider range of organisms to ingest the plastic debris.

An important next step is to determine the amount of microplastic ingested by these detritivores. Ingestion of plastic debris has been shown to have deleterious effects on an organism's health. Ingested plastics have the potential to accumulate in the gut of an organism (Browne et al., 2008; Murray and Cowie, 2011; Gall and Thompson 2015) and has been shown to reduce the ability of sediment-dwelling polychaete worms to store energy, and induce metabolically demanding inflammatory responses (Wright et al., 2013). The accumulation of plastic in the gut of organisms can also lead to blockages and nutrient dilution, in which nutritious food is replaced by less nutritious foods (McCauley and Bjorndal, 1999). Cruz-Rivera & Hay, (2000) found that the amphipod *E. levis* fed on low nutritional diets experienced reduced growth, fecundity and survivorship (Cruz-Rivera and Hay, 2000).

Plastic debris can accumulate potentially harmful chemicals, such as DDT, PCBs (Rios et al., 2007) and trace metals (Holmes et al., 2012) from seawater (Teuten et al., 2009; Mato et al., 2001). Plastics items can also contain additives such as plasticisers, flame retardants and antimicrobials agents. These can be present in high concentrations and are potentially toxic. The shredding of plastics by organisms may therefore facilitate the transfer of chemical additives to biota (Rochman et al., 2013). The potential for toxicological effects is not clear since plastics may release these chemicals prior to ingestion. Our experiments indicate that shredding of larger items of plastic by biota could lead to relatively rapid formation of microplastics, and may therefore increase the availability of newly generated microplastics to a wider range of organisms.

Our study also demonstrates the relevance of further work to examine the influence of plastic type and thickness on ingestion in order to establish whether other common types of debris such as bottles, packaging, rope and netting are also ingested and shredded by biota. It would also be useful to establish the generality of these findings with regard to other detritivores and scavengers, both marine and terrestrial. For example, anecdotal reports describe the fatal impacts of plastic ingestion by much larger organisms including camels and cows (Al Arabiya News, 2008).

5. Conclusion

Plastic carrier bags made of HDPE, degradable and biodegradable material were shredded by *O. gammarellus* in the laboratory with significantly greater ingestion/ shredding of fouled plastics compared to non-fouled plastics. There was also clear evidence of similar shredding of plastic bags on the shoreline. Our results indicate the shredding of larger plastic items by detritivores generates microplastics. A variety of other organisms also have the potential to shred plastic, thus when considering likely

rates of environmental degradation, it is important to consider biological factors alongside physical and chemical degradation.

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Figure 1:

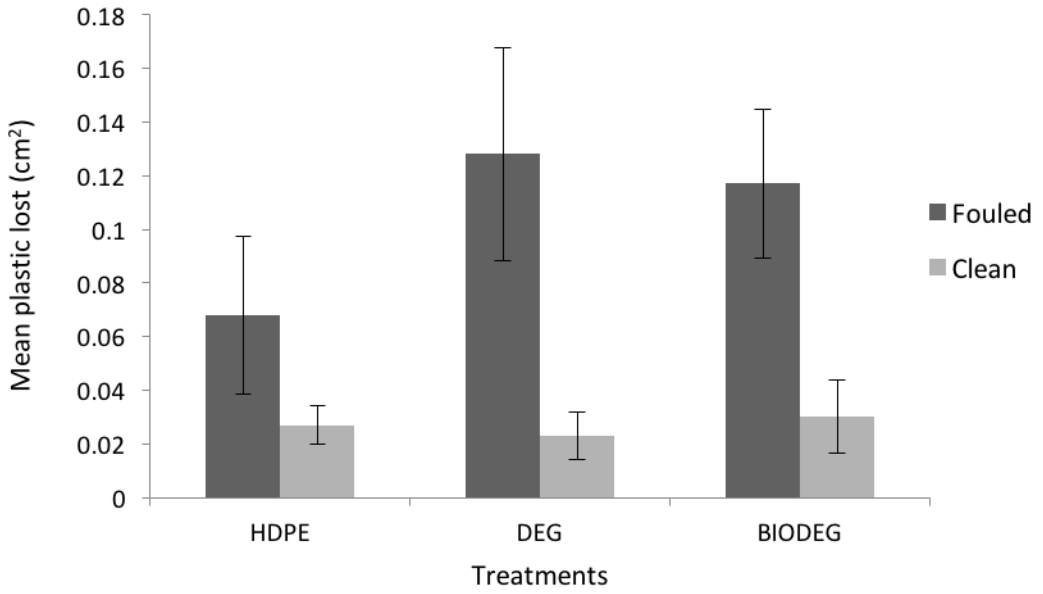

Fig. 1: Amount of plastic sample area lost (cm^2) during 7-day laboratory exposure of *O. gammarellus* across the treatments (HPDE= High Density Polyethylene/ DEG= Degradable/ BIODEG= Biodegradable)

Figure 2:

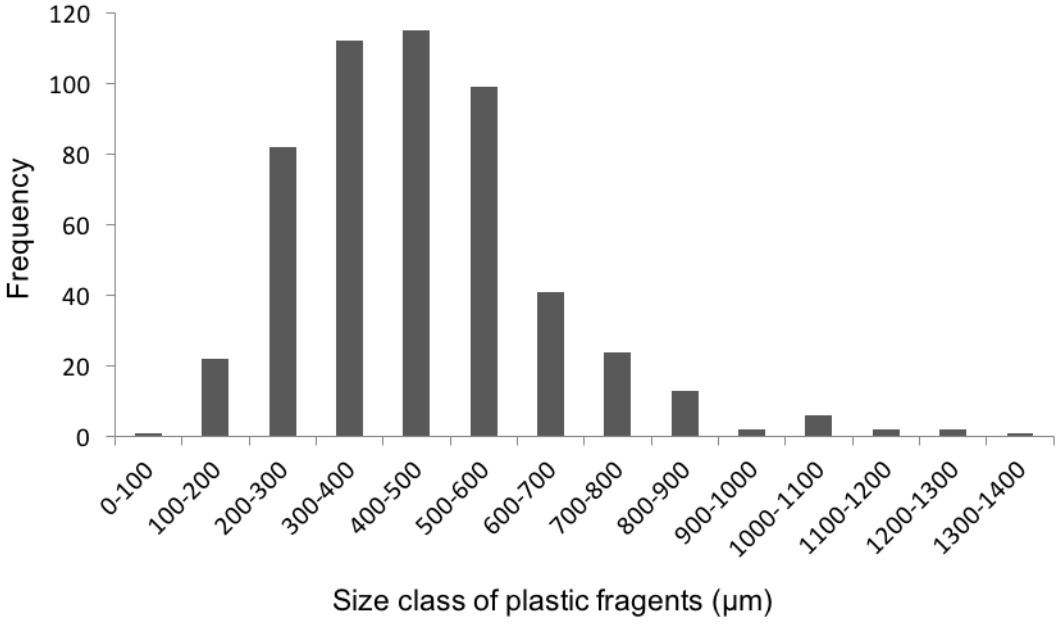

Fig 2: Size range and frequency of plastic fragments created by *O. gammarellus* during the 7-day laboratory exposure.

