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Plastic fragmentation in the environment

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A thesis submitted to Plymouth University in partial fulfillment of the requirements for the degree of:

Doctor of Philosophy

January 2016
To

Alan Johnson,
Jean-Pierre Biber,
and David Senn,

who inspire me
Abstract

Plastic fragmentation in the environment

Nicolas Francis Alexandre Biber

Microplastics are small pieces of plastic debris < 5 mm in diameter, which arise and enter the environment either as a consequence of the direct release of small pieces of plastic debris or as a consequence of the fragmentation of larger items. They are widely distributed in the marine environment, on shorelines, at the sea surface, on the seabed. They are also ingested by a variety of marine organisms, and there is some evidence that this may cause adverse effects. This thesis investigates the potential sources, distribution and fate of this contaminant.

Deterioration of plastics in a variety of environments was examined as a potential source of microplastics. Plastics were exposed in light and shade, in air, fresh water and sea water. Their deterioration was monitored over six hundred days. The fastest rate of deterioration, by far, was observed in air, and this was further accelerated by natural sunlight. Degradation in water was much slower and it was therefore concluded that plastic debris only fragments slowly into microplastics while they are in the water, and much faster when they are washed ashore and/or remain exposed to air, for example on a beach.

A method to assess the contamination of beaches by microplastics’ likely predecessors - small items of plastic debris - was therefore developed. High spatial variability, which can impede comparison between surveys undertaken by random sampling was overcome by assessing deliberately selected debris accumulations. An attempt to utilise citizen science using this rapid survey approach returned too much variability between volunteers to obtain consistent results. Nevertheless, the survey method was successfully used to identify narrow openings towards the sea, small tidal range and several weeks of high on-beach waves as predictors for high contamination.

Evidence of harmful effects of microplastics on animals is primarily from laboratory studies and could be substantiated by exposure experiments in-situ. Cockles were exposed to fluorescent plastic particles in an intertidal mudflat. After two weeks, particles were still present in the sediment. However, the test material could not be identified in the cockles with enough certainty to validate the method.

The thesis concludes that fragmentation of larger items of debris on land is an important potential source of microplastics. Therefore, preventing plastics that are prone to deteriorate from entering the environment is likely the most effective way to prevent the generation of microplastics. Measuring the success of such preventive measures in the field returns highly variable results, as small plastic debris are elusive. Measuring effects in the environment demands large amounts of data, and highly specific methods are required to detect microplastics in animals. The shift of environmental plastic contamination towards small particles demands that plastic debris research adjusts to the elusiveness of these particles. Large volumes of variable data on plastics density and precise biological essays will be required.
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Acknowledgements

I have been able to pursue this doctorate thanks to the generous financial support from the Theodor-Engelmann-Stiftung, Marianne Klemm †, Erika Klemm Roniger, the Community Research Award, my parents Susette and Jean-Pierre and my sister Alison.

My attendance to the Fifth International Marine Debris Conference in Honolulu in March 2011 was financed with a travel award from the National Oceanic and Atmospheric Administration (US Department of Commerce) and a Grant-in-Aid from the Plymouth Marine Science and Education Foundation.

I also wish to thank Adam Westmacott from Olympus for generously loaning me an SZX16 stereomicroscope to screen tissue sections for fluorescent particles.

Richard Thompson and Andy Foggo have both inspired and supported me in pursuit of my ideas. They guided me through my research with patience and dedication. As my supervisors they brought out the best in me, and I feel privileged to have been able to work with two such brilliant minds.

The tireless help and the capacious experience of Ann Torr, Jane Akerman, Roger Haslam and Richard Ticthurst made the practicalities of conducting my experiments and my fieldwork possible. Andrew Tonkin, Mike Hockings, Terry Richards, Angela Harrop and Will Vevers took the time to explain and grant me access to all the equipment I required in order to conduct Fourier transform infrared spectroscopy, tissue processing and microtomy, tensile properties measurement, light measurement and fluorescence microscopy. For access to field sites I wish to thank Mayflower Marina and especially Michael Whiffen and the friendly staff at Queen Anne’s Battery Marina who also allowed me to run a trial on site for an extended period of time. Thanks also to Josh Bam, who made the wooden beam clamps to my specifications. Special thanks go to Rebecca Harris, Roger Crook, Michael Whelan and Chris Booker from the Plymouth University Marine Centre for recovering that wooden beam clamp, which became detached and ended up at the bottom of Queen Anne’s Battery Marina.

I am grateful for the initiative of Friends of Par Beach, through which we were able to conduct a great research project together, especially Jenny Thomas, Tricia Jones, and Mike Freemantle who actively participated in the research work.

For spending days on end counting plastic debris on beaches with me I wish to thank Jean-Pierre Biber, Giovanni Romagnoni, Teo Van der Kammen, Ben Standen, Sam Houston, Jessica Chadwick, Jana Rajnohova, Kathrin Bacher, Mariangela Dejana; as well as Danni Hodgson, who also spent hours testing tensile properties for me; Fabian Rudin and Alison Biber, who also helped me with the FTIR; Tabitha Rudin for also participating in the tissue processing. I would also like to thank Massimo Ponti and Elena Rigetti for helping to deploy the cockles, and Nadja Christen for dissecting them.

Thanks also to Rachel Bramgrove, Sophie Rush, James Hinchcliffe, Rob Stanford, Jake Richards, Andrew Rodulfo, Daniella Hodgson, Kat Bromhall for participating in the beach survey method validation, and especially to Alex Fraser who helped coordinating.
The data analysis was helped by Vaclav Hornik who kindly gave me permission to use a large amount of data from http://www.windguru.cz. My literature list would not be complete without the help of Bridget Flowers and Cassandra Biber-Flowers from the University of Colorado Denver, who kindly provided access to literature that was not available at the Charles Seale-Hayne Library.

Thanks also to Mike Gordon Oldfield for music that works for making me work.

Margrit Gloor was my first teacher. She shaped my enthusiasm to further my knowledge and education. Ruedi Benz was my teacher for the longest time. As my judo instructor he taught me to recognise and adapt to both my strengths and my weaknesses.

Finally I give a big thank you to all the people who have given me moral support throughout my PhD, especially my mom and dad, Susette and Jean-Pierre, my sister Alison and my wonderful wife Cassandra, who has shown unmatched patience and who has been my best friend throughout.
Authors declaration

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the Graduate Committee. This study was partially financed with the aid of the Theodor-Engelmann-Stiftung, Basel (Switzerland) and the Community Research Award, Plymouth.

Presentations and conferences attended:
I have presented the following talk and poster at the Fifth International Marine Debris Conference, Honolulu HI, USA, 20-25 March 2011; I have also presented this talk at the 4th Annual Plymouth Marine Science Education Fund Conference, Plymouth Marine Laboratory, 14 December 2011:

- Biber, Nicolas F A, Ponti, Massimo, Foggo, Andy and Thompson, Richard C. Ingestion of microplastics by common cockles (*Cerastoderma edule*) in an intertidal mud flat. (Poster)

Workshops and courses attended:

- Training Workshop, Plymouth, UK: 2-6 November 2009 on Analysis of Multivariate Data from Ecology and Environmental Science using PRIMER v6 taken by PRIMER-E and the Plymouth Marine Laboratory, and held at the Marine Biological Association, Citadel Hill
- Certificate of Professional Development: Learning and Teaching for General Teaching Associates, University of Plymouth, 21 March 2011

Contributions to public education:

- Monitoring of plastic debris load on Par Beach, St. Austell Bay, Cornwall and comparison to other beaches around Southwest England. Community research project with Friends of Par Beach, St Austell, February 2011 to January 2012
- Shoreline plastic debris pollution in St. Austell Bay. Presentation given to Friends of Par Beach, St. Austell, 8 February 2012
• Fragmentierung von Plastik in der Umwelt. Presentation given to the Federal Office for the Environment (Switzerland), InfoPick, 4 December 2012; also given to Model UN Basel, Switzerland, 4. February 2013

• Small plastic debris from shorelines provided to Museum.BL, Liestal (Switzerland) for the exhibition ‘Wildes Baselbiet. Tieren und Pflanzen auf der Spur’ (permanent)

• Ein Sandstrand voller Plastik. Interview given to Zoo Basel (Switzerland), 11 July 2014, https://www.youtube.com/watch?v=ULddpM59zW0

• Der weite Weg des Plastik. Interview given to Zoo Basel (Switzerland), 15 July 2014, https://www.youtube.com/watch?v=EUItPMhA9Cs

Word count of main body of thesis: 38637

Signed:

Date:

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Chapter 1

General introduction

Plastics are synthetic polymeric materials that have revolutionised our society since the mid 20th century. Plastics are versatile with a wide range of applications in industries such as building, construction and packaging. Nearly 300 Million tonnes of plastics are now produced annually, and short-lived applications for these materials, such as packaging, have given rise to environmental concerns about the accumulation of plastic. Monitoring the sources, distribution and environmental impacts of plastic waste that has entered the environment as litter has become central to our understanding of this environmental contamination.

Animals can become entangled in plastic debris, which may cause them to suffocate or to drown. They can also ingest plastic debris, which may disrupt their digestive system or poison them. A wide range of marine vertebrate species including birds, turtles, fish and mammals are known to encounter marine debris, and in many cases these encounters case physical harm. In recent years, microplastics which can form from deterioration of larger debris have been detected. These small pieces of plastic can potentially affect a different suite of marine organisms including filter feeders and detritivores.

Microplastics are difficult to trace in the environment, because they are small, and in some cases microscopic. Hence, traditional plastic waste monitoring methods may
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not be applicable to them. Nevertheless, their sources, distribution and impact need to be explored in order to fully understand the potential for harmful effects. This thesis examines a variety of ways leading to the deterioration of larger plastic items into microplastics, it explores shorelines as potential entry points of microplastics into the environment, and investigates an approach to quantify microplastics ingestion in the natural environment.

In the environment plastic debris can fragment due to exposure to sunlight, heat and mechanical energy. The fragmentation of plastics is an important source of microplastics, which are defined as solid plastic particles smaller than five millimetres (GESAMP 2010). Microplastic contamination has been reported at a number of locations and in a range of marine habitats. In order to further the knowledge of the emergence of microplastics and their impact upon the environment, this thesis addresses the fragmentation of plastics in the environment, it observes the dynamics of small plastic debris from which microplastics partially originate, and it seeks out ways to observe microplastics and how they interact with biota.

Ryan et al. (2009) proposed that the monitoring of plastic litter in the environment over time should be addressed by three key questions: What are the main sources of plastic litter? What are the abundance, distribution and composition of plastic litter in the environment? What are the environmental and economic impacts of plastic debris contamination?

This chapter will introduce current knowledge on plastics in the environment and will explain how the thesis will develop and examine the questions of Ryan et al. (2009) with respect to microplastics in the environment. An overview of plastic materials and their role in society will be given in the first section, and this will indicate the short service life of many plastic items that, via inappropriate disposal, can lead to environmental contamination. The second section will address the molecular constitution of plastics, how they maintain stability, and also how they can degrade in certain environmental conditions, which results in the formation of microplastics, which are then discussed in the subsequent section. The increasing presence of microplastics in the environment will be examined, emphasising the need for research on plastics fragmentation as an important source of microplastics contamination.
Thereafter the presence of plastic debris in the environment will be discussed in a more general way focusing on their distribution patterns and pathways, and on the broad range of marine habitats where plastics can now be found, often making contamination difficult to assess. Due to their wide distribution in the marine environment, plastics have the potential to cause harm to a great number of marine organisms, which is discussed in the section thereafter. An explanation of the potential harm microplastics can cause to invertebrates is discussed in relation to the need for ecotoxicological research methods specifically addressing microplastics.

The following section discusses different methods to monitor plastics in the environment, and it addresses the challenges these are confronted with due to the dispersal of plastics debris in the environment. A section on monitoring will highlight the need for ‘on-target’ methods to overcome the challenges presented by the distribution of plastics in the environment. It will also introduce the use of volunteers in environmental research and how volunteers can potentially return large amounts of data on plastic debris in the environment.

### 1.1 Plastics: an overview

Plastic is an umbrella term for materials that are constituted from hydrocarbon polymers. Plastics are moldable at relatively low temperature. Plastics can be divided into two broad categories: (1) Thermoset plastics, which feature a complex three-dimensional structure and a high cross-linked density and thereby prevent molecular movement. Examples for thermosets are epoxy resins, silicones and polyurethanes. Thermosets account for 10% of all plastic production. (2) Thermoplastics, such as polyethylene, polystyrene or polyesters are linear organic molecules and can be softened by heating for reshaping and solidified by cooling (Carraher 2003).

Plastic is defined by its ability to be molded. It can be flexible and hard, and crystalline to any extent. Plastics exhibit elasticity that is typical for elastomers, but they have better tear resistance, a property that is more common with fibres. Thermoplastics have a low cross-linked density, which allows them to be used below their glass transition temperature, for amorphous species, or below their melting temperature if they are crystalline (Carraher 2003).
Many plastics float because they are lighter than water. Most plastics and organic liquids have specific weights of about 0.7 to 1.0 g cm$^{-3}$. Specific weights of most typical polyethenes are 4 to 9% lower than water. However some plastics are heavier than water. For example polystyrene, which is most commonly known in its expanded form and is used for buoyancy, is typically 4 to 20% heavier than water in its compacted form. Polyvinyl-chloride always has a greater specific weight than water (Carraher 2003).

Synthetic plastics were invented in the early twentieth century (Gorman 1993; Carraher 2003), with further development during World War II when they were used as wire insulation (Carraher 2003). Development of a wide spread of material types and fields of application for plastics took place in the 1960s (Allsop et al. 2006). Global plastic production growth increased by around 9% per annum since the 1950s; and amounted to 288 million tonnes in 2012 (PlasticsEurope 2013). Currently 5% of fossil fuel use goes towards plastic production (PlasticsEurope 2012).

Previous work has shown that there has also been an increase of plastic debris found on beaches (Barnes et al. 2009), ingested by birds (Moser and Lee 1992) and in microscopic debris found in plankton samples (Thompson et al. 2004). These observations suggest that there may be a link between the increase of plastic production and an increase of plastic debris in the environment. In the 1990s the effects and the status of marine litter were still poorly understood (Ryan and Moloney 1993), and no data on environmental safety and accumulation of degraded plastics were available (Klemchuck 1990).

Most plastics resist to biodegradation due to their high molecular weight and their complexity (Klemchuck 1990), but over time they are subject to mechanical and chemical breakdown (Pegram and Andrady 1989a; Searle 2003; Singh and Sharma 2007; Cooper and Corcoran 2010). Plastics degradation is slow (Pegram and Andrady 1989a) and many sources suggest that plastics can persist for centuries (Hansen 1990; Gorman 1993; UNESCO 1994; Goldberg 1995, 1997).

Today the most widely known application of plastics is for packaging, which has contributed to tremendous progress especially in the food industry where the shelf-life of foodstuffs has substantially increased by protection through plastic packaging, thus reducing food waste. Pack-
aging materials, however, typically have a service life that is much shorter than their molecular lifespan and go to waste within a year. Through inappropriate waste management plastics can end up in the environment, and packaging constitutes a substantial proportion of environmental plastics contamination.

In the developed world, plastics constitute approximately 10% of household waste by weight (Department of the Environment 1994a,b; Jones et al. 1996; Golder Associates 1999; Bai and Sutanto 2002; Dehoust et al. 2002; US EPA 2003; Poll 2004; Burnley 2007; Sokka et al. 2007). Plastics are recognised as a valuable commodity, and recycling methods have made substantial progress, however some plastics (e.g. polyvinyl-chloride) contain additives that enhance the material properties but render them incompatible with recycling (Klemchuck 1990; Carraher 2003). Another obstacle to plastic recycling has been the need for consumers to assume the responsibility of separating plastics from other waste (Carraher 2003). Even when suitable plastic recycling measures that would prevent the landfilling of these materials are in place, consumers do not always make sufficient use of them (Loughlin and Barlaz 2006). In 2012 almost 40% of post-consumer plastic waste (almost ten million metric tons) ended up in landfills (PlasticsEurope 2013).

1.2 Plastic debris in the environment

First reports of plastic debris in the marine environment were published in the early 1970s (Carpenter and Smith 1972; Carpenter et al. 1972; Colton et al. 1974). Over the last few decades, shoreline surveys (Ryan and Moloney 1993; Ribic et al. 1997; Torres and Jorquera 1999; Thompson et al. 2004; Barnes 2005), ocean surface trawls (Day and Shaw 1987) and bird gut content analyses (Day et al. 1985; Moser and Lee 1992) attested to an increase in plastic contamination. This increase could be attributed to the growth of worldwide annual plastic production from fifty million tonnes in 1976 (PlasticsEurope 2010) to its current amount of about 288 million tonnes (PlasticsEurope 2013).

50 to 80% of all waste detected in the marine environment is plastics (Gregory and Ryan 1997; Derraik 2002; Barnes 2005; Morishige et al. 2007; Barnes et al. 2009). The standing stock of plastic debris will continue growing if plastics are discarded in the environment. As plastics
CHAPTER 1. GENERAL INTRODUCTION

degradate at a substantially slower rate than they are added to the environment, they are persistent and are accumulating (Colton et al. 1974; Gregory and Ryan 1997; Barnes et al. 2009). Even if the introduction of plastic debris into the environment were stopped immediately, the existing legacy of plastic debris would persist for a long time (Ng and Obbard 2006; Barnes et al. 2009).

1.2.1 Where does plastic debris accumulate?

Large amounts of marine plastic contamination are observed near human population epicentres. The abundance of this debris correlates with human population within ten degrees latitude (Barnes 2005). However, even remote shores such as the Antarctic are affected (van Franeker and Bell 1985; Convey et al. 2002). Usually the litter found there does not originate from these remote locations, but is carried there by ocean currents. Over the last decade accumulations of plastic debris in oceanic gyres far from the nearest land fall has also been observed (Moore, Moore, Leecaster and Weisberg 2001; Law and Thompson 2014). Hence it is now clear that plastics can travel with ocean currents and end up far away from their point of entry (Barnes et al. 2009). Plastics have also been reported on beaches worldwide. According to Wilber (1987) oceanic islands intercept ('filter') floating debris from the open sea, and it has been suggested that the amount of debris in the open sea may be extrapolated from the debris burden measured on shorelines (Dixon and Dixon 1981; Merrell 1985).

Some areas in the marine environment have higher plastic debris contamination than others. For instance enclosed seas like the Mediterranean exhibit a higher density of plastic debris than the larger oceans (Barnes et al. 2009; Suaria and Aliani 2014) and once in the open sea, eddies and convergences cause debris to accumulate (Shaw and Mapes 1979). An accumulation of plastic debris in the marine environment that has given rise to a large amount of public attention is located in the North Pacific Gyre. Here debris densities as large as 5114 g km$^{-2}$, composed of mostly thin films and monofilament lines were recorded by Moore, Moore, Leecaster and Weisberg (2001). These findings show that plastic waste that likely originated on land can travel far from its point of entry with ocean currents, and once in the environment it can degrade into smaller fragments.

Various sizes of plastic debris can be found in the water column and on the seabed (Ryan et al.
1.3. PLASTIC DURABILITY AND DEGRADATION

2009); some plastics have a greater specific weight than water (Carpenter et al. 1972), while buoyant items of plastic debris will only sink if they become overgrown by organisms after prolonged environmental exposure that has allowed organisms to settle on them (Moore, Moore, Leecaster and Weisberg 2001). Debris accumulations on the seabed are therefore sometimes far away from the coast (Gregory 1978; Gregory and Ryan 1997; Barnes et al. 2009).

An analysis of recent global surface trawls exhibited a plastic debris abundance that was orders of magnitude lower than the abundance expected from production and input rates (Cózar et al. 2014). Possible explanations for this low detected abundance were submersion, washing ashore, ingestion by organisms or further fragmentation. Recent estimates based on amounts of plastic waste generated, population size and quality of waste management systems in 192 coastal countries amount to 4.8 to 12.7 × 10⁶ t entering the ocean per year (Jambeck et al. 2015).

1.2.2 Sources

The sources of plastic debris are numerous and cannot be quantified with certainty. Plastic debris contamination can therefore not be prevented by controlling just one source, and so it is difficult to organise effective mitigation campaigns (Ryan et al. 2009). Improper waste management and careless behaviour are the two main causes of the release of plastic debris to the environment. For instance, the disposal of plastics in landfills cannot be considered terminal. Films (e.g. from carrier bags) are the most likely to be carried into the environment after disposal as they are light weight and have a large surface area facilitating transport by wind (Barnes et al. 2009). Sewage is also considered to be an important sources of plastic debris (Lucas 1992). Sewage plants can act as a pathway of debris into waterways and ultimately into the ocean. Indeed, a high abundance of micro-debris was recorded in the Los Angeles and San Gabriel Rivers (CA, USA) by Moore et al. (2007), and sewage plants in the state of New York (US) have been found to release up to 100000 plastic particles per day (Chaskey et al. 2014).

1.3 Plastic durability and degradation

Plastics are robust and withstand deterioration by design. Their complex polymeric structure and their high molecular weight ensures that these materials maintain their quality (Carrasco
et al. 2001), but plastics still deteriorate due to mechanical stress over time (Carpenter and Smith 1972; Plotnikov 1988; Klemchuck 1990; Contat-Rodrigo and Ribes-Greus 1998).

Due to their non-polar surface plastics do not chemically interact with water (Carragher 2003). This also makes plastics largely insensitive to digestion, and they are typically non-biodegradable (Klemchuck 1990). In a society that is becoming increasingly aware of the environmental implications of plastic waste there has been an increasing demand for degradable plastics. The prefixes ‘bio’ and ‘degradable’ have become powerful marketing instruments for plastic products with a short service life. A lot of effort has gone into the development of biodegradable plastics (Wolf and Feldman 1991; Gorman 1993).

The biodegradability of a material should theoretically be measured by its ability to act as a food source for organisms. Some polymers are indeed biodegradable under laboratory conditions and after thorough photo-oxidation (Klemchuck 1990). Some strains of microbes are able to digest polyethene or polyvinyl-chloride (Orr et al. 2004; Shah et al. 2008; Sivan 2011). Microscopic plastics can be further degraded by microbes and integrated into biomass. If this process is completed it is called complete mineralization (Eubeler et al. 2009).

However, rates of degradability are highly variable. While some sources see long term potential for the advancement of biodegradable plastics in the addition of natural polymers to thermoplastics (Dave et al. 1997), it would appear that other ‘biodegradable’ plastics, such as starch-filled plastics, are falsely referred to as biodegradable materials, as only the starch portion biodegrades. The plastic portion does not mineralise (Andrady 2011). Biodegradable plastics have been found to remain in the environment as non-degradable residues, for which no data on environmental safety or accumulation was available (Klemchuck 1990). Biodegradation was only observed on materials with a molecular weight of 500 g mol$^{-1}$ or less (Andrady 2011), and even highly embrittled plastics often exhibit molecular weights of $10^5$ g mol$^{-1}$.

The contribution of sunlight to plastic degradation has been known for many years (Amin and Scott 1974; Kuzina and Mikhailov 1998; Fernando et al. 2007). Ultraviolet light drives the deterioration of plastics by reducing their molecular weight. In this process called photo-oxidation, ultraviolet light renders carbon bonds unstable and causes them to oxidise, which breaks the
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polymer at its backbone (Day and Wiles 1972; Blais et al. 1973; Otsu et al. 1979; Ranby 1989; Andrady 1990). The more reactive groups the molecule has (the more unsaturated it is), the higher the rate of degradation (Singh and Sharma 2007). Due to its high degree of unsaturation polystyrene, for instance, is particularly susceptible to this effect (Ghaffar et al. 1975; Kuzina and Mikhailov 1993; Israeli et al. 1994). Photo-oxidation plays an important part in the physical deterioration of plastics because the lower the molecular weight, the more brittle the material becomes (Andrady 2011).

Plastics owe their mechanical stability to their molecular weight and to their bond density. Contrary to mechanical deterioration, photo-oxidation is enhanced by higher molecular weight and higher bond density; due to a higher degree of unsaturation they are more susceptible to oxidation. Likewise, lower molecular weight materials degrade at a slower rate than higher molecular weight materials because they have a lower crosslink density and therefore a lower degree of unsaturation (Chirinos-Padrón et al. 1987). As a consequence the rate of photo-oxidation of plastics is expected to slow down the further the material deteriorates, and it is uncertain whether the material’s molecular weight ever drops to a level that is suitable for biodegradation.

1.3.1 Microplastics

Reports of large numbers of plastic resin pellets - the primary material used to make all plastic items, 0.1 to 0.5 cm diameter - on the East coast of North America in the early 1970s (Colton et al. 1974) and the detection of polystyrene spherules in zooplankton in southern New England in 1971 (Carpenter et al. 1972) are perhaps the first reports of the tiny plastic pieces that are now called microplastics. Microplastics have been detected in subtidal sediments (Thompson et al. 2004; Ng and Obbard 2006), surface waters (Thompson et al. 2004; de Lucia et al. 2014), subsurface waters (Desforges et al. 2014), mangroves (Nor and Obbard 2014) and even in arctic sea ice (Obbard et al. 2014) and on the seabed (Woodall et al. 2014). Analysis of plankton samples over the last four decades of the twentieth century showed that microplastics have been present in the environment since at least 1960. Their abundance has gradually increased since then (Thompson et al. 2004), and their composition and distribution has shifted from mostly plastic resin pellets to fragments of end products (Vlietstra and Parga 2002; Ryan 2008; Ryan
et al. 2009).

Microplastics enter the environment from everyday products. For example, hand cleansers now contain microscopic plastic particles as scrubbers (Gregory 1996; Cox 2014). Synthetic fibres can also end up in the waterways from washing machines via sewage (Browne et al. 2011). Microplastics have also been detected in intertidal sediments as a result of ship-breaking (Reddy et al. 2006), and plastic particles are also used in air-blasting instead of sand (Gregory 1996). The gradual degradation of large plastic items through photolytic, biological and mechanical action (Klemchuck 1990; Andrady et al. 1998; Searle 2003) has also been identified as a source of microplastics to the environment (Thompson et al. 2004). Indeed the most abundant plastic fragments detected in the North Pacific Gyre likely resulted from the fragmentation of films such as those used in plastic carrier bags (Moore, Moore, Leecaster and Weisberg 2001). The numerous sources of microplastics create a large potential for accumulation of these particles in the environment (Thompson et al. 2004), and like larger debris microplastics may be transported to remote locations (Zarfl and Matthies 2010).

Due to fragmentation large plastic debris will only remain large for a limited period of time, as it gradually degrades into smaller debris. It is possible that the amount of large debris in the environment will decrease, if fragmentation happens faster than introduction. However, the amount of small debris will continuously increase, because degradation is slower the smaller the particle is (Christensen 1992). It is likely that microplastics will become the predominant solid contaminant over large debris. Microscopic plastic particles already account for 80% by number of all plastic debris found in the Tamar Estuary (UK) (Browne et al. 2007).

Some particles recorded on beaches around Plymouth, UK were as small as 20 µm. Microplastics may be suspended in water along with natural sediment like other anthropogenic particulates (Jedwab 1980). Microplastics, therefore, may be more difficult to detect than large debris. Due to their small size it is also difficult to identify the original use of these microplastics. Material types can be determined by gas chromatography mass spectrometry (Moore et al. 2007) or by infrared spectrophotometry (Carpenter et al. 1972), and molecular identification can give some indication of the original use of the material (Thompson et al. 2004).
Microplastics research has made considerable progress in recent years, but a substantial amount of uncertainty remains as to the sources, fate and the potential effects of this contaminant in the environment. Microplastics are difficult to observe and identify, however monitoring slightly larger plastic debris items that could yield valuable information on the sources of microplastic particles. Since it is beyond this size that plastic debris becomes more difficult to control and is more likely to remain permanently in the environment.

The timescale for larger plastic debris fragmentation into microplastics is determined by their stability, but also by the environment they are located in. Environments where plastics are exposed to mechanical stress or high oxidising radiation will accelerate their deterioration; such environments may be a substantial source of microplastics. Plastics contaminate the environment on land, in fresh water systems and at sea; and while the highest amount of plastics is likely in the ocean (because the ocean is the ultimate sink for most watercourses), it is likely that deterioration occurs more rapidly on land, due to higher UV radiation and better oxygen availability. Nevertheless, microplastics are common in the ocean, and one could ask if the land-ocean interface could cause the deterioration of marine debris. This thesis will therefore take a look at the role of beaches in the formation of microplastics.

1.4 Adverse effects of plastics in the environment

Plastic debris can cause harm to a broad range of marine vertebrate species. Turtles (Balazs 1984), seals (Shaughnessy 1980; Fowler 1987; Page et al. 2004), whales (Perkins and Beamish 1979), birds (Schrey and Vauk 1987) and fish (Degange and Newby 1980) can become entangled in plastic debris. They can suffer lacerations from cutting edges on the debris they are entangled in, or may suffocate or drown. Turtles (Balazs 1984; Bjorndal et al. 1994), birds (Bourne 1976; Day et al. 1985; Furness 1985; Ryan 1987) and fish (Lusher et al. 2013) ingest plastics and could suffer toxicological effects as well as internal lacerations. Some of the earliest accounts of marine debris report plastics in seabird gut content and also in plankton samples (Kenyon and Kridler 1969; Buchanan 1971). Gregory and Ryan (1997) and Derraik (2002) predicted that the impact of plastic debris on the environment would increase over time due to the longevity of the material and poor waste management, and indeed, more species of
various taxa seem to be affected now (Secretariat of the Convention on Biological Diversity and the Scientific and Technical Advisory Panel - GEF 2012) than two decades ago (Laist 1997). However, the impact of marine litter is difficult to estimate because interactions with biota are diversely distributed, and victims may not be found before they sink or get eaten (Wolfe 1987).

This thesis addresses the fragmentation of plastics in the environment; it therefore focuses on small and microscopic plastic debris whose potential harm to animals likely comes via ingestion. Ingestion of plastic debris by seabirds, marine mammals, turtles, fish and squids is well documented (Blight and Burger 1997; Laist 1997; Bugoni et al. 2001; Cadée 2002; Tomas et al. 2002; Eriksson and Burton 2003; Mascarenhas et al. 2004; Mallory 2008). Due to their shape and their occurrence pattern, plastic fragments can be mistaken for food by various animals (Moser and Lee 1992), and they are frequently ingested by marine organisms. For instance, plastic pellets resemble fish eggs, and this may increase their ingestion by fish (Carpenter et al. 1972).

Plastic debris can cause blockage, ulceration and damage to the digestive system of birds (Bourne 1976; Day et al. 1984, 1985; Fry et al. 1987) and also fish (Carpenter et al. 1972). For example petrels have ingested debris that they are unable to pass through their pyloric sphincter (Furness 1985). The debris uses up free space and thus reduces effective stomach volume, which reduces appetite (Ryan 1988) and ultimately causes the animal to starve with a full stomach.

The size of the debris and the size of the organisms ingesting them may correlate, as has been shown for birds (Furness 1985; Ryan 1987b). Logically large plastic items cannot be ingested by small animals. However, small fragments are of particular concern, because they can potentially be ingested by a wider range of organisms than large ones (Thompson et al. 2004; van Franeker et al. 2005).

Microscopic plastic particles may resemble plankton and can be ingested by filter feeders (Moore, Moore, Leecaster and Weisberg 2001). There are now numerous reports of invertebrates eating microplastics. Detritivores such as amphipods (Chua et al. 2014), deposit feeders such as lugworms (van Cauwenbergh et al. 2012) and filter feeders such as barnacles (Gold-
stein and Goodwin 2013) and mussels (Browne et al. 2008) have been shown to readily ingest microplastics. There is evidence of this happening in the natural environment (Mathalon and Hill 2014; van Cauwenberghhe and Janssen 2014), and laboratory studies have looked at harmful effects on animals from the ingestion of microplastics. For example, the ingestion of microplastics has been found to cause oxidative stress and death in lugworms (Browne et al. 2013).

Once ingested, microplastics do not necessarily remain in the animal’s gut lumen; they may transfer into the vascular system (Volkheimer 1975; Hussain et al. 2001; Browne et al. 2008), where they can remain for periods much longer than a digestive cycle. There are also reports of ingested plastics being integrated into organisms’ tissue (Moore, Moore, Leecaster and Weisberg 2001). This may facilitate the transfer of the plastic particles along the food chain from prey to predator, which has been observed between mussels and shore crabs (Watts et al. 2014) and which may also happen in fish, as anchovies - a common prey - have been found contaminated with microplastics (Kripa et al. 2014).

A further concern arising from the ingestion of plastics in general is the leaching of toxic chemicals into organisms. This aspect is particularly pertinent for microplastics, as organisms can potentially retain them in tissues, also because microplastics have a much larger surface area per volume, and they therefore present a much larger interface for chemical reactions (Browne et al. 2008).

