Understanding species-microplastics interactions

A laboratory study on the effects of microplastics on the Azorean barnacle, *Megabalanus azoricus*

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Declaration

I hereby confirm that I am the sole author of this thesis and it is a product of my own academic research.

________________________________________________________________________

Student’s name.
Abstract

Understanding the impact of microplastics on the marine environment, wildlife and humans is a complex issue. Effects of contaminated microplastics (polyvinylchloride (PVC), mean size 1.5 µm) on the Azorean barnacle (Megabalanus Azoricus) were investigated within a global research project (GAME), in which akin experiments were conducted simultaneously at seven different sites worldwide in order to obtain comparable data for a range of benthic invertebrates. During a six weeks laboratory experiment individuals of M. azoricus were exposed to different microplastic density levels and the effects of these treatments on barnacle survival, respiration, motility and survival under hypoxia stress were measured. The results do not allow clear statements on a negative effect of microplastics on barnacles. Cirral activity decreased under medium plastic densities, with barnacles showing no respiratory pumping and beating, but at higher densities the behavior of the barnacles was normal. A similar pattern was observed for the respiration rates in the medium plastic density treatment group, although no statistical difference emerged between this and all other groups. At high plastic densities barnacles may have protected themselves from exposure, while barnacles at lower densities did not manage to do this, maybe because the reflex of feeding was still intact at medium particle densities. Although this experiment did not give clear answers, a comparison with all other studied species showed that under similar conditions some were clearly affected, indicating that some species might be more susceptible to microplastic exposure.

An additional investigation of sediment samples from a beach (Praia Formosa) in Southern Madeira should contribute to the understanding of actual microplastics abundance of the surrounding habitat of Megabalanus azoricus. The results suggest a rather low concentration with a mean microplastic abundance of 4 particles per kilogram sediment.

This study illustrates how marine science deals with uncertainty and complexity. While some microplastic-species interactions produce inconclusive results, other studies deliver first evidence of the negative influence of microplastics. This study helps to understand species-level impacts of microplastic pollution for range of marine organism from the base of the marine food web.

While trying to understand its effects on complex biological systems, it should be highlighted that microplastic pollution is irreversible, meaning there is no method suitable for removing it. There is abundant evidence of the presence of this contaminant in the ocean and the level of pollution is expected to grow. Thus, the precautionary approach is urged to be applied and research supporting mitigation of plastic pollution and decision-makers should be prioritized.
Our own interest lay in relationship of animal to animal. If one observes, in this relational
sense, it becomes apparent that species are only commas in a sentence, that each species is at
once the base and the point of a pyramid. All life is relational [...] And then not only the
meaning but the feeling about species grows misty. One merges into another, groups melt into
ecological groups until the time when what we know life meets and enters what we think of as
non-life: barnacle and rock, rock and earth, earth and tree, tree and rain and air. And the
units nestle into the whole and are inseparable from it[...]. All things are one, and one thing
is all things[...] It is advisable to look from the tide pool to the stars, and then back to the tide
pool again.

John Steinbeck, *The Log from the Sea of Cortez*
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Acronyms

µl  micro liter
µm  micrometer
ANOVA  analysis of variance
Cm  centimeter
DF  deposit feeder
Dw  dry weight
EPS  expanded polystyrene
FF  filter feeder
h  hour
HDPE  high density polyethylene
HPLC  high performance liquid chromatography
Kg  kilogram
L  liter
LDPE  light density polyethylene
M  meter
Mg  milligram
Ml  milliliter
Mm  millimeter
MP  microplastics
Mt  metric ton
NGO  Non-Governmental Organization
Nm  nanometer
NPSG  North Pacific Subtropical Gyre
PA  polyamide
PAH  polycyclic aromatic hydrocarbon
PBAT  polybutylenadipat-terephthalat
PBDE  polybrominated diphenyl ether
PBS  polybutylensuccinate
PCB  polychlorinated biphenyl
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>PCL</td>
<td>polycaprolactone</td>
</tr>
<tr>
<td>PE</td>
<td>polyethylen</td>
</tr>
<tr>
<td>PET</td>
<td>polyethylene terephthalate</td>
</tr>
<tr>
<td>pH</td>
<td>measure of hydrogen ion concentration</td>
</tr>
<tr>
<td>PHA</td>
<td>polyhydroxyalkanoates</td>
</tr>
<tr>
<td>PLA</td>
<td>polylactide</td>
</tr>
<tr>
<td>POP</td>
<td>persistent organic pollutant</td>
</tr>
<tr>
<td>PP</td>
<td>polypropylen</td>
</tr>
<tr>
<td>PS</td>
<td>polystyrene</td>
</tr>
<tr>
<td>PTT</td>
<td>polytrimethylene terephthalate</td>
</tr>
<tr>
<td>PVC</td>
<td>polyvinylchlorid</td>
</tr>
<tr>
<td>rpm</td>
<td>rounds per minute</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SPSG</td>
<td>South Pacific Subtropical Gyre</td>
</tr>
<tr>
<td>UNEP</td>
<td>United Nation Environmental</td>
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<tr>
<td>US EPA</td>
<td>US Environmental Protection Agency</td>
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1 Introduction

With the import of plastic to the marine environment, a new pollution has been created by humans. Plastic accumulation in the ocean is a global and rapidly growing problem. While the consequences of macroplastics for marine life are increasingly known, many uncertainties surround the impact and fate of microplastics. This raises questions about how plastic particles, which are smaller than sand grains, can affect organisms at the base of the food web.

1.1 Plastic – A success story?

As one of the largest manufacturing industries, the plastic industry has expanded significantly since the start of commercial mass production in the early 1930s and 1940s. Between 1975 and 2012, production of global plastics increased from 47 to 299 metric tons (Mt) per year - an increase of more than 80% (Figure 1) (PlasticsEurope, 2015). Asian Pacific countries dominated the global market in 2013 with 44.6% of the total market volume. China stands as the largest plastics producer, accounting for 24.8%. While European and North American market are relatively stable, plastic production in Asian-Pacific and Central and South America are expected to grow (Grand View Research, 2014).

Plastics’ success is based on their functionality and they cannot easily or economically be replaced by other materials. Plastics derive from oil and gas monomers that develop into a molecule of long carbon chains in a process of polymerization. Additives are joined with the polymers to improve their specific characteristics. Plastics are cheap, light, strong and durable, with high thermal and electrical insulation properties. Polymers exists with different
properties allowing a wide range of application that bring technological advantages, energetic savings and various societal benefits (Andrady and Neal, 2009). The most utilized polymer type is polyethylene (PE) (ca. 140 million tons/year) with 30% thermoplastic productions, followed by polyethylene terephthalate (PET), which accounts for 20% of thermoplastic produced. Other important polymers are Polypropylene (PP) (18%), polyvinyl chloride (PVC) and polystyrene/expanded polystyrene (PS/EPS) (European Commission, 2011a; Sivan, 2011). The variety of polymer types offers versatile usage, mostly for packaging, the sector which produces the largest amount of waste at around 60% of municipal solid waste in the European Union (European Commission, 2011b). Other usages include construction, as well as replacing metals in manufacturing goods such as computers and car parts. Plastics also play an important role in the health and medical sector, where disposable products such as syringes, catheters and cannulas are needed on a daily basis.

1.2 Plastics in the marine environment

From millions of tons of trash produced every year a high proportion is entering the marine environment, from which plastic is the primary constituent (Jambeck et al., 2015; Moore, 2003; Ryan et al., 2009; Thompson et al., 2004).

It is estimated that 20% of marine plastic waste come from ships through loss of fishing gear (“ghost nets”) and containers or through illegal waste dumping. It was only in 1989 that an international agreement prohibited waste disposal at sea. Nowadays it is estimated that 80% of the marine plastic debris originates from land, mainly due to poor waste management through run-offs via rivers and storm surges. The land-based share varies largely in space depending on local or regional waste management and population size. One estimation claims between 4.8 to 12.7 million metric tons of plastic waste entered the oceans in 2010, equivalent to 1.7 to 4.6% of the global production (Jambeck et al., 2015). The top twenty countries’ of “mismanaged plastic waste” account for 83% of the total mismanaged waste worldwide and China with 8.82 million metric tons per year is the biggest contributor (27.7%), followed by Indonesia (10.1%) (Jambeck et al., 2015). As the worlds’ population continues to grow, so will waste production and it is not expected to “peak” this century (Kennedy et al., 2013).

Plastics accumulation and fragmentation has recently changed the earth surface in a long-lasting manner. All plastic (with exception of incineration) that has been produced for 60 years of mass production is still existing as a whole or in fragments and it is too early to say how long the mineralization process takes (Barnes et al., 2009). Once in the marine
environment it underlies complex transport mechanism. They are washed ashore or float with ocean currents where plastic items may end up in so called gyres which are huge circular ocean currents that accumulate waste and keep it in the same area. Since discovery of a widespread plastic garbage contamination in the North Pacific, five other similar gyres have been reported from the North Atlantic, South Pacific, South Atlantic and Indian Ocean. In the North Pacific the plastic fragments are estimated at 300,000 items/km² and 200,000 in the North Atlantic garbage patch (Moore, 2003). In the framework of the United Nations Environment Programme (UNEP) (2006) the floating plastic concentration of the ocean was estimated to be around 18,000 pieces each km². Denser polymer types (e.g. PVC) tend to settle where they are released, but lighter polymers may also sink when developing microbial films changing their physiochemical properties (Lobelle and Cunliffe, 2011). Ultimately, the seabed can be seen as the repository of all plastics that are not stranded on the beaches. Here, the longevity of plastic is likely to be far higher than on land; presumably hundreds to thousands of years (Barnes et al., 2009)

Whilst plastic is convenient and indispensable, it is problematic when it comes into the ocean. There it encounters wildlife, causing a risk of entanglement and ingestion, distributing non-native and potentially harmful organisms, absorbing toxic chemicals and degrade into microplastics that may be ingested by a broader range of organisms (Sigler, 2014).

Large plastic items, such as ropes, cargo straps, fishing lines and traps are the main contributor for entanglement of sea turtles, whales, seals, bird, fishes or crustaceans. Nets and lines can also harm coral reef and sponges that get snagged by them, causing them to break. Ingestion of both, macro and microplastics, has been reported among at least 170 marine vertebrate and invertebrate species, causing life threatening complications such as gut perforations, reduced food intake, and transfer of toxic compounds (Avery-Gomm et al., 2013; Bond et al., 2014; Smith et al., 2014; Wegner et al., 2012).