Plate 1:

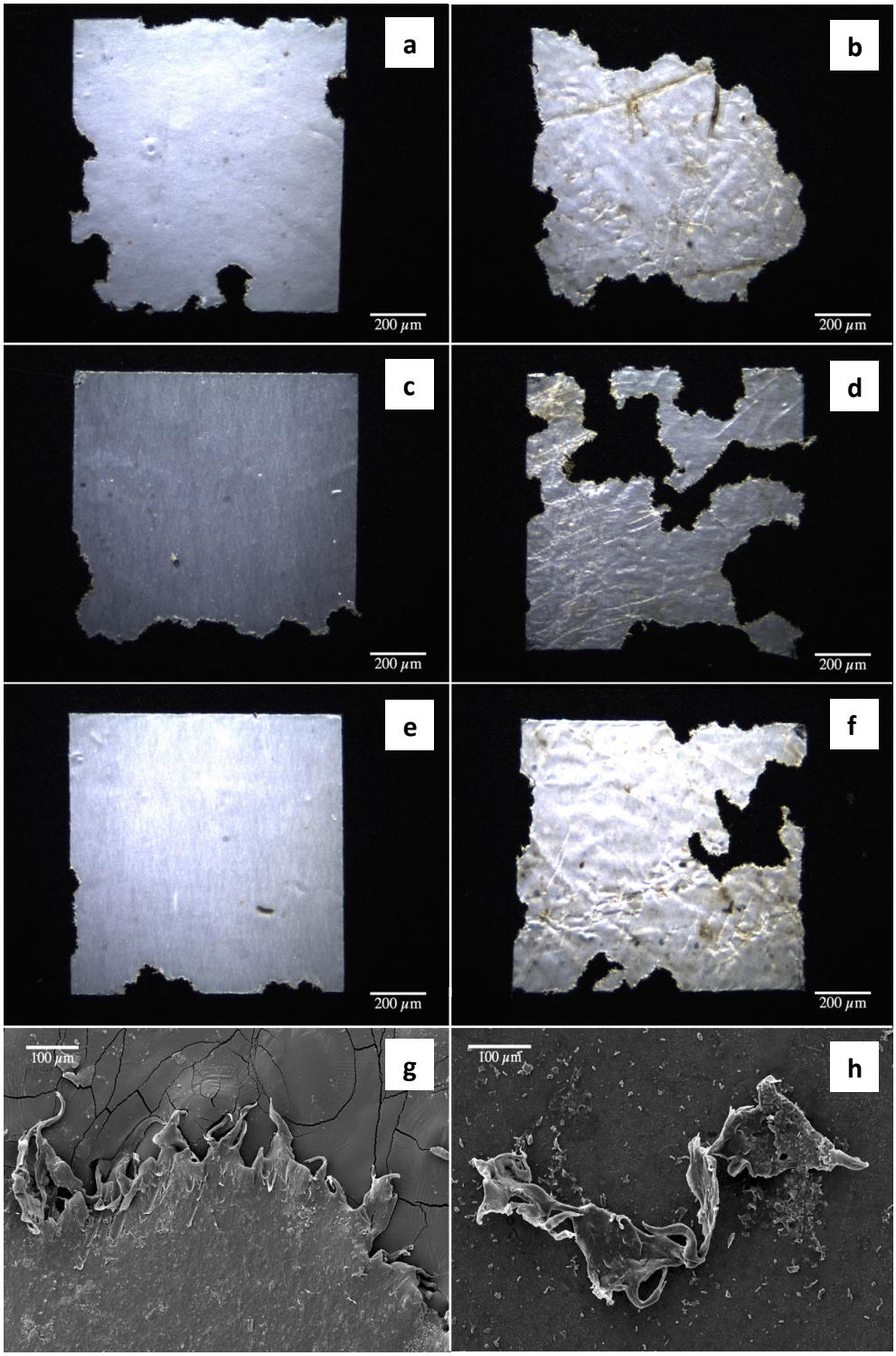

Plate 1: Plastic samples after 7-day laboratory exposure to *O. gammarellus* (a) Clean HDPE; (b) Fouled HDPE; (c) Clean degradable (d2w); (d) Fouled degradable (d2w); (e) Clean biodegradable; (f) Fouled biodegradable; (g) Foul plastic sample edge; (h) Ingested plastic fragment. *With credit to Plymouth University Electron microscopy Centre*.