Plastics have a hydrophobic surface and constitute an adsorbent for the trace enrichment of organic compounds such as poly-chlorinated biphenyls, nonylphenol and phenanthrene in water (Rice and Gold 1984; Moore et al. 2007) as well as trace metals like lead or copper (Holmes et al. 2012). The adsorption coefficient of plastics in water for persistent organic pollutants amounts to $10^5 - 10^6$, and long banned chemicals such as chlordanes have been found attached to plastic resin pellets (Moore et al. 2007). Meanwhile many plastics contain toxic substances such as poly-chlorinated biphenyls and nonylphenol as innate additives that can potentially be transferred to animal tissue if ingested (Koch and Calafat 2009; Oehlmann 2009; Talsness et al. 2009). Adsorption has been observed in polypropylene (Mato et al. 2001), which adheres aldehydes, ketones, esters, acids, peresters and peracids (Rjeb et al. 2000) and polyethylene (Pascall...
et al. 2005; Teuten et al. 2007), two material types that are very common in marine debris. Napper et al (2015) detected adsorption of phenanthrene and DDT by plastic particles that were extracted from cosmetic products, where they serve as scrubbers. The interaction (binding, transport, release) of plastics with toxic substances (Carpenter and Smith 1972; Derraik 2002) could cause them to become important for chemical transports across geographic areas (Teuten et al. 2007) but also into animals (Carpenter and Smith 1972; Ryan et al. 1988; Mato et al. 2001).

Indeed chemicals that have been absorbed by plastic particles in the water column are released much faster in gut conditions than in sea water, and even a few micrograms per gram of pvc in sediment can cause significant accumulation of phenanthrene in lugworms (Teuten et al. 2007). The reason for faster desorption of chemicals in gut conditions versus sea water may be attributed to lower pH, higher temperature or a combination of both (Bakir et al. 2014 a). Microplastics can be considered a potential transporter mechanism for toxins into the food web and therefore require particular attention especially in the view of possible biomagnification. Meanwhile whether microplastics generally cause harm when ingested remains unclear, for example the impact they had on the larva of a sea urchin was limited (Kaposi et al. 2014). However, experiments on microplastics impacts have mostly been conducted in the laboratory. Conducting an ingestion experiment with microplastics in the natural environment is difficult, because potential test organisms feed from a medium with a high turnover, e.g. the blue mussel, which obtains its nutrients from tidal water. Nevertheless, toxicological experiments should also be conducted in the natural environment, because the animal’s full biological response cannot be reproduced in a tank (Underwood 1995). In order to understand the health impacts of microplastics better, suitable methods to bring them to a potential test organism in its natural habitat have to be devised.

With this in mind, this thesis explored the possibility to conduct microplastics toxicity experiments in the natural environment. For this, the elusiveness of these particles and the high turnover in marine environments had to be overcome. Sediment-dwelling organisms live in a habitat that allows the inoculation of a solid contaminant and its recovery even after several
weeks. The common cockle is a sediment-dweller that is suitable for marking, releasing and recapturing; it was therefore chosen as a model to test the feasibility of in-situ microplastics toxicity experiments.

### 1.5 Monitoring

Along with the observation of impacts on organisms and the environment and the identification of sources, the assessment of distribution, abundance and composition constitute key approaches in the monitoring of plastic debris in the environment (Ryan et al. 2009).

The methods used to survey plastic debris in the marine environment are as varied as plastic debris contamination itself. Long-range and rapid visual surveys have been conducted on the ocean surface and on beaches to monitor large debris (Weiwei and Juying 2011; Fisner and Turra 2011; Leejarkpai et al. 2011; Hanke 2011). Debris has been collected in beach cleaning campaigns and in ocean surface trawls focussing on smaller plastic items (Moore et al. 2005; Marine Conservation Society 2014). The abundance of small plastic debris has also been indirectly monitored through investigation of stomach contents of beached birds and their home range (Harper and Fowler 1987; van Franeker and Meijboom 2002; Mallory et al. 2006; Ryan 2008). Birds typically travel far to forage; their stomach contents can therefore be representative of the plastic contamination over a large area. Estimates of plastic abundance at sea have meanwhile been made based on the frequency of animal interaction with plastic debris (Day et al. 1985; van Franeker 1985; Spear et al. 1995; van Franeker et al. 2005; Mallory et al. 2006).

Monitoring both the amount of plastics in the environment and the rate at which plastics are introduced into the environment constitutes a powerful way to illustrate to the public that any effort in reducing environmental contamination is making a difference (Sheavly and Register 2007), but also helps to identify sources of marine debris more accurately. For example the observation of a decrease of pellets on beaches and in birds stomach contents versus other debris suggest that actions taken by the plastic industry in the early 1990s, to prevent the loss of pellets, were successful (Gregory and Ryan 1997; Vlietstra and Parga 2002; van Franeker et al. 2005).
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The assessment of the amount of plastic debris in the environment is complicated by its spatial and temporal heterogeneity (Ng and Obbard 2006; Ryan et al. 2009). An accurate estimate of the abundance and the effects of plastic debris in the environment can only be acquired by monitoring across geographic areas, environments and debris sizes (Ng and Obbard 2006; Barnes et al. 2009). Plastic debris in the environment has been observed for decades and data are now abundant enough to observe seasonal, annual and long-term trends, such as the effect of El-Niño on the debris deposition on beaches (Morishige et al. 2007).

Surveys of plastic debris in the marine environment essentially constitute either counting the debris at sea, on the beach, or measuring the introduction rate. At-sea surveys conducted from a boat as opposed to beach surveys are free from potentially confounding factors, such as anthropogenic introduction and removal of debris or the physical parameters of the shoreline that is being assessed (Sheavly 2007). However, they require the consideration of a large area, as plastic debris often occurs in aggregations and are therefore difficult to accurately monitor in small scale surveys, and so requires more time and infrastructure than beach surveys. Beach surveys also offer the possibility to measure the introduction rate by periodic removal of debris, which is not possible on the ocean surface.

Beach surveys are therefore a widely used approach of marine plastic contamination monitoring, and they have returned valuable information on the distribution of plastic debris in the ocean. Surveys on remote beaches have provided proof of long range transport of plastic debris (Ryan and Moloney 1993), and there have been attempts to extrapolate the debris abundance in the open ocean from the debris burden measured on beaches (Dixon and Dixon 1981; Merrell 1985).

With the emergence of microplastics, beach surveys may attain even higher importance in environmental plastics research. As stated above, beaches may constitute a hot spot for microplastics generation, as they have a high turn-over of marine debris and present conditions that are favourable to photo-oxidation. Especially plastic debris that are likely to deteriorate into microplastics, such as debris that exhibit prior weathering but also small debris, which present a large surface per volume, should be gaining attention in beach debris surveys.

Beach surveys are often conducted in conjunction with cleaning campaigns (Marine Conserva-
1.6. AIMS OF THESIS

Based on the literature reviewed coastal environments are likely to play a pivotal part in the formation of microplastics in marine environment due to their high transition rate in plastic debris and their high solar irradiation.

Microplastics - as do larger plastic debris - constitute a potential threat to animals through ingestion. An overriding objective of the thesis is therefore to examine the formation of microplastics, the distribution of small plastic debris and the bioavailability of microplastics in coastal environments. The aims of this thesis are:

- To quantify the difference in rate of deterioration of polyethene, polystyrene, poly(ethylene terephthalate) and a degradable kind of plastic in sea water, fresh water and on land. This
is addressed in Chapter 2.

- To develop and test a method to quantify contamination of beaches by small plastic debris that can be applied on a great number of beaches by using a rapid survey approach and by making it transferable to volunteers. This is discussed in Chapter 3.

- To identify physical factors (morphology, wind and wave action) appropriate to each beach that influence the contamination of beaches by small plastic debris. This was addressed in Chapter 4 using weather records and data from a beach morphodynamics survey of England.

- To examine the common cockle (*Cerastoderma edule*) as a model organisms for *in-situ* experiments on ingestion in coastal habitats in Chapter 5.

- Chapter 6 will then (i) summarise the major findings of the thesis regarding sources of fragmented plastic debris, (ii) identify multi-pronged solutions to prevent small plastic debris from entering the environment and (iii) propose methods to measure the effectiveness of such solutions.
Chapter 2

Deterioration of plastics in air, fresh water and sea water

Plastic is one of the most abundant solid marine contaminants. Despite their durability, plastics gradually deteriorate into small fragments as a consequence of exposure to UV radiation and mechanical stress. This contributes to the accumulation of microplastics, which are defined as plastic particles smaller than 5 mm. Microplastic particles have been detected in the marine environment on a global scale. However, as of yet there is limited evidence that they pose a direct threat to marine organisms. The occurrence of microplastic contamination can result from direct introduction of microplasticised pieces, for example form their use as abrasive particles in cosmetics, and from the fragmentation of larger items in the environment. A number of possible deterioration processes, such as mechanical wear and oxidation have previously been described. However, the rate and extent of deterioration of plastics in the natural environment remains largely unknown. This chapter aimed to describe deterioration of some commonly used types of plastics in a range of natural environments. Samples of polymers (biothene™, polyethene, polystyrene and poly(ethylene terephthalate)) were deployed in seawater, fresh water and air. Subsamples of each material were collected
CHAPTER 2. DETERIORATION OF PLASTICS

from these environments at intervals over a twenty month period. Their deterioration was measured through changes in surface area, tensile properties and molecular composition. Deterioration occurred much more rapidly in air than in either sea water or fresh water, which was attributed to the reduced UV radiation in water, together with a further reduction resulting from fouling by organisms which was substantial in the marine environment. Deterioration in air led to changes in the tensile properties. With materials becoming more brittle their capacity for tensile extension was reduced. Tensile extension also decreased in material samples that were exposed in sea water even though no oxidation was measured. This suggests that the deterioration of plastics in marine environments can result from factors other than UV radiation.

2.1 Introduction

Plastic contamination presents a major environmental problem and can pose a threat to wildlife through ingestion and entanglement, and has also given rise to aesthetic concerns. As a consequence, plastic manufacturers have been investigating degradation processes of plastics with respect to material durability and quality, but also with regards to manipulating material degradability, as a way to reduce the amount of litter that is visible in the environment. Recent studies have shown that even at advanced stages of deterioration very small pieces of plastic could pose a threat to animals in the marine environment (Teuten et al. 2007; Browne et al. 2008; van Cauwenberghe et al. 2012; Goldstein and Goodwin 2013; Lee et al. 2014; Cole et al. 2014).

Accumulation of plastic debris in the environment is gaining increasing attention, not only in the scientific community, but also in the media and from the general public. It presents challenges to wildlife, the economy and human health. Numerous papers describe the abundance of debris, e.g. (Galgani et al. 2000; Moore, Moore, Leecaster and Weisberg 2001; Claereboudt 2004; Barnes 2005; Martinez-Ribes et al. 2007), and deterioration of plastics has been the object of research too. For example the reaction of materials to radiation or heat has been tested in laboratories (Israeli et al. 1994; Gardette et al. 1995; Nagai et al. 1999; Copinet et al. 2004). These studies, however, have been conducted as quality assessments on material stability. Some research has also gone into deterioration of plastics in natural environments, focusing on environmental health. The deterioration of plastics has been observed in sea water (Pegram and
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Andrady 1989a; O’Brine and Thompson 2010), in waste treatment plants (Gilmore et al. 1993), and in air (Pegram and Andrady 1989a; Satot et al. 1997). This chapter addresses the thesis’ aim to quantify the difference in rate of deterioration of polyethylene, polystyrene, poly(ethylene terephthalate) and biothene - a degradable kind of plastic - in sea water, fresh water and on land.

Even though one of the key attributes of plastic items is their durability, plastics will degrade in the environment over time due to UV radiation and mechanical abrasion (Colton et al. 1974; Gregory 1978; Andrady 2003; Thompson et al. 2004). Contamination associated with plastic deterioration has indeed been a concern in environmental sciences since the 1970s (Colton et al. 1974; Gregory 1978). Recently, attention has been drawn to the discovery of microplastics (Thompson et al. 2004). A considerable factor in plastic deterioration is the breaking of polymer chains at a molecular level (Klemchuck 1990). Crosslinks of long polymer chains provide the material’s cohesion. The lower the molecular weight, the less the material is mechanically stable (Sawai et al. 2006). Light, especially UV radiation, causes polymer chains to break (Allen et al. 1983; Ranby 1989; Gardette et al. 1995). This reaction is called photo-oxidation, and has been cited as the most important environmental cause of polymer deterioration (Carrasco et al. 2001). For example polyethylene and polypropylene lose extensibility, mechanical integrity and molecular weight when exposed to UV radiation (Hamid and Prichard 1991; Andrady, Pegram and Tropsha 1993; Obadal et al. 2006; Marek et al. 2006; Singh and Sharma 2008).

Photo-oxidation is initiated by the cleaving of a hydrogen atom off a polymer in the presence of oxygen. The polymer forms a peroxide. The high reactivity of the peroxide propagates this reaction throughout the material. The reaction is terminated by an oxygen radical cleaving its own polymer and forming a methylene group at one end and a carbonyl group at the other end (Figure 2.1) (Kuzina and Mikhailov 2001).

The photo-oxidation of polyesters such as poly(ethylene-terephthalate) terminates in a slightly different way. The polymer chain is broken by the cleaving of a carbon-oxygen bond, which ultimately leaves a carbonyl-group (-C=O) at one end and a carboxyl-group (-COOH) at the other end (Figure 2.2) (Fechine et al. 2002).

These characteristic polymer chain end groups offer the possibility to detect plastic deteriora-
CHAPTER 2. DETERIORATION OF PLASTICS

(a) Initiation: the backbone is radicalised, then peroxidised; $h\nu$: High frequency (UV) light.

(b) Propagation: the peroxide continues to radicalise the backbone.

(c) Termination: ultraviolet radiation splits the hydrogen-peroxide, which cleaves the backbone, as the oxygen radicals claim a single bond and a hydrogen atom respectively.

*Figure 2.1: Photo-oxidation of polystyrene (Kuzina and Mikhailov 2001).*

tion at a level that is undetectable from the perspective of mechanical strength. The carbonyl or hydroxyl density can be detected by infrared spectroscopy or electron spectroscopy as an indicator of chain-ends that are typical for photo-oxidation (Allen et al. 1994; Nakayama et al. 1996).
The durability of plastic items, degradation and degradability have been widely researched with a focus on material properties and how plastics withstand environmental stress. The degradability of plastics has also been the focus of extensive research in the field of chemistry with the objective to facilitate waste disposal and resource reclamation (Karaduman 2002). Considerable research effort has gone into the development of various types biodegradable plastics (Singh and Sharma 2008). However, biodegradable polymers can still leave a non-degradable residue (Klemchuck 1990; Ryan et al. 2009). Plastic deterioration can be quantified by the material’s tensile properties (International Organization for Standardization 2010). As the material degrades it sheds fragments and its surface area will consequently decrease. Loss of surface area has therefore also been used as a measure of plastic deterioration (O’Brine and Thompson 2002).
CHAPTER 2. DETERIORATION OF PLASTICS

2010).

In the context of environmental contamination, the fouling of a material by organisms could play a part in slowing material deterioration as fouling will attenuate the amount of UV radiation reaching the surface, which would otherwise cause photo-oxidation. The UV absorption of the fouling layer on plastic has therefore also been measured as a predictor of the amount of photo-oxidation on the sample (O’Brine and Thompson 2010).

While some plastics (e.g. polyethylene blends with polycarbolactone or starch) can indeed be completely digested by bacteria and fungi (Tilstra and Johnsonbaugh 1993; Chiellini et al. 2003), the definition of 'photodegradability' and 'biodegradability' can be misleading, especially at a consumer level, and interpreted as a justification for disposing of plastics negligently. In reality degradable plastics rely on ideal conditions, which can vary, especially between photodegradable plastics, which require sunlight, and biodegradable plastics that need composting conditions. Also many plastics that were designed for enhanced degradability have been found to remain in the environment as non-degradable residues (Klemchuck 1990). Plastics with enhanced degradability might therefore actually accelerate the formation of microplastics.

The total demand for plastics in Europe was 47 million metric tonnes in 2011. The six most widely used resin types in Europe in 2011 were polyethene 29 % (low density 17 %, high density 12 %), polypropylene 19 %, polyvinyl chloride 11 %, polystyrene 7.5 %, poly(ethylene terephthalate) 6.5 %, and polyurethane 7 % (PlasticsEurope 2012). Polyvinyl-chloride and polyurethane are mostly used in construction, automotive and other industries. 39.4 % of the total plastics demand in Europe goes towards packaging. The material types that are mainly used in packaging are polyethene, polypropylene, polystyrene and poly(ethylene-terephthalate), which is almost exclusively used for packaging (PlasticsEurope 2012). This study will therefore focus on polyethene, polystyrene and poly(ethylene terephthalate). In recent years there has been increasing production of plastic materials with enhanced degradability. A biodegradable material was examined in this study along with traditional materials in order to compare deterioration and thus indicate the potential environmental effectiveness of such materials.

Polyethene (also 'Polyethylene' or 'Polythene') is a simple organic polymer. It is made by the
polymerization of ethene, two carbon atoms linked with a double bond (two shared orbitals) and two hydrogen atoms attached to each. In polyethene one electron from the ethene double bond is used to bind the next carbon atom. Thus, each carbon atom has one shared orbital with each of its neighbouring carbon atoms. The other two bonds remain shared with either hydrogen atom. The polymer is terminated with a methyl group (one carbon atom and three hydrogen atoms) at each end. 13.6 million metric tonnes (29% of total plastic) of polyethene were produced in Europe in 2011.

Polystyrene (IUPAC: Poly(1-phenylethane-1,2-diyl)) is the polymer of styrene. Polystyrene is synthesised in an exothermic reaction of styrene that is initiated by temperature or a peroxide (PlasticsEurope 2008). In 2012 the demand for polystyrene in Europe was 3.6 million metric tonnes (8% of the market for polymers). Polystyrene ranks fourth in demand with Poly(ethylene terephthalate) behind polyethene, polypropylene and polyvinyl chloride. PlasticsEurope (2013) reported that Europe contributes 26% to the world market for polystyrene. The worldwide annual demand is therefore approximately 13.8 million metric tonnes. The main application for polystyrene in Europe is packaging (more than a third). Further applications are consumer electronics (13%), appliances (12%) and construction (11%). Only 1% is used in medical application, and the remaining 26% of usage is listed as ‘other’ (PlasticsEurope 2013).

Poly(ethylene terephthalate) (also ‘polyester’) differs from the other tested materials as it has ester groups in the main chain. Poly(ethylene terephthalate) is synthesised from terephthalic acid (TPA) and therefore also features a benzene ring. The synthesis of poly(ethylene terephthalate) is a two-step process (ChemSystems 2009). Bis-hydroxyethyl terephthalate (bis-HET), a prepolymer of poly(ethylene terephthalate) is produced from either terephthalic acid or dimethyl terephthalate and ethylene glycol. The bis-HET is then polymerised in an endothermic reaction. In 2011, poly(ethylene terephthalate) constituted 6.5% of the European demand for plastic (3 million metric tonnes). Even though it is a highly versatile material (PlasticsEurope 2013) it is almost exclusively used in packaging (PlasticsEurope 2012, 2013).

Biothene™ is licensed by Biothene, 27 Old Gloucester Street, London, England, WC1N3AX. It is based on a concept developed by the Royal College of Forestry, Stockholm, Sweden. Bio-
CHAPTER 2. DETERIORATION OF PLASTICS

Biothene is not a plastic itself, rather it is an active metal ion based additive that can be added to plastics, typically polyethylene to form a degradable material. Degradation is activated by UV light and triggers the breakdown of polymer chains that continues even when the material is no longer exposed to sunlight. The manufacturer’s product specification states that depending on environmental conditions the material completes degradation into hydrogen, carbon and a small amount of inoffensive mineral oxides within eighteen months after first exposure to ultraviolet light. Heat accelerates the degradation (http://www.biothene.co.uk, Apr. 29 2012). The data sheet provided on the Biothene website stresses that all the residues are non-toxic. While there is some literature on the deterioration of oxo-degradable plastics (O’Brine and Thompson 2010), there is no mention of Biothene in the peer-reviewed literature. Osawa (1988) states that metal ions that have been photo-activated can excite organic bonds and thus trigger a disintegrative chain reaction. However Osawa (1988) also argues that metal ion-polymer interactions are highly complex. The carrier material for Biothene is polyethylene. The degradation cascade is therefore assumed to be the same as that described for polyethylene. At the time of writing the company website did not provide any evidence to illustrate their statements about degradation time or degradation products.

Plastic debris is abundant in the ocean, and the rising amount of microplastics suggests that this debris is deteriorating and generating microscopic fragments. But plastic deterioration is likely to be slower in the ocean than on land. UV radiation - a main driver of plastic deterioration - is reduced by water and also by colonisation of the material by organisms; additionally, lower temperatures and the salinity can reduce the rate of chemical reactions that cause plastic deterioration (Gregory 1999; Gregory and Andrady 2003).

Plastics are frequently washed ashore from the ocean but may previously have been transported for extended periods of time. This is evidenced by remote beaches with no anthropogenic activity being littered with plastics (Lucas 1992; Barnes 2005; Bond et al. 2014). Marine debris may therefore spend significant amounts of time on land, and they may thus alternately be exposed to two environments that would affect their deterioration rate in different ways. In order to identify variations in the generation of marine microplastics, the deterioration of plastics on
2.2 METHODS

land has to be compared to the deterioration of plastics in water.

While the deterioration of various types of plastic has been studied in the laboratory (Sammon et al. 2000; Singh and Sharma 2008; Müller et al. 2012), little is known about the deterioration of plastics in the natural environment. With the emergence of microplastics from material deterioration, exploring these deterioration processes is crucial to the understanding of the microplastics generation rates.

This chapter addresses the thesis’ aim to quantify the difference in rate of deterioration of polyethene, polystyrene, poly(ethylene terephthalate) and biothene - a degradable kind of plastic - in sea water, fresh water and on land, as well as in illuminated versus shaded locations. The overarching aim was to verify that plastics indeed deteriorate faster in air than in water.

In order to quantify the the deterioration of the four selected materials, they were exposed to a variety of environments, and their degree of deterioration was measured at regular intervals. Indicators for deterioration were loss of tensile strength and extension, loss of molecular bond density and surface area loss.

A possible causal link between surface area loss and tensile properties was also examined in order to ascertain the independence of surface area loss as an indicator of material deterioration.

Whether beaches are a hearth for rapid plastic deterioration in marine environment is tested by comparing the rate of deterioration of the four selected materials in air versus fresh and sea water and also in light versus shade.

The deterioration rate of each material in each environment was observed to see if some materials transition into microplastics at a faster rate than others.

2.2 Methods

2.2.1 Material

To represent the four plastic types described in Chapter 1, the following plastics were sourced as carrier bags or films:

**Biothene** 500 375 ØE 450 + 75 mm White Biothene Carrier Bags were purchased from http:
CHAPTER 2. DETERIORATION OF PLASTICS

Polyethene 500 375 OE 450 + 75 mm White Polyethene Carrier Bags were purchased from http://www.midpac.co.uk, see address above.

Polystyrene One roll of 300 mm wide ST311050 Polystyrene Film 50 ㎛ thick was purchased from BANDO CO., LTD., 704-900, 358-66, Galsan-dong, Dalseo-gu, Daegu, 704-900 KOREA.

Poly(ethylene terephthalate) One roll of 300 mm wide Polymex PI700 matte translucent untreated polyester film 50 ㎛ thick was purchased from http://www.polymex.co.uk, Polymex Limited, Meridian House 62 Station Road, North Chingford, London, E4 7BA, United Kingdom.

2.2.2 Sample preparation

Each material was cut into 180 strips, each 5 by 30 cm. Each strip was then labeled with the material type and a unique three-digit-number. No more than one sample was extracted from each carrier bag to ensure independence of each replicate. The thickness of each sample was measured. It was weighed and photographed against a scale background (Figure 2.3).

2.2.3 Sample exposure

To quantify the difference in plastic deterioration rate, samples were exposed in three environments: sea water, fresh water and air. In each environment one well illuminated location and one shaded location was selected. One set of samples was kept in a cardboard box in the laboratory as a control. The locations are listed in Table 2.1.

There were 180 replicates of each material so that five samples could be removed in each of the seven locations on each of five sampling dates, leaving five samples per type of material for initial measurements. Sampling was conducted after 100, 240, 360, 480 and 600 days exposure. Therefore, five cohorts of material samples were exposed to each of the seven treatments. The 100 days cohort was exposed exactly one year after the other four sets. This sampling date was added to the experiment after the observation of rapid (less than 240 days) deterioration, especially of polystyrene, when exposed in illuminated air.
Table 2.1: Exposure locations in Plymouth, UK and corresponding mean variables ± standard deviations. Light intensity in air measured at ground level and at sample height.

<table>
<thead>
<tr>
<th>Environment</th>
<th>Site Description</th>
<th>Lighting</th>
<th>Location</th>
<th>Salinity at 100 cm depth ($10^{-3}$)</th>
<th>Temperature at 100 cm depth (°C)</th>
<th>Visibility (m)</th>
<th>Light intensity (µmol·s$^{-1}$·m$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sea-water</td>
<td>Queen Anne’s Battery Marina, visitor pontoon (50.365°N, 4.132°W) in 1 m of water (Figure 2.5c)</td>
<td>Illuminated</td>
<td>Along the West side of the pontoon, Underneath the pontoon</td>
<td>0.1 ± 0.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14.49 ± 0.55</td>
<td>1.63 ± 0.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>31.14 ± 24.71</td>
<td>31.05 ± 18.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Air</td>
<td>Skardon Garden (50.378°N, 4.137°W) (Figure 2.5a)</td>
<td>Shaded</td>
<td>Underneath the pontoon / 0.1 ± 0</td>
<td>14.59 ± 0.53</td>
<td>0.84 ± 0.17</td>
<td>38.69 ± 35.99</td>
<td>20.51 ± 17.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Illuminated</td>
<td>South-facing wall, 3 m above ground, North-East-facing wall, 1.5 to 2 m above ground</td>
<td>29.49 ± 2</td>
<td>11.84 ± 0.37</td>
<td>3.38 ± 0.71</td>
<td>33.04 ± 38.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Shaded</td>
<td>North-East facing wall, 1.5 to 2 m above ground</td>
<td>29.56 ± 2</td>
<td>11.81 ± 0.37</td>
<td>3.94 ± 0.65</td>
<td>1.4 ± 0.72</td>
</tr>
<tr>
<td>Fresh water</td>
<td>Drake Reservoir, central strip (50.37°N, 4.137°W) in 1 m of water (water level upon exposure date, Figure 2.5b)</td>
<td>Illuminate</td>
<td>Off the South edge of the strip, Off the North edge of the strip</td>
<td>51.30 ± 67.39</td>
<td>98.55 ± 55.57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Plymouth University, Davy Building Room 702</td>
<td>Shaded</td>
<td>Off the North edge of the strip</td>
<td>4.95 ± 1.77</td>
<td>11.28 ± 11.48</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER 2. DETERIORATION OF PLASTICS

Figure 2.3: Material samples (in this figure polyethene terephthalate) ready to be attached on wooden beam clamps.

Wooden beam clamps were used to deploy the samples in a manner following O’Brine and Thompson (2010). Each set of material samples contained five replicates of each of the four material types. Each set was attached to a wooden beam clamp in an irregular order that assured a non-repetitive sequence of material types to minimise spatial confounding. The replicates of each material type were distributed evenly across sequence numbers to eliminate effects of material quality between sets of samples. Samples were cushioned with several layers of the same material originating from the same bag or area on the coil respectively in order to protect the samples from being torn off along the edge of the wooden beam clamp. Copper staples were used to attach the samples. The two wooden beams forming the clamp were attached together using brass screws. Copper and brass were selected for their excellent robustness in sea water (Figure 2.4).

The wooden beam clamps were placed in each environment. In aquatic environments they were weighed down with a building brick at each end of the beam to prevent floatation and were deployed at a depth of 1 m. The beams assigned to air were attached to a wall on 30 cm shelf brackets to prevent the samples from being abraded against the wall (Figure 2.5).
2.2. METHODS

(a) Wooden beam clamp with attached samples.

(b) Detailed schematic of sample attachment method showing the brass screw, the upper beam, the cushioning material layers, the sample, the copper staples and the lower beam.

Figure 2.4: Illustration of wooden beam clamps for material sample exposure.

In order to relate the exposure response to environmental parameters, temperature, salinity, light intensity and visibility were measured in both lighting conditions, in all three environments and the control environment (where applicable, see parameters in Table 2.1). The light intensity was measured at 50 cm depth in the aquatic environments, and at sample height in air. Additionally the light extinction in metres was measured using a secchi disk in fresh water and sea water.
2.2.4 Measuring deterioration

Prior to and after exposure, samples were collected and weighed using a balance accurate to 10 mg, their thickness was measured in five sectors using a mechanical calliper that was accurate to 20 µm and their surface area was assessed visually as a percentage of their original area. The samples were photographed against a scale background. Material samples that had been exposed in the marine environment were also photographed submerged in seawater to better visualise settled organisms.

Surface area loss on some of the samples retrieved from the wooden beam clamp in the illuminated air location was due to the samples tearing off rather than to deterioration; these samples were not used in the analysis as they could no longer be identified with any certainty. An examination of the causal link between tensile properties and the surface area loss was, therefore, needed. In the case of this causal link a loss of tensile extension or strength would be the cause for reduced surface area in the subsequent sampling cycle (e.g. the tensile extension of biothene had decreased on the 240 days sampling cycle, therefore the surface area would be reduced in the 360 days sampling cycle). A linear model of the means of all material samples in each of the treatments of surface area loss versus inverse standardised tensile extension and inverse standardised tensile strength \((1/(0.2 + x \times 0.8))\) was calculated. The factor 0.8 was introduced to avoid infinite or extremely large values) in the subsequent and in the same cohort was run.
2.2. METHODS

**Tensile properties**

Measurements of tensile properties were conducted on all four materials. The specimens were cut using a Swann-Morton stainless steel surgical blade 11 ref. 0303, on a cutting mat. Tensile properties were assessed with an apparatus that complied with ISO 5893 (Instron, system ID 3345 k1669, force transducer model 2519-104, capacity 500 N, Figure 2.6). The elongation rate was set to 40 mm min$^{-1}$. BSI (1996) suggests a set of different specimen shapes for tensile properties tests. A preliminary test of these specimen shapes suggested that specimen type 2 (Figure 2.7) was the most suitable shape for the measurement of tensile properties for all four materials. The specimen dimensions used in this experiment were $b = 10\text{ mm}$, $l_3 = 150\text{ mm}$ and $l_0 = 50\text{ mm}$. In accordance with British Standards Institution (1996) $l$ was set 50 mm as the maximum elongation of biothene and polyethene at $l = 100\text{ mm}$ was nearly exceeding the instrument’s range. In the data evaluation the mean measured thickness of each sample was inserted for $h$. The applied measuring method returned the extension and the tensile strength of the sample at breakage. Both are indicators of the material’s ability to withstand mechanical stress. Extension indicates elasticity and tensile strength indicates resistance.

**Fourier transform infrared spectroscopy (FTIR)**

In order to monitor deterioration effects on the molecular composition of the materials, FTIR was conducted on five replicates of each material sample.

Prior to FTIR any accumulations of loose material or organisms were removed from the area on the sample where the FTIR specimen would be extracted using a cotton bud soaked in absolute ethanol. Absolute ethanol was selected for its excellent evaporation properties, which eliminate the risk of FTIR recording alcohol residues. FTIR scans before and after the application of absolute ethanol confirmed that this procedure did not affect the FTIR readings of the samples. For consistency the cleaning procedure was conducted on all samples irrespective of any fouling for consistency. The condenser of the microscope was adjusted for maximum amplitude of transmitted energy as recorded by the Bruker OPUS TM 5.5 software package.

Replicates of each material on each sampling occasion were extracted from two locations (prox-
IMAL and distal to where the wooden beam clamp was attached): on both edges of the strip and from the centre of the strip, where the specimen for tensile properties measurement was extracted. The replicates were placed on a two-part diamond window microscope slide. The specimen was pressed to minimum thickness between the two slides for best transmission. The specimen was positioned as to ultimately leave at least half of the diamond window open, so a background scan could be conducted. The scans were conducted with a Bruker Hyperion 1000 FTIR microscope and analyzed in OPUS TM 5.5. The shutter field of view was then directed
2.2. METHODS

Figure 2.7: Specimen type 2 (British Standards Institution 1996) applied in the tensile properties test. $b$: width, $h$: thickness, $l$: grip distance, $l_0$: gauge length, $l_3$: overall length.

on an open area of the slide. 32 background scans were performed. The shutter field was then directed at the sample, and the software was prepared for the sample scan. A preliminary real-time display of the absorption spectrum allowed optimal adjustment of the shutter field so as to enable maximum transmission. Finally, 32 sample scans were performed. Absorption spectra that showed an attenuation coefficient equal to or greater than six were repeated, as the peak of the reading was off the chart due to variable specimen thickness and could no longer be quantified. The microscope slide was wiped with a tissue, soaked in absolute alcohol after each sample.

A baseline correction was applied to the FTIR reading in order to compensate for any overall slope of the spectrum. The baseline was calculated as the slope between two areas of the spectrum that did not show any elevation. Two such areas could be defined that were valid for all four material types. They were 3900 to 3750 and 2250 to 2100 waves per centimetre (cm$^{-1}$). The slope between the mean absorptions of these two areas was subtracted from the spectrum rendering the baseline level. The minimum reading was subtracted from the whole spectrum to eliminate any negative readings resulting from the previous transformation.

The resulting spectra of non-exposed material samples and samples exposed to illuminated air (exposed to the highest UV radiation) were examined visually to identify absorption bands that had changed over time. These bands were selected for statistical analysis (Figure 2.8).
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Figure 2.8: FTIR spectra (attenuation coefficient vs. wavenumber (cm\(^{-1}\))) of non-exposed (black solid line) and exposed in illuminated air environment for 600 days (28 days for polystyrene) (red solid line). The peaks of the red vs. the black line were selected for further examination.