1.3 Microplastics

While the impacts of macroplastics have been well documented, microplastic research is in its infancy and recently, research examining the occurrence of microplastics in the marine environment has been increased substantially. In the last 15 years more than hundred laboratory and field studies documented the abundance of microplastic debris in oceans, freshwater systems, sediments and studied the effects of microplastic ingestion by fish, birds, whales, invertebrates and microorganisms (Browne et al., 2008; Vegter et al., 2014).
1.1.1 What are the sources of microplastics?

Per definition microplastics are plastic particles smaller than 5 mm (Arthur et al., 2009), occurring as primary and secondary microplastic in the environment.

Secondary microplastics are a result of different fragmentation processes of large plastic items. Due to the high molecular weight most widely used plastic types are not biodegradable (Shah et al., 2008). However, once in the ocean they will suffer under oxygen and sunlight (photo-oxidative degradation), which dominates the fragmentation (Andrady, 2011). Sunlight oxidizes the chemical structure, causing loose bonds and reduction of the polymer weight and resulting a brittle and disintegrative plastic (Browne et al., 2007). Other processes are thermal and/or chemical degradation, microbial degradation and mechanically effects of wave actions as well as abrasion by sand (Andrady, 2011).

Other microplastics enter the environment in form of small pellets used for production of goods or abrasives in industrial and domestic application, for example in toothpaste and facial cleaner (Fendall and Sewell, 2009). These are so-called primary microplastics. By washing synthetic garments huge amount of small fibers are released via sewage into the environment (Browne et al., 2011b). Davison and Asch (2011) have shown a novel source of microplastic pollution; the boring isopod Spaeroma terebran can release thousands of particles when burrowing polystyrene floats at aquaculture facilities and docks

1.1.2 How much microplastic is in the environment?

Irrespective of their origin, once in the environment, microplastics persist and accumulate, which is a particular concern as they are impossible to remove. Since four decades studies have identified microplastics in almost all marine habitats around the globe (Ivar do Sul and Costa, 2013).

High quantities of plastic pellets were first recorded in the 1960s during plankton sampling in the western North Atlantic (Carpenter and Smith, 1972). There, microplastics increased in abundance from the 1960s to the 1990 (Thompson et al., 2004). Despite a large increase of plastic production, no trend of increasing accumulation was observed during an extensive ship-survey in the North Atlantic Ocean and Caribbean Sea between 1986 and 2008, underlining how poorly constrained the sources and sinks of plastic debris are in the ocean (Law et al., 2010). Possible sinks for floating plastic debris include fragmentation, sedimentation, shore deposition, and ingestion by marine organisms. Microplastic tend to mix
vertically during wind events and by changes in wind force and wind direction drive long-term shifts in circulation pattern of plastic (Collignon et al., 2012; Law et al., 2010). The highest concentration of plastic items was associated with the subtropical convergence, illustrating how plastics can act as a tracer for ocean currents (Law et al., 2010). Most neuston samples from the South Pacific Subtropical Gyre (SPSG) contained mainly high- and low-density PE, PP and 88% of plastics were smaller than 10mm (Eriksen et al., 2013b). Similarly, most of the plastic fragments found in the North Pacific Subtropical Gyre (NPSG) were under 5 mm and plankton abundance was around five times higher than that of plastic particles, whereas the mass of plastic was roughly six-fold that of plankton (Moore et al., 2001).

Furthermore, microplastic has been found in freshwater systems, such as rivers (Dubaish and Liebezeit, 2013; Lechner et al., 2014), lakes (Faure, Corbaz, Baecher, & De Alencastro, 2012, Eriksen et al., 2013; Free et al., 2014) and sediments of lakeshores (Imhof et al., 2013; Zbyszewski and Corcoran, 2011). Most recently a microplastic reservoir was found in the Arctic sea ice (Obbard et al., 2014). Eriksen et al., 2013 found an average abundance of 43,000 plastic particles km\(^{-2}\) in the Laurentian Great Lakes of the USA, with highest densities occurring around the urban areas (Detroit and Cleveland), where 466,000 particles km\(^{-2}\) were reported. Plastic beads, used in facial cleanser or other personal products, predominated the microplastic pollution and may become a major source in the ocean (Fendall and Sewell, 2009). Even in areas with low human populations, poor waste management can heavily contribute to the pollution of freshwater systems (Free et al., 2014). High densities with an average 20,264 particles km\(^{-2}\) were found in a large, remote mountain lake Hovsgol in Mongolia, where plastic fragments and films were most abundant (Free et al., 2014).

Surveys on microplastic pollution in marine sediments are far less numerous. Generally, microplastics are more abundant in sediments than in the water column (Hidalgo-Ruz et al. 2012a). Occurrence in sediments found first evidence in the late 70ies, when translucent pellets, 2-5 mm in size, have been related to spillages at major ports in New Zealand and Canada (Gregory, 1983, 1978, 1977). Nowadays, microplastics are being reported globally, with higher amounts commonly related to higher populated areas. Often the majority of plastic types are fibers suggesting an input by sewage effluents, including wastewater from washing machines (e.g. Browne et al., 2011, 2010; Claessens et al., 2011; Thompson et al., 2004).
Contrastingly, on Hawaiian beaches, influenced by the North Pacific Subtropical Gyre, the majority of plastics were fragments (87%) (McDermid and McMullen, 2004). Highest reported pollution was from the Kamilo Beach on a Hawaiian Island, with an average of 3.3% plastic by weight in the surface layer (Carson et al., 2011). Carson et al. (2011) state that sediments with plastic warm slowly (16% maximum decrease in thermal diffusivity) and reached lower maximum temperature, with potential effect on beach organism, for example sea turtles, whose sex-determination is temperature dependent.

Analysis of microplastic in the subtidal sediment at the Lagoon in Venice revealed a tendency of accumulation of particles in areas with low hydrodynamics (Vianello et al., 2013). Studies mostly reported microplastic in surface sediment samples giving less information about the three dimensional distribution. Turra et al. (2014) criticize that standing-stock estimated by sampling protocols can be largely underestimated. They found pellets in deep of 2 m, with surface layer accounting for <10% of total pellets in the sediment column (Turra et al., 2014).

1.3.1 How microplastics interact with marine biota?

Because of the small dimensions of microplastics, they are interacting with a wide array of species throughout the marine food web. A potential uptake of the particles is possible via normal ventilation processes (Watts et al., 2014), direct ingestion of microplastics (e.g. Thompson et al., 2004; Besseling et al., 2013) or through trophic transfer (e.g. Eriksson and Burton, 2003). Microplastics are ingested by whales (Fossi et al., 2014), sea birds, fish and marine invertebrates, often with negative health consequences (Besseling et al., 2012; Rochman et al., 2013b; Wright et al., 2013b).

For at least four decades the interaction of seabird with plastic has been monitored allowing a quantification and composition of ingestion over time (e.g. Azzarello & Van Vleet, 1987; Colabuono, Barquete, Domingues, & Montone, 2009; Moser & Lee, 1992; P.G. Ryan, 1988). Seabirds mistake small plastics (macro- and microplastic) of specific shape and color for food items (Moser and Lee, 1992). In five seabird species stomachs’ with plastic content the proportion of virgin pellets has decreased over the last 20 years, indicating a change in the composition of small plastic debris from pellets to user plastics in the Atlantic and southwestern Indian Oceans (Ryan, 2008). Similarly, Bond et al. (2014) reported a low proportion of industrial pellets (7%) compare to user plastics fulmar and shearwaters in Nova Scotia, Canada.
Most affected species are the surface-feeding tubenoses (order procellariids) with several recent studies reporting between 80 and 100% incidences of plastic ingestion in all examined individuals (Auman et al., 2004; Kinan and Cousins, 2000; Van Franeker et al., 2003; Young et al., 2009). In the North Sea, Northern fulmars (*Fulmarus glacialis*) are used to indicate the state of the pollution level of the marine ecosystem (Van Franeker et al., 2011). There, an Ecological Quality Objective (EcoQO) has been established, with a target of no more than 10% of fulmars having >0.1 g of plastic (OSPAR, 2008), but 48–78% of fulmars exceed this goal (Van Franeker et al., 2011). Other studies documenting plastic ingestion by fulmars have been conducted in British Columbia, Alaska, the Canadian Arctic, the western North Atlantic, Iceland, the Faroe Islands, and Jan Mayen (e.g. Bond et al., 2014; Kühn and van Franeker, 2012; Mallory, 2008; Moser and Lee, 1992) The range of studies on seabird give increasing evidence of negative effects of plastic ingestion for individuals and populations, since plastic increases the contaminant burden (Tanaka et al., 2013), reduce fledging success, lower body mass and are able to damage the gastrointestinal tract.

Microplastics are readily ingested by many fishes species (e.g. Boerger et al., 2010; Choy and Drazen, 2013; Davison and Asch, 2011; Lusher et al., 2013), but the effects on fish health remain uncertain. They possibly include gut blockages (Choy and Drazen, 2013) and transfer of chemicals (Teuten et al., 2009). In the North Pacific Subtropical Gyre plastic fragments have been found in approximately 9 to 32% of sampled stomachs, from mesopelagic and planktivorous fish (Boerger, Lattin, Moore, & Moore, 2010, Davison & Asch, 2011). Another extensive study from this region indicates that commonly consumed mesopelagic fishes show the highest incidences of debris ingestion, between 30% to 58 % of sampled fishes (Choy and Drazen, 2013). Since they are unlikely to come into contact with surface waters, this suggests that plastics are transported into the deep ocean through interconnected epi- and mesopelagic food webs or sinking processes (Choy and Drazen, 2013). A lower proportion of plastic in fishes has been found in the North Sea (2.6%), but in the English Channel more than 33% of cod contained plastics (Foekema et al., 2013).