Ultraviolet absorption

UV absorption was introduced as a parameter in the later course of the experiment upon collection of the 360 days cohort. UV absorption was used as a potential predictor rather than an indicator of deterioration, as the amount of UV light that passes through any fouling, which may have accumulated on the sample could affect the material properties. UV absorption was measured in a room with constant fluorescent light to avoid any disturbance by natural light. The UV radiation transmitted from a 365 nm UV light through the material sample to a UV-A sensor reading 400 to 315 nm was measured. In order to only read the light transmitted through the sample, the sensor was contained in a chamber with a window that was entirely covered by the sample (Figure 2.9).

The readings were taken in mW cm\(^{-2}\). For each material sample one background reading (no
sample, $34.08 \pm 2.94 \text{ mW cm}^{-2}$) and two sample readings (one in the least and one in the most fouled area, if obstructions could be found) were generated. Absorption was recorded as one minus the quotient of the background reading and the mean sample reading. The background reading in mW cm$^{-2}$ translates into $1.04 \pm 0.09 \mu\text{mol s}^{-1} \text{ m}^{-2}$ after $E = hci$ where $E$ is energy, $h$ is Planck’s constant ($6.626 \times 10^{34}$), $c$ is light speed ($3 \times 10^8 \text{ m s}^{-1}$) and $l$ is the wavelength for reference with the irradiance readings in Table 2.1.

2.2.5 Experimental design

The experiment was laid out in a three-way design. The 3 fixed factors were 'material', 'environment' and 'lighting'. Each level in the orthogonal combination of factors was replicated five times (five samples of the same material on each wooden beam clamp). The array was initially set up in four cohorts to be collected and analysed 240, 360, 480 and 600 days after deployment. A second (asynchronous) series was deployed exactly one year after the first in order to obtain a 100 days cohort. Finally, a third series was deployed after polystyrene deteriorated in air before the 100 days cohort was collected. This third series only accounted for polystyrene
in illuminated air. Samples were collected at seven days intervals over a 28 days period. A time zero control was conducted for each series, and each series was represented in the control environment.

2.2.6 Data analysis

Due to the large number of tested treatments, sample analysis, the sample size per treatment had to be kept small in order to keep the experiment manageable \((n = 5)\). Therefore, the data analysis was designed to accommodate data with non-normally distributed residuals. PRIMER v6 (Clarke and Gorley 2006) and its PERMANOVA+ (Anderson et al. 2008) package are not affected by non-normal distribution of residuals as they use a similarity index between samples within and across groups. Rather than the distribution of values within groups of data points, the similarity of pairs of data points is used to establish differences between groups.

Changes in material properties over time were tested by observing deterioration metrics (tensile properties, molecular constitution and surface area) between samples of the same material in the same environment at different exposure times. Continuous change of these metrics over time (uniform increase or decrease) would indicate that materials do in fact deteriorate over time. Non-continuous change (alternating increasing and decreasing of an effect) would indicate that factors other than time (such as quality differences) affect material stability. The significance of any differences between exposure times was tested using permutational multivariate analysis of variance (PERMANOVA).

The effect of light was tested by pairing each set of samples that were exposed in well illuminated environment with its counterpart in shaded environment. A significant difference between samples exposed in illuminated and shaded environment that would uniformly increase along sampling dates would indicate that light influences material deterioration.

2.3 Results

Of the 840 material samples that were deployed, 663 were recovered. FTIR could only be conducted on 603 where a sufficient amount of the exposed part of the samples was still available. Tensile properties were measured on the 530 samples that had sufficient remaining surface area
to extract an appropriate specimen. UV absorption was measured on the 501 samples that still had sufficient remaining surface area and whose absorbance had not already been altered by fouling removal, as UV absorption measurements were only introduced upon collection of the 360 days cohort.

The 600 days cohort in the shaded sea water environment had become detached from the pontoon several months before its scheduled collection date, and it had remained on the sea floor for an undetermined amount of time. Due to construction works in Queen Anne’s Battery Marina, the samples could not be recovered until a few days before the scheduled collection date. These samples may have suffered additional physical strain e.g. from abrasion on the seabed, but they may also have been better protected from ultraviolet radiation than the other samples. The affected samples were included in the analysis, but the results were interpreted in the view of a possible effect of the samples becoming detached from their original location.

Over the whole 600 day period surface area loss of all four material types was observed in sea water and air, and both lighting conditions. FTIR was conducted on biothene, polyethene and poly(ethylene terephthalate). These still had a sufficient amount of the relevant surface area left in sea water and air, in both light and shade. The remaining surface area on these three materials was also sufficient to test tensile properties on the samples that were exposed in the shaded location of both sea water and air.

The 20 polystyrene samples that were exposed in air over a 28 days period underwent almost complete surface area loss in the first two weeks of exposure (Figure 2.10). UV absorption and tensile properties were therefore only measured on the samples that were collected after 7 days. Surface area loss and FTIR were measured on all 20 samples.

2.3.1 Change in material properties over time

The most pronounced effect over time was surface area loss in illuminated air (Figure 2.9). Biothene, polyethene, polystyrene and poly(ethylene terephthalate) lost nearly all their surface area after 600 days exposure (biothene: $t = 179.95$, $p < 0.001$; polyethene: $t = 238.95$, $p < 0.001$; polystyrene: no statistics were calculated as the denominator was zero (100 % lost) and poly(ethylene terephthalate): $t = 108.08$, $p < 0.001$) in illuminated air. Polystyrene lost almost
all its surface area after 14 days, and very little polystyrene was left after 100 days in shaded air. However, the other materials retained almost all their surface area until 600 days exposure in the shaded air location. Polystyrene was also the only material that began to lose surface area in sea water. This occurred in both the illuminated and the shaded locations after 360 days exposure. However, in the air location, detached but largely undamaged material samples were found on the ground below the wooden beam clamps.

Surface area loss significantly increased with decreasing tensile strength and extension. Tensile strength and extension were reliable predictors of surface area loss in the next sampling event, and materials that had lost surface area exhibited lower tensile strength and extension on the
2.3. RESULTS

Table 2.2: Collinearity of surface area loss versus inverted tensile extension and strength in previous and same sampling event.

<table>
<thead>
<tr>
<th>Sampling event</th>
<th>Metric</th>
<th>Coefficient B</th>
<th>Significance p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous</td>
<td>Extension</td>
<td>0.22</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Strength</td>
<td>0.19</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Same</td>
<td>Extension</td>
<td>0.19</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Strength</td>
<td>0.99</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

remaining sample in the same sampling event (Table 2.2). However, these linear models exhibited highly non-normal residuals (Anderson-Darling test $A = 12.62, 12.82, 13.10$ and $13.18$ and $p < 0.001$ for all four materials). A non-parametric approach was therefore used to verify the results of the collinearity analyses.

The non-parametric test (Primer v6 RELATE statistics) confirmed that surface area loss was significantly correlated with tensile properties in the previous and in the same cohort (previous cohort: $r = 0.36, p < 0.001$, same cohort: $r = 0.32, p < 0.001$). The sample tearing off due to reduced tensile strength and extension was therefore a more likely explanation for surface area loss than material disintegration. The remaining surface area was thus largely determined by the breaking point of the sample upon tearing, which is arbitrary considering that tensile properties changed throughout the sample. The proportion of samples remaining intact was therefore a more reliable metric for material deterioration than the remaining amount of surface area.

Tensile properties also underwent substantial change over time in all materials. For biothene tensile extension (Figure 2.11) was almost entirely lost in illuminated air, between 0 and 360 days exposure ($t = 9.79, p = 0.003$), after 240 days with polyethene ($t = 9.08, p < 0.001$) and after seven days with polystyrene ($t = 3.67, p = 0.006$, Figure 2.10). Only one sample of poly(ethylene terephthalate) was left in the illuminated air environment after 360 days. Its tensile extension was also substantially lower than at initiation; no significance could be established for this effect as only one sample was left. Tensile extension of biothene significantly decreased from a factor $6.38 \pm 0.96$ to $4.55 \pm 0.58$ of the original length in the control environment ($t = 3.86, p = 0.005$ between 0 and 600 days exposure) indicating that biothene deteriorates in air with no measurable light. Meanwhile, the decrease of biothene tensile extension was still faster in the natural environments. It was slow at first in shaded air, and shaded and
illuminated fresh water with no significant change between 0 and 100 days, and a continuous
decrease thereafter. The decrease was faster in illuminated air than in shaded air, and faster in
the first 240 days in both illuminated and shaded seawater than in air; however, after 240 days
in both seawater locations there was no further change (Figure 2.11).

Polystyrene also rapidly lost tensile extension in shaded and illuminated sea water in the first
100 days exposure, but continued to fluctuate throughout the rest of the experiment (Figure
2.11). In shaded air, tensile extension of all materials, except poly(ethylene terephthalate),
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decreased. The decrease occurred after 100 days in polystyrene and after 600 days in biothene and polyethene (Figure 2.11).

Tensile extension proved to be a good indicator of material stability. It divides materials and treatments, and it could show the mechanic effects of molecular changes. All materials lost stability most rapidly in illuminated air, polystyrene being the most extreme with almost complete loss of tensile extension within a week. Polyethene (and presumably polystyrene, based on samples tearing off) became unstable at a significantly faster rate in shaded air than in water. Biothene and poly(ethylene terephthalate) retained the same stability in shaded air as in water; light was therefore their main driver for stability loss.

Contrary to tensile extension, tensile strength of polyethene and polystyrene remained within the non-uniform fluctuations that were also observed in the control environment, due to which effects over time remain uncertain, even if they were statistically significant (Figure 2.12). An ad-hoc examination of control samples revealed that there was actually a difference in tensile properties between the original exposure set and the exposure set that was added to obtain a 100 days cohort one year later, in biothene and polystyrene (see Figure 2.13). With this in mind biothene and poly(ethylene terephthalate) both gradually lost tensile strength between 0 and 600 days exposure in shaded ($p = 0.04$ and $p = 0.01$ respectively, see Figure 2.12) but not in illuminated sea water. Biothene and poly(ethylene terephthalate) lost tensile strength at a similar rate in illuminated air ($p = 0.02$ and $p = 0.08$ (due to only one remaining sample) respectively), but poly(ethylene terephthalate) deteriorated at a substantially lower rate than biothene in shaded air (Figure 2.12). Light, therefore, has a stronger effect on poly(ethylene terephthalate) than on biothene.

Another property that showed a substantial change over time was the molecular constitution, foremost of polystyrene, but also of biothene and polyethene (Figure 2.15). As measured by the infrared light absorption at the specific band, the density of hydroxyl (3450 cm$^{-1}$), carbonyl (1713 cm$^{-1}$) and other photo-oxidation products (1100 cm$^{-1}$) of polystyrene all increased 2 to 4 times more within 28 days exposure in illuminated air than within 100 days in shaded air. The density increase of these oxidation products was uniform but became slower over
Figure 2.12: Maximum tensile strength of all four materials in all environments and both lighting conditions and standard errors plotted against exposure time (days, x-axis). Open symbols: illuminated; filled symbols: shaded; circles: sea water; squares: fresh water; triangles: air; shaded area: standard error interval of controls. The development of tensile strength in controls only differed from samples in the environment with biothene and with poly(ethylene terephthalate) in illuminated air. The data series are shifted along the x-axis for clarity.

... time in illuminated air (Figure 2.14). Changes in the attenuation coefficient of the 1100 cm\(^{-1}\) absorption band between the 21 days and the 28 days cohort were not statistically significant.

In sea water the density of hydroxyl and other photo-oxidation products (but not carbonyl) increased in the first 100 days. However, the interpretation of this reading cannot be directly compared with samples from other deployment dates as the 100 days exposure samples were deployed one year after the rest. The further development of these density readings is not uniform, and it seems that time was not the main driver of oxidation in sea water.
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Figure 2.13: Difference in tensile strength and extension of biothene and poly(ethylene-terephthalate) between the controls from the first exposure set (plot 1) and the controls from the second exposure set (plot 2, deployed 1 year apart), solid bar: median, box: interquartile range, outliers: outside 4 interquartile ranges, error bars: range not counting outliers, summary ANOVA results annotated.

2.3.2 Difference between environments

The previous analyses indicated a strong effect of light on deterioration, therefore it was important to characterise the light intensities (Table 2.1) in the various environments. The visibility in the water environments is also important, as it reduces light reaching the sample surface. For example illuminated air had by far the highest irradiance, and in air, the contrast between the illuminated and the shaded location was the largest. Irradiance in illuminated fresh water was less than a third of that in illuminated air, but shaded fresh water had a higher irradiance than shaded air. In fresh water the visibility was lower than in sea water, but irradiance in sea
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Figure 2.14: Evolution of the oxidation absorption bands of polystyrene in illuminated air environment. The solid line shows the spectrum of non-exposed polystyrene. Exposure time in days is annotated.

water was by far the lowest. 4.37% of this irradiance would be UV ($\lambda < 400$ nm at terrestrial global 37 South facing tilt according to ASTM Standard (2008)). Due to the limitations of the measurement equipment, light intensity could only be measured at maximum cord extension (50 cm), while samples were deployed at 100 cm. The light intensities in fresh water and sea water are therefore likely to be overestimates.

Biothene, polyethene and polystyrene exhibited considerable oxidation as measured through
the carbonyl content in both illuminated and shaded air. No significant change of hydroxyl or carbonyl was recorded in any of the other environments except for a marginal carbonyl increase with polyethylene in illuminated sea water. However, polystyrene lost almost all its tensile extension after 100 days in both light and shaded sea water, and the uniform loss of tensile extension of biothene was also recorded to be faster in both light and shaded sea water than in the control environment. In fact the loss of tensile extension was faster in sea water than in air in the beginning. While biothene tensile extension continued to steadily decrease in air, it did not significantly change between 240 and 600 days in sea water.

The relationship between the molecular constitution and tensile properties was not the same in air as in sea water, as shown by the RELATE analysis in PRIMER-v6 (Table 2.3). While tensile extension and molecular constitution were strongly linked in biothene, polyethylene and polystyrene that were exposed in air, there was no such effect in sea water. The RELATE statistics compares the spearman rank correlation of the pairs of tensile extension and carbonyl absorption band values to the correlation of random permutations of these pairs as illustrated in Figure 2.16 and Figure 2.17. The analysis showed that in air there was a significant correlation between the number of polymer chain ends (carbonyl groups) and the materials stability as measured by tensile extension. In sea water, where material stability was also reduced over time (see Figure 2.11) this effect was not linked to an increase in molecular chain ends. In sea water a factor other than chemical deterioration caused the materials to destabilise.

Another parameter that differed substantially between environments was the UV absorption of the materials, and while materials differed as illustrated in Figure 2.24 and evidenced in a pairwise PERMANOVA, they seemed to follow a similar change over all tested treatments (Figure 2.18). In both illuminated and shaded sea water the UV absorption of all materials rose to 100% after 100 days exposure, which coincided with the growth of a substantial amount of fouling on the samples. The UV absorption dropped again after 600 days in shaded sea water in coincidence with a decrease in fouling. UV absorption also uniformly increased in fresh water, but at less than half the rate, and one to two orders of magnitude more slowly in air.
Figure 2.15: Continued on next page, see caption at end.

(a) Biothene, 3450

(b) Biothene, 1713
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(c) Polyethene, 3450

(d) Polyethene, 1713

Figure 2.15: Continued on next page, see caption at end.
Figure 2.15: Continued on next page, see caption at end.
Figure 2.15: Change in the FTIR attenuation coefficients at isolated wavenumbers (cm$^{-1}$) over time (days). Open shapes indicate illuminated, solid shapes shaded locations. Squares for fresh water, triangles for air, circles for sea water. Error bars indicate standard errors. The lines that represent the environments were shifted against each other along the x-axis to prevent the error bars from overlapping. The shaded area shows the standard error interval of the controls. The grey line shows the FTIR absorption spectrum of the material before exposure with indication of the attenuation coefficient interval (horizontal grey dotted lines) and the wavenumber (vertical grey dotted line) reference for the effect versus time plot. There is no scale for the spectrum.
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Table 2.3: RELATE statistics from PRIMER v6 showing Spearman’s Rho for the correlation of tensile extension and the Carbonyl absorption band value and its significance level based on 9999 random permutations of the values.

<table>
<thead>
<tr>
<th>Environment</th>
<th>Material</th>
<th>Spearman’s Rho</th>
<th>Significance (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>Biothene</td>
<td>0.614</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Polyethene</td>
<td>0.554</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Polystyrene</td>
<td>0.293</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Sea water</td>
<td>Biothene</td>
<td>0.034</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>Polyethene</td>
<td>0.006</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Polystyrene</td>
<td>0.048</td>
<td>0.15</td>
</tr>
</tbody>
</table>

To quantify the absorption of UV light, the proportion of a UV irradiance of $1.04 \pm 0.09 \mu\text{mol s}^{-1} \text{m}^{-2}$ that passed through the material sample was measured. The irradiance used in the absorption measurement corresponded approximately to the UV irradiance in the tested environments (4.31 in illuminated, 0.49 in shaded air, 1.36 in illuminated, 0.90 in shaded fresh water, 0.11 in illuminated and 0.02 in shaded sea water). While UV absorption measurement was therefore a slight overestimate in air, it corresponded to conditions in fresh water and produced an underestimate on the sea water samples. The absorbed proportion of the UV irradiance present at the sea water exposure site was likely higher than measured, and the amount of UV light passing through the samples during the most part of their exposure was zero.

The tested environments, of course, vastly differed in the presence or absence of air and UV radiation, and so a variety of factors may have resulted in the measured differences (i.e. change in molecular constitution, change in tensile properties and loss of surface area) between environments. Structural integrity of all materials declined substantially faster in air than in fresh water or sea water. The effects were also not uniformly dependent on the environment. In sea water the materials’ tensile properties changed while their molecular constitution remained constant.

2.3.3 Difference in deterioration processes between materials

Materials differed in surface area loss, UV absorption, tensile strength and extension, hydroxyl and carbonyl absorption bands. They also differed in the pattern of change in these effects between treatments (i.e. air, fresh water and sea water, light and shade). One treatment versus another did not have the same effect on one material as on the other material. For instance, the change in tensile strength between fresh water and air was not the same with biothene as with
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In terms of tensile properties, molecular constitution and surface area loss, polystyrene had a much faster deterioration than the other materials. This was clearly illustrated in Figure 2.11, Figure 2.15 and Figure 2.19. The tensile extension of polystyrene decreased from $2.92 \pm 1.04$ times its original length to $0.19 \pm 0.02$ in shaded air, $0.44 \pm 0.17$ in shaded sea water and $0.52 \pm 0.46$ in light sea water in 100 days, and to $0.54 \pm 0.32$ in the illuminated air location, in 7 days...
Figure 2.17: Visualisation of the PRIMER V6 RELATE analysis per material type exposed in sea water. The vertical dotted line indicates spearman’s Rho of the actual pairs of tensile extension and carbonyl absorption band values. The bars indicate the frequency distribution of spearman’s Rho of 9999 random permutations of these value pairs. In (b) the vertical dotted line.

(polystyrene tensile extension was highly variable also within the control environment (2.42 ± 0.71)). With the other materials such dramatic loss of tensile extension only occurred after 240 (polyethylene) and 360 days (biothene and poly(ethylene terephthalate)), and while biothene uniformly lost tensile extension in all environments including the control, polyethylene started losing tensile extension only 240 days exposure in shaded air. Poly(ethylene terephthalate) remained within the variation of the control over the whole duration of the experiment in all
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Figure 2.18: Surface area loss, UV absorption, tensile strength and extension for the third series of polystyrene that was exposed in illuminated air for 28 days. The error bars indicate standard errors. The shaded area is the error range of the controls.

environments, except illuminated air, where both tensile strength and extension appeared to abruptly drop between 240 and 360 days exposure (no statistical analysis as \( n = 1 \) at 360 days).

The substantial increase in the hydroxyl absorption band in the illuminated air location, compared to either aquatic habitat clearly distinguishes polystyrene from biothene and polyethene. The hydroxyl absorption band did not significantly change in either biothene or polyethene. The hydroxyl absorption band changed from 0.01 ± 0.003 polystyrene to 0.38 ± 0.033 after 28 days in the illuminated air location, and 0.16 ± 0.056 in shaded air after 100 days, showing a much faster increase in hydroxyl content with polystyrene in the illuminated air location than in the shaded air location. The rate was therefore 8.62 times faster in light than in the shaded air.
location. While there appears to be an increase in the hydroxyl absorption band of biothene and polyethene over 600 days exposure in both illuminated and shaded air, the change remains well within the statistical variation among measurements, and the rate was five orders of magnitude lower than with polystyrene.

Biothene, polyethene and polystyrene all underwent a substantial increase in the attenuation coefficient of the carbonyl absorption band in both light and shaded air, but also the effect over time and of light versus shade was more substantial in polystyrene than in biothene and polyethene with respect to carbonyl (see Figures 2.15b, 2.15d and 2.15f). The rate was two
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orders of magnitude higher in polystyrene than in biothene and polyethene (illuminated air), but also the difference in rate between light and shaded air is a factor of 14.3, while it is only a factor of 2.6 in biothene and a factor of 1.9 in polyethene. This means that the effect of light on deterioration was far greater on polystyrene than on biothene and polyethene.

Biothene and polyethene showed an increase in the attenuation coefficient of the carbonyl absorption band at a much lower rate than polystyrene (0.15 ± 0.011 and 0.15 ± 0.025 per day respectively after 600 days in illuminated air, Figure 2.15), and the contrast between illuminated and shaded air was also not as strong in these materials (0.07 ± 0.008 and 0.09 ± 0.006 for biothene and polyethene respectively after 600 days in shaded air). The change in the attenuation coefficient of the hydroxyl absorption band of biothene and polyethene remained within a much smaller range (0.056 and 0.051 respectively) over 600 days exposure, and the variability of the data points concealed any possible uniform change over time.

Materials differed significantly in the rate of increase in change in tensile properties and the FTIR absorption bands for hydroxyl and carbonyl. Polystyrene showed the strongest effects overall while biothene and polyethene behaved in a similar way. Poly(ethylene terephthalate) most noticeably showed a loss of tensile extension in the illuminated air location, but very little effect in other treatments. Materials also differed in how the various treatments affected the rate of these effects. Light versus shade in air caused a larger effect on polystyrene than on biothene or polyethene.

2.3.4 Effect of light

Air was the only environment that showed a uniform difference of the measured properties between the light and the shaded locations. It also had the greatest difference in irradiance (98.55 ± 55.57 versus 11.28 ± 11.48 µmol s⁻¹ m⁻²). The most substantial effect was surface area loss in biothene, polyethene and poly(ethylene terephthalate), all of which had lost nearly all of their surface area, from the test rig, after 480 days exposure in illuminated air, while the samples in shaded air were still complete after 600 days (no statistics calculated, as denominator was zero). Note, however, that the decrease of surface area in the illuminated air location occurred abruptly (from 0 % to 100 % between two sampling events). This and samples of plastic
that were found on the floor beneath the wooden beam clamp in the air environment, indicate that the loss of area was likely due to detachment of the sample rather than disintegration. The abrupt loss of almost 100% surface area (Figure 2.19) supports this indication, as surface area loss due to material deterioration would be more continuous. The loss of tensile extension was also distinct in air between illuminated and shaded treatments.

The tensile extension of polyethene had dropped to $1.82 \pm 1.26$ times its original extension.
after 240 days in illuminated air versus 6.29 ± 0.72 in shaded air (t = 6.8867, p = 0.008).
The tensile extension of biothene and poly(ethylene terephthalate) in illuminated air were last
measured after 360 days (0.73 ± 0.22 versus 3.55 ± 0.11 in shaded (t = 25.008, p = 0.017) and
0.16 (n = 1) versus 0.87 ± 0.03 (no significant difference due to n = 1 in light)). In shaded
air biothene and polyethene continued to decrease while the tensile extension of poly(ethylene
terephthalate) remained constant (Figure 2.11).

The molecular constitution of polystyrene presents a substantial difference between illuminated
and shaded air (as already mentioned above). The factor of 14.3 difference in the rate of increase
of the attenuation coefficient of the carbonyl absorption band is almost proportional to the dif-
fERENCE in irradiation between the light and shaded air location. The evolution of the carbonyl
absorption bands of biothene and polyethene both also were significantly different in illumi-
nated and in shaded air (factor 2.6, t = 12.84, p = 0.01 and factor 1.9, t = 5.12, p = 0.0085
respectively after 600 days). A further difference between illuminated and shaded air with these
two materials was the continuity of the change in molecular constitution (Figure 2.20). In il-
luminated air the absorption coefficient increased continuously over time, while it alternately
increased and decreased in shaded air.

### 2.3.5 Settlement of marine organisms

Over time, the samples that were exposed in marine environments became heavily colonised by
organisms, reaching a wet mass of up to 600 g per sample. The data (Figure 2.21) showed a
substantial seasonal variation, especially in illuminated locations, which also had more biomass
than in the shaded locations. The wet mass of the samples retrieved from the shaded location in
sea water was almost equal to its initial mass in the 600 days cohort. The wooden beam clamp
became detached from the pontoon and sank to the seabed, which may have caused colonizing
organisms to be dislodged from the samples.
Figure 2.21: Wet mass (g) of material samples exposed in marine environment versus exposure (days), months are annotated.
2.4 Discussion

The results of this study showed that oxidation as measured by carbonyl and hydroxyl content of biothene, polyethene and polystyrene occurred much faster in air than in water and was accelerated by light. While the content of oxidation products was not measured in poly(ethylene terephthalate), light has an indisputable effect on the material’s stability, as tensile extension decreased substantially in illuminated air. There was no evidence of oxidation, but this observation suggested that oxidation also occurred in poly(ethylene terephthalate) in illuminated air, and that this caused the material to destabilise.

2.4.1 Deterioration of plastics in illuminated air

All tested materials in this study had one reaction in common: they deteriorated much faster in illuminated air than in any of the other environments. With the exception of biothene and polystyrene there was no loss of tensile extension in sea water. For marine debris this means that they begin to deteriorate and consequently to shed microplastics at an increased rate on shorelines as opposed to when in the sea. Andrady, Pegram and Tropsha (1993) suggested that the more rapid deterioration in air was due to higher temperatures and higher UV irradiance partially due to absence of fouling.

Andrady, Pegram and Tropsha (1993) monitored environmental conditions in their exposure sites in much more detail than the present study. In this study light was taken into account as a factor, and 2 observations suggested that material deterioration was more linked to light than to temperature: (a) samples that were exposed in illuminated air deteriorated much faster than samples that were exposed in shaded air in the same outdoor environment, and (b) deterioration (as measured by tensile extension) was strongly linked to oxidation, which is caused by UV irradiation. While chemical reactions such as oxidation occur more rapidly at higher temperatures, light was clearly a primary factor.

The absence of light appears not to be the only factor that reduced deterioration in water. Samples that were exposed in fresh water had similar irradiation as samples in shaded air, yet they exhibited no oxidation at all, while samples in shaded air did. Water has a much lower oxygen
content than air, and it is possible, that reduced oxygen availability in water limited these oxidation processes. This is important because deterioration of marine debris that floats at the surface would also be reduced. To confirm the hypothesis that lack of oxygen slows oxidation in water with enough certainty, the experiment of Andrady, Pegram and Tropsha (1993) would have to be repeated, and the oxidation product contents of the samples would have to be measured. To quantify the effect of all three factors, temperature, UV irradiance and oxygen concentration on plastic deterioration, an experiment in controlled environment may be more suitable. Materials could be exposed in tanks with water and air with various oxygen concentrations, UV irradiance levels and at various temperatures.

This study does not primarily look at individual factors that contribute to material deterioration, but at the difference in deterioration rate between sea water and air. Biothene deteriorated twice as fast in illuminated air as in sea water, polystyrene about 50 times as fast. For polyethene and poly(ethylene terephthalate) there was no estimate, as no measurable deterioration occurred in sea water. While the other materials remained stable for months even in illuminated air, polystyrene lost almost all its tensile extension in a matter of days. Polystyrene has a strong reaction to UV radiation, because it has tertiary bonds that accelerate oxidation propagation and hinder termination (Amin and Scott 1974). This means that a piece of polystyrene that is washed up on a beach by one spring tide after possibly years of being in the ocean could become extremely brittle before the next spring tide, and very little mechanical stress would be needed to cause it to shed microplastics. Gregory and Andrady (2003) also reported that plastics in the marine environment degrade on beaches as the temperature, the friction and the UV irradiance are higher than in water.

This study provides a qualitative illustration of deterioration on beaches versus water, indicating a difference of two orders of magnitude for polystyrene. Since no significant loss of tensile extension was recorded on polyethene and poly(ethylene terephthalate) that were exposed in sea water, this factor may be much greater for these materials and requires longer exposure durations to be assessed more exactly. While the deterioration of biothene in sea water kept up with illuminated air at early exposure stages, it became stagnant later, and it cannot be said
with any certainty, what the difference in deterioration rate between the two environments is in this material. Biothene, polyethene and poly(ethylene terephthalate) do not deteriorate as rapidly in illuminated air, but also at an accelerated rate versus sea water. Items that are made from these materials that are washed ashore repeatedly or for long periods of time may also produce microplastics. It is therefore possible that a large portion of microplastics now present in the ocean may have been formed by fragmentation out of water, for instance on the shoreline.

2.4.2 Mechanical destabilisation in sea water

Very little oxidation of biothene, polyethene and polystyrene was observed in either fresh water or sea water. Nevertheless, tensile extension decreased substantially with polystyrene and also biothene in sea water. Contrary to air, in sea water this loss of tensile extension was not related to oxidation. While materials destabilise much faster in air than in water, it seems that they can deteriorate in water due to reasons other than oxidation. This finding is also supported by the fact that samples in fresh water were exposed to higher irradiance than samples in sea water, yet sustained less loss off tensile extension.

Polystyrene, which had the lowest tensile extension to begin with, deteriorated the fastest of all four tested materials. Especially in air, high oxidation rate led to a rapid loss in tensile extension. In sea water polystyrene lost more tensile extension than could be explained by oxidation. Polystyrene has generally poor environmental stability, especially in air. This is important, because polystyrene is a widely used packaging material (PlasticsEurope 2013) and is thus an important contributor to plastic waste.

As in this study, Andrady, Pegram and Tropsha (1993) observed a higher rate of deterioration measured by tensile extension on land than in sea water, while their sea water samples were exposed floating at the surface and not submerged as in this study. Pegram and Andrady (1989b) attributed this observation to lower temperatures in water than on land in an earlier study and to fouling of the samples by microorganisms, which inhibits photo-oxidation through irradiance (Andrady, Pegram and Tropsha 1993). This study also examined molecular alterations of the materials, and it established that loss of tensile extension in sea water was in fact not linked to oxidation.
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Andrady, Pegram and Tropsha (1993) suggested that some organisms that colonise plastics mechanically alter the material (boreholes), especially materials whose degradability is enhanced with starch addition. Biothene and polystyrene which both exhibited significant loss of tensile extension in sea water do not contain such additives, but given the degree of fouling on the samples in this study this is a possible explanation. Sea water and fresh water differ vastly: sea water is more alkaline, saline and - in this study - likely had higher wave action. Which factor affected tensile extension the most, cannot be concluded from the present results. The primary aim of this study was not to observe such ‘bio-deterioration’, but it produced information on type and abundance of organisms colonising plastics, and analysis of this data may conclude that certain organisms do accelerate plastic deterioration.

2.4.3 Effectiveness of enhanced degradability

This chapter challenges the environmental relevance of plastics with enhanced degradability. Some of these materials may simply present a faster way for microplastics to form from larger debris. But microplastics aside, observations made on biothene suggest that it may not even deteriorate faster than polyethene.

Andrady, Pegram and Tropsha (1993) observed the deterioration of conventional plastic and plastic with enhanced degradability in sea water versus air. They tested three kinds of plastics with enhanced degradability; the type that compared perhaps the best to biothene, which was used in this study, is a type of low density polyethene with metal compound prooxidant additives. They observed that the effect of enhanced degradability (versus traditional materials) was stronger in sea water than in air. This means that sea water slows down deterioration of degradable plastics more than deterioration of traditional plastics.

O’Brine and Thompson (2010) used a similar method as in this study to compare the deterioration rate of regular plastic bags and plastic bags with two different degradability enhancement methods in sea water. They discovered that materials with compostable additives had significantly improved deterioration properties over regular plastic, while oxo-biodegradable bags deteriorated at a similar rate as polyethene.

The results of both studies are consistent with the observations made in the present study: There
was very little difference between biothene, which was made degradable using metal ions, and polyethene in oxidation rate in any of the treatment groups. Tensile extension of polyethene decreased faster than biothene in air overall. Though biothene lost tensile extension more rapidly in the beginning, it stabilised later during exposure, and it was also more resistant against surface area loss than polyethylene. Only in sea water did biothene lose tensile extension more rapidly than polyethylene. Biothene was not significantly more degradable than polyethylene. Andrady, Pegram and Tropsha (1993) registered a two orders of magnitude loss of tensile extension with polyethylene with metal compound prooxidant additives after 40 days exposure. Tensile extension of biothene was only reduced 50% after 240 days in the present study. The concerns of Osawa (1988) that the effect of metal ions on the degradability of plastics are unpredictable, are thus supported by the present study.

2.4.4 Surface area loss as a measure of deterioration?

O’Brine and Thompson (2010) reported that a large proportion of the compostable material had deteriorated after 16 weeks; nothing was left after 24 weeks. The metric for deterioration was surface area loss. The present study used a similar method for material exposure. The samples were attached to wooden beam clamps. The samples that were exposed in air exhibited rapid surface area loss, but surface area loss in this case was a measure of samples tearing off the wooden beam clamp, as was evidenced by remainders found in the exposure site. Because no such remainders could be found in sea water, it is unclear whether the surface area loss observed there was actual deterioration or also just detachment. To measure surface area loss caused by deterioration, an exposure method that excludes this confounding factor needs to be applied.

The incidence of samples breaking off the exposure element can be interpreted as a measure of brittleness that is perhaps more pragmatic than measuring tensile strength and extension. It would indicate that - in the given environment - the material would actually break apart and become smaller pieces. A process that - if perpetuated - leads to the emergence of microplastics. For any statistical validity the sample size would have to be substantially increased, as the binary event of breaking is much more variable than the metrics used in this trial. More and smaller material samples would adequately represent this effect. It may also be useful to design a test
2.5 Conclusion

Out of the three widely used materials polyethene, polystyrene and poly(ethylene terephthalate) and biothene as an example for degradable plastics, polystyrene showed the most rapid rate of deterioration in air, especially when exposed to sunlight. Polystyrene is widely used in packaging (as Styrofoam cushioning or seal-wrapping for food items), it therefore has a short service life and is likely to end up in the environment more frequently than other polymers. It is therefore advisable to further examine polystyrene as a source of microplastics in the environment.

The experiment showed that the deterioration of all four tested polymeric material types occurred more quickly in air than in one metre of sea water or fresh water. This was evidenced by measurements of loss of material, loss of tensile extension and changes in molecular composition within an exposure period of six hundred days. The molecular composition of the samples exposed in fresh water and sea water did not change. In fresh water sunlight was likely still at similar levels as in shaded air, and it is possible that water reduces photo-oxidation regardless of the UV radiation through a low concentration of solved oxygen. According to the findings of the present study and many sources cited above, the marine environment where plastics are likely to degrade the fastest is the beach. Marine debris may drift in the sea, cooled by water shielded from UV radiation and exposed to little oxygen. As a consequence plastic items may remain intact for years, before they wash ashore. Once ashore, they may degrade much more rapidly. The present experiment showed that plastic deterioration is by far the fastest in well illuminated, air environments; this is a characteristic that is exhibited on the surface of beaches. It is therefore likely that beaches play an important role in the formation of microplastics through deterioration in the marine environment. Therefore, beach clean-ups may be a substantial way to reduce the formation of microplastics in marine environments.
Are you here with me?
Just looking out on the day
Of another dream

- Gorillaz

Chapter 3

Development and evaluation of a rapid survey approach to assess plastic contamination on beaches

Since the 1960s researchers have employed a variety of methods to study plastic contamination in the marine environment; these include surface trawls with nets, beach surveys and studies on entanglement or ingestion by animals. Due to their relative simplicity the easiest method is some form of the beach survey. Beach surveys have been applied widely with the help of volunteers in beach cleanup campaigns. Small plastic debris, which may be a precursor to microplastic formation, have often been inadvertently omitted by beach surveys, as they are too small to be easily observed. They are also often not evenly distributed on beaches, which causes traditional survey methods such as transects to return highly variable data. In order to reduce this variability, a rapid survey method for small plastic debris focusing on debris accumulations was developed. Using semi-logarithmic contamination ratings small plastic debris accumulations were aggregated on each beach. This returned an overall score, which permitted a comparison of small plastic debris contamination between beaches. A trial
with volunteers assessing small plastic debris contamination showed that the variation between observers was substantial. The study concluded that the method could further be optimised using computer simulation, which would also allow the integration of variability between observers. Surveys using this or a similar method could potentially allow surveying a large number of beaches rapidly by volunteers. Hence the approach could generate a larger volume of data than conventional surveys and so help overcome the natural variability that is typically observed in intertidal litter data.

3.1 Introduction

Environmental contamination by plastic debris has been the topic of nature conservation efforts and scientific research since the late 1960s (Kenyon and Kridler 1969; Carpenter and Smith 1972; Rothstein 1973; Colton et al. 1974). Three main approaches to the assessment of plastic contamination in the marine environment have been employed: beach surveys, at sea surveys and estimates of amounts entering the sea (Rees and Pond 1995). At-sea surveys are free of many of the confounding factors such as sediment size, vegetation, slope or litter removal campaigns that are encountered in beach surveys. However, they have higher time investment and infrastructure requirements, and they will only assess standing stocks (Ryan et al. 2009). Like other visual survey methods, visual assessments at sea are constrained by a number of factors such as: item visibility, general visibility conditions and personal detection skills. These constraints have to be accounted for in the study design and in the data analysis (Ryan and Cooper 1989; Skipp and Brownfield 1993; Willoughby et al. 1997). At-sea surveys require an extraordinarily large sample size because of the spatial overdispersion of debris densities, and are therefore quite elaborate. Opportunistic trawls and remote cameras constitute ways to improve the efficiency of at-sea surveys (Ng and Obbard 2006; Ryan et al. 2009). Aerial surveys are also valuable in detection of spatial patterns (Skipp and Brownfield 1993; Willoughby et al. 1997; Ryan et al. 2009). Surface trawls cover a very small area, which can be problematic as marine debris tends to be clustered; a considerable length of transect therefore has to be assessed in order to obtain a reliable mean (Skipp and Brownfield 1993; Willoughby et al. 1997; Ryan et al. 2009). However trawls have proven successful in indicating increase of plastic debris abundance over time (Day et al. 1990; Ogi et al. 1999; Moore, Moore, Leecaster and Weisberg 1999).
Beach surveys constitute a method that can readily be applied with minimum infrastructure and on a low budget (Rees and Pond 1995). Moreover, changes in debris composition on beaches have been shown to reflect changes in the composition at sea (Ryan et al. 2009). They are resilient, straight-forward methods that allow volunteers with minimal training to conduct the work and rapidly collect a large amount of data (Storrier et al. 2007; Bravo et al. 2009). Beach surveys also address an aspect of environmental plastic contamination that is immediately appreciated by a broad audience: the cleanliness of beaches has been recognized as the most influential criterion in tourists’ perception of habitat quality (Ballance et al. 2000). Beach surveys have successfully been used by organisations such as the UK Marine Conservation Society and internationally by Ocean Conservancy, both of whom have generated comprehensive datasets from beach-clean campaigns conducted by large numbers of volunteers over many years. This chapter addresses the thesis’ aim to develop and test a method to quantify contamination of beaches by small plastic debris that can be applied on a great number of beaches by using a rapid survey approach and by making it transferable to volunteers.

Beach litter surveys can be subdivided into standing stock surveys and rate of arrival surveys (Ryan et al. 2009). Standing plastic debris stock on beaches is determined by a number of physical (currents, beach structure etc.) and interactive (land-based sources, cleanups) factors (Sheavly 2007). Rate of arrival surveys are therefore considered to give a better representation of the amount of plastics at sea (Ryan et al. 2009); however, they are much more elaborate than standing stock studies, as each sampling event requires complete removal of all recorded debris. Frequent sampling of the standing stock returns information on changes in the composition of beach litter (Ryan and Watkins 1988), but can also compensate for rapid turnover (Day et al. 1990; Ogi et al. 1999; Ryan et al. 2009) and may be a suitable replacement for rate of arrival surveys.

3.1.1 Beach surveys and small debris

Most beach litter surveys focus on large items (Ryan et al. 2009). While tow net surveys in the open sea have obtained extensive data on small debris, thus far, few beach surveys have consid-
erated small debris (Ivar do Sul et al. 2009). Even fewer beach surveys consider microplastics. A possible reason for this is that beach surveys typically employ transects (Gabrielides et al. 1991; Golik and Gertner 1992; Garrity and Levings 1993; Rees and Pond 1995; Velander and Mocogni 1999; Moore, Gregorio, Carreon, Weisberg and Leecaster 2001; Abu-Hilal and Al-Najjar 2004; Alkalay et al. 2007; Storrier et al. 2007; Oigman-Pszczol and Creed 2007; Silverton 2009; OSPAR Commission 2007; Oigman-Pszczol and Creed 2007), which may be unsuitable for detecting small debris, due to the extent of the area, which demands a superficial overview (Ivar do Sul and Costa 2007; Moore 2008; Costa et al. 2010). The detection of small debris requires more detailed screening, which can be achieved by surveying a smaller area. Visual assessments have been known to miss very small debris (Kusui and Noda 2003). Because of their size, microplastics constitute a challenging target for beach litter surveys and cleanup campaigns. Typically, debris that are smaller than two centimetres have been ignored or overlooked (Gabrielides et al. 1991; Golik and Gertner 1992; Corbin and Singh 1993; Madzena and Lasiak 1997; Abu-Hilal and Al-Najjar 2004; Alkalay et al. 2007; Storrier et al. 2007; Santos et al. 2009; Topçu et al. 2013) or studies make no specific reference to size categories in their results or discussion (Moore, Gregorio, Carreon, Weisberg and Leecaster 2001). Other surveys do record small debris but focus strictly on the driftline. Traditional beach survey protocols (Ribic et al. 1992) may not be applicable to small fragments, as these show different dynamics and different dependence on environmental factors to larger debris (Browne et al. 2010), for example small debris are susceptible to being widely transported across the beach by wind (Thornton and Jackson 1998). Wilber (1987) reported that small fragments accumulate near the uppermost wrack lines; they therefore potentially occur everywhere between the driftline and the upper limit of the beach.

A large number of beach surveys have focused on the composition of debris in relation to their origin and use (Ribic et al. 1992; Moore, Gregorio, Carreon, Weisberg and Leecaster 2001; Otley and Ingham 2003). As plastic debris degrades, these characteristics may become beyond recognition. Meanwhile, small debris will constitute an increasingly large portion of beach litter (Klemchuck 1990; Ryan and Moloney 1990) due to degradation. At the present time the largest
3.1. INTRODUCTION

The proportion of beach debris cannot be readily identified and is reported simply as 'fragments' (Martinez-Ribes et al. 2007; Santos Silva-Cavalcanti et al. 2009; Topçu et al. 2013). Storrier et al. (2007) even stated that small debris were omitted from their study because they were too numerous to count. Hence, due to the likely shift in the focus of environmental debris surveys towards small debris (e.g. Morét-Ferguson et al. (2010)), methods of monitoring small debris will become important in the context of future beach litter surveys. Furthermore, small debris monitoring is important due to the potential impacts of small debris upon animals, through ingestion (Carpenter et al. 1972; Bourne 1976; Pettit et al. 1981; Day et al. 1985; Fry et al. 1987; Copello and Quintara 2003; Mascarenhas et al. 2004; Boerger et al. 2010; Lindborg et al. 2012; Rodríguez et al. 2012; Lusher et al. 2013; Donnelly-Greenan et al. 2014).

3.1.2 High variability

In the light of the limitations of traditional beach surveys for assessing small plastic debris (necessarily spatially extensive, yet superficial and inherently variable), a method is required that records small plastic debris abundance and accounts for the within-site variability caused by patchy distribution of debris.

Beach surveys in general have encountered high variability within sites (Costa et al. 2010). This high variability can be attributed to spatially random sampling, which does not consider the frequently encountered statistical overdispersion of plastic debris densities over the beach (Jones 1995; Madzena and Lasiak 1997; Willoughby et al. 1997; Thornton and Jackson 1998; Debrot et al. 1999; Martinez-Ribes et al. 2007). This high variability constrains comparisons between beaches, and it makes changes over time less detectable. It may be overcome by surveying a larger proportion of the beach; beach surveys have traditionally been conducted in transects or as a by-product of beach clean-ups, which both aim to cover as large an area as possible. This practice in particular can result in an underestimate of the actual amount of litter, as debris is still likely to be missed because of the extent of the scope of observation.

Small plastic debris occur in patchy aggregates. Similar to organisms that only occur in certain habitats (e.g. in rock pools within a rocky shore), their quantification outside these habitats makes no sense. However, the occurrence of small plastic debris is not predictable; unlike rock
pool dwellers they cannot be targeted in their specific habitat. The occurrence pattern of small plastic debris is not only patchy, but also unpredictable. The quantification of small plastic debris on a beach therefore demands (1) the identification of small plastic debris accumulations and (2) the assessment of small plastic debris abundance in these accumulations. Due to this, beach surveys targeting small plastic debris still rely on a superficial overview of the beach, which is unsuitable for the detection of small plastic debris.

The quantification of small plastic debris on beaches is impaired due to a vast dimension gap between the target and the environment in which it is being recorded; this is an obstacle to the systematic and repeatable identification of accumulations. This study therefore examined the use of ‘walks on the beach’ looking out for accumulations.

The purpose of this approach was to reduce the within-site variability that has previously been encountered in beach plastic surveys. The first aim of this study was to validate the use of a quick visual plastic contamination scoring system (‘burden rating’, rather than detailed counts), on deliberately selected debris accumulations (‘dirty quadrats’), as a method to measure the plastic contamination on beaches.

The proposed method was based upon the premise that the majority of all possible 1 m$^2$ quadrats on the beach have a low burden score. The frequency distribution of the burden ratings 0 to 5 would, therefore, be right-skewed like a log-normal, Poisson or negative binomial distribution. Measuring the contamination of accumulations of small plastic debris raises the question whether there is an appropriate way to aggregate measurements to reflect the contamination of the whole beach; what is measured is the tail end of a distribution. What is also measured is the count of occurring burden ratings in descending order, and it is plausible that a beach where 10 quadrats with a burden rating of 4 can be found is more contaminated than a beach where 6 such quadrats can be found, and the remaining quadrats have a lower burden rating. An aggregated value will reflect this difference between two beaches. An aggregated value that is appropriate for the distribution of burden ratings on a beach would therefore allow to compare multiple beaches or the outcome of multiple survey events on the same beach. The log-linear family of distributions are best described by the geometric mean (Galton 1879), which is commonly
used in distributions with extreme values as it is less influenced by the latter, and nearer the median than the arithmetic mean (Olivier et al. 2008). In addition the geometric mean has a smaller chance of tied ranks than the arithmetic mean or the median (3003 possible geometric means vs. 51 possible arithmetic means or 6 possible medians over all burden rating permutations). The present study, therefore, uses the geometric mean of the quadrats’ burden rating plus one (as any occurrence of the burden score zero would render the geometric mean zero) to describe small plastic debris burden scores for the whole beach. The geometric standard deviation to mean ratio over heavily contaminated, "dirty" quadrats was then used as a measure of within-site variability versus the geometric standard deviation to mean ratio over the random quadrats.

Metrics for the validity of this method were (a) correlation of the burden rating with quantitative measures of small plastic debris contamination in the surveyed area (debris count, cumulative mass and cumulative size), (b) lower variability of the burden rating within a beach versus random sampling and (c) collinearity of the beach burden score from dirty quadrats versus random quadrats. Random sampling was represented by five quadrats per beach, which were selected randomly from the area that contained the previously selected debris accumulations. The representativeness of these five random quadrats was then tested by sampling and comparing with data from thirty random quadrats on three beaches.

### 3.1.3 Use of volunteers

In order to obtain robust results, large scale surveys over a substantial number of beaches are required. The use of volunteers could be invaluable to this end (Rees and Pond 1995). Beach surveys are conducted following various methodologies, and often they are not comparable among each other (Ribic et al. 1992). A survey approach that relies on few constraining factors (such as survey intervals, transect width, beach size, number of volunteers or time investment) could help to overcome this problem and thus provide a large amount of robust data. The second aim of this study therefore was to tailor the proposed method for volunteers of differing backgrounds and prior knowledge of the subject.

Volunteer participation in beach plastic surveys has found wide application, especially within
beach cleaning programs (Storrier and McGlashan 2006). For example, thousands of volunteers work with the UK Marine Conservation Society to collect and record plastics from beaches (Marine Conservation Society 2014). There have also been efforts to involve volunteers in the monitoring of small plastic debris (Hidalgo-Ruz and Thiel 2013; Roosevelt et al. 2013). Surveys that are supported by volunteers typically collect the plastics and produce a detailed account of the collected items. The method presented in this study relies on visual assessments, which can be influenced by individual perception. Many studies outside this field have experimented with volunteer participations, such as behavioural surveys (Williams et al. 2012), or surveys of insect populations (Prysby and Oberhauser 2004). Volunteer observations tend to be unreliable if they require specialised equipment, e.g. for water quality testing, or if the volunteers are required to identify species (Robson 2012). Meanwhile, recording quantitative data correctly is less of a concern (Williams et al. 2012). More often than misreporting observations, volunteers tend to not follow protocol correctly (Williams et al. 2012; Hidalgo-Ruz and Thiel 2013), keeping the sampling protocol simple is therefore essential.

A volunteer trial was conducted to establish whether the method presented in this study would be suitable to be applied by volunteers. In order to conduct the survey effectively, observers have to (1) correctly identify small plastic debris accumulations and (2) correctly assess the abundance of small plastic debris in these accumulations. Testing the correct identification of small plastic debris accumulations would have required each volunteer to screen the same beach for accumulations independently. Time limitation of this trial would not have allowed the consecutive screenings of a beach by a sufficient number of volunteers. This part of the evaluation was therefore omitted.

Volunteers were instead asked to assess the small plastic debris abundance in a set of preselected quadrats. The agreement among volunteers on the burden rating of each quadrat and the consistency of each volunteer throughout all observation cycles were used as a measure of repeatability of the assessment.
3.2 Methods

76 surveys were conducted on 51 beaches along the coast of Southwest England (Figure 3.1). 5 beaches were surveyed 6 times as they were part of a project that also observed the change of the small plastic debris contamination over the course of one year. All sampling events are listed in table A.3 in appendix A.

The beaches were screened for clusters of plastic debris that was smaller than 5 cm within a stretch of one hundred to two hundred metres of continuous, sedimentary shoreline, covering the region between the driftline and the top of the beach. The ten clusters with the perceived largest amount of small plastic debris per unit area were selected for screening (dirty quadrats). Each cluster was marked out with a one by one metre quadrat around its epicentre, covering as much of the debris as possible. Each quadrat was then assigned a burden rating from 0 to 5. This scale was inspired by the SACFOR scale that is used for abundance estimates of sessile organisms (SACFOR abundance scale 1990). It assigns a semi-logarithmic rating to the perceived number of pieces of plastic in the quadrat (burden rating, see Table 3.1 for details and Figure 3.3 for examples). Each quadrat was photographed from a vertical angle facing away from the water. Additionally the geodetic position (decimal degrees) was measured with a Garmin Geko 301 GPS receiver.

3.2.1 Accuracy

5 random quadrats were selected within the sampling area that was defined as the minimum convex polygon around the 10 dirty quadrats, as illustrated in Figure 3.2. The minimum convex polygon around the ten dirty quadrats was established by using a coordinate system consisting

<table>
<thead>
<tr>
<th>Burden score</th>
<th>Number of pieces</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>1000</td>
</tr>
<tr>
<td>5</td>
<td>&gt;1000</td>
</tr>
</tbody>
</table>

Table 3.1: Burden scores assigned to quadrats and corresponding numbers of plastic pieces (orders of magnitude).
Figure 3.1: Map of Southwest England showing the sampling sites used for the shoreline plastic contamination survey, numbers match table A.1 in Appendix A.
3.2. METHODS

Figure 3.2: Schematic of small plastic debris cluster sampling and selection of random quadrats. Red open squares: selected clusters; red dashed line: minimum convex polygon around selected clusters; grid: coordinate system for random quadrat selection (x and y axis annotated); emphasised grid cells: random quadrats; blue shaded area: sea; black shaded area: non-sedimentary area.

of the number of paces walked parallel to the water line (X) and the number of paces walked perpendicularly to the water line (Y), walking to the quadrat from the lower left corner of the investigated area. The number of possible quadrats in the sampling area was calculated. Thus all quadrats received unique numbers running from the top left to the bottom right of the sampling area. 5 numbers from this list were selected randomly to determine the random quadrats, thus all quadrats in the minimum convex polygon - including the dirty quadrats - had the same chance to be selected. The random quadrats were oriented in line with the driftline, with their lower left corner on the determined coordinates.

The representativeness of 5 random quadrats, of the frequency distribution of burden ratings on a beach was tested by a validation on a subset of beaches that were represented by 30 random quadrats instead of 5. 100 random sets of 5 ratings and 100 random sets of 10 ratings were sub-selected from the 30 random quadrats. The distributions of the random sets of 5 and 10 quadrats were fitted to the distribution of the original 30 quadrats using a reduced chi-squared statistic. A
good fit between the majority of the random sub-selections and the original set would indicate that the small plastic debris contamination on the beach is equally well represented by 5 as by 30 random quadrats; sampling 30 instead of five random quadrats would not add information to the data.

To test the validity of the burden rating, it was compared to quantitative measurements of small plastic debris abundance. Therefore on eleven beaches debris was collected from the five random quadrats and from five dirty quadrats that were randomly selected out of the original set of ten. Each quadrat was divided into sixteen subquadrats. In dirty quadrats, all debris within the four most contaminated subquadrats and in random quadrats all debris in four randomly selected subquadrats, was collected for counting, weighing and measuring.

The size and mass of each collected piece of debris was recorded. Debris count, cumulative mass and cumulative size was used as quantitative measures of the quadrats’ contamination. A linear regression analysis between the burden rating and the log-transforms of the small plastic debris count, cumulative size and cumulative weight was conducted to test the burden rating’s representativeness of the quantified contamination.

In order to show that sampling clusters returns lower within-site variability than sampling random quadrats, the geometric standard deviation to mean ratios of the dirty quadrats versus those of the five random quadrats’ burden ratings were calculated for each site. A low ratio would indicate that the contamination of the sampled quadrats was similar, and that the geometric mean would allow for good comparability between surveys.

### 3.2.2 Applicability for monitoring by the public

In the view of the method’s intended use by volunteers, transferability was tested with the help of nine volunteers. Ten quadrats were laid out on a beach covering as many burden scores as possible. The quadrats were photographed and assigned a burden score by the author according to Table 3.1. The volunteers were given a printed methods description (Figure B.1, Appendix B), and a verbal reiteration of the methods at the site. The volunteers were informed of the goal of the survey event, and were asked not to exchange information during the survey in order to maintain independence. Figure B.2 in Appendix B shows the data collection sheet used in the
3.2. METHODS

Figure 3.3: Photo references of quadrats, each representing a different burden rating. Burden category 5 has never been recorded.

In order to check the consistency of each participant, the survey was conducted three times in total. In the second run the order in which the quadrats were surveyed was reversed, in the third run it was randomised in order to minimise the bias from previous observations.

After the first two runs some participants expressed uncertainty whether some fragments were considered plastics or not. Therefore they were given extra information for the third run. They
were made aware of very small pieces of plastic, and plastic was defined more accurately also to include all manmade polymeric materials (e.g. also polystyrene and rubber).

The consistency of the burden rating has two levels, (a) several volunteers assigning the same burden rating to the same quadrat and (b) the same volunteer assigning the same burden rating to the same quadrat on separate survey events. A mixed model approach using the lme4 package (Bates et al. 2014) in R (R Core Team 2015) was used to test the consistency of this method across volunteers. Volunteers and survey run were included as random factors to test the effect of each.

3.3 Results

3.3.1 Accuracy

The burden ratings of the quadrats, in which material samples were collected, were in good agreement with the number of pieces, their cumulative size and their cumulative mass, as shown by a linear regression (Table 3.2 and Figure 3.4). All relationships were highly significant. The goodness of fit showed that there was an amount of overlap between burden ratings. The burden rating was intended to reflect the debris count, for which the goodness of fit was \( R^2 = 0.46 \). The burden ratings assigned to quadrats accounted for just under half of the variability encountered in the number of pieces collected in the subquadrats of both dirty and random quadrats. Meanwhile, the goodness of fit of the burden rating against the cumulative size was \( R^2 = 0.53 \).

The beach burden score calculated from the burden rating of the dirty quadrats was three times less variable than the burden score calculated from the burden ratings of the random quadrats as shown by the geometric standard deviation to mean ratio (Figure 3.5b). Analysis of variance

<table>
<thead>
<tr>
<th>Model</th>
<th>DF</th>
<th>B</th>
<th>( R^2 )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log count</td>
<td>71</td>
<td>0.44±0.06</td>
<td>0.46</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Log cumulative mass</td>
<td>71</td>
<td>0.24±0.04</td>
<td>0.28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Log cumulative size</td>
<td>71</td>
<td>0.40±0.05</td>
<td>0.53</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
3.3. RESULTS

(a) Count

(b) Cumulative size

(c) Cumulative weight

Figure 3.4: Visualisation of the regression of burden score versus log transformed particle count, cumulative weight and cumulative size.

Table 3.3: Analysis of variance of the geometric standard deviation to mean ratio of the burden ratings of random versus cluster quadrats per survey.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Df</th>
<th>Sum of squares</th>
<th>Mean squares</th>
<th>F value</th>
<th>Pr(&gt; F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect</td>
<td>1</td>
<td>0.10</td>
<td>0.10</td>
<td>5.67</td>
<td>0.02</td>
</tr>
<tr>
<td>Residuals</td>
<td>74</td>
<td>1.33</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

between the two sets showed that the difference was significant (Table 3.3) The two outliers with a high geometric standard deviation to mean ratio in the survey of small plastic debris clusters were Dawlish (18) and Teignmouth (19); both beaches are set apart from the remaining beaches by their curvature, which is convex against the sea instead of concave. Differences between survey events are therefore much more likely to be picked up by sampling dirty quadrats, as random quadrats would produce a within-site variation that would conceal between-site variation.
Out of 30 random quadrats, the burden score distributions of random sets of 5 and 10 quadrats were compared to the burden score distribution of all 30 quadrats in order to validate the representativeness of the sample size of 5. This random validation was conducted on three beaches, Broadsands, Harlyn and Par Beach (Table 3.4 and Figure 3.6). Generally, random subsets of 10 quadrats were a better match for both the predicted poisson distribution and the distribution of the original 30 random quadrats over burden ratings, but Par Beach presented an exception in this regard: Random sets of 10 quadrates were less likely to match the distribution of 30 random quadrats than random sets of 5. Meanwhile, the poisson prediction was more frequently matched by random sets of 10 than 5. Par beach had a higher overall burden score by random quadrats than the other two beaches; burden ratings 1 and 2 were in fact both encountered more frequently than burden rating 0, and the subsets of random quadrats were an inferior match to both the poisson prediction and the actual distribution of the 30 random quadrats over burden scores than on the other two beaches.
3.3. RESULTS

3.3.2 Applicability for monitoring by the public

The volunteer trial revealed that the initial instructions were not sufficient for the volunteers to correctly identify all target debris. The burden ratings would therefore be expected to be higher in the last survey run, when volunteers had improved information. However, the burden rating did not significantly change across cycles (Table 3.5a). Volunteers were consistent in their burden rating assignment, and their experience level did not affect the result. Consistent underestimates due to lower experience level could also be ruled out, as the volunteers’ burden ratings were not generally lower than the author’s (Figure 3.7).

While each volunteer gave consistent burden ratings, the volunteers disagreed on the burden rating for the same quadrat (Table 3.5a). The order of magnitude estimate of the small plastic

![Figure 3.6: Frequency distribution of burden scores in the random validation on Broadsands, Harlyn and Par Beach. Black bars: Distribution across all thirty random quadrats, Grey bars: Mean (extrapolated) distribution of 100 random sets of 5 random quadrats, Error bars: Standard deviations, Dotted line: Poisson prediction.](image)
Table 3.4: Chi squared statistics for the random validation on each of the 3 sites. p: Average chi squared probability of the distribution of 100 random sets of 5 and 10 quadrats vs. (3.4b) the Poisson-predicted distribution based on 30 assessed quadrats and (3.4a) the distribution of the 30 assessed quadrats standard deviation, % p > 0.05: Proportion of random sets of 5 and 10 quadrats with p > 0.05.

(a) Poisson prediction

<table>
<thead>
<tr>
<th></th>
<th>Broadsands</th>
<th>Harlyn</th>
<th>Par Beach</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 5</td>
<td>p</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.22 ± 0.20</td>
<td>0.36 ± 0.31</td>
<td>0.11 ± 0.22</td>
</tr>
<tr>
<td>%p &gt; 0.05</td>
<td>65</td>
<td>58</td>
<td>27</td>
</tr>
<tr>
<td>n = 10</td>
<td>p</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.55 ± 0.28</td>
<td>0.56 ± 0.30</td>
<td>0.46 ± 0.35</td>
</tr>
<tr>
<td>%p &gt; 0.05</td>
<td>93</td>
<td>94</td>
<td>83</td>
</tr>
</tbody>
</table>

(b) Measured

<table>
<thead>
<tr>
<th></th>
<th>Broadsands</th>
<th>Harlyn</th>
<th>Par Beach</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 5</td>
<td>p</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.27 ± 0.25</td>
<td>0.43 ± 0.37</td>
<td>0.28 ± 0.34</td>
</tr>
<tr>
<td>%p &gt; 0.05</td>
<td>76</td>
<td>71</td>
<td>50</td>
</tr>
<tr>
<td>n = 10</td>
<td>p</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.53 ± 0.29</td>
<td>0.49 ± 0.32</td>
<td>0.13 ± 26</td>
</tr>
<tr>
<td>%p &gt; 0.05</td>
<td>96</td>
<td>96</td>
<td>27</td>
</tr>
</tbody>
</table>

Table 3.5: Analyses of variance of two pairs of models for quadrat burden score, a: including vs. not including survey run as a factor, b: including vs. not including participant as a factor. P-values were generated using likelihood ratios of the Chi-Squared distribution.

(a) fmc: Model for 'Burden' including 'Quadrat' and 'Participant'; fm1: Model for 'Burden' including 'Quadrat', 'Participant' and 'Run'

<table>
<thead>
<tr>
<th>Model</th>
<th>Df</th>
<th>AIC</th>
<th>logLik</th>
<th>Chisq</th>
<th>Pr(&gt;Chisq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>fmc</td>
<td>12</td>
<td>283.34</td>
<td>-129.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fm1</td>
<td>13</td>
<td>284.55</td>
<td>-129.28</td>
<td>0.7934</td>
<td>0.3731</td>
</tr>
</tbody>
</table>

(b) fmp: Model for 'Burden' including 'Quadrat' and 'Run'; fm1: Model for 'Burden' including 'Quadrat', 'Participant' and 'Run'

<table>
<thead>
<tr>
<th>Model</th>
<th>Df</th>
<th>AIC</th>
<th>logLik</th>
<th>Chisq</th>
<th>Pr(&gt;Chisq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>fmp</td>
<td>12</td>
<td>299.27</td>
<td>-137.63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fm1</td>
<td>13</td>
<td>284.55</td>
<td>-129.28</td>
<td>16.718</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

debris count is significantly influenced by individual perception. The burden ratings for 3 out of 10 quadrats ranged over 3 orders of magnitude (Figure 3.7).
3.4 Discussion

3.4.1 Representation by outliers

This study aimed to find a way to quantify the contamination of beaches by small plastic debris, which occur in patchy aggregates. This aim is constrained by two obstacles: (a) small plastic debris are too numerous and too small to be detected and accurately quantified on the scale of an entire beach, and (b) they occur in patchy aggregates, which are an unlikely target for random sampling, and while they are the main drivers of the amount of contamination. The proposed method addresses these obstacles by targeting the patchy aggregates specifically.

This approach goes against statistical intuition, which would call for strict random sampling.

Figure 3.7: Ranges of burden ratings for each quadrat across volunteers. Open squares indicate burden rating by author.
Venturing outside random sampling also raises the question what might be a good way to aggregate the values of such deliberately selected samples, as random samples would call for the arithmetic mean. In this study, the geometric mean was used because (1) it is recommended for right-skewed distributions (Galton 1879) and (2) there are more possible geometric means than medians or arithmetic means, which limits tied ranks and allows for better comparability between beaches.

Plastic debris surveys have favoured more highly contaminated areas of the beach over random areas. Storrier and McGlashan (2006) and Hidalgo-Ruz and Thiel (2013), who targeted small plastic debris specifically, as well as Rosevelt et al. (2013) who distinguished between size ranges have focused on the drift line, which typically contains small plastic debris, but does also not represent the entirety of the beach. In fact, seven out of ten methods to sample beach litter tested by Velander and Mocogni (1999) deliberately focused on areas where litter tends to accumulate: the vegetation line and/or various generations of the drift line. Two of the methods that gave a less determined representation of the beach (1 m and 2 m sampling strip) were very time-consuming. The last remaining sampling method used random quadrats, which returned high variability, as has also been shown in this study.

Velander and Mocogni (1999) rightly suggest that sampling the vegetation line will return an overestimate of the effective amount of litter on the whole beach, and that because different methods have such different estimates, studies using different methods cannot be consolidated. What is left is to use an approach that returns consistent results, that has low within-beach variability; and focusing on small plastic debris accumulations rather than sampling random quadrats does exactly that.