Microplastic ingestion by invertebrates has been shown for planktonic organisms, amphipods and decapods (Besseling et al., 2014; Chua et al., 2014; Cole et al., 2013; Haemer et al., 2014; Kaposi et al., 2014), as well as for other invertebrates such as worms, mussels, sea cucumbers, lobster, crabs and goose barnacles (e.g. Besseling & Wegner, 2012; Browne, Niven, Galloway, Rowland, & Thompson, 2013; Goldstein & Goodwin, 2013; Murray & Cowie, 2011; Watts et al., 2014; Wright, Rowe, Thompson, & Galloway, 2013). A commonly studied
filter feeder, the blue mussel *Mytilus edulis*, retains particles in its gills and transports them into the stomach and the digestive glands (von Moos et al., 2012). There they accumulate in the lysosomal system and can cause an inflammatory response (von Moos et al., 2012). Furthermore, microplastics have been translocated from the digestive tract to the circulatory system of mussels where they persisted for over 48 days (Browne et al., 2008). Wegner et al. (2012) observed a reduced filtration rate quantified as reduction of valve opening and an increase in pseudofaeces production when feeding *M. edulis* with nanoparticles. Farrell and Nelson (2013) showed first trophic transfer of microplastics from mussels to crabs, in which they were translocated in the haemolymph and tissue. In feeding experiments of microplastic to different zooplankton taxa ingestion has been shown after 3 h of incubation and indicates the potential of microplastic transfer via planktonic species (Setälä et al., 2014). Furthermore, in nature the presence of microplastics has been detected in invertebrates. For instance in mussels from Nova Scotia and the North Sea and in oysters from France, which are commercially cultured for human consumption (Mathalon and Hill, 2014; Van Cauwenberghe and Janssen, 2014).

However not all studies found negative effects of microplastics. A marine isopod (*Idotea emarginata*) was not effected in mortality or growth and was able to prevent intrusion into mitgut gland where plastics normally accumulate (Haemer et al., 2014).

### 1.3.2 How do microplastics interact with chemicals?

As mentioned before, plastic may act as vector for pollutants because they adsorb polychlorinated biphenyl (PCBs), polycyclic aromatic hydrocarbons (PAHs), metals, and other petroleum hydrocarbons, some of which may even desorb in acidic stomachs resulting in uptake by the animals (Teuten et al., 2009; Van et al., 2012). Furthermore, the toxic additives – such as flame retardants, antimicrobials, and plasticizers – may leach from the plastics. Pesticides and organic pollutant were found on plastics in harmful concentration, which were 100 times higher than those found in the sediment and 1 million times higher than those occurring in sea water (Teuten et al., 2009).

In nature, complex mixtures of plastics and pollutants often pose different hazards compared to a single pollutant. It has been shown, that sorption kinetics of chemicals to microplastic highly depends on the combination of pollutant and polymer type (e.g. Bakir et al., 2012; Rochman et al., 2013; Teuten et al., 2007). Few laboratory studies investigated the transfer of chemicals from microplastics to marine organism, such as to fish (Koelmans et al., 2014;
Rochman et al., 2013b), seabird chicks (Teuten et al., 2009), lugworm (Besseling et al., 2012; Browne et al., 2013; Koelmans et al., 2014) and an amphipod (Chua et al., 2014). Fish exposed to a mixture of chemicals that has absorbed from the marine environment to PE suffered from liver toxicity and pathology (Rochman et al., 2013b). Bioaccumulation of chemicals were shown for lugworm (Arenicola marina), which resulted in compromised ecophysiological functions, such as burrowing activity (Besseling et al., 2012; Browne et al., 2011b).

1.4 Importance of this study

The number of investigations on the impacts of microplastics on marine biota increased rapidly during the last 10 years, showing that all of the studied organisms are able to ingest microplastics depending on size, and some of them were negatively effected (Browne et al., 2008; von Moos et al., 2012). Nevertheless, there is still a lack in our understanding of the species-level impacts of microplastic pollution (Vegter et al., 2014), especially whether they act as a vector for contaminants. Furthermore, most studies have focused on vertebrates; mainly on birds and fishes. Less than twenty studies have been conducted on invertebrates and of these only four investigated potential pollutant effects: three on the lugworm Arenicola marina (Besseling et al., 2012; Browne et al., 2013) and one on the marine amphipod Allorchoestes Compressa (Chua et al., 2014). Studies were performed with different polymer types, sizes and densities. Applied microplastic densities often exceeded natural concentrations. Besseling et al., (2012) exposed lugworms to densities up to 7.4% PS per dry weight sediment.

Another constraint of understanding impact by microplastic pollution is the short experimental duration; most exposure experiments ranged from three hours to some days (Browne et al., 2008; Setälä et al., 2014; Wegner et al., 2012) and only three of them lasted more than three weeks (Farrell and Nelson, 2013; Haemer et al., 2014; Wegner et al., 2012). These experiment durations are very short compared to the lifetime in which different species may experience exposure to microplastics. The diversity of study designs offer limited comparability and results cannot be easily transferred to other species.

The complexity of microplastic pollution and urgency of understanding the species-impacts of plastic debris, in order to enable effective mitigation, calls for further research. It is important to understand, which species are more susceptible to plastic particles and which are species related thresholds of microplastic concentrations.
1.5 Aim of the study

The aim of GAME XII was to conduct experiments closely to natural conditions on a variety of benthic invertebrates to allow an estimation of consequences of microplastic pollution in their natural habitat. Carrying out research on multiple species over several climate zones under the same conditions in the framework of GAME allows a deeper understanding on absence or presence of effects. Highest concentrations was 3% microplastic per dry weight sediment, because highest concentrations found in sediments reported a concentration of 3.3% microplastic per weight of sediment (Carson et al., 2011). Other concentrations were set in a logarithmic scale (0.003%, 0.03% and 0.3%) to allow identification of possible thresholds if effects occur. The duration of the experiment will last between 1 to 3 months to allow a better outcome in comparison to other, shorter studies. Another aspect of interest was the role of microplastics to become a vehicle for organic pollution. Here, we need to restrict us to one concentration level and one pollutant (fluoranthene) in order to prevent a mixing up of too many factors.

The objective of this research is to present and analyze the results of a six weeks laboratory investigation of the impact on the Azorean barnacle *Megabalanus azoricus* exposed to different densities (from natural to exaggerated concentrations) of contaminated microplastic in the course of parallel experiments conducted on fourteen species of two feeding types (filter- and deposit feeders) within a global research project (see The GAME-Approach).

A side investigation answered how abundant are microplastics in Madeira by investigating sediment samples from the beach Praia Formosa in Southern Madeira.

1.6 Research Questions

The research questions of this study are based on the results of responses of *Megabalanus azoricus* that have been measured during an exposure experiment and under hypoxia. Subsequently, a microplastic monitoring served as a reference of the current microplastics pollution in Madeira. Furthermore, the aim is to put responses in a bigger picture to explain what factors might play a role for the presence or absence of effects of microplastic as well as in combination with a pollutant. Finally, I want to know in which management context the research on microplastics has taken place and provide answers to the question how this emerging pollutant can be mitigate among various stakeholders.
1. How do different microplastic densities affect the Azorean barnacle *M. azoricus* under laboratory conditions in terms of a) cirral activity, b) respiration or c) survival under hypoxia?

2. Does fluoranthene contamination of microplastic play a role for the effect on the Azorean barnacle *M. azoricus*, and if so, how?

3. How abundant are microplastics in Praia Formosa, a beach in Southern Madeira? Is there a difference in microplastic abundance between samples from the high tide line and the intertidal line?

4. What is the current status of management of microplastic pollution in the marine environment? Which stakeholders are involved in mitigating it?

### 1.7 Data and Methods

In order to answer the first two research questions a laboratory study was conducted which will be introduced in the following subchapters. For the third question sediment samples were taken from Praia Formosa, a beach in Southern Madeira. To answer the fourth research question about the management of microplastic, I conducted a literature analysis, which was primarily based on recently published scientific papers. These papers reviewed on the plastic problem in the Ocean and provided advice in summing up current mitigation efforts and research priorities. Beyond screening scientific papers, examples of mitigation efforts, which were related to current public engagement and political debate, were found in newspapers and official websites.

#### 1.7.1 Experimental design of the exposure experiment

Not many long-term laboratory studies investigating microplastic effects on marine benthic organisms include various density levels, which allows identify at which concentrations microplastic might be problematic. Furthermore only few studies incorporated the pollutant aspect in their experimental design.

Only three laboratory studies on microplastic impacts had lasted longer than three weeks, which is still little compared to the lifespan organisms might expose to plastic pollution. Farrell and Nelson (2013) studied trophic transfer of crabs that were fed up to three weeks with mussels that previously ingested microplastic. Besseling et al. (2012) exposed lugworms for four weeks to a PCB contaminated sediment/plastic mixture with a logarithmic scale from 0, 0.074, 0.74 and 7.4% PS per DW sediment. And the longest microplastic exposure
experiment was 6 weeks with the isopod *Idotea emarginata* with plastic-supplemented food (Haemer et al., 2014).

Highest concentration found in sediments in nature were 3% microplastic per weight of sediment in a highly polluted beach in Hawaii (Carson et al., 2011). A 3% microplastic treatment group was the highest pollution density level of our study. As most studies report microplastic abundance by particle numbers, they were not directly comparable to treatment concentrations in our study, which were based on mass percent (%) of sediment weight. During a six exposure experiment with contaminated microplastic particles the physical and chemical effects of microplastics on barnacles were examined. One treatment level with no microplastic served as a reference (0%), three levels entailed contaminated microplastic in an increasing logarithmic density (0.003% - 0.03% - 0.3% - 3%) and one level 3%+ microplastic particles which were not contaminated with fluoranthene (the asterisk (+ indicates no contamination with fluoranthene). Each level had a replication of 13 animals (Table 1).
Table 1: Experimental design with different particle density levels and fluoranthene (*)

<table>
<thead>
<tr>
<th>Group ID</th>
<th>Microplastic density per kg sediment (percent by weight)</th>
<th>Fluoranthene</th>
<th>Pipette (ml plastic solution*)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>No</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>B</td>
<td>0.003%</td>
<td>Yes</td>
<td>0.012</td>
<td>13</td>
</tr>
<tr>
<td>C</td>
<td>0.03%</td>
<td>Yes</td>
<td>0.12</td>
<td>13</td>
</tr>
<tr>
<td>D</td>
<td>0.3%</td>
<td>Yes</td>
<td>1.2</td>
<td>13</td>
</tr>
<tr>
<td>E</td>
<td>3%</td>
<td>Yes</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>F</td>
<td>3%*</td>
<td>No</td>
<td>12</td>
<td>13</td>
</tr>
</tbody>
</table>

*a the microplastic solution entailed 100g PVC in 500 ml seawater

During tidal change organic and inorganic material is resuspended. This scenario was imitated two times per day. For two hours in the morning and after six hours in for another 2 hours in the evening the water in the experimental unit was agitated to resuspend the microplastic in the water column. With this experimental design, I wanted to test the following null hypotheses:

I) \( H_0^1: \) Possible effects on M. azoricus do not change with microplastic density.

II) \( H_0^2: \) Possible effects of microplastic particles on M. azoricus do not change with the presence of fluoranthene.

As all participating student teams used the same experimental design but different animals, a global analysis was possible.

1.7.2 Procedure of the experiment

The experiment was divided into two parts, 1) the laboratory exposure experiment, investigating the effects of contaminated microplastics applied at different levels of particle density on *Megabalanus azoricus* and 2) the investigation of microplastic pollution in the field (microplastic monitoring). This practical part was done during five months (from May until September 2014) on Madeira Island, where all data were collected in teams and in cooperation with the Marine Biological Station of Funchal (‘Estação de biologia marinha do
Funchal’). My team partner Laura Nogueira agreed on using the data that were collected for this thesis. After the investigations in Madeira, tissue samples and microplastic were tested for fluoranthene contamination at the Ecotoxicology Department of the University of Kiel, Germany, in November 2014 (Figure 2).

The study was conducted within the 12th project of the GAME program (2014). GAME is an international approach to conduct marine ecological experiments on the effects of global change on a diverse range of marine organisms (see next chapter Figure 3).

1.7.3 The GAME-Approach

The international research and student training program GAME (Global Approach by Modular Experiments), located at GEOMAR Helmholtz Centre for Ocean research, aims at conducting identical experiments in marine ecology on the effects of marine global change beyond climate and biogeographical borders. It is one of the few initiatives that scale up ecological studies from a regional to a global scale and consequently reduce the system-specific noise in data. This facilitates to find general principles, which is often a weak point in today’s ecological research (Lenz, 2014). Moreover, young scientists are trained and become part of international network of marine research institutions and alumni students, which has been formed within 11 years and currently includes 33 partners in 24 countries.
In GAME XII, 14 students worked in 7 bi-national teams that conducted two experiments (one filter- and one deposit feeding organism) each (see next page Figure 3 and Table 2). For the preparation and post-processing of the experiments all participants came to GEOMAR (Kiel). In the post-processing all data were analyzed and results were presented at different universities in Germany.

Table 2: Overview of study organisms (with scientific name and class) of all GAME XII (2014) stations

<table>
<thead>
<tr>
<th>GAME-Station</th>
<th>Deposit feeder</th>
<th>Filter feeder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chile (Coquimbo)</td>
<td>Spoon worm, <em>Ochetostoma baronii</em> (Echiura)</td>
<td>Chilean mussel 'chorito', <em>Perumytilus purpuratus</em> (Bivalvia)</td>
</tr>
</tbody>
</table>

1.7.4 Important aspects of the experiment

Even though I will focus on *Megabalanus azoricus*, I will discuss my results with regard to the results of other teams that participated in the project. It was the second GAME project in which the effects of contaminated microplastics were investigated, although last year’s experiments (GAME XI, 2013) differed with regard to organism, plastic type, pollution type...
and treatment levels (Table 3). A natural scenario, including realistic densities of microplastic pollution, was still realized, but now a logarithmic scale enables to identify at which density an effect might occur. Equally, we wanted to include another group of organisms (filter feeders) to test whether different functional groups differ in their sensitivity to microplastics or their sensitivity to pollution with PAHs. Furthermore, we changed the contaminant and plastic type. Fluoranthene is more toxic and is more likely to attach to polyvinylchloride (PVC) than phenanthrene on polystyrene (PS). The plastic particle size is smaller, because we needed to find small particles for the filter feeders that only ingest particles of a specific size.

Table 3: Comparison of the two GAME projects (2013 and 2014) with regard to differences in the experimental design

<table>
<thead>
<tr>
<th>Aspects of the study</th>
<th>GAME XII (2014)</th>
<th>GAME XI (2013)</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organisms</td>
<td>Deposit (DF)- and Filter feeders (FF)</td>
<td>Deposit feeders</td>
<td>Possible difference in sensitivity to microplastic between DF and FF</td>
</tr>
<tr>
<td>Plastic Type</td>
<td>Polyvinyl chloride (PVC) powder</td>
<td>Polystyrene (PS) beads</td>
<td>PVC has a higher density and thus good for the DF; higher affinity to absorb fluoranthene than PS</td>
</tr>
<tr>
<td>Plastic size</td>
<td>170 µm for Deposit feeders, 1.5 µm for Filter feeders</td>
<td>&gt;200 µm</td>
<td>FF ingest smaller particles</td>
</tr>
<tr>
<td>Pollutant</td>
<td>Fluoranthene 2 µg/L&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Phenanthrene 2µg/L</td>
<td>Higher potential of harming marine organisms</td>
</tr>
<tr>
<td>Microplastic densities</td>
<td>Logarithmic scale in percent of the weight sediment (0%, all polluted: 0.003 %, 0.03 %, 0.3 %, 3 % and nonpolluted: 3%*)</td>
<td>400 (low) and 800 (high) beads per kg sediment</td>
<td>Range from low density to high densities allow identification of possible threshold</td>
</tr>
</tbody>
</table>

<sup>a</sup> in Madeira we had concentration of 20 µg/L due to a calculation mistake. All other teams had 2µg/L.
1.8 Structure of the thesis

The introduction provided background information on plastic production and the problem plastics cause in the marine environment. Sources of microplastics and the plastic break down into microplastics in the marine environment were explained. Furthermore, I provided an overview on studies that have proofed microplastic ingestion by various marine species and their effects. The way how microplastic can work as a vector for pollutant was introduced. Finally, I explained why there is a need of further understanding species-microplastics interactions, especially on benthic invertebrates and in comparable experimental designs. Subsequently, I introduced the aim of this study as well as the four research questions. This was followed by the overview on the material & methods, in which I explained the process of the experiment and provided a timeline with important steps. Further, I introduced the GAME-approach, participating countries and species, in which my experiment was embedded. Finally the subchapter about the scope and delineation explains my focus on *Megabalanus azoricus* within the global research and compare important aspects (scope) of this year’s GAME XII (2014) to GAME XI (2013). After having explained the scope of this research and within the GAME framework, the chapter concludes with delineations of this study.

In material and methods I will present more, followed by an introduction of the study site Madeira and the study species. I explain in detail how we loaded the plastic material with fluoranthene, the experimental design and set-up. I will illustrate how the response variables are measured, which statistic was used, as well as the method used to analyze fluoranthene. The chapter will be concluded by describing the additional monitoring of microplastics at the beach Praia Formosa.

In the results sections, all findings will be provided and visually illustrated.

In the discussion I will explain observed effects and compare them with findings in literature. Absence of effects and their possible meaning will be discussed. Moreover, I will put them in relation to the complexity of research on microplastic. In this context I will furthermore highlight limitations of this study and give suggestions for future research.

The last chapter is dedicated to the third research question on how we can manage microplastic pollution, which is shown to be embedded in managing marine plastic litter in general. Examples of recent activity show how decision-makers, scientists, production sector and the general public are intertwined in face of the global problem of plastic pollution in the oceans.
2 Material and Methods

2.1 Study area

Madeira Islands are a Portuguese archipelago in the Northwest Atlantic (32°38’N, 016°57’W); around 400 kilometers north of the Canary Islands and around 700 kilometers west of the marocain coast. Madeira is the biggest (741 km²) and most populated island (~350.000 inhabitants) of the archipelago, which includes the smaller island Porto Santo (~3000 inhabitants) and the non-inhabited Desertas Islands (Figure 4ab).

Figure 4: a) Political regions of Portugal (blue): Portugal, the Azores and Madeira (red circle) b) Madeira Island, Porto Santo and Desertas Islands c) Southern coast of Madeira including the different activities of the experiment. a) and b) produced with www.stepmap.de, c) modified from Google Earth 2014 (accessed 13.01.2015).
The volcanic origin created mountains which often steeply fall into the sea, building rocky shorelines with few black sandy beaches. The influence of the Gulf Stream brings moderate climate with constant water temperature between 17° to 24°, while the salinity varies between 36 ‰ and 37 ‰. Madeira’s open-ocean location makes the coastal waters oligotrophic, meaning there is little availability of nutrients and low primary production. Due to the limited availability of sandy beaches and the low phytoplankton production, there is a low biodiversity of bivalves (Segers et al., 2009).

This is also the reason, why we worked with a Cirripedia species as a filter feeder, which we collected from Ilhéu do Gorgulho, a rock island, located around 130 meters in front of the station (Figure 5). The Marine Biological Station is located in Lido, a district in the west of the capital Funchal. It is built in an open bay surrounded by hotels and influenced by sea-based tourism. Because of the absence of sandy beaches at the station the investigation of the abundance of microplastics was done at the Praia Farmosa, the closest sandy beach (see Figure 4c).

2.2 Study organism

As mentioned above this year’s GAME project investigated the effects of microplastic on filter and deposit feeders. In this thesis I constrain myself to one species, which I will put in context with other results in the discussion.