Deliberate selection of samples, which is better suited to return non-zero values, has been applied in other fields of environmental sciences. The total of a quantitative variable is best estimated by putting the least sampling effort into areas where value is zero or small, and the most sampling effort into areas where the value is large or variable (Cormack 1988). Quadrat surveys have often been used to examine the species communities on rocky shores (Hawkins and Hartnoll 1983; SACFOR abundance scale 1990). Surveys on rocky-shore species communities are
confronted with similar problems as plastic debris surveys on beaches; i.e. they also examine patterns that exhibit a high spatial variability, and it can therefore be difficult to determine a single ‘right’ way to do it (Hartnoll and Hawkins 1980). In quadrat sampling the number of replicates that are required to pick up differences between surveys varies; in a survey of intertidal species communities ten to twenty quadrats were determined to be a suitable sample size (Hawkins et al. 1986). Variation of species communities on a rocky shore over time were successfully picked up by examining the same quadrats at set time intervals (Hawkins and Hartnoll 1983). Rock pool dwellers are an extreme example. Their abundance on a rocky shore is driven solely by their abundance in each rock pool on the rocky shore. Surveying their population outside their cluster does not make sense, because it would be zero.

Organisms that are limited to rock pools occur in static and highly defined clusters. The topology of a rocky shore is static, and rock pools are delimited, but waterfowl wintering in Florida, which also occur in clusters, are highly mobile, and it is not predictable where in a larger survey area these clusters are. Systematic assessment of the population density would require counts even in areas with no presence of the animal. Smith et al. (1995) determined that selecting areas with higher population density out of a random sample, then sampling the neighbouring areas could result in higher precision than simple random sampling. This method is called adaptive cluster sampling (Thompson 1990). Adaptive sampling selects data points that meet a criterion of interest out of a random set. The neighbouring data points of this initial sample are included. The calculation of the estimator of the population as well as the variance based on these data points includes the detection probability of the selected data points.

The method proposed in this study has a less systematic approach to selecting data points that meet a criterion than was presented by Thompson (1990). The initial random set would be the observer’s trail. The initial sample would be the identified small plastic debris accumulations. At this stage - while adaptive cluster sampling would use an exact account of the population of the neighbouring areas - the proposed method gives an estimate of the order of magnitude of the population density in the accumulation. Adaptive cluster sampling weighs this account with detection probability. A way to transfer this approach from quantitative accounts to estimates
of orders of magnitude has yet to be identified.

Smith et al. (1995) used the best effort of a full count of waterfowl in a 5000 km$^2$ area to calibrate this method for their purpose with a simulation of adaptive cluster sampling. Even a best effort of a full count of small plastic debris on a beach will result in a substantial underestimate, as pieces can be buried in an indefinite depth of sand (Velander and Mocogni 1999; Kusui and Noda 2003). Meanwhile, computer simulations could be used to measure the effectiveness of a survey method. Distribution patterns of small plastic debris on beaches could be artificially generated. Virtual surveys could then be conducted multiple times on the same distribution pattern; the consistency of the survey method could then be quantified.

3.4.2 Inter-observer variability

The volunteer trial in this study falls short of supporting the use of volunteers to count small plastic debris via surveys on beaches for two reasons: (a) volunteers were not allowed to select the dirty quadrats themselves, and (b) assessments of the same quadrat by different volunteers were not in sufficient agreement to give the same representation of the same beach.

Point (a) was omitted in this study, because a corresponding test would have required each volunteer to screen the same beach independently and identify dirty quadrats. This was not possible within the amount of time available. Identifying the dirtiest quadrats is an integral part of this method, and it is essential that volunteers pick not the same quadrats, but a set of quadrats that amounts to the same overall burden score of the beach.

An effect that is typically a concern in volunteer surveys could bias this method: Over-reporting of more conspicuous and less common events. In a tree stand survey, volunteers (grade 3 through 10 students) over-reported rare pines and larger oaks versus professionals (Galloway et al. 2006). In underwater visual surveys the difference in count between inexperienced observers and professionals would be larger for cryptic species than for conspicuous species (Williams et al. 2006). In abundance surveys, volunteers are more interested in surveying areas with higher abundance than ones with lower abundance (Robson 2012). This sampling bias is unwanted, as it leads to overestimates.
Adaptive cluster sampling is in itself a complex method that requires a series of logical steps, but in a simplified way it can also be looked at as the intuitive selection of samples with high abundance. Volunteers may in fact be better suited for this approach than professionals, who are conditioned to random sampling.

The measure of repeatability of the proposed method is that volunteers identify dirty quadrats that aggregate to the same burden score for the beach. This has to be tested. A set of volunteers would be taken to a beach. Each volunteer would take a turn at seeking out the ten dirtiest spots. Both the volunteer and a professional would independently rate the selected spot. This would be repeated for multiple beaches.

Point (b) shows that, in this trial, surveys conducted by different volunteers resulted in different burden ratings. In past volunteer-based beach debris surveys, volunteers would return collected debris or an exact account of the number of pieces found. The UK Marine Conservation Society provides a form volunteers use to log the exact number of a specific type of debris they collect (Marine Conservation Society 2014). In beach surveys, much effort has gone into identifying the original purpose/use of debris items (Ribic et al. 1992; Rosevelt et al. 2013; Marine Conservation Society 2014; Ryan et al. 2014), and volunteers have successfully contributed to the collection of such data (Storrier and McGlashan 2006; Marine Conservation Society 2014). Small plastic debris can often not be counted (as it is too numerous) or categorised (as the original purpose can no longer be identified). Surveys therefore rely on estimates of debris abundance.

Estimates are highly affected by individual perception, unless there are detailed protocols, and detailed protocols are precisely what volunteers struggle with. Hidalgo-Ruz and Thiel (2013) have worked with volunteers using methods that had some similarities with the methods proposed in this study. They identified sampling quadrats that were likely to contain a large number of small plastic debris, and they quantified the amount of small plastic debris found in them. The amount was not an estimate but an account of each detected piece, which is more accurate but also more time consuming. While their volunteers reached similar results as subsequent calibration counts by professionals, the results were affected by lack of protocol compliance. Debris
was misidentified as plastic, and data was not communicated correctly. Asking a volunteer to categorise quadrats on a logarithmic scale is much more complex, and this study has shown that observations vary substantially between volunteers. Meanwhile the volunteers in this trial remained consistent throughout the three observation cycles, even after they were given additional instructions concerning the identification of debris. This indicates that the source of variation between volunteers is the complex process of converting a quick visual assessment into a quantitative estimate.

Progress in technology has given rise to new ways to involve citizen scientists in research. Smartphones loaded with an appropriate app can become sensors and data loggers. This has also been applied in marine debris research with the Marine Debris Tracker app (Jambeck et al. 2011), which allows the user to log items they find in a central database. A promising feature of smartphones is georeferenced photography. In Creek Watch, volunteers documented water levels and flow rates of streams with photographs that would then be analysed by professionals (Robson 2012). When a visual assessment is sufficient data, a photograph can serve as a proxy, and while the data collection is conducted by volunteers, the data can still be analysed by the same, qualified person. Photographs may not contain enough detail to measure up to a visual assessment on site, but they allow controlling inter-observer variability.

### 3.5 Conclusion

Adaptive cluster sampling presents a set of survey and analytical methods that suits the spatial distribution of small plastic debris on beaches. While an even more exact quantitative assessment than the method proposed in this study would still return an underestimate of the actual contamination, it would also return a more reproducible estimate to compare beaches and sampling events. Future studies could investigate if (a) adaptive cluster sampling can be adapted for this purpose, and (b) if such an adaptation could preserve the rapidity of the proposed method.

Velander and Mocogni (1999) have tested 10 beach survey methods and evaluated their effectiveness serving different purposes on 16 beaches. Validating survey methods is essential, and doing so in the field is a substantial time commitment. Computer simulations of beach surveys could be used at least initially to evaluate the variability and the accuracy of a method,
especially because - in contrast to practical examples - the total number of debris is known.

The method developed and evaluated here returns a measure of the dirtiness and the frequency of small plastic debris accumulations on a beach. This is a long way from an accurate account of the amount of small plastic debris on a beach, but beaches with a high abundance of small plastic debris are likely to return dirtier quadrats and more such quadrats. Dirty quadrats are much less variable than random quadrats, and low variability allows for better comparison between beaches or between sampling events on the same beach.

The selection of dirty quadrats constitutes an integral part of the proposed method, and a corresponding volunteer trial has yet to be conducted. It is possible that intuitive volunteer behaviour will return a sufficiently accurate result. A quick estimate of small plastic debris abundance in a quadrat is a complex task, and observations vary between volunteers. Volunteers could photograph debris accumulations in addition to or instead of making the estimates themselves. A more comprehensive study of volunteer participation using this method is required before it can be productively disseminated.

Once this method can be applied consistently not just by professional staff but also by volunteers, it presents a good way to frequently survey a great number of beaches, which can return a highly defined account of small plastic debris contamination and allow for more extensive monitoring over time.
CHAPTER 3. RAPID SURVEY APPROACH
Chapter 4

Physical factors affecting plastic contamination on beaches

Beach surveys present an efficient way to assess the abundance of plastic in the marine environment. However, they are susceptible to variation from local factors other than actual quantities of at-sea debris abundance, such as local littering and litter removal, beach morphodynamics (e.g. slope, wave climate), beach exposure and weather. Small debris has been reported to accumulate on the shoreline. It is therefore important to understand the physical factors influencing accumulation. This chapter aims to identify environmental factors that promote the accumulation of small plastic debris in order to better understand the dynamics of the debris load on the shoreline. Using the method developed in Chapter 3, the contamination by small plastic debris of 52 beaches was measured. These beaches were characterised according to their environmental variables extracted from a weather database, and a survey of beach morphodynamics. Beach curvature and apex towards the open sea were assessed cartographically. Principal component analyses were used to summarise these suites of highly collinear variables, and a stepwise linear model approach was then used to test the power of environmental variables in explaining debris load patterns. The findings showed that small plastic debris contamination changed over time and was linked
CHAPTER 4. BEACH FACTORS

to hydrodynamic parameters more strongly than to wind. Results also showed that two
overreaching factors, retention and deposition, influenced small plastic debris contam-
ination in the same way, but that these factors may be conversely linked to physical
parameters such as beach exposure or wave action.

4.1 Introduction

Plastic constitutes 50 to 80% of solid waste washed up on beaches (Gregory and Ryan 1997;
Derraik 2002; Barnes 2005; Morishige et al. 2007). In the late 1980s and the early 1990s plastic
was shown to constitute an increasingly large portion of beach debris (Gabrielides et al. 1991;
Gilligan et al. 1992). Accumulation of plastics on shorelines gives rise to aesthetic concerns, and
wildlife may be harmed by the ingestion of debris in particular fragments (Carpenter et al. 1972;
Ryan 1987a). Ingestion of plastics may also lead to the transfer of potentially toxic chemicals
to organisms. While the arbitrary upper size boundary for microplastics is five millimetres
(GESAMP 2010), plastic fragments larger than that may also result from deterioration of larger
debris, and they are also likely to be ingested by animals. Beach surveys often do not consider
small debris, as this size class is difficult to detect at the scale the surveys are usually conducted
(hundreds of metres of transect) (Moore 2008). Considering the putative role of beaches as
important points of origin for microplastics as a consequence of UV accelerated fragmentation
(see Chapter 2), plastic debris smaller than five centimetres should also be considered as a
likely predecessor of microplastics; in this respect, debris smaller than five centimetres would
also gain significance. As such, the present study considers that the debris classed smaller than
five centimetres calls for particular attention.

Beach debris can have considerable economic impacts, for example in countries that rely on
tourism (Gabrielides et al. 1991; Ofiara and Brown 1999), since the cleanliness of beaches
has been recognised as the most important criterion for tourists (Morgan et al. 1993; Ballance
et al. 2000). Communities in the United Kingdom spend almost 18 million pounds each year
to clean up beaches (Mouat et al. 2010). Understanding factors affecting the distribution of
plastic fragments will help inform predictions as to areas that will experience the highest impact.
Based on this information, precautions could be taken to protect these locations and also to

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4.1. INTRODUCTION

guide beach cleaning campaigns. This chapter addresses the thesis aim to identify physical factors (morphology, wind and wave action) that influence the contamination of beaches by small plastic debris in order to begin building a model consisting of static (beach morphology) and dynamic (wave action, wind) factors to explain the contamination of a beach by small plastic debris.

Changes in local plastic debris abundance on beaches may be caused by weather, but also by changes in beach topology and vegetation over time (Whiting 1998). Ocean currents seem to play a critical role in the deposition of plastic debris on islands. Wilber (1987) and Lucas (1992) for instance describe archipelagos as "filters" or "sieves" for plastic debris, whereby the debris are deposited on the beaches by the passing currents.

There is substantial scientific literature to support the role of on-shore winds in causing plastic debris to accumulate on beaches (Dixon and Cooke 1977; Golik and Gertner 1992; Garrity and Levings 1993; Debrot et al. 1999; Ivar do Sul et al. 2009). Browne et al. (2010) showed that downwind sites are potential sinks for the accumulation of small debris. Browne et al. (2010), however, also state that small plastic fragments have a different dynamic, and are likely influenced by different environmental factors than larger debris. In their study Browne et al. (2010) established the number of particles per unit volume of sediment and differentiated between material types. However, the effort per site was substantial, and consequently only few sites within a single estuary were assessed. Hence the conclusions of this paper need to be verified on a larger geographic scale.

In the present study a rapid survey approach (Chapter 3) was used in order to observe a larger number of sites and examine the explanatory power of physical factors for which data were available; material types were not distinguished. Therefore, potential effects of debris properties themselves, such as density, on distribution are not considered.

Plastic debris accumulations on the ocean surface have been discovered in current convergences (Moore, Moore, Leecaster and Weisberg 2001; Law and Thompson 2014). Beaches that border a more heavily contaminated body of water may be more contaminated than beaches that border less contaminated parts of the sea. The present study examines local physical effects on
the abundance of small plastic debris on the shoreline; however, the at-sea abundance of small plastic debris and its effect on the shoreline abundance of small plastic debris may have a more significant effect. Therefore, the consequences of larger scale effects were addressed by comparing the small plastic debris burden on shorelines of one body of water against another. High variation within a body of water versus between bodies of water would attribute local factors of individual beaches a higher importance than differences between bodies of water. Southwest England adjoins the Celtic Sea to the North and the English Channel to the South and East (separated by Cape Cornwall). It has been suggested that the debris burden measured on shorelines could predict the amount of debris in the open sea nearby (Merrell 1980a; Dixon and Dixon 1981), and a significant difference in small plastic debris contamination between beaches on the North Coast and beaches on the South Coast could indicate that the Celtic Sea and the English Channel carry different amounts of small plastic debris.

As previous studies have linked wind to the amount of plastic on beaches (Gabrielides et al. 1991; Corbin and Singh 1993; Debrot et al. 1999; Abu-Hilal and Al-Najjar 2004, 2009; Ivar do Sul et al. 2009; Browne et al. 2010), wind direction and speed were both considered here. However, small debris have a smaller area subject to windage than large debris. Large debris generally protrude further from the water surface and are therefore exposed to stronger winds. Consequently, hydrodynamics may be more important than wind for the transportation of small debris, hence the influence of wave direction, height and period were also considered.

The consideration of parameters that are variable over time requires the definition of a reference time frame. Beaches are dynamic environments that undergo substantial turn-over. To establish the potentially relevant time period for the effect of wind and wave action, prior to the assessment of the small plastic debris burden, the effects of wind and wave conditions over the three days, three weeks and three months prior to the assessment on small plastic debris burden score were examined. For the assessment of the potential effect of physical parameters that change over time on the small plastic debris burden on beaches, archived regional atmospheric and sea condition data (wind, wave climate) were obtained. The surf forecast website http://www.windguru.cz provides archived forecast data for a large number of coastal sites.
4.1. INTRODUCTION

around Southwest England. The archived data for these sites over the entire survey period was downloaded.

Wind and wave action are considered as factors that could carry items of small debris from the sea onto shorelines, therefore the extent to which the beach was enclosed and its exposure to the open sea was also considered. The beach enclosure and its contact area were quantitatively expressed by the length of the beach, its curvature radius, the distance to the outmost obstructions to the open sea and the angle between them. (Abu-Hilal and Al-Najjar 2004) stated that unprotected (less enclosed) beaches are more susceptible to plastic debris contamination than protected beaches. However, it is also plausible that the opposite may be true, where enclosed beaches entrap plastic debris through a small apex angle towards the open sea.

The beach interface with the sea is also characterised by its tidal conditions and its topography. A large tidal range provides a large contact area that could retain small plastic debris, while a steep slope may reduce the retention rate, as small plastic debris are more likely to fall back in the water. Sediment size may play a role in the retention of small plastic debris as coarse sediment may provide more friction than fine sediment and prevent small plastic debris from being carried away. Data on morphodynamics of beaches was extracted from a survey conducted by Scott (2009). The mean levels, relative to the mean sea level, of the height and the low tide, at spring and neap, and the slope of these levels, as well as the median sediment size on the lower and the upper shore, were extracted from Scott (2009).

Tide levels are often similar within a geographic area, however, the possibility that they are a regional confounding factor, and that an effect of tidal range on small plastic debris contamination is actually a regional effect, had to be considered. The north coast and the south coast of Southwest England exhibit quite different tidal ranges (Table 4.1); the comparison between the two coasts was therefore also used to eliminate this possible confounding factor.
Table 4.1: Analysis of variance of tidal ranges between the North and the South coast of South-west England. Tidal ranges [m] extracted from Scott (2009).

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coast</td>
<td>1</td>
<td>36.05</td>
<td>18.023</td>
<td>80.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Residuals</td>
<td>39</td>
<td>8.74</td>
<td>0.224</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In order to answer the question of whether beaches can be expected to have consistent small plastic debris burden, this study also aimed to establish whether the small plastic debris burden of a beach changes over time and whether wind and wave action drive the small plastic debris burden on a beach to a larger extent than parameters that do not change over time. While the effects of wind and waves will be discussed separately, they were tested combined in a principal component analysis.

4.2 Methods

A rapid survey method to assess an index of plastic contamination on beaches was developed in Chapter 3. This method was used to survey 52 beaches (Table A.1 Appendix A) with known environmental parameters around Southwest England. The beaches were selected from a set of 89 beaches for which morphodynamic parameters had been assessed by Scott (2009) (‘UKBC beaches’). 5 beaches were selected outside the UKBC study (St. Austell Bay) due to an opportunity to conduct repeated surveys on a set of beaches in cooperation with Friends of Par Beach, a local community group. These five beaches (IDs 22 through 26, Table A.1 Appendix A) were each surveyed six times at two month intervals in 2010 and 2011.

To ascertain the relevance of the local physical factors of the surveyed beaches over their proximity to two different bodies of water (the Celtic Sea and the English Channel), the small plastic debris burden score of beaches on the North Coast and beaches on the South Coast of Southwest England were compared in an analysis of variance using R (R Core Team 2015).

Wind and wave data were obtained from http://www.windguru.org. Windguru.cz uses a Weather Research and Forecasting (WRF) model (Skamarock et al. 2001) at a 9 km resolution. The three-hour interval data on all available locations was retrieved from the archive for the period
from June first 2009 to November tenth 2011 thus covering all sampling events. Due to the large volume of data, the dynamic variables were entered into an ORACLE database and extracted as required using Structure Query Language in a Hypertext Preprocessor (PHP) interface on a local Apache web server.

Wind and waves are directional variables (two components). Hence they needed to be broken down to scalar variables for statistical analysis. Both the wind and the wave directions were broken down into North and East components. The wind vector components were multiplied with the wind speed, as stronger winds are likely to have more of an impact on the transportation of debris than weaker winds. Wave direction was considered a dimensionless vector. Each of the surveyed beaches was associated with the nearest windguru.cz forecast location (Table A1, Appendix A). For each beach the mean wind, wave direction, wave period, wave height and the maximum wind speed at the corresponding windguru.cz forecast location were aggregated over the three days, three weeks (21 days) and three months (90 days) prior to the beach survey date.

Two facing angles were measured for each beach: the apex and the beach facing (see Figure 4.1). Wind and wave directions were considered relative to both (orthogonal projection, see Figure 4.1 for schematic). This resulted in a scalar value that would be positive for on-beach and on-apex wind and waves and negative for off-beach and off-apex. The MySQL code used for these operations is documented in Figure 4.2.

Thus, the 3 day, the 21 day and the 90 day periods before the small plastic debris burden assessment were each described by the following variables, for each survey event: mean on-beach wind component, mean on-apex wind component, on-beach component of the maximum wind, on-apex component of the maximum wind, mean on-beach waves component, mean on-apex waves component, mean wave height and mean wave period. The parameters that characterise the beach enclosure (i.e. radius of curvature, apex angle, length and distance to obstructions towards the open sea) were determined cartographically. Aerial photographs of each beach were extracted from Google Earth™ and imported into Géométrix (http://geometrix.free.fr). The beach enclosure was constructed according to Figure 4.1 and the parameters were returned by the program. Mean levels of high and low water at neap and spring tide, the slope at these
Figure 4.1: Schematic of beach geometry and wind and wave calculation. \( l \): Beach length; \( d_1, d_2 \): Distance to obstruction towards open sea; \( \alpha \): Apex angle; \( r \): Beach curve radius; \( \vec{a} \): apex facing; \( \vec{b} \): beach facing; \( \vec{v}_{\text{wind}} \): wind speed and direction; \( \vec{v}_{\text{waves}} \): wave direction; \( \vec{v}_{\text{wind}, \vec{a}}, \vec{v}_{\text{wind}, \vec{b}}, \vec{v}_{\text{waves}, \vec{a}}, \vec{v}_{\text{waves}, \vec{b}} \): orthogonal scalar projections of wind and waves on apex and beach facing respectively.
4.2. METHODS

/* Code to quantify the orientation of beach and apex in sine and cosine of angle against north arrow */
UPDATE BEACHES SET APEXFACVECX=sin(APEX_FACING*3.14159/180), APEXFACVECY=cos(APEX_FACING*3.14159/180)
UPDATE BEACHES SET BEACHFACVECX=sin(BEACH_FACING*3.14159/180), BEACHFACVECY=cos(BEACH_FACING*3.14159/180)

/* Code to quantify wind and wave direction in sine and cosine of angle against north arrow */
UPDATE TEMPDATA SET WINDVECX=sin(winddir*3.14159/180)*windspeed, WINDVECY=cos(winddir*3.14159/180) WHERE windspeed IS NOT NULL AND winddir IS NOT NULL
UPDATE TEMPDATA SET WAVEVECX=sin(wavedir*3.14159/180), WAVEVECY=cos(wavedir*3.14159/180) WHERE wavedir IS NOT NULL

/* The code sections below show how wind and waves data of the 3 previous days was associated with each survey event. This was repeated for 21 days and 90 days data */
UPDATE BEACH_SURVEYS SET WINDX03=(SELECT AVG(TEMPDATA.WINDVECX) FROM BEACHES,TEMPDATA WHERE BEACHES.ID=BEACH_SURVEYS.LOCATION_REF AND TEMPDATA.LOCATION=BEACHES.WINDGURU_REF AND TEMPDATA.WINDVECX IS NOT NULL AND TEMPDATA.DATETIME<BEACH_SURVEYS.DATETIME AND TEMPDATA.DATETIME>=BEACH_SURVEYS.DATETIME-3);
 UPDATE BEACH_SURVEYS SET WINDY03=(SELECT AVG(TEMPDATA.WINDVECY) FROM BEACHES,TEMPDATA WHERE BEACHES.ID=BEACH_SURVEYS.LOCATION_REF AND TEMPDATA.LOCATION=BEACHES.WINDGURU_REF AND TEMPDATA.WINDVECY IS NOT NULL AND TEMPDATA.DATETIME<BEACH_SURVEYS.DATETIME AND TEMPDATA.DATETIME>=BEACH_SURVEYS.DATETIME-3);

/* Code to assign the mean x and y components of wind in the nearest Windguru reference point to each survey event */
UPDATE BEACH_SURVEYS SET WAVESX03=(SELECT AVG(TEMPDATA.WAVEVECX) FROM BEACHES,TEMPDATA WHERE BEACHES.ID=BEACH_SURVEYS.LOCATION_REF AND TEMPDATA.LOCATION=BEACHES.WINDGURU_REF AND TEMPDATA.WAVEVECX IS NOT NULL AND TEMPDATA.DATETIME<BEACH_SURVEYS.DATETIME AND TEMPDATA.DATETIME>=BEACH_SURVEYS.DATETIME-3);
 UPDATE BEACH_SURVEYS SET WAVESY03=(SELECT AVG(TEMPDATA.WAVEVECY) FROM BEACHES,TEMPDATA WHERE BEACHES.ID=BEACH_SURVEYS.LOCATION_REF AND TEMPDATA.LOCATION=BEACHES.WINDGURU_REF AND TEMPDATA.WAVEVECY IS NOT NULL AND TEMPDATA.DATETIME<BEACH_SURVEYS.DATETIME AND TEMPDATA.DATETIME>=BEACH_SURVEYS.DATETIME-3);
 UPDATE BEACH_SURVEYS SET WAVESH03=(SELECT AVG(TEMPDATA.WAVEHEIGHT) FROM BEACHES,TEMPDATA WHERE BEACHES.ID=BEACH_SURVEYS.LOCATION_REF AND TEMPDATA.LOCATION=BEACHES.WINDGURU_REF AND TEMPDATA.WAVEHEIGHT IS NOT NULL AND TEMPDATA.DATETIME<BEACH_SURVEYS.DATETIME AND TEMPDATA.DATETIME>=BEACH_SURVEYS.DATETIME-3);
 UPDATE BEACH_SURVEYS SET WAVESP03=(SELECT AVG(TEMPDATA.WAVEPERIOD) FROM BEACHES,TEMPDATA WHERE BEACHES.ID=BEACH_SURVEYS.LOCATION_REF AND TEMPDATA.LOCATION=BEACHES.WINDGURU_REF AND TEMPDATA.WAVEPERIOD IS NOT NULL AND TEMPDATA.DATETIME<BEACH_SURVEYS.DATETIME AND TEMPDATA.DATETIME>=BEACH_SURVEYS.DATETIME-3);

/* Code to assign x and y components of maximum wind to each survey event */
UPDATE BEACH_SURVEYS SET WINDMAXX03=(SELECT AVG(TEMPDATA.WINDVECX) FROM TEMPDATA WHERE TEMPDATA.LOCATION=BEACH_SURVEYS.WINDGURU_REF AND TEMPDATA.DATETIME<BEACH_SURVEYS.DATETIME AND TEMPDATA.DATETIME>=BEACH_SURVEYS.DATETIME-3 AND TEMPDATA.WINDVECX IS NOT NULL AND TEMPDATA.WINDSPEED=(SELECT MAX(TEMPDATA.WINDSPEED) FROM TEMPDATA WHERE TEMPDATA.LOCATION=BEACH_SURVEYS.WINDGURU_REF AND TEMPDATA.DATETIME<BEACH_SURVEYS.DATETIME AND TEMPDATA.DATETIME>=BEACH_SURVEYS.DATETIME-3);
 UPDATE BEACH_SURVEYS SET WINDMAXY03=(SELECT AVG(TEMPDATA.WINDVECY) FROM TEMPDATA WHERE TEMPDATA.LOCATION=BEACH_SURVEYS.WINDGURU_REF AND TEMPDATA.DATETIME<BEACH_SURVEYS.DATETIME AND TEMPDATA.DATETIME>=BEACH_SURVEYS.DATETIME-3 AND TEMPDATA.WINDVECY IS NOT NULL AND TEMPDATA.WINDSPEED=(SELECT MAX(TEMPDATA.WINDSPEED) FROM TEMPDATA WHERE TEMPDATA.LOCATION=BEACH_SURVEYS.WINDGURU_REF AND TEMPDATA.DATETIME<BEACH_SURVEYS.DATETIME AND TEMPDATA.DATETIME>=BEACH_SURVEYS.DATETIME-3);

/* Repeated for 21 and 90 days data */

Figure 4.2: MySQL code used to transform wind and wave data. See explanations commented ('/*', '*/') inline.
CHAPTER 4. BEACH FACTORS

levels and the sediment grain size ($D_{50}$) on the lower and upper shore were extracted from Scott (2009).

The 5 beach geometry variables, the 10 morphodynamics variables and the 8 dynamic variables that were aggregated over 3 different time periods represent 49 variables that were to be tested in a model explaining the small plastic debris burden in 47 data points. Such a model could potentially be over fitted with a highly collinear suite of variables, which would render it invalid for any data points outside the set that was used to create it.

Principal component analysis constitutes a way to reduce the number of variables and the weight of collinear variables in a model. The two first principal components of a set of variables may represent a large proportion of their variability. The first and second principal components of five groups of variables (geometry, morphodynamics, 3 days, 21 days and 90 days) were therefore used as independent variables for the model that was to be built. These selections of variables also allow estimating the importance of beach geometry, morphodynamics and wind and wave action over the days, weeks or months in general.

The principal components were calculated using the ‘princomp()’ function (calculation of principal components) in the statistical package R (R Core Team 2015). The results of the principal component analysis are listed in Table 4.2 and illustrated in Figure 4.3.

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Figure 4.3 (facing page): Visualisation of the principal component analysis of beach factors. Circles show scores of each survey event (or each beach for geometry and morphodynamics) on the first and second principal component (referring to bottom and left axis). Arrows show eigenvectors of each factor (referring to top and right axis). $d_1$: distance to right obstruction, $d_2$: distance to left obstruction, $l$: beach length, $r$: beach curvature radius, $\alpha$: apex angle, mlws: mid low water level at spring tide, mlwn: mid low water level at neap tide, mhws: mid high water level at spring tide, mhwn: mid high water level at neap tide, $D_{50l}$: median sediment size at lower beach, $D_{50u}$: median sediment size at upper beach, $s_{lt}$: slope at spring tide low water line, $s_{ln}$: slope at neap tide low water line, $s_{ht}$: slope at neap tide high water line, $s_{hb}$: slope at spring tide high water line, $f$: wave period, $h$: wave height, $\nu_{waves,a}$: mean on-apex waves component, $\nu_{waves,b}$: mean on-beach waves component, $\nu_{wind,a}$: mean on-apex wind component, $\nu_{wind,b}$: mean on-beach wind component, $\nu_{wind,a}$: on-apex component of maximum wind, $\nu_{wind,b}$: on-beach component of maximum wind.

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The relationship of the observed small plastic debris burden with the resulting ten principal components was tested in a linear model in R. Successively, the variables with the largest p-value were removed, while observing the model’s corrected Akaike information criterion (AICc). The AIC is a measure of the quality of a model based on the number of parameters and the maximised likelihood of the model (Akaike 1974). For small sample sizes (i.e. when the sample size n is not many times larger than the squared number of tested parameters $k$) a penalty of $\frac{2k(k + 1)}{(n-1)n}$ is added to the AIC to give the corrected AIC. With a sample size 47 and 10 tested parameters, this rule applied. The model with the lowest AICc was retained for interpretation.

4.2.1 Importance of factors that change over time

In order to test whether the plastic debris burden score of a beach changes over time, and whether parameters that change over time have more explanatory power for the plastic debris burden score on beaches than static parameters, the set of repeated observations from the five beaches around St. Austell Bay was used to establish whether local static factors or dynamic factors, e.g. wind and waves, were better predictors of the plastic debris burden on beaches. The burden score was tested against the first and the second principal component of the wind and wave data of each of the time intervals 3, 21 and 90 days to determine the fit of a dynamic factor model. An analysis of variance of the six observations of burden score on each of the five beaches was used to determine whether the variation between beaches was greater than within beaches. Finally an analysis of variance of the burden score over the six observation cycles was used to see if the burden score variation was greater between than within cycles, i.e. all beaches show similar burden scores at any given time, which would rule out any local effects within the area of St. Austell Bay.
4.2. METHODS

Table 4.2: Coefficients of beach variable eigenvectors on the first and second principal components of the variable sets 'Geometry', 'Morphodynamics', '3 days', '3 weeks' and '3 months'. Percent variation accounted for by each principal component listed in the second rows, n: number of data points.