2.2.1 Species screening and sampling

The first pilot study explored which filter feeding organisms might be suitable for the experiment. Unlike the other study locations, we could not work with mussels (see Study area, previous chapter). Not only little studies have been exploring the effects of microplastic on filter feeders, up to now there is no research on the effects of microplastics to Cirripedia. Species requirements for this experiment were abundance of species, robustness under laboratory conditions and the ability to ingest microplastic in order to identify effects of microplastics.
The gooseneck barnacle (*Lepas anatifera*) (Figure 6), a common pedunculated species that lives on buoys and floating objects, was tested. Commonly feeding on plankton and larval fishing, it has been shown that they ingest microplastics: In the North Pacific Subtropical Gyre (NPSG), microplastic was found in the gastrointestinal tract of 33.5% of 385 dissected barnacles (Goldstein and Goodwin, 2013), making them a suitable test organism. A floating buoy with around 200 individuals was found. In order to separate the individuals from each other, we carefully cut the buoy with a pruner into parts. To avoid injuring the barnacles, we kept the attachment of the peduncle to the piece of buoy. The individuals (n=30), with a capitulum length between 1.5 - 2.3 cm, were placed in 1000 ml containers. There they hang in a grid, which was attached to a tube to make them float. This tube resuspended the microplastic particles via air flow twice a day to imitate a tidal event (Figure 7). However, even with additional air stones and frequent water exchange (4 times per day with an average temperature of 24.5 °C) over one third of the individuals died after one week. Because of the difficulties to maintain the gooseneck barnacles under laboratory conditions and due to their limited availability, we decided to work with the Azorean barnacle (*Megabalanus azoricus*), an acorn barnacle species that appeared to be more robust under the same conditions.
2.2.2 Megabalanus azoricus

Around 150 different barnacle species exist almost everywhere in the marine environment where you can find hard substratum: on rocks, harbor walls, coastal protection, on floating objects and even on other species such as mussels and turtles. In adaptation to the different challenges of the life history two fitness-increasing strategies evolved in the animal kingdom: r-strategy and K-strategy (Southwood et al., 1974). With a high reproduction rate and a relatively short larval development (between 10 - 45 days) barnacles show the r-strategy.

It was only in the second half of the 19th century that Thompson (1830) found out that barnacles are crustacean arthropods, meaning they are distantly related to crabs, lobsters and shrimps. As all crustaceans they have a hard exoskeleton made of chitin and during their free swimming larval stage as nauplii, they closely resemble the larvae of crabs (Thompson, 1830). In their pelagic stage, barnacles usually progress through six larval phases and the cyprid, which is the last stage, finally undergoes a metamorphosis into the sessile adult form (Figure 8).

For additional protection against predation and physical impact, the adults develop an outer calcareous “shell”, called test, that distinguishes them from other crustacean (Lohse and Raimondi, 2007). This is made of several plates that are either directly attached to the substratum with a basal plate or to a fleshy stalk (such as L. anatifera). The legs are forming into a cirral net, which does not serve for locomotion, but for filtering food from the water column. The cirri come out of an opening, the aperture, which is covered by two plate pairs, the operculum. For species identification the basal plate and the operculum are the most important features, as the phenotype can be variable in shape as a result of dense growth (Luther, 1987). Most barnacles are hermaphrodites, meaning they have both male and female reproductive organs. Prior to mating, the male is tapping with its elongated penis, whether nearby barnacles carry eggs to inseminate them. The internal fertilization and brooding of the eggs within the test could be an important feature of the revolutionary success of barnacles (Buhl-Mortensen and Høeg, 2006).
Our species is an eastern warm-temperate and tropical Atlantic species (Southward, 2008). Apart from the Azores, today it is only known on the Canaries, Madeira (Wirtz et al., 2006; Southward, 2008) and St Helena, inhabiting a range from 0 to 40 m depth (Southward, 1998). In Madeira, observed barnacles form bands just below the low tide line on exposed rocky shores (Wirtz et al., 2006). The name “Megabalanus” was proposed by Hoek (1913), because it is the subdivision with the largest forms of existing balanids. It is classed as endemics for the Azores, but their ability to be carried by ships or floating objects might explain their occurrence on Madeira, St Helena and the Canaries (González et al., 2011) (Figure 9). This species inhabits rocks within a narrow subtidal area, about 1 m below the waterline down to 5 m, exceptionally to 15 – 40 m, and is commonly in places that experience wave action (Southward, 1998).

On the Azores the species has long been collected as a food resource, causing the risk of overexploitation. The OSPAR convention and WWF demand for urgent action to safeguard the species with concentration on regulation of the fishery (OSPAR, 2010; Santos, Hawkins, Monteiro, Alves, & Isidro, 1995). There is no regulation of fishing activities up to date. To relief fishing pressure López, López, Pham, & Isidro (1994) proposed a discussion about artificial farming and investigated the suitability of Megabalanus azoricus recruiting on artificial substrates. Further threats for the species are the degradation of suitable habitats and its vulnerability to contamination by oil pollution (OSPAR, 2010). Microplastic could also harm this species as it is very sensitive due to the restricted habitat in which it occurs.

### 2.2.3 Microplastic ingestion

For the experiment it was important that the test organism ingests microplastic particles. Barnacles are relatively unselective omnivores feeders, feeding on phytoplankton and zooplankton and they ingest food items ranging from 2 µm up to 1 mm (Southward, 1955). In a stomach of a lepadid barnacle, collected in the north pacific a fragment of 6.7 mm was found (Goldstein and Goodwin, 2013). It was therefore very likely that M. azoricus were able to ingest the plastic material used in the investigation that had a mean size of 1.5 µm. A

![Figure 9: Study organism Megabalanus azoricus. Young barnacles grow on an old individual. Source: Author](image)
fragment of 6.7 mm was found in a Lepadid barnacle collected in the North Pacific (Goldstein and Goodwin, 2013). On the one hand, the applied microplastic particles were small enough to ensure ingestion, but on the other hand it was not possible to clearly distinguish them from plankton and gonads when dissecting the barnacles. Furthermore, fecal pellets as a means of analyzing food intake (Anderson, 1994) were not visible with naked eye, possibly because of the small size of our individuals. Thus, I have not direct proof of particle ingestion. However, I observed typical feeding behavior, when barnacles were exposed to microplastics.

2.3 Contamination of microplastics

2.3.1 Plastic Material

_Megabalanus azoricus_ were exposed to polyvinylchloride (PVC) powder with particles in a size range from 1.3 µm to 50 µm, but an average size around 1.5 µm (PyroPowders, Erfurt, Germany) to simulate microplastic contamination during a tidal event (Figure 10). The PVC particles are irregularly shaped and have no coating additives. Their density of 1.38 g/l makes them heavier than seawater (1.025 g/l), which is needed for this experiment, since they sink to the bottom of the tank and become available for the barnacles during a re-suspension event (see Experimental set-up).

In last year’s project, polystyrene (PS) was used, but it did not sink effectively was furthermore not available in such a small size. With around 20% of the total plastics produced, PVC is expected to be widely found in marine environment. It is used in packaging, such as plastic films, bottles and cups and as a construction material (Brien, 2007; PlasticsEurope, 2013).
2.3.2 Loading of microplastics with fluoranthene

As microplastics can act as a vector for pollutants, the PVC in this experiment was pre-treated with fluoranthene (Figure 11), a poly aromatic hydrocarbon (PAHs) emerging from fossil fuel and incomplete combustion processes. It commonly occurs in the aquatic environment as part of complex mixtures of PAHs. PAHs do not degrade easily in natural environment and therefore have raised increasing concern (Haritash and Kaushik, 2009).

Fluoranthene has been shown to be acutely toxic to some marine species (US EPA, 1978). The median effect- and lethal concentrations for saltwater species range from 40 to 500 µg/L. For example, the median time to death (LC50) for Artemia salina (brine shrimp) was 1 hour at a concentration of 40 µg/L (Irwin and National Park Service, 1997). Furthermore it has been shown to be carcinogenic and mutagenic to marine animals (Haritash and Kaushik, 2009).

In order to achieve a concentration of 20 µg fluoranthene/L seawater a stock solution was prepared in a serial dilution with acetone as a solvent (Figure 12). From the last solution (80 µg fluoranthene in 20 ml of acetone) 12.5 ml (50 µg fluoranthene) were added to a round glass aquarium with 2500 ml seawater and 500 g PVC powder. The total sorption time was three weeks and during this period the seawater/fluoranthene solution was renewed every fourth day to make sure that the concentration of fluoranthene stayed constant. Two water pumps ensured constant mixing of the plastic material in the water and a lid avoided evaporation.
After the plastic was loaded with fluoranthene, it was washed with fresh seawater once and stored in 500ml seawater per 100g PVC powder in the fridge.

### 2.3.3 Calculation of the microplastic treatment

The calculation of the different plastic treatment levels is based on the assumption that 5% of the volume of each tank entailed sediment (Table 4). However, the sediment was not in the tanks, because the resuspension would not work due to technical reasons. First, because the resuspension with air was not strong enough to fully resuspend sediment. Second, due to the small container size the animal would be too close to the sediment and probably harmed by such amount of resuspending sediment. The volume of the bottle filled with hypothetical sediment (with a density of 1.6 kg/cm³) of the total tank volume (1000ml) is 50ml with a weight of 80 g. From 80g sediment the microplastic densities were calculated which reflects a density ranging from 0.0024 to 2.4 g plastic material added (see Table 4). The plastic content per tank in g was then converted into ml, because the plastic particles were added as a plastic-seawater mixture with a density of 0.2g PVC/ml.

*Table 4: Scheme and calculation of theoretical sediment volume of 5% per one liter tank volume, as a basis to calculate different microplastic treatment levels and a plastic-seawater solution of 0.2g/ml*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plastic content (g)</th>
<th>PVC/water solution (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3%</td>
<td>2.4</td>
<td>12</td>
</tr>
<tr>
<td>0.3%</td>
<td>0.24</td>
<td>1.20</td>
</tr>
<tr>
<td>0.03%</td>
<td>0.024</td>
<td>0.12</td>
</tr>
<tr>
<td>0.003%</td>
<td>0.002</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Based on 5% Sediment (density 1.6 kg/cm³) of total volume
A precise estimation of the amount of particles during resuspension could not be calculated, because the amount of microplastic decreased during the week and the water became clearer with each resuspension. Some of the plastic material was lost during regular water exchange, although we tried to avoid this by stopping the water flow during and after one hour of resuspension. Another reason was the particle behavior in the experimental unit. Particles of different sizes tended to aggregate and accumulated on exposed surfaces and in corners of the exposure bottle, aeration stones and tubes. An estimated amount between 60 – 80 % of the initial plastic material was bioavailable during resuspension and that this amount decreased during the week by around 50% before the microplastic was exchanged.

2.3.4 Experimental set-up

On 25th July, big individuals of *M. azoricus* on which smaller individuals grow, were collected from the rock island Ilhéu do Gorgulho (32°38'3.46"N, 16°56'3.49"W) and were immediately transported to the marine station (see Study area). In the laboratory we separated 78 individuals by dividing bigger once with a screwdriver. Subsequently, they were cleaned from algal on growth and placed into 1.5 L-plastic water bottles (filled with 1000 ml seawater), which served as experimental containers (Figure 13).