(a) Geometry (n=49)

<table>
<thead>
<tr>
<th>% variation</th>
<th>Comp.1</th>
<th>Comp.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radius</td>
<td>-0.394</td>
<td>0.427</td>
</tr>
<tr>
<td>Apex angle</td>
<td>0.02</td>
<td>0.845</td>
</tr>
<tr>
<td>Length</td>
<td>-0.548</td>
<td>0.123</td>
</tr>
<tr>
<td>Distance to left obstruction</td>
<td>-0.532</td>
<td>-0.214</td>
</tr>
<tr>
<td>Distance to right obstruction</td>
<td>-0.511</td>
<td>-0.205</td>
</tr>
</tbody>
</table>

(b) Morphodynamics (n=40)

<table>
<thead>
<tr>
<th>% variation</th>
<th>Comp.1</th>
<th>Comp.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope at spring high water line</td>
<td>-0.308</td>
<td>0.134</td>
</tr>
<tr>
<td>Slope at neap high water line</td>
<td>-0.357</td>
<td>0.052</td>
</tr>
<tr>
<td>Slope at neap low water line</td>
<td>-0.361</td>
<td>0.164</td>
</tr>
<tr>
<td>Slope at spring low water line</td>
<td>-0.306</td>
<td>0.33</td>
</tr>
<tr>
<td>Mid high water spring</td>
<td>0.308</td>
<td>0.403</td>
</tr>
<tr>
<td>Mid high water neap</td>
<td>0.301</td>
<td>0.384</td>
</tr>
<tr>
<td>Mid low water neap</td>
<td>-0.293</td>
<td>-0.29</td>
</tr>
<tr>
<td>Mid low water spring</td>
<td>-0.309</td>
<td>-0.405</td>
</tr>
<tr>
<td>Median sediment size upper</td>
<td>-0.308</td>
<td>0.364</td>
</tr>
<tr>
<td>Median sediment size lower</td>
<td>-0.303</td>
<td>0.391</td>
</tr>
</tbody>
</table>

(c) Temporal (n=76)

<table>
<thead>
<tr>
<th>% variation</th>
<th>3 days</th>
<th>3 weeks</th>
<th>3 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Comp.1</td>
<td>Comp.2</td>
<td>Comp.1</td>
</tr>
<tr>
<td>onbeachwind03</td>
<td>-0.431</td>
<td>-0.006</td>
<td>-0.472</td>
</tr>
<tr>
<td>onbeachwaves03</td>
<td>-0.385</td>
<td>0.141</td>
<td>-0.34</td>
</tr>
<tr>
<td>onapexwind03</td>
<td>-0.422</td>
<td>0.004</td>
<td>-0.461</td>
</tr>
<tr>
<td>onapexwaves03</td>
<td>-0.381</td>
<td>0.143</td>
<td>-0.351</td>
</tr>
<tr>
<td>maxwindonbeach03</td>
<td>-0.41</td>
<td>-0.109</td>
<td>-0.378</td>
</tr>
<tr>
<td>maxwindonapex03</td>
<td>-0.417</td>
<td>-0.101</td>
<td>-0.383</td>
</tr>
<tr>
<td>WAVESH03</td>
<td>0.011</td>
<td>0.679</td>
<td>0.143</td>
</tr>
<tr>
<td>WAVESP03</td>
<td>0.02</td>
<td>0.69</td>
<td>0.127</td>
</tr>
</tbody>
</table>


4.3 Results

4.3.1 Regional differences

The small plastic debris burden of beaches on the Celtic Sea coast of Southwest England was overall the same as on the English Channel coast (Analysis of variance Table 4.3). The adjacent body of water was therefore not a reliable predictor for the amount of small plastic debris on the beaches therein. This also produces an argument against regional confounding factors. For instance, tidal levels, which are regional and whose effects can be confounded with other regional factors, e.g. notoriously higher regional contamination levels, could gain more explanatory power.

4.3.2 Physical factors as predictors for small plastic debris contamination

The model of physical factors explaining small plastic debris contamination was built using stepwise linear regression. The model’s explanatory power was improved by successively removing the parameters with the least contribution. The first parameters to be removed were wave height and period over 90 days prior to the survey event (represented by the second principal component of 90 days data, significance level of coefficient $p = 0.77$, relative likelihood of after vs. before exclusion $q_{rel} = 7.41$) followed by the amount of on-beach wind and waves over 90 days (second principal component of 90 days data, $p = 0.75$, $q_{rel} = 6.37$). This shows that wind and wave events over a period of three months are a poor predictor for small plastic debris contamination.

On-beach wind and waves over three days before the survey event were also eliminated from the model (first principal component of the three days data, $p = 0.62$, $q_{rel} = 5.12$). Decreasing slope and sediment size combined with increasing tidal intervals were also removed from the model (first principal component of morphodynamic data, $p = 0.57$, $q_{rel} = 4.37$). Decreasing on-beach

Table 4.3: Analysis of variance of the burden scores between beaches on the North and on the South coast of Southwest England.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coast</td>
<td>1</td>
<td>0.71</td>
<td>0.3561</td>
<td>0.675</td>
<td>0.512</td>
</tr>
<tr>
<td>Residuals</td>
<td>73</td>
<td>38.52</td>
<td>0.5276</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.3. RESULTS

Table 4.4: Coefficients of the best fitting model for the beach small debris burden score. Residual standard error: 0.68 on 35 degrees of freedom. Multiple R-squared: 0.41, Adjusted R-squared: 0.34, F-statistic: 6.13 on 4 and 35 degrees of freedom, p-value: <0.001.

|                          | Estimate | Std. Error | t value | Pr(>|t|) |
|--------------------------|----------|------------|---------|----------|
| Intercept                | 1.83     | 0.11       | 16.42   | < 0.001  |
| Geometry PC2             | -0.22    | 0.10       | -2.25   | 0.031    |
| Morophodynamics PC2      | 0.24     | 0.09       | 2.73    | 0.010    |
| 3 days PC2               | -0.40    | 0.16       | -2.58   | 0.014    |
| 21 days PC2              | -0.33    | 0.11       | -2.94   | 0.006    |

Wind and waves combined with increasing wave height and period in the 3 weeks leading up to the survey event were also removed from the model (first principal component of 21 days data, \( p = 0.60, q_{rel} = 4.05 \)). The last factors to be excluded were beach length, radius of curvature and distance to obstruction (first principal component of geometry, \( p = 0.41, q_{rel} = 2.95 \)).

With the available physical parameters, the model that best explained the small plastic debris burden on beaches included the second principal component of beach geometry, morphodynamics, 3 days and 3 weeks wave and wind data (\( AICc = 92.15, \) Table 4.4. It was therefore selected for further examination.

Small plastic debris burden increased with increasing wave height and period as well as predominant on-beach wave direction during the three weeks before the survey event. By contrast, strong on-beach wind events seemed to have a negative effect on burden. This is shown by the negative relationship between burden score and the second principal component of the 21 days data, which is in turn positively related to the on-beach component of maximum wind and negatively related to wave height and period as well as on-beach wave direction.

Small plastic debris burden was higher, the larger the tidal intervals were. Also, larger burden was measured on beaches with larger sediment size. This is demonstrated by the positive relationship between the small plastic debris burden score and the second principal component of morphodynamics data. The second principal component’s main difference from the first (which was eliminated from the model early in the stepwise process) was the sign of the coefficients for sediment size and the reduced coefficients for slope.

In the 3 days preceding the survey, small plastic debris burden was negatively related to wave
height and period, which is a contrast to the 3 weeks preceding the survey event. This is described by the second principal component of the 3 days data. The main difference from the first principal component, which was excluded from the model early in the stepwise process, was a strong positive relationship with wave height and period.

Regarding the beach geometry, small plastic debris burden increased the smaller the apex angle towards the sea and the smaller the curvature radius of the beach was. These two factors were positively related with the second principal component of beach geometry, to which burden was negatively related.

4.3.3 Importance of factors that change over time

The small plastic debris contamination on the five beaches around St. Austell Bay varied more over time on each beach than it differed between beaches (analysis of variance, \( df = 4, F = 1.331, p = 0.286 \)). This means that parameters that changed over time did not only affect the small plastic debris burden on beaches, they also outweighed local constant physical factors. This suggests a strong involvement of factors that changed over time in the amount of small plastic debris contamination detected on a beach. Indeed, wind and wave events preceding the survey seemed to explain to some extent the small plastic debris contamination on the 5 beaches that were observed every two months for one year. Linear model of burden score versus principal components 1 and 2 of 3, 21 and 90 days data: \( F = 2.43, df = 23, p = 0.058 \). Meanwhile, with all burden scores combined, there was no significant difference between survey cycles (analysis of variance of burden score of 5 beaches between 6 survey cycles: \( F = 1.87, df = 24, p = 0.138 \)). Even though these 5 beaches were within few kilometres of each other, their small plastic debris burden did not respond to wind and weather events in the same way.

4.3.4 Key findings

This study showed that wave action and tide, which change over time, are better predictors of beach contamination by small plastic debris than geometry or morphology, which are fixed. Specifically wave action was an important predictor of small plastic debris burden, and its influence changed depending on the observation interval: 3 weeks trends of high waves with
4.4 Discussion

Many studies have examined plastic debris on beaches, but most have focused on the composition of these debris, which may point to the possible sources of environmental contamination. Only a few have looked at links between environmental factors such as weather, topology or geographic location and the amount of plastic debris found on beaches; none have as thoroughly as the present study. There is substantial evidence that weather conditions can influence the amount of plastic on beaches (Dixon and Cooke 1977; Golik and Gertner 1992; Garrity and Levings 1993; Debrot et al. 1999; Ivar do Sul et al. 2009), and it has also been suggested as a predictor of the abundance of microplastics (Browne et al. 2010). Additionally, the physical constitution of a beach has been linked to the amount of plastic (Abu-Hilal and Al-Najjar 2004), and changes in topology and vegetation on beaches have been linked with changes in plastic debris abundance (Whiting 1998).

By considering such a large suite of parameters, the present study has succeeded in linking the small plastic debris burden on beaches with hydrodynamics more than with winds. The repeated screening of five beaches around St. Austell Bay also revealed that small plastic debris burden changes significantly over time, and that wind and wave events rather than prevailing conditions predict the small plastic debris burden on these beaches.

With a shift of marine debris from large, identifiable items to smaller fragments identification of beach debris may become less feasible, and beach surveys will have one less factor that could potentially explain debris dispersal patterns. Beach debris that could still be identified as fishing line, carrier bags or sunscreen bottles have been linked to the proximity of major population centres or river sheds (Lee et al. 2013), shoreline activities (Kordella et al. 2013) or fishing routes (Jang et al. 2014). As beach debris are becoming increasingly dominated by fragments that cannot be identified as such items, linking them to potential sources in this manner is no longer possible.
4.4.1 A model to predict small plastic debris contamination

There are reasons other than proximity of human settlements and activity that some beaches gather more plastic debris than others, and these have only been explored to a limited extent. A handful of studies have linked higher amounts of plastic debris to prevailing on-shore winds (Corbin and Singh 1993; Debrot et al. 1999; Abu-Hilal and Al-Najjar 2004; Ivar do Sul et al. 2009) while sometimes mentioning, that wave action that is related to wind may also be a factor or that ocean currents have the same direction as the prevailing winds.

By contrast, the present study takes a great number of physical parameters into account. Most importantly, short-term effects of wind and wave events are considered in the model that was built here. Many of these parameters have an effect on the small plastic debris burden on beaches. But due to the sample size, which was not much higher than the number of parameters, no single parameter could be identified as a main driver.

Even with parameters aggregated into principal components, less than 10 data points per parameter remained in the model containing all principal components. This put the stepwise linear regression on a relatively limited basis. The model that was ultimately selected contained 4 parameters, which returned a higher degree of freedom. But the models used in the stepwise selection were potentially oversaturated, which may have affected the selection criteria.

This study indicates that some of the considered environmental factors influence the amount of small plastic debris on beaches, and it is worth further exploring these parameters using larger datasets. This means that small plastic debris burden needs to be assessed on more beaches, for which the same parameters are known or can easily be measured. This study suggests that small plastic debris burden is mostly driven by (a) tidal range, (b) wind and wave events and (c) beach geometry. (a) is regionally known worldwide, and detailed information is available for many beaches. (b) can be extracted from weather records, and (c) can be measured cartographically.

Thus a next step would be to apply the survey method developed in Chapter 3 on more beaches and to thus expand the dataset to better inform the model. With enough data points this model can also move past using principal components and be narrowed down to relevant physical
Currently this model is fitted solely to data that was collected in Southwest England, which only covers a limited range of physical parameters. Considering areas with e.g. stronger wave action or slower wind would give the model a larger descriptive range. Expanding the dataset geographically would generally improve the model’s applicability, also due to possible regional differences in small plastic debris concentration at sea. No regional differences in small plastic debris burden on beaches was detected between the North and the South coast of Southwest England, but this comparison can only discard regional confounding factors in this study and is not representative of wider scale regional differences.

From the modelling here it would appear that small plastic debris burden on beaches is strongly influenced by two main factors, (a) the amount of plastic debris washed on to the beach (deposition) and (b) the amount of plastic debris that is not washed away (retention). An active interface between the beach and the ocean works both ways and can potentially influence the small plastic debris burden positively and negatively at the same time. E.g. a small apex angle may shield the beach against small plastic debris being washed in from the open sea, but it may also prevent small plastic debris from being washed out. In this study a small apex angle caused more retention than it prevented deposition.

Similarly, high waves may carry in more debris, but they may also remove more, and a narrow opening towards the open sea may shield the beach from debris being carried in, but it may also prevent existing debris from being washed out. These trade-offs make a model more complex; parameters have to be considered in combination with each other, as one parameter may influence another parameter’s trade-off.

### 4.4.2 Hydrodynamics and small plastic debris

The amount plastic debris on beaches has often been related to wind (Golik and Gertner 1992; Debrot et al. 1999; Ivar do Sul et al. 2009). The present study investigated both wind and hydrodynamics as predictors of small plastic debris burden, and it showed that hydrodynamic events contributed more to the model based on these observations than wind.
In this study, both a positive and a negative effect of high wave action were observed. During the 3 weeks before the survey, greater wave height and periods incurred higher small plastic debris burden. Deposition was more important than retention. During the 3 days before the survey it was the opposite.

This result may be due to the survey timing. Surveys were consistently conducted at neap tide in order to maximise the observable area above the drift line; it seems therefore that the coincidence of events of wave action with stages in the tidal cycle affect the small plastic debris burden. Apart from wave action, the tidal range of beaches has been linked to the small plastic debris burden. Links between tidal patterns and the amount of debris deposition have been observed previously by Storrier et al. (2007), although they do not specify what these links might be; their study focused mainly on debris composition. This study suggests that the higher the tidal range is, the higher the small plastic debris burden. High tidal ranges constitute a strong interface between sea and land, which suggests that deposition is overall more important than retention. This theory is also supported by the observation that small plastic debris burden and wave action are negatively related around neap tide (when the tidal range is the smallest) and positively related in the longer time interval, which includes a spring tide.

The observation of opposite effects of wave action in different time intervals also has to be examined with the nature of the parameters in mind: The model fits against principal components, which means that the good fit with wave action may be confounded with another physical parameter in the same principal component. But wave height and period have strong coefficients within both the 3 days and the 21 days principal component. Another explanation could be that wave height and period are statistically bound to reach higher maxima in 21 days than in 3 days. If these maxima are outliers, it is possible that they oppose the linear regression against small plastic debris burden.

While small plastic debris burden on beaches seems to be more strongly related to waves than to wind, this distinction has to be made with caution. Both wind and wave data were obtained from the archive of a forecasting website; they are therefore not direct measurements (which would not be possible at such a high spatial and temporal definition) but derived from climate data.
4.4. DISCUSSION

data. Wave data itself is in part derived from wind data using the Wavewatch III model (Tolman 2014) by NOAA. The independent variables in the temporal data are therefore not altogether independent. Wind forecasts from different locations and times may be derived from the same measurement, and wave data is therefore even a secondary derivative.

Wind and Wave data do show a good mutual fit, albeit with a large amount of residual variation. Going into the detail of wave modelling exceeds the scope of this study, but this observation shows that waves are only in part driven by wind, which also makes them unequal predictors for small plastic debris burden. The conclusion that small plastic debris are more directed by water than by air therefore stands. The reason for this phenomenon could be, that small debris protrudes less from the water surface than large ones. Wind speed, however, increases with increasing distance from the water surface. Small debris are therefore exposed to weaker winds than large debris.

4.4.3 Off-shore winds

Perhaps the most curious observation in this study, which is contrary to the findings of many previous studies, is that small plastic debris would be lower within three weeks after strong wind onshore toward the beach. Previous studies indicate that beaches facing prevailing winds exhibit higher plastic contamination than beaches facing the other way (Corbin and Singh 1993; Debrot et al. 1999; Ivar do Sul et al. 2009; Browne et al. 2010). Corbin and Singh (1993) conducted their study on St. Lucia and Dominica in the Caribbean Sea. They state that beaches facing trade winds and the Equatorial Current exhibit higher contamination than beaches on the other side of the island. Current and wind may be confounding. Ivar do Sul et al. (2009) also attribute higher debris contamination to on-beach winds; wave action is not taken into account.

The present study focused on small debris, which may not react to wind the same way as larger debris. But Debrot et al. (1999) as well as Ivar do Sul et al. (2009), who considered the size distribution of debris, found that smaller debris were more prevalent versus large debris on windward than on leeward beaches, which contradicts the observations in the present study.

Debrot et al. (1999), who expressed that this observation may be attributed to higher wave action on windward than on leeward beaches, conducted their study on the island of Curaçao in
the Caribbean Sea. Ivar do Sul et al. (2009) conducted their study on the island of Fernando de Noronha in the Equatorial Western Atlantic. It is possible that wind and waves are more collinear in these insular environments as they are in the Celtic Sea and in the English Channel, where the present study was conducted. The present study may be in a better position to distinguish between wave and wind direction.

Browne et al. (2010) specifically examined the effect of prevailing winds on spatial patterns of microplastics contamination. They found that downwind sites had more contamination than upwind sites. However, their study sites were all located in an estuary, which likely has less wave action than beaches facing the open sea. As in insular environments - as described above - in an estuary, wave and wind direction may be more strongly linked than on the beaches examined in this study, and wind as observed by Browne et al. (2010) may have an effect by waves as an intermediate. Dekiff et al. (2014) observed that small and large plastic debris do not follow the same spatial distribution patterns. Beaches with no large plastic debris may be contaminated with small plastic debris. The paradigms for spatial distribution of large plastic debris may not apply to small plastic debris. This positive relationship between small plastic debris burden and off-beach wind may mean either of two things: 1) Stronger on-beach winds remove debris from the observed area, or 2) Stronger off-beach winds move more debris into the observed area. Again, there is a trade-off between deposition and retention.

Given the nature of the model built in this study, the role of these strong wind events in causing higher small plastic debris contamination cannot be considered in an isolated way, but only in the context of the other parameters. The effect of these wind events is merely indicative, but the early exclusion of wind related parameters from the linear regression reinforces a model without wind to predict small plastic debris burden.

A further distinction between this and previous studies is that previous studies looked at general prevailing winds, while this study looked at conditions specific to each survey event. This study is thus able to document the immediate response of small plastic debris burden to wind and wave conditions, which change over time. In fact, data that would be most representative of prevailing winds (aggregated over 3 months) did not contribute to the model at all. Instead of
predicting small plastic debris burden solely on location, the model created in this study can thus also predict changes in the same location over time.

4.5 Conclusions

This study has shown that small plastic debris burden on beaches depends much more on hydrodynamic events than on wind. This was evidenced by the dominant parameters representing wave action and tidal ranges in the principal components that are part of the model. A larger sample size has to be generated to further develop the model and to possibly identify single physical parameters rather than principal components of sets of parameters.

Previous studies have attributed the amount of debris found on beaches to prevailing winds and waves. Prevailing waves and winds were factored out against shorter-term events in this study. The wind and wave data that was aggregated over three months offered no explanation for the amount of small plastic debris on beaches. Wave events rather than prevalence are better predictors for this effect. This makes small plastic debris burden a phenomenon that is not only localised in space but also in time.

This temporal fluctuation of parameter interaction also illustrates that parameters may be co-dependent and affect each other if they change over time. At neap tide increasing wave action reduces the small plastic debris burden, while it generally has the opposite effect over the whole tidal cycle. The trade-off between retention and deposition in changing the small plastic debris burden on beaches has to be considered when causes for this contamination are examined.
Chapter 5

Ingestion of microplastics by common cockles (*Cerastoderma edule*) in an intertidal mudflat

In order to investigate the potential effects of microplastics on animals, exposure experiments in the natural environment are needed. The common cockle was examined as a model organism for such an experiment, as it lives in a habitat that allows controlled exposure to microplastics. Cockles were deployed in designated quadrats in an intertidal mud flat, which was then inoculated with fluorescent microplastic particles. Sediment samples showed that the plastic particles were still present in the sediment after two weeks, when the cockles were collected. The digestive tracts of the cockles were extracted and sliced with a microtome for examination with a fluorescence microscope. The animals contained fluorescent particles; however, these could not be identified as the test particles with certainty, as fluorescent particles were also found in control animals. Furthermore, a separate trial showed that the tissue processing method that was applied on the stomachs reduced the fluorescence of the plastic particles. The study concluded that objectives of in-situ microplastics exposure trials need
to be determined more precisely, so they can be addressed by more specific methods
in order to minimise sample processing.

5.1 Introduction

Microplastic particles arising from the degradation of large plastic debris or direct introduction into the environment are widespread in marine habitats (Colton et al. 1974; Gregory and Ryan 1997; Thompson et al. 2004) and have raised concerns about potential threats to marine organisms. Microplastic particles may be ingested, and their physical presence may affect invertebrates in a similar way to that shown for large debris affecting other animals, e.g. birds (Ryan 1988; van Franeker et al. 2005). Plastic particles have also been shown to absorb persistent organic pollutants from water (Mato et al. 2001; Teuten et al. 2007; Bakir et al. 2014b), which can be transferred to organisms as a consequence of ingestion (Yamashita et al. 2011; Browne et al. 2013; Tanaka et al. 2013).

Laboratory experiments on the blue mussel (*Mytilus edulis*) have shown that microplastic particles are ingested and remained in the organism in excess of 48 days (Browne et al. 2008). Microplastics have been shown to be ingested by various species of zooplankton (Cole et al. 2013) specifically copepods (Lee et al. 2013), sand hoppers (Ugolini et al. 2013) and gooseneck barnacles (Goldstein and Goodwin 2013). Microplastics may also be transferred from lower to higher trophic levels via predation (Farrington et al. 1983; Watts et al. 2014). Microplastics have been detected in pelagic and demersal fish (Lusher et al. 2013) and also freshwater fish (Sanchez et al. 2014). There is evidence from laboratory studies of negative effects on animal health by ingested microplastics (Browne et al. 2013; Wright et al. 2013).

Microplastics are difficult to identify in the field, and measurements are easily affected by other environmental variables. Controlled laboratory environment may also employ higher doses of contaminants than found in field conditions. Therefore, toxicological experiments have so far only been conducted in laboratories. However, toxicological experiments in the field are also important in order to establish the full extent of any biological response (Underwood 1995). Both field and laboratory studies of microplastics rely upon identifying plastic particles ingested and potentially translocated within the study organisms’ tissues, therefore advances in
techniques to assist in quantification of these processes are of considerable interest. This chapter therefore sets out to test the common cockle (*Cerastoderma edule*) as a model for microplastics ingestion by animals in coastal habitats.

A study of bioturbation by the common cockle (*Cerastoderma edule*) (Barone 2012) presented a timely opportunity to examine animals that were exposed to microplastics in their natural habitat. Barone (2012) observed the effect of cockles on the vertical movement of sediment. For this purpose cockles were deployed in trial quadrats on an intertidal mudflat that were previously inoculated with luminophore particles. These cockles were exposed to a high concentration of these luminophore particles for two weeks, which sets an ideal premise for the ingestion of microplastics. Luminophore particles are a fluorescent polyamide powder. Fluorescent particles have been used in feeding behaviour studies in the past (Hudson et al. 2004; Browne et al. 2008), and they constitute a useful way to trace ingested particles in histological studies. Luminophore particles of two size fractions were used: D50 = 41 µm (red) and D50 = 129 µm (blue).

Browne et al. (2008) showed that the blue mussel ingests microplastic particles and also translocates them to the circulatory system in a laboratory trial. The blue mussel lives on rocky shores, that are exposed to wave action and have a high medium turnover environment, which presents a considerable challenge for *in-situ* exposure experiments. Hudson et al. (2004) conducted an *in-situ* feeding experiment using luminophore particles on the deposit feeding holothurian *Stichopus tremulus* and concluded that the applied method was a significant improvement in the study of deep sea deposit feeders.

The common cockle (*Cerastoderma edule*) is a suspension-feeding sediment dweller and is common in temperate waters in the North-West Atlantic Ocean. Cockles have a large capacity to ingest particles 60 to 500 µm in size (Karlsson et al. 2003). Their habitat is easily accessible at minimum logistical investment. Therefore, they represent a potential model organism for *in-situ* experiments on the ingestion and translocation of microplastic particles.

For the cockle luminophore model to succeed the luminophore particles must be specifically detectable. This means that the detection method will not show any positive readings in control samples. The screening method with the best discrimination of the luminophore particles
against any disturbances has to be identified and tested against controls. The luminophore particles must maintain this specificity throughout all steps of sample processing, as in the histological examination by Browne et al. (2008). To test the suitability of the cockle luminophore model for in-situ microplastic ingestion studies, this study established whether luminophore particles could be detected in animals that were exposed to them, and whether these particles could be identified with enough certainty to be valuable in particle uptake field trials.

5.2 Methods

5.2.1 Animal exposure

Twenty cockles were exposed to 'Luminophore' polyamide powder in each of 4 quadrats in fine sediment and 4 quadrats in coarse sediment in an intertidal mudflat following a method previously used by Montserrat et al. (2009).

Luminophore particles of two different granularity distributions, D50 = 41 µm and D50 = 129 µm were mixed with the natural sediment in proportions to reflect the granularity distribution of the natural sediment (Table 5.1). The mixture was frozen into eight 16 by 21 cm tiles. 180 common cockles (*Cerastoderma edule*, shell length (± sd): 3.1 ± 0.4 cm) were marked with red nail polish and deployed in the eight 16 by 21 cm quadrats on coarse and fine sediment, in an intertidal mudflat, in the Plym estuary, Devon, England (50.37°N, 4.14°W) at low tide. The quadrats were marked with sticks at each corner to facilitate recovery. The animals were left to settle in the sediment. After one day the sediment-luminophore-mixture tiles were placed on these quadrats at low tide (Figure 5.1).

5.2.2 Sample preparation

After two weeks the animals were recovered and placed in a freezer to euthanise them. All instruments and receptacles that were subsequently used were kept dust-free and cleaned frequently.

The animals were dissected. The stomachs were extracted and placed in buffered formol saline for five days for fixation. They were then processed in a Leica TP1020 V1.13 tissue processor following the cycle in Table 5.2. The processed cockle stomachs were then cast into a paraffin
Table 5.1: Natural composition of coarse and fine sediment and proportion of blue and red luminophore added.

<table>
<thead>
<tr>
<th>Size (µm)</th>
<th>Proportion in %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coarse</td>
</tr>
<tr>
<td>&gt;1000</td>
<td>20</td>
</tr>
<tr>
<td>&gt;500</td>
<td>24</td>
</tr>
<tr>
<td>&gt;250</td>
<td>25</td>
</tr>
<tr>
<td>&gt;63</td>
<td>12</td>
</tr>
<tr>
<td>&lt;63</td>
<td>19</td>
</tr>
<tr>
<td>Blue</td>
<td>4.3</td>
</tr>
<tr>
<td>Red</td>
<td>6.4</td>
</tr>
</tbody>
</table>

Figure 5.1: Exposure quadrat at cockle deployment and at inoculation with the luminophore tiles.

block to suit a Leica RM2335 microtome.

The prepared stomachs were then sliced into 10 µm thick sections (Figure 5.2). Only every fifth section was further processed, as the targeted particles were larger than 50 µm. The remaining sections were preserved in appropriate-sized ziploc plastic slips by groups of four. The selected sections were transferred to the surface of a 30% alcohol in de-ionised water solution, then to the surface of a 47°C de-ionised water bath and finally to a microscope slide coated in an egg-white glycerol mixture.

5.2.3 Sample analysis

Tissue sections were examined using an Olympus SZX16 stereomicroscope fitted with an SZX2-FUV, an SZX2-FGFPHQ and an SZX2-FRFP2 filter set (specifications see Table 5.3), as well as an
Table 5.2: Leica TP1020 V1.13 tissue processing cycle. Time per step in hours.

<table>
<thead>
<tr>
<th>Step</th>
<th>Medium</th>
<th>Time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Formol saline</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>50% alcohol</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>70% alcohol</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>90% alcohol</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Industrial methylated spirit</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>Absolute alcohol</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>Absolute alcohol</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>Histolene</td>
<td>1.5</td>
</tr>
<tr>
<td>9</td>
<td>Histolene</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>Histolene</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>Paraffin</td>
<td>2</td>
</tr>
<tr>
<td>12</td>
<td>90% alcohol</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>24.5</strong></td>
</tr>
</tbody>
</table>

Figure 5.2: Cockle stomach sections in paraffin medium.

Olympus CAM-XC10 camera at sevenfold magnification. Filters with the best specificity for the luminophore particles were identified.

Each section was photographed under the selected wavelength and under the full light spectrum for reference at the appropriate exposure time (Table 5.3). Images were recorded at a resolution of 1376 by 1038 pixels and at a 16-bit grey scale. Each pixel thus had a value from 0 through 4095. The observations of luminophore-matches were enumerated.
5.2. METHODS

Table 5.3: Specifications of filter sets fitted to the Olympus SZX16. Wavelengths in nm and appropriate exposure time in s

<table>
<thead>
<tr>
<th>Designation</th>
<th>Wavelengths (nm)</th>
<th>Exposure time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exciter</td>
<td>Barrier</td>
</tr>
<tr>
<td>SZX2-FUV</td>
<td>330-385</td>
<td>420</td>
</tr>
<tr>
<td>SZX2-FGFPFHQ</td>
<td>460-480</td>
<td>495-540</td>
</tr>
<tr>
<td>SZX2-FRFP2</td>
<td>545-580</td>
<td>610</td>
</tr>
<tr>
<td>No filter</td>
<td>Full spectrum</td>
<td>-</td>
</tr>
</tbody>
</table>

5.2.4 Controls

To identify the best method to detect luminophores, and to verify the specificity of the detection method for luminophores, several controls had to be conducted.

To test the specificity of fluorescence microscopy for the luminophore particles, a set of common cockles were collected from both coarse and fine sediment from a location up-stream from the exposure site in the Plym estuary and analysed alongside the exposed animals. Readings that resemble luminophore particles in these animals would indicate that the detection method is not specific to luminophores in this context, and that particles present in the environment mimic luminophore.

To identify an adequate method for luminophore detection, luminophore particles were applied to a microscope slide and screened with the filters available on the fluorescence microscope. The filters with the best specificity for blue and red luminophores respectively were selected to screen the tissue samples. The specificity was calculated by the ratio of the mean luminescence of the luminophore particles versus background. The metric for luminescence was the pixel value (0 = black, 4095 = white). Luminophore particles covered less than half of the images. The median pixel value of the image was therefore considered background luminescence. Pixels that were at least twice as luminescent as the background were considered particles.

To determine a luminescence threshold for luminophore particles against tissue samples, the particles luminescence was measured against a paraffin block. A paraffin block containing blue luminophore particles, one containing red luminophore particles and one block containing no luminophore particles at all were sectioned and photographed using the appropriate filter. The
maximum pixel value in the paraffin sections that did not contain luminophore particles was selected as the lower boundary of the grey scale range.

To establish if luminophore particles kept their properties in tissue processing, red and blue luminophore particles were run through a simulation of the tissue processing cycle in order to detect any possible loss of fluorescence along the cycle. The luminescence of the particles was measured after each simulated tissue processing step to register potential changes in fluorescence properties.

5.3 Results

Of eight quadrats that were marked in the intertidal mudflat, seven were recovered. In order to maintain balanced design three fine sediment and three coarse sediment quadrats with luminophore inoculation were included in the analysis. 24 cockles were analysed, 3 from each quadrat, as well as 3 from coarse and three from fine sediment in an area removed from the experiment site in the same mudflat.

Once they were processed, the cockles’ stomachs yielded $108 \pm 21$ sections each. The sections were 10 µm thick and 40 µm apart.

5.3.1 Luminophore particle fluorescence

The RFP filter showed the best specificity for the red luminophore particles. UV slightly improved the visibility of the blue particles (Details in Table 5.4).

The paraffin sections showed a high specificity of RFP for the red luminophore particles. The maximum pixel value in the paraffin sections that did not contain luminophore particles was 431, leaving 432 to 4095 to indicate red luminophore particles. The threshold to indicate lu-

<table>
<thead>
<tr>
<th>Filter</th>
<th>Red particles $\pm$</th>
<th>Blue particles $\pm$</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFP</td>
<td>$1.20 \pm 0.22$</td>
<td>$1.78 \pm 0.49$</td>
</tr>
<tr>
<td>RFP</td>
<td>$3.62 \pm 3.39$</td>
<td>$1.66 \pm 1.58$</td>
</tr>
<tr>
<td>UV</td>
<td>$1.66 \pm 0.49$</td>
<td>$2.12 \pm 0.83$</td>
</tr>
<tr>
<td>Full spectrum</td>
<td>$1.61 \pm 0.45$</td>
<td>$2.03 \pm 0.72$</td>
</tr>
</tbody>
</table>

Table 5.4: Ratio of particle luminescence (pixel value) versus background luminescence (specificity +/- standard deviation) of red and blue luminophore particles under different filters.
5.3. RESULTS

Table 5.5: Analysis of variance of count of fluorescence readings between trial quadrats.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quadrat</td>
<td>5</td>
<td>309.6</td>
<td>61.92</td>
<td>0.762</td>
<td>0.594</td>
</tr>
<tr>
<td>Residuals</td>
<td>12</td>
<td>974.7</td>
<td>81.22</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

minophore particles was conservatively chosen at a pixel value of 1000. UV light showed the best specificity for the blue luminophore particles, but the maximum pixel value in the paraffin sections that did not contain luminophore particles was 4095 using the UV filter. Blue luminophore particles were therefore not further considered in this experiment.

The simulation of the tissue processing cycle on the red luminophore particles showed that the distribution of pixel values was significantly different ($p \leq 0.001$ for the chi-squared distribution) between each pair of steps of tissue processing. Initially the median was 1156, 1171 after three hours in formol saline, 1001 after three hours in 50% alcohol, 871 after three hours in 70% alcohol, 663 after two and a half hours in 90% alcohol, 674 after two hours in IMS, 874 after four hours in absolute alcohol and 585 after four hours in histolene.