![Figure 13: Left – Overview of experimental set-up (3% treatment) during resuspension, Right – Sideview of experimental unit (0% treatment). Explanation: 1) water supply, 2) resuspension tube and 3) additional air supply, 4) *Megabalanus azoricus* of 0% treatment attached to a plastic grid via a cotton string and 5) overflow at 1 L. Source: Author](image)
To minimize a possible bias by the size of the animals, some bigger barnacles were distributed evenly among the different treatment levels. Barnacles ranged from 0.6 – 0.8 cm in basal diameter. For analyzing the effects of size on barnacle performance and to standardize the measured response variables to body size, animal size was calculated as shell ‘volume index’ (VI = b * r * h where b and r are basal diameter and approximated minor basal diameter (0.75 * b) and h the height of the shell in cm was measured as suggested by Foster (1987).

Before starting the experiment, barnacles were acclimatized for seven days. Each container was equipped with air supply and a resuspension tube that circulated the microplastic via air bubbles twice a day. The set-up was connected to a sea water tank, from which the water in the experimental units was completely renewed six times a day by a flow-through-system. As the water was unfiltered and contained plankton, the animals did not need additional food provision.

During the resuspension and one hour after, there was no flow-through to reduce the loss of plastic particles from the experimental containers. Once a week we cleaned the bottles and added new microplastic particles. The used water and all used microplastics were filtered to avoid any contamination of the sewage water with plastic. The water quality was monitored in the beginning of the experiment with an ammonium test kit. As there was very frequent water exchange, ammonium was not a problem. Every day during the first week of the experiment, we measured oxygen with the oximeter (WTW Oxi 197) which was calibrated before each usage. Average water temperature was 24.5 °C. Because the set-up was located close to a window barnacles experienced normal circadian rhythm.

Mortality of barnacles was observed every day by lifting up the tube the animal was attached to (Figure 14). Once outside I carefully looked if the barnacle moves by slightly blowing at it. If it did not react the barnacle was placed into a container with fresh seawater and was observed under stereo-microscope.

Figure 14: Mortality control by lifting the resuspension tube. Here: Lepas anatifera, but comparable to mortality control of Megabalanus azoricus. Source: Author
2.4 Response variables

2.4.1 Cirral activity

The bioactivity of barnacles, i.e. the cirral activity, is the basis for respiration and feeding and is evoked mainly by water currents (Crisp and Southward, 1961). Often barnacles have a burst of movements being interrupted by phases of non-activity, which is referred to by Crisp and Southward (1961) as “activity rhythm”. Variations in cirral activity are closely linked to environmental factors (flow, temperature, pH, oxygen, presence of food etc.). Most important factors are temperature and water velocity. Up to a certain point increasing temperature and flow leads to an increase in activity (Anderson and Southward, 1987). To a smaller degree activity depends on the size of barnacles. The rate of beating decreases with increasing size and age (Anderson and Southward, 1987).

Crisp & Southward (1961) described five different activity types of barnacles

“(1) testing, in which the valves hardly open and the cirri are not protruded; (2) pumping, in which strong rhythmic movements of the operculum occur, but the cirri are protruded only slightly and not extended; (3) normal beat, a development of pumping, but with the cirri fully extended and withdrawn in rhythm with the opercular movements; (4) fast beat, with less opercular movement, but strong and fast rhythmic cirral movements; (5) extension, in which the cirri are held outside the shell for varying periods without rhythmic movements.” (Crisp & Southward, 1961, p. 271).

For measuring motility, I placed the barnacles in a small container (500ml) with fresh sea water and without plastic. A submerged tube connected to an air pump provided a slight and constant moderate water flow, to reduce the variance in withdrawal times. Firstly, the barnacle recovered for one minute to adapt to their new environment before movements were counted for another minute. I assumed that each movement lasts one second, and thus the counts of movements were expressed in percent of activity per minute. The frequency was assigned to ranks (0 – 5%, 5%< , 6 – 15%, 15%<) of three different cirral behaviours (testing, pumping and beating) based on the classification by Crisp and Southward (1961) (Table 5). For example, when counting 0 – 3 movements (0 – 5% of activity per min) the barnacle was assigned to “No activity”; 4 – 9 movements represent an activity 6 – 15% and 10< movements represent more than 15% activity per minute. I did not distinguish between normal and fast pumping or normal and fast beating, but only the frequency at which pumping and beating
occurred per minute. Extension was not considered, because *Megabalanus azoricus*, does not often extent their cirri, which is common for *Lepas anatifera* for predation (Crisp and Southward, 1961).

Table 5: Illustration of behavior categories based on the activity (number of movements expressed in % per minute) and behaviors (no, testing, pumping and beating) of *M. azoricus*

<table>
<thead>
<tr>
<th>Behavior Category</th>
<th>Activity (%) per min</th>
<th>Explanation of behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td>No activity</td>
<td>0 - 5%</td>
<td>No or little activity</td>
</tr>
<tr>
<td>Testing</td>
<td>5% &lt;</td>
<td>Aperture is parted slightly, small movements of the body inside the mantle cavity, cirri not protruded</td>
</tr>
<tr>
<td>Pumping –</td>
<td>6 - 15%</td>
<td>Body performs rhythmic movements to produce an inflow of water into the mantle cavity; cirri protruded; rise and fall of operculum with reduced or closed aperture between beats</td>
</tr>
<tr>
<td>Pumping +</td>
<td>15% &lt;</td>
<td></td>
</tr>
<tr>
<td>Beating –</td>
<td>6 - 15%</td>
<td>Basic events as in pumping, but long cirri are unrolled and spread out and then curled up and withdrawn, aperture reduce but not closed</td>
</tr>
<tr>
<td>Beating +</td>
<td>15% &lt;</td>
<td></td>
</tr>
</tbody>
</table>

2.4.2 Respiration

Barnacles respire by pumping water into the mantle cavity with their operculum. The respiration of barnacles is regulated by changing their pumping activity (Crisp and Southward, 1961). Therefore, respiration is a crucial indicator of the physiological state of the animals. In this study, the respiration rate (RR) was measured three times: in the beginning (1st week), in the middle (4th week) and in the end of the experiment (after 7th week) before the hypoxia stress test. For the measurements, an airtight container (50 ml volume) was filled with fresh seawater and in its lid an oxgensensor was mounted. It measured the oxygen level (in mg/L) before (oxy1) and after (oxy2) a time span of 20 minutes. A magnet stirrer (VARIOMAG®, Thermo Electron LED GmbH) provided constant mixing of the water at 300 rpm. In the measurement chamber entailed no microplastic (Figure 15). After each measurement, the water inside the chamber was replaced by oxygen saturated seawater. Additionally, the water temperature was noted, since it is one of the most important factors influencing metabolic rates in marine invertebrates (Nishizaki...
and Carrington, 2014a). The mean temperature during the first two measurements was 26.2°C, while it slightly increased during the third measurements to 27.2°C.

For defining a background respiration (BR), which might be due to bacterial activity, only seawater was measured for four times (BR 0.08mg/L, SD =0.0374, SE= 0.0167).

The oxygen consumption was than calculated as follows:

\[ RR = (oxy_1 - oxy_2 / 20 \text{ minutes}) - BR \]

Moreover, the size dependency of respiration has to be considered. The relationship of respiration for most organisms can be explained algorithmically.

\[ RR = a \cdot VI^x \]

where RR is the oxygen consumption per hour, VI = volume index (size) and a and x are constants. We used a mass exponent (x) of 0.75, which has been established for small invertebrates by Banse (1982).

As a size measure a ‘volume index’ of the barnacles is a non-destructive size assessment of barnacle shell:

\[ VI = l \cdot b \cdot h \]

where l and b are the major and minor basal diameter and h the height of the shell. The VI assumes barnacle shell is cuboid. It is closely proportional to volume and a better criterion of size than wet weight and ash-free dry weight, because of the variations in the shell thickness and seasonal changes in tissue weight (Southward and Crisp, 1965).

### 2.4.3 Survival during hypoxia

The habitat of adult barnacles, determined by the fall and rise of the tide, is comprised of a complex variation of physic-chemical factors. In showing tolerance or using protection mechanism, barnacles adapt to extreme conditions (Desai and Prakash, 2009). The sensitivity to stress of *Megabalanus azoricus* formerly exposed to microplastics should be tested. I decided on hypoxia-stress, as it is common to the intertidal animals to be living partly under oxygen depletion during low tide. Figuring out the suitable oxygen concentration and how often deoxygenation and water exchange is needed, I collected 15 animals for a pilot study, placed them in reversed bottles (500ml) and applied three different levels (0.5, 1.0 and 1.5 mg
oxygen/L). Dry nitrogen gas deoxygenated the seawater under continuous bubbling. To minimize deoxygenation via the surrounding atmosphere a plastic foil, commonly used in the household, was carefully placed on the surface and stick to the side of the bottle walls (Figure 16). The oxygen and temperature was controlled every 8 h for five days. The water quality was recorded each day. The pilot study indicated that deoxygenation was needed every 24 hours, as it was not possible to fully seal the bottle, as a consequence the water re-oxygenized up to 3 mg/L. The mortality did not differ between the groups, half of all test organisms died after 48 hours and one survived in the higher levels (1 and 1.5 mg/L) until the end of the pilot study (day 5).

I decided on an oxygen level of 0.75 mg/L, a value between moderate (1mg/L) and severe hypoxia (0.5mg/L) (Nilsson and Rosenberg, 1994), and deoxygenation every 24 hours. A water exchange every third day was sufficient and there was no extra food provision. The mortality was checked every 8 hours. The hypoxia stress test lasted for 192 hours (8 days).

2.5 Chemical analysis

The fluoranthene analytic instructed by Dr. Hans-Jörg Martin and Daniel Appel was carried out in the institute of toxicology institute in Kiel in October 2014. With the HPLC (High-performance liquid chromatography) fluorescence detection we wanted to find out, whether fluoranthene accumulated on the microplastic particles and if they were able to transfer into the tissue of M. azoricus.