5.3.2 Cockle stomach section screening

In the cockle stomach sections overall 104 readings of pixel values over 1000 (fluorescence readings) were detected, which could have been luminophore particles inside the cockle’s stomach, 7 of which were detected in control animals. 115 such readings were detected that were on the area of the paraffin section outside the stomach or caused by a disturbance in the specimen (e.g. folded over), 32 of which were detected in control animals.

The mean count of fluorescence readings (Figure 5.3) was $5.67 \pm 9.72$ per individual in coarse sediment ($1 \pm 1$ in controls) and $5.11 \pm 8.12$ in fine sediment ($1.33 \pm 1.15$ in controls). The count of fluorescence readings per individual was not significantly different between trial quadrats (Table 5.5).

While the observation suggests that individuals that were exposed to luminophore particles exhibited more fluorescent readings, this effect was not significant according to an analysis of variance between exposed animals and control animals. (Table 5.6).
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Figure 5.3: Cockle stomach section with fluorescence reading. Dashed line: tissue sample, red circle: fluorescence reading. Image enhanced to show tissue sample, contrast of fluorescent particle is normally stronger.

Table 5.6: Analysis of variance of treatment versus control count of possible in stomach luminoaphore readings per individual.

(a) Coarse

<table>
<thead>
<tr>
<th>EffCon</th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>49</td>
<td>49.0</td>
<td>0.646</td>
<td>0.44</td>
</tr>
<tr>
<td>Residuals</td>
<td>10</td>
<td>758</td>
<td>75.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(b) Fine

<table>
<thead>
<tr>
<th>EffCon</th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>32.1</td>
<td>32.11</td>
<td>0.606</td>
<td>0.454</td>
</tr>
<tr>
<td>Residuals</td>
<td>10</td>
<td>529.6</td>
<td>52.96</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5.4 Discussion

Based on the observations made in this study, that cockles that were exposed to luminophore particles exhibited a higher number of fluorescent particles than control cockles, it is probable that cockles do in fact ingest the particles. There was, however no conclusive evidence of this process, as the detection method may not have been sufficiently specific to the marker.

The Common cockle would constitute a useful complement to a list of animals that have already
been used for the observation of microplastics. Cockles and lugworms, which have also been the object of microplastics ingestion research, offer similar properties for *in-situ* such experiments. Both are intertidal sediment dwellers, and their habitat can effectively be inoculated with microplastics. They belong to different taxa and may therefore have different responses to potential microplastics toxicity. The common cockle could be a useful complement to a growing set of model organisms, and the possibility of an effect that was observed in this study encourages efforts to further explore this model.

This trial did not return sufficient evidence for microplastics ingestion, because the marker (fluorescence) was (a) not specific enough for conclusive distinction from other particles present in the organisms, and (b) not resistant against the histological preparation methods. The adequacy of fluorescence tracking and alternative methods to study microplastics ingestion on the common cockle are therefore further explored below.

### 5.4.1 Fluorescence for microplastics tracking

The luminophore particles used in this trial were designed for sediment tracing (Montserrat et al. 2009; *Environmental Tracing Systems Ltd: Tracers* 2015). Hudson et al. (2004) used a similar kind of particle also in a novel approach to observe gut throughput of holothurians. Their particles were sand-based rather than plastic, but they were also made fluorescent. This study attempted to locate fluorescent particles precisely within the cockle’s stomach. The stomach had to be fixed and sliced, which requires a lot of manual and chemical processing. Hudson et al. (2004) divided the animal’s intestine into segments and screened the contents for luminophore particles. The processing was minimal, but the particle localisation was limited to the length of gut covered. Highly defined localisation is important if the goal is to show particle translocation through animal tissue. But higher spatial definition comes at the cost of more processing, which - in this study - has rendered luminophore particles unreliable as markers. If highly defined localisation is not required, the approach of Hudson et al. (2004) would be adequate. They suspended the gut content in distilled water in a petri dish and screened for luminophore particles using a dissecting microscope and UV light.
CHAPTER 5. INGESTION BY COCKLES

Browne et al. (2008) fed mussels fluorescent polystyrene microspherules. The objective of that study was to observe the translocation of plastic particles from the digestive system into the animal’s tissue. Browne et al. (2008) used a similar tissue processing technique as in this study. The spherules could be identified with enough certainty to exclude false-positive readings, which refute evidence for microplastics ingestion in this study. Browne et al. (2008) conducted their experiment in a controlled environment, and contamination of control animals was possibly less likely than in-situ. This is important, as it suggests that markers may not be reliable when used in the field due to similar particles already present in the environment or cross-contamination.

Watts et al. (2014) studied microplastics intake through the ingestion of pre-exposed food - shore crabs were fed mussels that had previously been fed similar particles as used by Browne et al. (2008). These microspheres were successfully identified in tissue homogenisates, which as in (Hudson et al. 2004) only allows limited localisation of the particles. However, Watts et al. (2014) used a different technique to localise particles. Coherent anti-Stokes Raman Scattering (CARS) microscopy can penetrate hundreds of microns into biological tissue (Garrett et al. 2012) and detect polymeric materials as it is sensitive to bond density (Zumbusch et al. 1999), which also makes fluorescence obsolete. Cole et al. (2013) had previously used this technique to detect microplastics in zooplankton.

CARS microscopy happens to be specific to materials with high bond density such as plastics. It could therefore have great potential for the detection of microplastics in tissue. However, both Watts et al. (2014) and Cole et al. (2013) conducted their experiments in laboratory environments, and whether CARS microscopy is also successful with in-situ experiments has to be verified.

5.4.2 Microplastics ingestion trial without tracking

This study faced two obstacles: (a) there were false positive readings and (b) the fluorescent die was affected by tissue processing. The previous section discussed ways to avoid problems related to marker loss, but the risk of false-positive readings in in-situ experiments may be the most important one. It cannot be prevented by using more robust markers. Contamination of
the samples with materials that mimic the applied markers cannot be excluded. Fluorescence, which is a very common marker, may be more vulnerable to this effect than e.g. radioisotopes, but labels generally have a way of being faked.

Without labels there is no tracking, and without tracking there is no evidence for a pathway of microplastics from the digestive system into tissue. Detected particles may or may not be the inoculated ones - in the case of this study, luminophores.

A statement that is more robust against false positive readings than whether or not cockles ingested microplastics is that cockles that were exposed to luminophore particles exhibited significantly more fluorescence readings than control cockles. It is therefore likely that the presence of luminophores in the cockles’ substrate explains these additional readings and that consequently cockles do in fact ingest microplastics from their substrate. The examination of hundreds of microtome sections may, however, not have been an adequate approach to make this statement, for which the localisation of the particles is not necessary. Far simpler quantitative methods are available for this.

As mentioned earlier, suspending gut content or homogenising tissue samples limits the ability to localise particles in the organism, but as localisation is compromised by false positive readings, effectively no information would be lost against the method used in this study. van Cauwenberghe and Janssen (2014) detected microplastics in mussels’ tissue by homogenising the tissue. Particles that resembled plastics were isolated. A subset of these particles was verified using Raman spectrometry, which has been validated for this purpose by Garrett et al. (2012) (see also above). This examination of single particles is also quite elaborate, even though only a subsample was examined to calibrate the visual identification. Raman spectroscopy was also biased towards pigmented particles - a further indication that microplastics are difficult to identify.

The present study was conducted using field samples that were produced in, but not the target of a separate study (Barone 2012). As the experiment of Barone (2012) was ongoing at the time the present study started, the quality control and validation of the method used in the present study could only be conducted after the main experiment. While microplastics have previously been
detected in organisms in the natural environment (Goldstein and Goodwin 2013; Lee et al. 2013; Ugolini et al. 2013; Cole et al. 2014), the present study showed that experimental exposure of animals in their natural habitat for ecotoxicological purposes remains difficult. The reason for this is that the particles are difficult to track.

Sanchez et al. (2014) screened intestines of fresh water fish for microplastics. They attempted to identify plastic particles visually, and they had to concede that some particles were 'suspected' microplastics. It is conceivable that plastics deteriorate beyond recognition and could be mistaken for sediment or vice-versa. Lusher et al. (2013) also extracted microplastics from fish intestines. These particles were identified using FTIR. FTIR is very laborious and - if it had been used in this study - might even exceed microtome sections in this respect. There is also a lower size limit to particles that can be analysed using FTIR, as it depends on the surface area that can be scanned.

Studies on ingested microplastics have two things in common: 1. they are all very conscious of sample contamination; microplastics can be anywhere, even airborne. 2. the less effort that goes into identification, the less certain the authors are that they are actually looking at microplastics. Microplastics are cryptic, and visual identification can be inaccurate.

Matrices other than animal tissue or gut content have been screened for microplastics. Shoreline sediment has frequently been the object of microplastics research (Thompson et al. 2004; Ng and Obbard 2006; Browne et al. 2011). All three studies separated the microplastics from the sediment by floatation, hence they relied on the material’s specific gravity. Particles that were filtered from the supernatant of this procedure also have to be identified secondarily, typically using FTIR.

Microplastics research seems to stand or fall with the specificity of the detection method, and often a two-step process is required, separation and verification. Be the particles verified by Raman or FTIR spectroscopy, they are typically too numerous to consider the whole yield of whatever sample is being analysed. It is therefore advisable to verify a subsample to calibrate the less specific pre-selection method.
5.5 Conclusion

There is substantial need and potential for investigating the ingestion of microplastics by filter and deposit feeders, as this process has now been described on multiple species and potential risk for organisms becomes increasingly plausible.

Meanwhile, observing microplastics in organisms - or in any kind of natural environment for that matter - is difficult due to their size, and because they blend in with a great many natural particles. Marking microplastics with fluorescence, as was done in this study, limits histological analyses, as the marker may deteriorate in the process.

Due to these constraints, the objectives of microplastics ingestion studies have to be narrowed down to what can be achieved using one specific method. As this study showed, gut to tissue translocation cannot be studied in the same experiment as microplastics ingestion behaviour in-situ.
Chapter 6

Addressing plastic fragmentation in the environment

In recent years, 3 major sources of microplastics have been described in the environment: washing machine effluent, scrubbers from cosmetic products (microbeads) and deterioration of larger plastic debris. Washing machines and cosmetic products constitute sources, where microplastics could be intercepted directly and systematically. However, no such strategy could be devised for microplastics that emerge from fragmentation. The best way to prevent the emergence of such microplastics is to prevent materials that are prone to deteriorate from entering the environment. Measure to prevent plastics from entering the environment need to be supported by society, policy and industry. They include reductions in the use of plastic especially in applications with short service life such as carrier bags and packaging. In addition, international standards for ‘degradability’ of plastics need to be modified since at present they do not guarantee degradation will complete. Hence degradable products may fragment into microplastics rather than fully degrading. Any measures to prevent the emergence of microplastics from deterioration in the environment need to be quality-controlled. Monitoring microplastics in the environment requires different principles to those used to survey larger items of plastic debris: Exact numbers are hard to determine and it
may be more feasible to use estimates to compare contamination by small plastic debris and microplastics between locations and over time. Use of extensive data sets can compensate for the variability encountered in the natural environment and computer models can be used to calibrate data. Microplastics are hard to quantify and so methods to detect them in organisms in the wild have to be carefully selected to suit study objectives such as ingestion, translocation and toxicity. Contamination of the environment with plastics is undergoing a shift towards smaller debris, which are difficult to detect and to trace back to their sources. Hence strategies to prevent contamination and research to quantify the effects of microplastic more comprehensively are urgently needed.

This thesis addresses the environmental importance of plastic deterioration in the marine environment. Previous research has produced detailed findings on various aspects relating to microplastics. Several previous studies have addressed the generation of microplastics through the fragmentation of larger debris, its occurrence in the environment and its ingestion by animals. The purpose of this thesis was to obtain a better understanding of the pathways for microplastics from their generation to their encounters with wildlife. This chapter places the results of the research contained in the thesis into wider context, focusing upon synthesising the practical applications of the findings. It also discusses potential measures to prevent plastics that are prone to fragmenting from entering the environment and critically considers methods to monitor the effectiveness of such measures.

Microplastics have been traced to a variety of sources. In this Thesis, deterioration of larger plastic items into microplastics was examined as one mode of origin. Synthetic textile fibres from clothing have been detected in sewer sludge and traced back to washing machine effluent (Browne et al. 2011), which has been shown to contain substantial quantities of synthetic fibres, potentially explaining part of the fibre abundance detected in the environment (Browne et al. 2011). Plastic microbeads that are used as scrubbers in cosmetic products are also bound to travel through sewage systems and likely to end up in the ocean (Zitko and Hanlon 1991; Gregory 1996; Fendall and Sewell 2009).

Browne et al. (2011) found that microplastics in sewer effluent were dominated by synthetic tex-
tile fibres presumably from washing machine effluent rather than from fragmentation or from cleaning products. However, as these particles persist in the ocean and blend in with naturally occurring particles, Browne et al. (2011) determined that scrubbers and microplastics originating from fragmentation become more difficult to detect than fibres, which stand out through their distinct shape and colouration. Any account of microplastic provenance based on such a visual assessment is therefore likely biased towards fibres, making the contribution of each source of microplastics to environmental contamination difficult to quantify with any certainty.

Microbeads from cosmetic products have long been suspected to enter the environment, because they are washed off after they are applied and consequently end up in the sewer and ultimately the ocean. Unlike fibres, they cannot be effectively identified on the basis of their appearance. Eriksen et al. (2013) compared microplastics found in the environment to scrubbers in consumer products and found a putative match, but without new methodological approaches evidence for a link between the two is likely to remain elusive.

In practical terms, this elusiveness should be regarded as a potential threat whose extent can only be estimated through per capita use (17.5 ± 10.0 mg d⁻¹), (Gouin et al. 2015). Like many types of plastic particles examined previously, microbeads from cosmetics products have been shown to adsorb toxic chemicals such as phenanthrene and DDT (Napper et al. 2015). The potential effect of such contaminated microplastics was discussed in Chapter 5, and in the likely event that such scrubbers do reach the environment they are as potentially harmful as microplastics used in toxicology experiments (Wright et al. 2013).

The main distinction between microplastics from sewers (scrubbers and clothing fibers) and microplastics that emerge from deterioration (secondary microplastics) is that the microplastics from scrubbers and clothing can be controlled directly, as the sources can be localised, whereas fragments formed by deterioration can only be controlled by measures that reduce the entry of larger plastic items to the environment. In some locations microbeads are being banned from use in cosmetic products. Indeed legislation in Illinois in the United States (Time June 24 2014) was followed by actions in Maine, New Jersey, Colorado, Indiana and Maryland and similar bills were pending in California, Michigan, Minnesota, Washington and Oregon until recently
(Fighting Pollution From Microbeads Used in Soaps and Creams - The New York Times 2015)

Finally, a bill introduced in the US Congress in March 2015 was signed into law, banning microbeads in cosmetic products on a federal level (H.R.1321 - Microbead-Free Waters Act of 2015 114th Congress (2015-2016) 2015) in December 2015. Manufacturers of consumer products containing microbeads are coming under an increasing amount of pressure, and some have promised a moratorium on microbeads.

The other well defined source of microplastics, synthetic textiles and washing machine effluent, has proven more difficult to control, as Browne discovered (Browne et al. 2011; Inside the lonely fight against the biggest environmental problem you’ve never heard of - The Guardian 2015). Two ways to mitigate this source of microplastics are to design textiles that shed fewer fibres and to introduce filters for washing machines that intercept fibres. Synthetic fibres are much more widely used than microbeads (annual per capita demand 6.8 kg fibres (Shui 2013) vs 6.4 g microbeads (Gouin et al. 2015)), making their effective control even more pressing. While introduction of fibres to the environment can effectively be prevented in theory, the number of stakeholders that need to be on board is substantial, and any effective measures are likely further away for fibres than for microbeads.

Meanwhile, microplastics originating from deterioration of larger items offer differing opportunities for leverage to achieve prevention. Once in the environment, plastics can deteriorate anywhere UV irradiance and oxygen are present. Microplastics that originated from deterioration are elusive, and there is no way to tell when the items they originated from were introduced into the environment. Meanwhile, plastics, especially those with a short service life (packaging materials such as polystyrene) have been shown to deteriorate into microplastics the most rapidly in the environment (Chapter 2).

It is likely that beaches have an important role in the emergence of microplastics as small plastic debris break down under UV radiation. Removing plastics from beaches could therefore be considered a further way to reduce plastic fragmentation in the environment, but the capacity of any kind of beach cleaning efforts may be limited compared to the amount of plastics entering the environment (Wyles et al. 2016).
While plastics may deteriorate relatively rapidly on land when exposed to enough UV, they could be much slower to degrade in the ocean with little change in their mechanical stability over intermediate timescales, as shown in Chapter 2 of this thesis. It is not at all clear how long it has taken the particles we currently are recording as microplastics to form. All we can say with certainty is that we are mostly dealing with particles that have formed during the last 60 years i.e. the time since plastics were first mass produced (Figure 6.1). The cumulative amount of plastic entering the environment is predicted to increase by one order of magnitude by 2025 (Jambeck et al. 2015) (Figure 6.2). Based on production figures, estimates of plastics entering the environment and deterioration rates one can only speculate as to how much microplastic debris there will be in another 60 years.

Figure 6.1: Development of plastic production over time (PlasticsEurope 2013). Reprinted with permission from PlasticsEurope.
6.1 Leverage to reduce formation of microplastic by fragmentation in the environment

Preventing microplastic precursors from entering the environment requires action by society as a whole. Consumers should reduce their use of plastic packaging materials and dispose of them appropriately, and producers and retail should favour recyclable packaging. As was done for microbeads in cosmetic products, legislation to promote such a development needs to be put in place. Standards for ‘biodegradable’ or ‘degradable’ plastics are a further point of action, especially in this market that is becoming increasingly aware of the environmental implications of plastic waste. Current standards for labelling degradable plastics are not appropriate for the assessment of degradation in the environment (Briassoulis and Dejean 2010; Briassoulis et al. 2010).

Involving volunteers in research of microplastics contamination as introduced in Chapter 3 could help to further raise awareness of this environmental problem. It could promote the image of plastics as highly durable and versatile materials, as something we make cars, aircrafts and spaceships from, rather than single use packaging or cosmetic scrubbers, that plastics should be made not more, but less degradable, and that inappropriate disposal of plastics can lead to fragmentation and dispersal. Plastic also has to be perceived as a material that requires fossil
resources and should therefore be used sparingly, just like combustion fuels. On a higher level, better public awareness could change waste disposal practices that are not currently commensurate with what we have learned about the environmental threats from plastics contamination. Whilst research has shown that plastics are an environmental hazard they are still widely treated like all other types of waste (Rochman et al. 2013).

6.1.1 Plastic bags

Plastic bags have many attributes that make them likely to enter the environment. They are often single-use items with a short service life. They are very light, and if disposed of improperly they may be dispersed by wind. Even if plastic bags are discarded in the waste stream, many countries still operate open landfills, from which plastic such as bags can escape and enter the environment.

Plastic bags are often made of polyethylene film. Some of the materials used in Chapter 2 of this thesis originated from such plastic bags. Both polyethylene and biothene exhibited a rapid loss of tensile extensibility when exposed in illuminated air, which renders them prone to breaking apart into smaller fragments. Films present a large surface area per volume; they consequently adsorb much more oxidising radiation, and other destabilising agents than more compact plastic items and consequently are likely to deteriorate into fragments more rapidly. Bags and other plastic films may therefore be rapid sources of microplastics, and preventing them from entering the environment may reduce the amount of microplastics originating from fragmentation in the environment.

The United Kingdom has introduced a 5 pence charge for plastic carrier bags in grocery stores as of October 2015 (Charges for single-use plastic carrier bags - GOV.UK 2015). France has introduced a ban on supply of free plastic carrier bags in retail as of 2016 (Interdiction des sacs plastique en France - Ministère du Dévelopement Durable 2015). Morocco is going further by introducing a bill to ban manufacturing, importation, exportation, commercialisation and use of plastic carrier bags (Conseil de gouvernement du jeudi 29 Octobre 2015 | Maroc.ma 2015). France uses 17 billion plastic carrier bags annually, Morocco 26 billion. This could substantially reduce the quantity of bags entering the environment, which could effectively reduce the amount
of microplastics formed from fragmentation. Almost more importantly, these bans could reflect a societal realisation that has weighed the benefits of a plastic-free environment against the convenience of using single-use plastic bags.

### 6.1.2 Standards for material stability and degradability

Considerable effort has gone into improving the degradability of plastics in order to facilitate waste disposal (e.g. Karaduman (2002)). Consumers are given the confidence that the plastic bag they put in the general waste will not be an environmental issue, however as a consequence the incentive for recycling is taken away. The kinds of bioplastics used in these bags are becoming more and more interesting for environmental (Gross and Kalra 2002; Demirbas 2007) and also bioengineering (Anderson et al. 1998; Zhang et al. 2000) purposes. The worldwide production of bioplastics was estimated at about one million tonnes per year in 2011, and already the area of land needed for crops for the production of bioplastics has become an environmental issue (Giorni and Piemonte 2011).

Meanwhile, ‘Bioplastics’ - in fact all terms that somehow combine ‘bio’ with plastics, such as Biocycle, Oxo-biodegradable Plastic Technology (EPI Environmental Products Inc. 2016) or Bioplastics (European-bioplastics 2016) - remains an ambiguous term that may be understood as either bio-sourced or bio-degradable materials. Terms for plastics that are made from biological materials and plastics that are biodegradable are often confounded (Briassoulis and Dejean 2010). ‘Bio’ often seems to be used as a marketing instrument invoking the consumers’ sense of environmental responsibility. Conversely, such labels can lead to improper disposal of litter and ultimately increase rather than reduce environmental contamination with plastic debris (Kershaw 2015).

Biothene for instance, which was tested in Chapter 2 of the present thesis, is derived from polyethylene and contains metal ions that enhance photo-oxidation. Neither the origin of the material nor its degradability justify the prefix ‘Bio’. The base material of biothene is polyethylene, which may be synthesised from plant material or fossil resources and is thus no more or less biologically sourced than any other synthetic polymer. It is also not more or less suitable to be digested enzymatically than any other synthetic polymer; it merely has better oxidation
properties, which ultimately cause it to turn into microplastics at a faster rate than conventional synthetic polymers.

All industrial standards for biodegradation require the biodegradation to a certain percentage over a certain period of time (Briassoulis and Dejean 2010). For example, the American Standard for Testing Materials (ASTM) D6400 determines that a material is compostable if less than ten percent of dry weight is found, more than sixty percent (ninety percent for blends) of carbon conversion has occurred and ecotoxicological tests, in which any harm to a set of model organisms such as earthworms or blue algae is excluded, are passed after six months. They do not ascertain complete biodegradation, however, partial biodegradation can be achieved by blending in the required proportion of biodegradable substance. Thus materials that do not exceed a certain concentration of non-degradable substances still qualify for biodegradation by ASTM D6400.

Compostable materials should have to break down under organismic action to total mineralization in composting conditions. As a measure of compostability the conversion of plastic into CO$_2$ and biomass and the material fragmentation are quantified (Briassoulis and Dejean 2010). Guo et al. (2012) identified the EdK (Environmental Degradable parameter) that compares the carbon yield of materials versus starch as a reference material as a reliable indicator of potential degradability.

Environmental conditions such as temperature, humidity oxygen availability and the microbial population all affect the degradation of polymers (Orhan et al. 2004). For instance, microbial strains whose enzymes have the best oxidative properties and whose cell surface is the most hydrophobic should be favoured for industrial biodegradation of plastics (Sivan 2011). In general, the advertised degradability of materials should come with information as to what conditions are necessary in the recipient environment for the materials to degrade. Greene (2009) found that oxodegradable, UV-degradable and conventional polyethylene did not degrade in compost conditions. Artificial breakdown processes can enhance biodegradation. They generate a larger surface area for microbial colonization and they reduce the molecular weight, making the polymers easier to digest (Palmisano and Pettigrew 1992). Best results were obtained with molec-
ular weights below 620 g mol\(^{-1}\) (Haines and Alexander 1974; Yamada-Onodera et al. 2001). Some polymers can be ionized and thus rendered soluble at high pH (Chambliss 1983). This facilitates enzymatic and hydrolytic breaking of the polymer (Ishigaki et al. 1999).

The most widely used materials (i.e. polyethene, polypropylene) are also the most durable ones. Some experts are calling for further exploration of their biodegradability (Sivan 2011). Polypropylene in conjunction with ten, fifteen or fifty percent natural fibers was biodegradable to five to fifty percent after ninety days exposure depending on the fiber concentration (Chattopadhyay et al. 2011). Microcrystalline cellulose and polylactic acid biodegrade at a much faster rate than polyethene even if the latter is recomposed with starch (Leejarkpai et al. 2011). Reports on biodegradation into carbon dioxide and water of polyethene and polypropylene typically document this process to some percentage of the total mass and not the complete digestion or mineralization of the specimen. They therefore still leave room for a residue that is likely to comprise of a proportion microplastics.

With the abundance of microplastics increasing, traditional metrics for quantification of plastic’s degradation have become inadequate. For example most standards allow for deterioration into small size fragments, an ’out of sight, out of mind’ principle. Since the discovery of microplastics, and especially since their potentially harmful effects have been reported, new standards for plastics degradability that ascertain the complete mineralization of the material have to be devised. Current degradable plastics also depend on very specific conditions to degrade. They rely on the user to deliver the plastic to a waste stream providing an environment that exhibits a suitable climate of temperature, humidity and microbiota. Consumers may misinterpret the material’s proclaimed degradability and discard it in the environment. In order for degradable plastics to be successful, they would need to fully mineralize in any environment, rather than only in specific conditions.

For example, as Chapter 2 of this thesis showed, sea water slows the deterioration of plastics. This was in part attributed to the material’s colonisation by organisms, which shielded it from oxygen and UV radiation which are both a requirement for photo-oxidation. Nevertheless, materials in sea water lost tensile extensibility, and Andrady, Pegram and Song (1993) suggest
that this may be due to mechanical material alterations by organisms colonising the sample surface. (Ye and Andrady 1991) suggested that plastics that float initially may later sink due to fouling, then ‘de-foul’ due to changing habitat and resurface. However, the observations in Chapter 2 showed that plastics below the water surface are also colonised, which could have a variety of consequences: (a) plastics could sink further, (b) they could sink more rapidly, (c) materials could be mechanically altered influencing deterioration, (d) plastics could be ingested by predators targeting the colonising organisms.

Colonisation of plastic debris by organisms will vary between geographic areas and water depths. Photographs illustrating fouling on the samples of Andrady, Pegram and Song (1993), which were kept floating at the water surface, show very little fouling compared to the samples exposed in sea water for Chapter 2 of this thesis, which were submerged in 1 m of water. Samples floating at the surface of Biscayne Bay (FL, USA) showed different fouling to samples exposed at the surface of Puget Sound (WA, USA). Whether plastic debris float or sink depends not only on the density of the polymer but also on where they are. Polystyrene, which is an important food packaging material (PlasticsEurope 2013) with a short service life, lost 90% of its tensile extension in one year in sea water with little oxidation, while in fresh water, where very little fouling was observed for the duration of the experiment, tensile extension remained the same as in controls. This loss of tensile extension may be associated with reduced molecular weight, and could make the material become more prone to be eaten by animals. Most likely, however, reduced tensile extension renders the material more susceptible to tearing under mechanical stress, thus accelerating the generation of microplastics.

While improved standards for degradable plastics and replacing current materials with genuinely degradable alternatives would certainly reduce the formation of microplastics, as debris would minerlaise with no residue, such an approach would not allow society to evolve towards more rational use of plastics. Preventing plastics - especially plastics that leave non-degradable residues after deteriorating, such as films of polystyrene, polyethylene or many degradable materials - from entering the environment may be the most powerful way to prevent microplastics forming by deterioration. Legislation banning and industry and consumer commitment to re-
scind single use plastics may generate enough leverage to reduce environmental contamination. How powerful these measures are has to be assessed by monitoring plastic fragments in the environment.

6.2 Monitoring the efficacy of measures to reduce the quantity of plastic fragmenting in the environment

Ryan et al. (2009) proposed that assessment of changes in distribution, abundance and composition of marine debris as well as changes in their impact on organisms constitute key approaches in the monitoring of plastic debris in the environment. This thesis went some way in developing methods to apply this principle to plastic fragments, which are becoming increasingly abundant due to the deterioration of existing debris. This section of the general discussion aims to build a strategy to monitor the success of measures to prevent fragmentation of plastics in the environment building on the findings of this thesis.

6.2.1 A case for variable but abundant data

Do we need exact numerical abundance data for plastic debris, or are estimates sufficient? This question has to be answered in the view of the purpose of any particular plastic debris survey, and also from the standpoint of mitigating the problem. Estimates allow us to compare debris contamination in different geographic locations and environments as well as over time. They will help us address the problem where it is most pronounced, and will indicate changes in debris density over time. Measuring exact quantities of plastics is very challenging, because debris can often not be detected; here estimates and models may be invaluable to help direct our efforts to prevent environmental contamination by plastic debris.

The difficulty with assessing the abundance of small plastic debris on a beach is the wide dimension gap between the target and its environment. Beaches can be large areas to survey for very small and sometimes elusive items. Likely for this reason, there are no precedents for small plastic debris surveys, and Chapter 3 was dedicated to testing a possible method. The applied method relied on correctly locating accumulations of small plastic debris on a beach, which can be influenced by subjective perception of the observer.
When multiple observers contribute to the same dataset it is essential that the dataset is calibrated for inter-observer variability. In the method presented in Chapter 3, inter-observer variability could be due to observer’s experience in detecting small plastic debris or individual bias for certain accumulations over others. Using an aggregated value over multiple accumulations may help resolve this variability. Two observers who select independent sets of small plastic debris accumulations on the same beach at the same time may reach the same aggregated value, as the detection probability for each burden rating is related to its frequency in the same way for both observers.

Quantifying inter-observer variability between beach surveys is impractical. Observation conditions and debris visibility change over time. Multiple observers would have to operate in a very confined timeframe, and would therefore not remain independent. Independently measuring the same thing twice is challenging and so a theoretical estimate of inter-observer variability may be the best option.

A validated way to do this could be via computer simulation. Smith et al. (1995) did this to identify a suitable method for a waterfowl census. They randomly subsampled an actual population count using Monte Carlo simulation and applied two different adaptive cluster sampling methods as well as random sampling to estimate the simulated population. Under certain conditions one method would work better than the other and vice-versa. Computing power has improved vastly since Smith et al. (1995) conducted their experiment, and simulating realistic datasets is used e.g. in multivariate statistics (PRIMER).

The method presented in Chapter 3 works, but it is only one of many possible. And with computer simulation at hand, finding the best method may not be as far off as it would be in the field. Distributions of plastic particles on beaches with various abundances and various degrees of clustering can be generated artificially and assessed by an artificial survey algorithm many times such as to measure the variability of observations of the same phenomenon instead of estimating it. The effect of sampling 20 quadrats versus 10 or of excavating buried particles in accumulations versus superficial screening can be tested, and always against an exact count of particles.
Especially when involving volunteers, calibrating observations against each other is important, because experience levels vary vastly. But the use of volunteers in surveying plastics on beaches should not be discounted. Volunteers can be recruited globally and would allow to survey a much larger geographic area than trained researchers, who have to travel to each place they want to survey. Volunteers also inestimably increase the task force available to the surveys, and they can return much more data in a shorter amount of time, than trained volunteers who are fewer. This higher data yield can also compensate for any variability that may result from the volunteers not being trained to the same level as professional researchers.

In recent years there have been a large number of media reports on the contamination of the environment with plastics. The abundance of plastic debris in the environment has reached a level that, not only specialists, but also the public perceive as too high. The market for new self-proclaimed sustainable plastic products could be a reflection of society’s attention to this issue. While consumers should not renounce plastics, they need to be more responsible about plastic use and disposal, so as to move toward a plastic-free environment.

### 6.2.2 Using the momentum of public awareness

Public recognition of plastic contamination in the environment is also reflected in the substantial public involvement in beach cleaning campaigns. Each year the Marine Conservation Society mobilizes thousands of volunteers to collect hundreds of thousands of plastic items from British beaches (Marine Conservation Society 2014). With a foreseeable shift of environmental plastic contamination towards microplastics, each clean-up gains even more importance in the prevention of adverse effects of plastics on the environment; it is therefore more important than ever, to harness public awareness to prevent formation of microplastics by removing large debris items before they fragment. The willingness of volunteers to participate in preventing environmental plastic contamination is not limited to oceanic beaches. Within Thames21 the Thames River Watch coordinates numerous litter surveys powered by volunteers, which further demonstrates that volunteers can raise meaningful data (Thames River Watch - Litter | Thames21 2015). Involving volunteers in marine debris removal from beaches also has an educational dimension. Removing litter from a beach has a proven effect on the volunteer’s behaviour. Uneputty et al.
(1998) showed that beaches in Indonesia remained substantially cleaner after the local population had been involved in removing debris. Wyles et al. (2016) found that beach cleaning increased the marine awareness of participating individuals, more so than recreational activities on the coast.

This public enthusiasm for marine debris removal could also be used for research. The Marine Conservation Society beach clean-ups have a successful history of data collection. The debris were quantified and classified, and a large number of data have been generated over many years. Volunteers participate in beach debris surveys (Bravo et al. 2009; Hong et al. 2014), but the consistency of the data collected by volunteers has never been quantified. While the use of volunteers in beach surveys has tremendous potential, as far as the amount of data collected and in the geographic extent of a survey, there are concerns about the quality of the data collected, because the results from different surveys will only be comparable if the methods are consistently applied.