The following samples were tested:

- **Microplastic loading**: 3 x 3 samples of each 2g PVC powder, which was incubated in 2 µg fluoranthene/seawater solution for three weeks. As the experiment lasted for three month, the fluoranthene might have dissolved from the plastic during the storage. Thus, we took à 3 samples from the beginning, middle and end which were directly frozen in - 30 ° C.
• **Tissue samples:** We took 3 samples of each level, but needed to pool all polluted (n=12) and all non-polluted groups (n=6) together, as around 5 g of tissue was needed for the analysis, which was still more than we had.

**The standard curve and HPLC Conditions**

The quantification of fluoranthene is based on a standard curve, which allows identifying peak areas in relation to a standard. Firstly, the fluoranthene standard was diluted 10x to a concentration of 500 ng/µL. With a 0.5 ml Hamilton syringe and a 10 ml volumetric flask a serial dilution was done with 20x dilution in five steps up to a dilution factor of 1.600.000. Different volumes are injected in the HPLC in order to establish the standard curve. Under the area of the curve is the amount of fluoranthene that can be detected with the HPLC-software (“Clarity”, DataApex Ltd.).

The HPLC run with a Supercosil™ LC-PAH and had a flow rate of 1.5ml per minute and an injection volume of 20 µL per sample. The mobile phase consisted of a water and acetonitrile ratio of 2:3. Ultimately, fluoranthene was detected under a wavelength of 480 nm for excitation and 560 nm for the emissions.

**Fluoranthene detection on the polyvinylchloride powder**

Firstly, we weighted the sample of PVC, added 6 ml hexane and shook it gently for one minute. After that, we transferred only the solution into a glass tube and evaporated the solvent by using a stream of nitrogen. The residue was than dissolved with 500 µL acetonitrile, from which 20 µL was injected in the HPLC. The amount of fluoranthene was calculated by using the standard curve. The factor was multiplied by 25, since 20 µL of 500 µL was injected in the HPLC.

**Fluoranthene detection in the tissue**

Both samples, polluted and non-polluted, were weighted, homogenized and 8 ml of acetonitrile mixed with the tissue under shaking it for one minute with the centrifuge. After that, a bond Elut QuEChERS AOAC salt package was added and shaked again for one minute before it was placed into the centrifuge at 4000 rpm for 5 min. Consequently, 6 mL of the solution was transferred to a Bond Elut QuEChERS Dispersive SPE 15 ml tube and after shaking centrifuged again as before. At last it was filtered through a 0.45 um PVDF syringe filter and 20 µl extract to a HPLC sample vial.
As before the amount of fluoranthene was calculated with the standard curve and that value was multiplied by 400 (20 µl of 8000 µl were injected) and divided by the sample weight, which results in the amount of fluoranthene per gram tissue.

2.6 Abundance of microplastics *in situ*

The design of the sediment sampling was established to get an idea about the abundance and origin of microplastics in coastal system in the south of Madeira, with the possibility to compare it to last years’ study. Therefore sediment samples were taken at high tide- and intertidal line at Praia Formosa (32°38'30.30"N, 16°57'19.05"W), one of the few sandy beaches. In a distance of around twenty meters three replicates were taken with a metal core (10 cm diameter, ~12 cm height). One replicate consisted of 5 cores, which were placed close to each other, depending on the ground. The cores were divided in upper (0 – 5 cm) and lower layer (5 – 10 cm) via a metal plate and placed into bags (see Figure 17).

![Figure 17: Microplastic sampling in the high tide zone and in the intertidal. In both zones three replicates (1*1 meter) consist of five cores. Source: Author](image)

To standardize the samples by weight they were dried and subsequently sieved over a 5 mm sieve to exclude bigger stones. The sand and microplastic was separated via density separation with a hypersaline solution, in which plastic and lighter particles are separated. Therefore we mixed 360 g NaCl with 1000 ml of tap water, which dissolved with constant mixing on a heated magnet stirrer. One sample was filled into a glass filled with approximately 1 L of hypersaline solution and properly stirred so that light plastic particles can swim on the surface. The surface water was filtered over a 500 µm sieve, while the hypersaline solution was recovered. This procedure of stirring and filtering was repeated twice to ensure all plastic particles were trapped. After washing the sample with tap water, the
particles were poured over a cotton wool filter to identify microplastic by color, shape and consistency. All identified particles were classified according to their shape (fragments, fibers, spheres, films).

### 2.7 Statistical Analysis

All graphs and statistical analysis were conducted with the statistic program R and the graphical package ggplot2 (Wickham, 2008). Respiration standardized by the basal diameter of the *Megabalanus azoricus* individuals. For respiration a repeated measure one-way analysis of variances (ANOVA) was conducted to see whether sample means differed. Firstly, I visualized the data with dotcharts to identify outliers and then by box-whisker-plots. Influential data points were identified by Cook’s distance and were excluded when Cook’s distance exceeded 1. The assumption of normality was verified with histograms and by Q-Q-Plots and was further statistically tested with the Shapiro-Wilk’s-W test. In case the data were not normally distributed, Kruskal-Wallis ANOVAs instead of a parametric ANOVA were used. The assumption of homogeneity of variances was graphically verified by a fitted vs. residual plot and statistically by the Fligner-Killeen test. In case the test was significant, the variances were considered inhomogeneous, thus the data were transformed and plotted again before the ANOVA was re-run. For violation of the assumption of homogenous variances after transformation, the Welch’s F correction for ANOVA was used. When ANOVA was significant (p ≤ 0.05), a post-hoc test (Tukey’s HSD) was carried out to determine which group differed from each other.

For analyzing mortality rates during the main feeding experiment and during the hypoxia stress test, the ‘survival’ package for R was used. Kaplan-Meier-curves were produced, which show the survival in percent of total individuals (y-axis) over time (x-axis). Each death event is presented by a vertical line that creates a typical step pattern. Statistically, mortality rates were compared between groups by using Cox regressions for proportional hazards. The median time to death is defined as the time, when 50% of individuals of one treatment died. For testing for a pollution effect the 3% and 3%* non-polluted group were compared with the Peto-Wilcoxon test. The motility was not statistically analyzed. Instead, movements were counted and assigned to different classes (see Cirral Activity). The frequency of barnacles’ activity in each class was then graphically displayed by using frequency distribution vertical-bar graphs. Also for fluoranthene analysis and the microplastic monitoring in situ no statistical analysis were conducted.
3 Results

3.1 Cirral Activity

Over all groups, testing and respiratory pumping were the most observed activity modes (43% and 49% mean frequency) in both measurement occasions (after 4th and 5th week). With 11% frequency, beating behavior was less observed among all treatment groups. A high percentage of barnacles were inactive in the moment of observation (47% in the fourth week and 39% in fifth week).

Individuals in the group with 0.3% microplastics were the most inactive at both times. At this level, the highest numbers of inactive animals were counted and beating behaviors were not observable. When including testing into a “non-active behavior category” the percent of barnacles from the 0.3% group in this category was 92% and 88% (fourth and fifth week), that are 17 and 45% (fourth and fifth week) more barnacles with non-active and testing behavior compare to the 0% group. Compared to the average of all treatment groups the proportion of non-active behavior was 33% and 37% (fourth and fifth week) lower than in the 0.3% group. Besides the 0.3% group, a slightly reduced activity with no beating and with 50% non-active barnacles was observed at the second measurement for the 3% group with pollutant. All other groups did not show any conclusive behavioral pattern with regard to changes in microplastic density (see Figure 18).
Figure 18: Frequency of different cirral behaviours during 1 min observation for barnacle individuals exposed to different microplastic densities at two measurement times (4. and 5. week). Abbreviated behaviours are testing, slow and fast pumping, extended behaviours are slow and fast beating. aPercentages of microplastic densities per L are based on percent weight of 80 g sediment. One group marked with an asterisk (*) was not contaminated with fluoranthene
3.2 Respiration

Oxygen consumption, measured in absence of microplastic, did not change between groups formerly exposed to different microplastic densities (H0₁) nor with the presence of fluoranthene (H0₂) (Table 6 and Figure 19). Of all measured individuals, 72% consumed between 0.5 to 1.5 mg O₂ L⁻¹ h⁻¹ /VI₀.⁷⁵ (standardized by volume index), while 20% of the measured animals had a respiration higher than 1.5 mg and 8% did not respire at all.

There was a significant interaction between microplastic density and time. A Tukey’s test revealed that respiration in the 0.3% treatment group significantly decreased in between the first measurement to the second and third measurement. Within 20 days, the mean respiration rate of the 0.3% group decreased by 67% from 2.1 ± 1.7 mg O₂ L⁻¹ h⁻¹ /VI₀.⁷⁵ to 0.7 ± 1.8 mg O₂ L⁻¹ h⁻¹ /VI₀.⁶⁵⁸ and stayed low until the end of the experiment (0.7 ± 0.9 mg O₂ L⁻¹ h⁻¹ /VI₀.⁷⁵).

Table 6: Comparison between respiration rates (mg O₂ L⁻¹ h⁻¹ / volume index0.658) among all groups (H0₁) and between the 3% polluted and the 3%* non-polluted (H0₂) group. Results from repeated measures ANOVA. Treatment and time represent fixed factors, number of individuals is the random factor. numDF = numerator degrees of freedom, denDF = denominator degrees of freedom

<table>
<thead>
<tr>
<th>Tested null-hypothesis</th>
<th>Factor tested</th>
<th>Num DF</th>
<th>denDF</th>
<th>F-Value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>H0₁: Physical effect of microplastic</td>
<td>treatment group</td>
<td>5</td>
<td>126</td>
<td>1.529</td>
<td>0.185</td>
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<td>9.321</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td>treatment group : time</td>
<td>10</td>
<td>126</td>
<td>1.642</td>
<td>0.102</td>
</tr>
<tr>
<td>H0₂: Pollution effect</td>
<td>treatment group</td>
<td>1</td>
<td>24</td>
<td>0.226</td>
<td>0.639</td>
</tr>
<tr>
<td></td>
<td>time</td>
<td>2</td>
<td>46</td>
<td>0.056</td>
<td>0.945</td>
</tr>
<tr>
<td></td>
<td>treatment group : time</td>
<td>2</td>
<td>46</td>
<td>0.093</td>
<td>0.912</td>
</tr>
</tbody>
</table>
Figure 19: Respiration rate as a function of time (start, middle and end) and microplastic density (%) for Megabalanus azoricus. Boxplots represent median respiration with interquartile range and whiskers. One group marked with an asterisk (*) was not contaminated with fluoranthene
3.3 Survival during microplastics exposure

Survival rates during the 42 days of exposure to microplastics did not differ between groups of barnacles exposed to different microplastic densities (Likelihood ratio test = 5.03, df = 5, p = 0.413) (Figure 20). 82% of the individuals survived the exposure period. With five dead individuals, the treatment group with the lowest microplastic abundance (0.003 % contaminated microplastic) showed the highest mortality. The second largest mortality (3 dead individuals) was found in the group that was not exposed to microplastics. All other groups had low mortality rates ranging between one to two individuals. Furthermore, there was no effect of the pollutant, because the 3% and 3%* treatment group did not significantly differ from each other (Log Rang-Test: \( \chi^2 = 0.2 \), df = 1, p = 0.665).