Reports on such citizen science in other disciplines are already available. Especially in research projects that rely on a large amount of data collected on a wide geographic scale, for example in entomological surveys, citizen science protocols can help meet the required information volume. Oberhauser and Prysby (2008) have successfully established a network of citizen scientists that spans several regions of the United States and Canada to monitor temporal and geographic differences in the abundance of monarch butterflies. However, when the targeted data includes information as complex as species identification, there can be a significant quality gradient between scientists and even trained volunteers (Kremen et al. 2011). This thesis has addressed the use of volunteers using a rapid survey method for small plastic debris in Chapter 3. The method was simple so as to train the volunteers with minimum effort. However, the results from the surveys conducted by each volunteer diverged significantly, and at present they would not be suitable to compare the plastic debris burden of different beaches. Furthermore, only part of the methodology was executed by the volunteers, the quadrats whose burden rating was assessed were preselected. In real application the volunteers would select these quadrats themselves. The volunteer experiment conducted in Chapter 3 illustrates the difficulty of obtaining reliable
data from citizen scientists. A method that is adapted to being applied by multiple observers with various levels of experience needs further development. With computer simulation, large portions of this development process can be conducted without putting volunteers in the field. Volunteers can later be used to test a set of methods that were identified as the most suitable ones.

Benefits of citizen science include the possibility for researchers to obtain larger amounts of data over a wider temporal and geographic extent than they would achieve on their own, a sense of community and the ability to contribute to science for volunteers. Additionally, it provides a way for science itself, to open up to non-scientists and acquire increasing acceptance (Oberhauser and Prysby 2008; Raddick et al. 2009). Citizen science in the field of plastic debris in the environment has already been applied, for instance, by Mato et al. (2001) with pelletwatch.org and by Jambeck et al. (2011) with the Marine Debris Tracker. Smartphones can be used as data collection devices with no further involvement of the volunteer than carrying the device around and allowing it to transfer data to a server (Newman et al. 2012). In the method presented in Chapter 3, photographing the quadrat in addition to estimating its burden rating and uploading the photograph into a database would present a more quantitative assessment of small plastic debris contamination and could also help calibrate the observer’s assessment against others. Delaney et al. (2008) quantified the accuracy of volunteers’ crab species identification through double-checking. Comparing photographs against volunteers estimates in a beach survey could indicate the observer’s accuracy in the same way. Using playful incentives such as ‘levelling up’, this could even be implemented as a software-only application, where users assign burden ratings based on the photograph. Yu et al. (2010) developed a way to incorporate the observers level of expertise in the analysis of bird observations that people could volunteer on a website. They based their assessment of expertise on the likelihood of observation of a species and on their visual similarity to others. In that way observations of a rare species in an area, where a similar looking species is common, could indicate lack of expertise. This would actually translate into a simpler concept for small plastic debris. Burden is a continuous, while species ID is binary (an individual does or does not belong to a species). An observers burden ratings could simply be compared to an expert’s opinion of the corresponding photograph.
A further benefit of employing volunteers via their smartphone is that applications support two-way data transfer, and citizen scientists could be allowed to monitor the analysis of the data they just submitted (Aanensen et al. 2009; Graham et al. 2011) allowing them to become more involved in the research. Going further than a mere reward, the data on small plastic debris on beaches submitted by volunteers can be tested against the physical parameters characterising the beach, as was Chapter 4 of this thesis. Each data point would be added to a model that can predict which beaches should be surveyed or cleaned and when, in order to obtain the most complementary set of data or to have a maximum bearing in the control of microplastics in the environment by removing small plastic debris.

6.2.3 Observing the distribution of plastic debris in the ocean

In order to validate the possibility of relating the abundance of small plastic debris at sea, with beach surveys as suggested by Merrell (1980b) and Dixon and Dixon (1981), at-sea surveys, in conjunction with beach surveys are necessary. If a link between the at-sea abundance and the small plastic debris burden on beaches is detected, beach surveys could indeed be used to monitor the global distribution of small plastic debris in the ocean.

Chapter 4 presented a crude, but promising model to predict the amount of small plastic debris based solely on physical factors appropriate to the beach and to the time period before the survey as opposed to linking it to nearby population centres or shipping routes. This is important, because the more plastic debris deteriorate into small fragments, the less they can be identified and attributed to sources. It seems that small plastic debris dispersal is above all directed by hydrodynamic factors such as wave action and tides. But the model that revealed this connection is based on (a) orthogonal projections of correlated variables, (b) a limited number of data points that may not satisfy the requirements for an oversaturated model and (c) a geographically limited survey area. We can therefore not relate small plastic debris burden on beaches to a single effect. Indeed results are likely to vary if areas outside the English Channel and the Celtic Sea are included.

The English Channel and the Celtic Sea are two independent bodies of water, but they may exhibit similar hydrodynamics. Both are equally confined by land, and their wave models are
therefore likely more complex than they would be e.g. on an oceanic island (Cavaleri and Bertotti 2004). Corbin and Singh (1993), Debrot et al. (1999) and Ivar do Sul et al. (2009) surveyed beaches on oceanic islands and linked the amount of debris on beaches to currents and winds. In such an insular setting it is possible that wind and waves are strongly correlated. They were not in the data used in Chapter 4, as was shown by their different representation in separate orthogonal projections. Perhaps the study in Chapter 4 illustrates the importance of separating hydrodynamics and wind as possible causes for plastic debris being washed on to beaches or off beaches. Contrary observations in studies on oceanic islands strongly suggests the need to expand the model geographically, since it currently only applies to Southwest England. Regions with different correlations between the model parameters need to be included in order to obtain a more general view. This endeavour is constrained by the availability of the model parameters for beaches globally. Many of the parameters were assessed in field surveys by Scott (2009). Relying solely on beaches for which these parameters are available substantially limits the number of candidate beaches; surveying beach morphodynamics on a sufficient number of beaches constitutes a monumental task. Some model parameters, however, are readily available. Tidal levels are known for numerous places globally, beach geometry can be assessed cartographically, and wind and wave data can be extracted from existing meteorological databases. Above all, what is required to geographically expand this model is small plastic debris burden assessment on beaches for which the model parameters are known. For this purpose, developing a minimalistic method that can be applied widely by volunteers is paramount. Volunteers can be mobilised for marine debris related activities on beaches, and independent actions that are coordinated via the internet, social media or mobile apps such as the International Pellet Watch (Ogata et al. 2009) and the Marine Debris Tracker (Jambeck et al. 2011) are gaining momentum. Before deploying volunteers to collect field data, an attempt to geographically expand this model using existing data could be attempted.

The model of physical factors is indeed independent from the method that is used to assess the small plastic debris burden; it can even be fitted to data for all, not just small plastic debris, as long as the survey method is consistent. Many studies such as Storrier et al. (2007) or Martinez-Ribes et al. (2007) conducted multiple surveys whose results could be fitted to the
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model. This would require some data mining, especially if temporal data are to be included. The survey dates would have to be acquired, and the corresponding wind and wave forecast need to be available. Details permitting, it may even be possible to consolidate results from multiple studies by accepting a high variability between surveys, which would also be the case using the method presented in Chapter 3. Obtaining a large number of data points is key in small plastic debris research. The margin for error of each survey is wide, trends can therefore only be identified using a large sample size.

6.2.4 Microplastics in the food web

The effect of measures to reduce the formation of microplastics by fragmentation in the environment ultimately has to be assessed through the health of organisms that are affected. An in-situ method to quantify microplastics ingestion, as was attempted in Chapter 5, could be used to link possible health effects to contamination. A conclusion from Chapter 5 was that studies on microplastics ingestion may only address one aspect of the phenomenon at a time, as different aspects require different experimental approaches. Aspects of microplastics ingestion are uptake quantity (Browne et al. 2008; Lusher et al. 2013; Ugolini et al. 2013; Sanchez et al. 2014), gut to tissue translocation (Browne et al. 2008; van Cauwenbergh and Janssen 2014) and effects on the animal (Browne et al. 2013; Wright et al. 2013). Microplastics are difficult to detect and to identify with certainty (Goldstein and Goodwin 2013; van Cauwenbergh and Janssen 2014; Sanchez et al. 2014). The analytical approach has to be tailored to the objective of the study. For an assessment of uptake quantities, particles need to be separated from the organism. To observe gut-tissue translocation, particles that can be localised in tissue cross-sections are required. None of these principles were followed in Chapter 5 due to the opportunistic nature of the study. Microplastics ingestion studies on the common cockle are novel, but the common cockle could be developed as a model, and the first step would be an exposure experiment in a controlled environment. Browne et al. (2008) introduced the blue mussel as a model. A similar experimental design could be used for the common cockle. Cooke et al. (1979) exposed cockles to Cadmium in an artificial environment. A similar setup could be used for microplastics. Cooke et al. (1979) acclimatised the animals in sediment-free water for several days prior to the
experiment; thus the gut contents of the animals could be controlled as in van Cauwenberghe et al. (2012). At this experimental stage, fluorescent particles are adequate, as the environment can be controlled for potential false positive readings, and uptake quantities can be measured by simple gut content screening.

In a first step toward a model for *in-situ* microplastics ingestion research, cockles could be exposed to a range of concentrations of microplastics. The concentrations that cockles were exposed to in Chapter 5 were high compared to concentrations that are observed in natural environment. In order to perform as a model for microplastics ingestions, cockles need to be exposed to naturally occurring concentrations in a controlled environment. Once there is evidence for microplastics ingestion at environmentally relevant concentrations, wild cockles could be checked for microplastics. Goldstein and Goodwin (2013) screened gooseneck barnacles for microplastics. Their approach of extracting the intestine and screening the contents for plastic particles visually under a dissecting microscope could be suitable for this.

Gut to tissue translocation of microplastics is best examined in a laboratory controlled environment, where false positive readings can be avoided, and microplastics can be applied in a targeted way as was done by Browne et al. (2008) and Watts et al. (2014). Marked or non-marked plastic particles could be used, and tissue samples could be examined either by fluorescence microscopy, which would be more specific than it was in Chapter 5, as false positive readings could be excluded, or by CARS microscopy. Neither screening method is advisable if gut to tissue translocation is monitored *in-situ*, due to the risk of false positive readings. In this case a suitable alternative for CARS and fluorescence microscopy could be to free the animals of any plastic particles in the gut lumen, to homogenise sections of tissue and to attempt extracting potential microplastic particles from the homogenate.

In uncontrolled environments, the difficulty remains that we do not know where the microplastics came from. Unlike a laboratory setting, where each reading can be attributed to a parameter set in advance, in the natural environment, particles can occur, which may or may not be plastic, and the only way to know is by detailed analysis. FTIR is limited in the size of sample that can be examined. Infrared is long-waved; the spectrum operates around 1000 cm$^{-1}$ (10 µm). Espe-
cially particles that are able to translocate from the gut lumen into the tissue may be too small to be captured by this approach.

Effects of microplastics on animal fitness have been observed on the lugworm. Browne et al. (2013) examined the role of microplastics in carrying organic pollutants into the organism and described effects caused by these pollutants. Wright et al. (2013) observed direct effects of microplastics themselves. The lugworm has a set of fairly well established biomarkers such as phagocytic cell count, energy reserves, oxidative status and survival. While some biological essays have been conducted on cockles looking at lethal and estrogenic effects (Marin et al. 2008) or immunotoxicity (Matozzo et al. 2008), cockles are not yet established models for toxic effects (Malham et al. 2012).

Biological essays on the lugworm have been conducted in controlled environments (Browne et al. 2013). Controlled environments have fewer factors that may influence animal fitness than found in the natural environment, and in-situ assays are required to observe the full biological response to a contaminant (Underwood 1995). The translation of these toxicological experiments from the laboratory to the field is potentially much easier than for studies on ingestion of gut to tissue translocation.

Lugworms and cockles occupy similar habitats, and given the better availability of references on biological response to toxic pollutants and contaminants, lugworms currently seem like a better choice for the observation of biological effects of microplastics. Conversely, cockles are more practical for marking, releasing and recapturing, because they have a shell, which can be marked and which can also be collected long after death. Given the increasing presence of microplastics in the environment their potential for harmful effects should be researched further. Cockles potentially present a valuable model for this, as their habitat allows for effective exposure to microplastics and they are suitable for tracking in-situ. The objectives of future studies should be (a) to examine the bioavailability of microplastics to cockles as described above, and (b) to further establish a set of toxicity biomarkers for use with cockles.

There is potential for a suite of methods to be developed to monitor microplastics in the environment following the principles proposed by Ryan et al. (2009) to monitor changes in distribu-
tion, composition, abundance and effects of microplastics over time. Such methods would be a powerful instrument to evaluate measures to reduce the accumulation of plastics in the environment. This is important, because policy can only be enforced if reliable performance indicators are available.

6.3 Conclusions

This thesis shows that widely used plastics can degrade into microplastics when discarded in the environment. Especially in air, plastics can physically deteriorate quite rapidly. Hence in the marine environment the shoreline may be the site of greatest deterioration compared to the water column and seabed for example. Plastics as an environmental contaminant are gaining increasing public attention, and many consumers are keen to help resolve this environmental issue.

Environmental plastic contamination is undergoing a shift from large debris to smaller microplastics. This shift must be considered in environmental plastic mitigation as well as in research strategies. Quantitative observations of small plastic debris in the environment are invariably quite inaccurate, because small particles can be elusive. Be it ingestion, geographic dispersal, contamination of beaches, identifying trends will therefore always require large sample sizes. Increasing the amount of detail on any kind of observation may well not actually improve the data quality and resources may be better invested in replicates than in detail.

It is becoming evident that the contamination of the environment with plastic debris (small and other) is a case for 'Big Data'. Big data can be made available in two ways: 1) By limiting studies to essential observations. E.g. the exact location of a plastic particle on a beach is not relevant. Finding it on the shoreline will do. 2) By focusing on the head count of the task force rather than on the qualification. Volunteers that set out for an hour to find ten small plastic debris accumulations on a beach and to rate them on a scale from 0 to 5 will return a very crude result. But they can repeat this effort many times, and there are many volunteers that can do this. Observers can be calibrated against each other, and numerous albeit variable data may well show trends that smaller more detailed surveys would not.
6.3. CONCLUSIONS

With the deterioration of plastics and the emergence of small plastic debris and microplastics the impact of environmental contamination with plastics shifts. Different sets of organisms will be affected in different ways; rather than at entanglement of birds and ingestion by turtles we are looking at clogged up filter-feeders contaminated with trace metals and endocrine disruptors. Another aspect of plastic pollution that is changing is our ability to identify sources and causative factors. The portion of plastic debris that can be recognised and linked to certain sources, manufacturers or consumers is becoming smaller, and it is thus harder to link the contamination to specific measures. Preventing environmental plastic contamination at the source - while it is not anonymous - remains the preferential action, as punctual action is easier than blanket measures. The same goes for direct sources of microplastics. They are best intercepted where they emerge, for example in the production of cosmetic products with scrubbers, at washing machine drains, and in locations where larger debris items might deteriorate into microplastics. In preventing the emergence of microplastics, beach cleans may play a pivotal part and they are gaining increasing importance in addressing environmental plastics contamination.
**Table A.1:** Surveyed beaches, frequency surveyed \((n)\), geodetic coordinates (decimal degrees, E, N), associated measuring point in the Windguru database (WG) and its distance from the beach in decimal degrees \((d_{WG})\).

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Table A.2: Coefficients of the best fitting model for the beach small debris burden score. Residual standard error: 0.7044 on 35 degrees of freedom. Multiple R-squared: 0.3657, Adjusted R-squared: 0.2933, F-statistic: 5.046 on 4 and 35 degrees of freedom, p-value: 0.002564.

|                | Estimate | Std. Error | t value | Pr(>|t|)  |
|----------------|----------|------------|---------|----------|
| Intercept      | 1.90683  | 0.11171    | 17.070  | < 2 × 10^{-16} |
| Geometry PC2   | -0.23150 | 0.10017    | -2.311  | 0.02685  |
| Morophodynamics PC2 | 0.24993 | 0.09174    | 2.724   | 0.00999  |
| 3 days PC2     | 0.27983  | 0.13839    | 2.022   | 0.05087  |
| 21 days PC2    | -0.24193 | 0.10459    | -2.313  | 0.02671  |

Table A.3: List of sampling events in the shoreline plastic pollution survey.

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<td>22/06/2011</td>
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<tr>
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<td>29/11/2010</td>
<td>Sennen</td>
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<td>24/01/2011</td>
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<td>10/07/2011</td>
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<tr>
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<td>24/02/2011</td>
<td>Par Beach</td>
<td>20/09/2011</td>
</tr>
<tr>
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<td>Treyarnon</td>
<td>24/02/2011</td>
<td>Boolie</td>
<td>20/09/2011</td>
</tr>
<tr>
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<td>38</td>
<td>Towan</td>
<td>25/02/2011</td>
<td>Caerhays</td>
<td>21/09/2011</td>
</tr>
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</table>

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Appendix B

Public documents
Shoreline plastic debris survey

Quadrat-based visual assessment method

Background
In the scope of a survey on plastic debris pollution a sampling quadrat based visual pollution assessment method was developed. This method should facilitate quick determination of a pollution index for a whole beach from the pollution estimates of deliberately selected plastic debris accumulation.

Goals
- To find the ten spots with the largest accumulation of plastic debris.
- To assign each of these ten spots a burden score from 0 to 5 based on the number of plastic pieces within one square metre.

Method
1. Select the ten spots with the largest number of plastic debris per square metre within a stretch of beach of at least 100 metres.
2. Apply 1 x 1 metre sampling quadrat on each spot so as to include a maximum number of plastic pieces.
3. Assign the sampling quadrat a burden score from 0 to 5 based on a quick visual assessment following Table 1 and Figure 1. The visual assessment should not take longer than two minutes. Leave the quadrat undisturbed. Do not dig for more debris in the sand or under seaweed. Only touch debris to make sure they are plastic.
4. Enter the burden score into the appropriate field in the datasheet provided.

<table>
<thead>
<tr>
<th>Burden score</th>
<th>You would say …</th>
<th>Number of pieces</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No plastic</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>Few pieces</td>
<td>1-9</td>
</tr>
<tr>
<td>2</td>
<td>Tens of pieces</td>
<td>10-49</td>
</tr>
<tr>
<td>3</td>
<td>Hundreds</td>
<td>50-499</td>
</tr>
<tr>
<td>4</td>
<td>Thousands</td>
<td>500-4999</td>
</tr>
<tr>
<td>5</td>
<td>Tens of thousands</td>
<td>5000 and more</td>
</tr>
</tbody>
</table>

Table 1: Burden score descriptions.

Figure B.1: Instructions for shoreline plastic debris survey used in the volunteer trial Chapter 3.
Figure B.2: Survey form used in the volunteer trial in Chapter 3. The letter A indicates the survey run.
List of references.


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6.c.6. Using a rapid survey approach to identify relationships between beach physical factors and micro- and meso-debris pollution

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KEYWORDS
shoreline, beach, debris, survey, method, environmental factors, morphodynamics, volunteer

BACKGROUND
Various approaches have been used to assess the abundance of plastic debris in the marine environment. These include beach surveys, at sea surveys and estimates of amounts entering the sea (Rees and Pond 1995). Beach surveys constitute a method that can readily be applied with minimum infrastructure and at a low budget. They are resilient, straight-forward methods that allow volunteers to conduct surveys and rapidly collect a large amount of data. They have successfully been used by organisations such as the UK Marine Conservation Society who have generated comprehensive datasets from beach-clean campaigns conducted by large numbers of volunteers over many years. However, beach-clean campaigns often target macro- and mega-debris only, and hence little is learnt about the occurrence of meso- and micro-debris. Studying meso- and micro-debris could yield important additional information. For instance it has been suggested that meso- and micro-debris reflect the at-sea abundance of plastic debris better (Ryan et al. 2009). This type of debris is also more likely to affect animals by ingestion. Also beach surveys to date have shown a large within-site variability that may be attributed to random sampling missing patchy accumulations of debris. There are different approaches to monitoring plastic debris on beaches: standing stock and accumulation surveys. Standing stock on beaches is determined by a number of physical factors (currents, beach structure etc.) and interactive factors (land-based sources, cleanups) (Sheavly 2007; Isobe et al. 2001). Accumulation studies meanwhile are considered to give a better representation of the amount of plastics at sea. However, these are more elaborate than standing stock studies, and cannot be applied in studies that target meso- and micro-debris as this would involve complete removal of all targeted debris in each repeated survey event. For a micro- and meso-debris survey it would therefore be beneficial to devise an alternative approach.

It is argued that repeated measurement of standing stock reflects the accumulation of debris on a beach (Ryan et al. 2009). As repeated measurements require a quick effective survey method that is not constrained by logistics, we examined a simple approach to monitoring the standing stock
of micro- and meso-debris on beaches returning temporally resilient semi-quantitative data with low within-site variability. We used this approach to identify physical factors that influence the plastic debris abundance on a set of beaches for which physical factors had previously been assessed (Scott, 2009). This work was conducted in close co-operation with a local community group with a strong focus on knowledge transfer and dissemination.

The aims of our work were to validate a quantitative rapid survey method, targeting meso- and micro-debris on beaches that could be applied by volunteers and to begin to identify morphodynamic factors that promote plastic debris accumulation on beaches.

**METHODOLOGY**

Sampling locations were selected from a list of beaches with known morphodynamic parameters (Scott, 2009). In addition, parameters describing beach geometry (i.e. radius of curvature, apex angle, length and distance to lateral obstructions) were determined cartographically.

A 100-200 meters transect was laid parallel to the waterline. The ten locations along the transect with the highest accumulations of meso- and micro-debris were flagged (hereafter referred to as ‘dirty’). In addition, five random locations were positioned at random coordinates within the area determined by the minimum convex polygon around the positives. All locations were then sampled using 1m² quadrats. Quadrats were photographed (Fig. 1) and assigned a plastic debris ‘burden score’ ranging from 0 (no debris) to 5 (more than 10⁴ pieces) by visual assessment. This burden score was validated by comparing it to actual counts, weights and measurements of plastic debris in a subset of quadrats.

In order to reduce noise caused by a large number of independent variables, the number of independent variables used to predict burden scores was reduced by grouping morphodynamic parameters into ‘context clusters’ summarised using principal components analysis. The context clusters were beach slope, sediment size, tidal characteristics and wave action. The first principal components (minimum eigenvalue = 0.85) for each cluster were used as independent variables, along with beach geometry measurements. The burden score variance to mean ratios of both random and dirty quadrats for each site were calculated in order to establish which method returned the lowest within-site variance, random sampling or the deliberate selection method. We tested for relationships between mode dirty burden and the independent variables.

**OUTCOMES**

The calibration of the visual quadrat burden scoring method showed a relationship with all three measured values (fragment abundance, cumulative size and weight). The visual scoring system can therefore be considered a reliable indicator of debris burden. The burden scores resulting from the deliberate selection method showed a variance to mean ratio 75 percent lower than random sampling. The strong reliability of the visual scoring system and the low within-site variation returned by deliberate selection sampling suggest that this method constitutes a useful instrument for the quick semi-quantitative indexing of plastic debris burden on beaches. The mode burden score of the dirty quadrats was therefore used as an index of the plastic pollution on a beach. None of the first principal components of the morphodynamic parameters (Scott, 2009) showed a significant relationship with the mode dirty burden. The regression analyses using the
beach geometry variables showed a significant negative relationship between the radius of curvature and the mode dirty burden (Table 1).

**PRIORITY ACTIONS**
The public awareness of the beach plastics issue and the willingness to tackle it is becoming more and more pronounced. Developing and disseminating simple and robust survey methods would further empower public campaigns to actively contribute to plastic debris research.

**FIGURES AND TABLES**

![Photo reference of a burden score 3 sampling quadrat.](image)

<table>
<thead>
<tr>
<th>Model</th>
<th>β</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radius</td>
<td>-0.436</td>
<td>0.022</td>
</tr>
<tr>
<td>Apex</td>
<td>-0.338</td>
<td>0.049</td>
</tr>
<tr>
<td>Length</td>
<td>-0.177</td>
<td>0.350</td>
</tr>
<tr>
<td>Obstruction</td>
<td>-0.087</td>
<td>0.597</td>
</tr>
</tbody>
</table>

**Table 1**: Linear regression of mode dirty burden vs. beach geometry, n=36.

**REFERENCES**
Scott, T. M. (2009), Beach morphodynamics and associated hazards in the UK, PhD thesis.
64.a. Ingestion of microplastics by common cockles (Cerastoderma edule) in an intertidal mud flat

AUTHORS
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Massimo Ponti, Centro Interdipartimentale di Ricerca per le Scienze Ambientali in Ravenna, University of Bologna, Italy
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KEYWORDS
microplastics, in-situ, exposure experiment, method, cerastoderma

BACKGROUND
Microplastic particles arising from the degradation of large plastic debris or directly introduced into the environment are widespread in marine habitats (Colton et al. 1974, Gregory 1996, Thompson et al. 2004) and have raised concerns about potential threats to marine organisms. Microplastic particles may be ingested, and their physical presence may affect invertebrates in a similar way to that shown for large debris affecting e.g. birds (Ryan 1988, van Franeker et al. 2005). Plastic particles have also been shown to absorb persistent organic pollutants from water (Mato et al. 2001, Teuten et al. 2007), which could be transferred to organisms as a consequence of ingestion. Laboratory experiments on the blue mussel (Mytilus edulis) have shown that microplastic particles are ingested and remained in the organism for in excess of 48 days (Browne et al. 2008). However, experiments in the field are also important in order to establish the full extent of any biological response (Underwood 1995). Both field and laboratory studies of microplastics rely upon identifying plastic particles ingested and potentially translocated within the study organisms’ tissues, therefore advances in techniques to assist in quantification of these processes are of considerable interest.

The aims of this study were therefore to determine the practicality of using fluorescent plastic tracers to monitor ingestion and uptake of microplastic particles in-situ and to identify a model system that is suitable for monitoring the effects of microplastics on organisms in-situ that could be used to complement the findings of laboratory experiments such as Browne et al. (2008).

METHODOLOGY
The common cockle (Cerastoderma edule) is a suspension-feeding sediment dweller and is common in temperate waters in the North-West Atlantic Ocean (FAO, 2011). Cockles have a large capacity to ingest particles 60 to 500 μm in size (Karlsson et al. 2003). They therefore represent a potential model organism for in-situ experiments into ingestion and translocation of microplastic particles.
Twenty cockles were exposed to ‘Luminophore’ polyamide powder in each of 4 quadrats in fine sediment and 4 quadrats in coarse sediment in an intertidal mudflat following a method previously used by Montserrat et al. (2009). The Luminophore particles were applied as a mixture in proportions that reflected the natural sediment (Table 1 and 2). After two weeks the animals were recovered and the stomachs were extracted, dehydrated and preserved in paraffin block. They were then cut into 10 μm thick sections using a microtome. The sections were examined using a fluorescence microscope, and luminophore particles were enumerated.

OUTCOMES
To date it has only been possible to make a preliminary examination of four animals. Scaling up from the 385 sections examined so far it would appear that each individual contained several hundred Luminophore particles. Retained Luminophore particles were often detected within the stomach tissue rather than the lumen (Fig. 1). From the initial Luminophore particle concentration and the particle intake rate observed by Karlsson et al. (2003) the retention rate of ingested Luminophore particles was less than 1 %.

Fluorescent particles can be traced in cockles’ stomachs after 14 days exposure. This system provides a possible method to address ingestion and uptake of microplastic particles in the field.

PRIORITY ACTIONS
The findings of this study give further evidence that organisms at a low trophic level can ingest microplastic particles. It is therefore important to quantify the biological effects on organisms that ingest microplastic particles and to look at transfer between trophic levels.

FIGURES AND TABLES

Table 2: Proportion of both Luminophore particle size fractions (head indicates median size) added to the natural sediment.

<table>
<thead>
<tr>
<th>Size µm</th>
<th>&gt; 1000</th>
<th>&gt; 500</th>
<th>&gt; 250</th>
<th>&gt; 63</th>
<th>&lt; 63</th>
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<tbody>
<tr>
<td>Coarse</td>
<td>20%</td>
<td>24%</td>
<td>25%</td>
<td>12%</td>
<td>19%</td>
</tr>
<tr>
<td>Fine</td>
<td>6%</td>
<td>5%</td>
<td>10%</td>
<td>20%</td>
<td>58%</td>
</tr>
</tbody>
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Table 1: Natural sediment composition.

<table>
<thead>
<tr>
<th>Sediment type</th>
<th>Luminophore</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>129 µm</td>
</tr>
<tr>
<td>Coarse</td>
<td>4.30%</td>
</tr>
<tr>
<td>Fine</td>
<td>3.40%</td>
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</table>
REFERENCES
Browne M A, Dissanayake A, Galloway T S, Lowe D M and Thompson R C (2008), 'Ingested microscopic plastic translocates to the circulatory system of the mussel, Mytilus edulis (L.), Environmental Science and Technology 42(13), 5026 –5031
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Underwood A J (1995), Toxicological testing in laboratories is not ecological testing of toxicology, Human Ecological Risk Assessment 1, 178 – 182
INTRODUCTION

Microplastic particles arising from the degradation of large plastic debris or directly introduced into the environment are widespread in marine habitats (Colton et al. 1974, Gregory 1996, Thompson et al. 2004) and have raised concerns about potential threats to marine organisms.

Microplastic particles may be ingested, and their physical presence may affect invertebrates in a similar way to that shown for large debris affecting e.g. birds (Ryan 1988, van Franeker et al. 2003). Plastic particles have also been shown to absorb persistent organic pollutants from water (Mats et al. 2001, Trenn et al. 2007), which could be transferred to organisms as a consequence of ingestion.

Laboratory experiments on the blue mussel (Mytilus edulis) have shown that microplastic particles are ingested and retained in the organism for at least 48 days (Brown 2003). However, experiments in the field are also important in order to establish the full extent of any biological responses (Underwood 1995).

Both field and laboratory studies of microplastics rely upon identifying plastic particles ingested and potentially translocated within the study organisms’ tissues, therefore advance in techniques to assist in quantification of these processes are of considerable interest.

AIMS

To determine the practicality of using fluorescent plastic tracers to monitor ingestion and uptake of microplastic particles in situ.

To identify a model system that is suitable for monitoring the effects of microplastics on organisms in situ, that could be used to complement the findings of laboratory experiments such as Brown et al. (2006).

APPROACH

The common cockle (Cerastoderma edule) is a suspension-feeding sediment dweller and is common in large-scale habitats in the North-West Atlantic Ocean (FAO, 2011). Cockles have a large capacity to ingest particles 60 to 500 μm in size (Karlsson et al. 2003). They therefore represent a potential model organism for in situ experiments into ingestion and translocation of microplastic particles.

OBJECTIVES

1. To establish whether microplastics are ingested by common cockles that are exposed to them in their natural habitat.

2. To determine whether ingested microplastic particles are retained after ingestion.

3. To detect possible effects of sediment size and plastic particle size on rates of ingestion.

METHODS

Ingestion of microplastics by common cockles (Cerastoderma edule) in an intertidal mudflat

Nicolas F A Biber1, Massimo Ponti2, Andy Foggo3, Richard C Thompson1

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Twenty cockles were exposed to ‘Luminophore’ polyamide powder (Fig 3) in each of 4 quadrats in fine sediment and 4 quadrats in coarse sediment (Fig 2 and 4) in an intertidal mudflat (Fig 1) following a method previously used by Montenweil et al. (2009).

The Luminophore particles were applied as a mixture in proportions that reflected the natural sediment (Table 1).

After two weeks the animals were recovered and the stomachs were extracted (Fig 6), dehydrated and preserved in paraffin blocks (Fig 7). They were then cut into 10 μm thick sections using a microtome (Fig 8).

The sections were examined using a fluorescence microscope (Fig 9 and 10), and Luminophore particles were enumerated.

RESULTS

To date it has only been possible to make a preliminary examination of four animals. Scaling up from the 365 sections examined so far it would appear that each individual contained several hundred Luminophore particles. Refined Luminophore particles were often detected within the stomach tissue rather than the lumen.

From the initial Luminophore particle concentration and the particle intake rate observed by Karlsson et al. (2003) the retention rate of ingested Luminophore particles was less than 1%.

CONCLUSIONS

Fluorescent particles can be traced in cockles’ stomachs after 14 days exposure. This system provides a possible method to address ingestion and uptake of microplastic particles in the field.

PRIORITY ACTIONS

The findings of this study give further evidence that organisms at a low trophic level can ingest microplastic particles. It is therefore important to quantify the biological effects on organisms that ingest microplastic particles and to look at transfer between trophic levels.

ACKNOWLEDGEMENTS

I wish to thank Amy Gurant, Andrew Tonkin, Elena Righetti, Joceline Triner, Michael Hawthins, Nadja Christen and Will Veevers without whose help this work could not have been conducted.

This research project was funded by Erika Klaam-Staninger and Marianne Klaam (Rheinsel-Gen, Switzerland), Theodor-Guertig-Stiftung (Basel, Switzerland) and my family.

REFERENCES


Table 1: Sediment composition. a) Natural sediment composition, size fractions, proportions in % vol. b) Proportion of both Luminophore particle size fractions (head indicates median size) added to the natural sediment.