Figure 20: Proportion of surviving Megabalanus azoricus (thirteen replicates in each group) during 42 days of exposure to different densities of polluted microplastic particles. One group marked with an asterisk (*) was not contaminated with fluoranthene.
3.4 Survival during hypoxia

After being exposed for 42 days to different microplastic density treatments, the survival in subsequent hypoxia stress test (192 hours) did not deviate significantly from each other (Likelihood ratio test = 4.6, df = 5, p = 0.467). Median time to death was lowest in the 0.03% treatment group with 32 hours, while highest median time to death was found in the 3%* non-polluted group with 88 hours (Table 7). Even the group without microplastics had a relatively high median time to death of 48 hours (Figure 21).

![Figure 21: Proportion of surviving Megabalanus azoricus during 192 hours of hypoxia. Before hypoxia barnacles were exposed 42 days to different microplastic density treatments contaminated with fluoranthene. One group, marked with an asterisk (*), was not contaminated with fluoranthene. The number of replicates varied from 7 - 11 between treatment groups.](image-url)
Table 7: Median time to death (in hours) of Megabalanus azoricus during hypoxia stress (without presence of microplastics) test after six weeks of exposure to different microplastic densities. In all groups the microplastic entailed fluoranthene, except 3%* non polluted.

<table>
<thead>
<tr>
<th>Microplastic density group</th>
<th>Median time to death (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>48</td>
</tr>
<tr>
<td>0.003%</td>
<td>72</td>
</tr>
<tr>
<td>0.03%</td>
<td>56</td>
</tr>
<tr>
<td>0.3%</td>
<td>64</td>
</tr>
<tr>
<td>3%</td>
<td>56</td>
</tr>
<tr>
<td>3%*</td>
<td>88</td>
</tr>
</tbody>
</table>

The pair-wise comparison between the 3% polluted and 3%* were marginally insignificant (Log Rank-Test: $\chi^2 = 3.2$, df = 1, $p = 0.0754$) (Figure 20). Two individuals of the 3%* group without contamination survived 192 hours of hypoxia, whereas in the 3% group all individuals died after 168 hours (Figure 22).

![Figure 22: Proportion of surviving Megabalanus. azoricus during 192 hours of hypoxia stress test in the 3 % polluted and 3%* non-polluted groups(asterisk)](image)

### 3.5 Fluoranthene contents

The loading of microplastic particles with fluoranthene was successful and led to a mean abundance of 25 ng fluoranthene / g microplastics with a standard deviation of ± 10 ng/g. After incubation with fluoranthene, directly taken microplastic samples entailed the similar mean amounts of fluoranthene (24 ±12.7 ng/g) than cooled samples after 3 (24.4 ±4 ng/g) and
6 (25.5 ± 13.7ng/g) weeks of storage, suggesting that no desorption of fluoranthene happened during storage. However, the amount of fluoranthene found on the PVC particles ranged from 13 ng fluoranthene/g to 41 ng fluoranthene/g microplastic. The amount of fluoranthene in the water during loading was constantly hold at 50.000 ng in 2.5 L seawater, reflecting an average sorption of 25% of fluoranthene to 500g microplastic during three weeks of loading. Inconclusive is result of the fluoranthene analytic of the barnacle tissue. In the pooled tissue of barnacles from the polluted groups no fluoranthene was detected, whereas 33 ng of fluoranthene were detected in the pooled tissue of non-polluted barnacles (Table 8 and Table 9).

Table 8: Fluoranthene content (ng / g PVC) from microplastic samples directly taken after microplastic loading with fluoranthene (Start), after 3 (Middle) and 5 (End) weeks of storage in the fridge

<table>
<thead>
<tr>
<th>Sample</th>
<th>ng fluoranthene / g PVC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start 1</td>
<td>37.84</td>
</tr>
<tr>
<td>Start 2</td>
<td>12.91</td>
</tr>
<tr>
<td>Start 3</td>
<td>21.15</td>
</tr>
<tr>
<td>Middle 1</td>
<td>24.32</td>
</tr>
<tr>
<td>Middle 2</td>
<td>20.32</td>
</tr>
<tr>
<td>Middle 3</td>
<td>28.55</td>
</tr>
<tr>
<td>End 1</td>
<td>16.16</td>
</tr>
<tr>
<td>End 2</td>
<td>41.22</td>
</tr>
<tr>
<td>End 3</td>
<td>19.03</td>
</tr>
</tbody>
</table>

Table 9: Fluoranthene content of pooled tissue samples. Tissues from three organisms from each microplastic treatment group were pooled together in two groups: polluted (n = 12; 0.003, 0.03, 0.3, 3%) and nonpolluted (n=6; 0.3*% nonpolluted). The percent is calculated as mass fraction of microplastic in sediment.

<table>
<thead>
<tr>
<th>Sample</th>
<th>ng fluoranthene / g tissue (dw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue (pooled polluted)</td>
<td>0,0</td>
</tr>
<tr>
<td>Tissue (pooled nonpolluted)</td>
<td>33,2</td>
</tr>
</tbody>
</table>
3.6 Microplastic monitoring

The sediment investigation has shown a presence of microplastic in Praia Formosa, a beach of the southern coastline of Madeira. In 60 collected cores (mean weight of 534 ±121 g sediment), containing a total of 28.6 kg of sediment, 120 microplastic particles were found (average 4.1 particles/kg sediment). However, more than one third of the cores did not contain any microplastics (Figure 23). The mean microplastic density was 50% higher in the high tide zone (5.3 particles kg\(^{-1}\)) than in the intertidal zone (2.3 particles kg\(^{-1}\)). However, a general high variability between replicates indicated that microplastics were not evenly spread (Table 10). In the intertidal, 4 cores contained between 9 – 12 particles per kg \(^{-1}\) (extrapolated) making 75% of the total particles, whereas the rest had none or between one and two particles. Alike, in the high tide zone, 5 cores of 15 entailed 65% of the total number of particles. With regard to the question if microplastic density changes with sediment depth, no clear pattern could be recognized (Figure 24). By looking at the lower layers (from 5-10 cm) the box plot graphically indicates a similar median (2 particles) at the high tide line and intertidal, but again the high variability shows that the particles were unevenly spread.

Table 10: Number of plastic particles per replicate (means ± standard deviation) at the different sites (high tide line and intertidal) and at different sediment depths.

<table>
<thead>
<tr>
<th>Depth</th>
<th>High tide line</th>
<th>Intertidal</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-5 cm</td>
<td>5.21 ±3.71</td>
<td>2.25 ±4.08</td>
</tr>
<tr>
<td>5-10 cm</td>
<td>5.34 ±6.59</td>
<td>2.57 ±3.17</td>
</tr>
</tbody>
</table>
Most of the particles found were fibers (> 60%) followed by fragments (25%) (Figure 25 and Table 11). Fibers were mostly found in agglomerations of 4 to 9 particles, indicating that they come from the same source. Samples from the intertidal (upper and lower layer pooled) contained slightly more fragments than fibers. Pellets or spheres were not detected.

Table 11: Total number of particles found in the high tide and intertidal (upper- and lower layers pooled) according to categories of microplastic types

<table>
<thead>
<tr>
<th>Sampling zones</th>
<th>Fiber</th>
<th>Film</th>
<th>Fragment</th>
<th>Foamed</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>High tide line</td>
<td>60</td>
<td>5</td>
<td>12</td>
<td>0</td>
<td>77</td>
</tr>
<tr>
<td>Intertidal</td>
<td>16</td>
<td>7</td>
<td>18</td>
<td>2</td>
<td>43</td>
</tr>
</tbody>
</table>
4 Discussion

4.1 Summary
This study investigated the effects of contaminated microplastics in different densities on the Azorean barnacle *Megabalanus azoricus*, in a six weeks laboratory study. By simulating a tidal scenario we generated water current to resuspend different microplastic densities (0, 0.003, 0.03, 0.3, 3%, 3%*) twice a day and each resuspension lasted two hours. The densities were calculated in mass percentage of theoretical sediment and correspond to a density from 0.0024, 0.024, 0.24 and 2.4 g microplastic per liter. One group (3%*) was not contaminated with fluoranthene to allow identification of effects caused by microplastic and the pollutant.

The tissue analysis for fluoranthene was inconclusive. Surprisingly, in the tissue of barnacles that were not exposed to contaminated microplastics 33 ng fluoranthene was detected, whereas no fluoranthene was found in barnacles that were exposed to contaminated microplastic. A methodological error might have been the reason for this finding.

Microplastic ingestion could not be documented, due to the lack of equipment (to identify the fecal pellets), as well as difficulties to distinguish very small particles of microplastic (~ 1.5 µm) from the tissue. But the observed feeding activity suggests the uptake of microplastics by barnacles. Variables measured to identify physiological effects were respiration, cirral activity, and survival during exposure and under hypoxia stress.

Important findings from the microplastic exposure were:

- Reduced cirral activities when exposed to 0.3 % microplastic concentrations by counting frequencies of different behaviors (not statistically tested). Most barnacles in the 0.3% that treatment group displayed more inactive behavior during the observation compared to other treatment groups and a ‘no beating’ behavior was observed in two measurement occasions.

- Respiration rates did not significantly differ among barnacles exposed to different microplastic densities. A reduced oxygen uptake over the course was identified in barnacles exposed in 0.3% microplastic density treatment group.
• No statistical difference between experimental groups was observed for survival during microplastic exposure and under subsequent hypoxia stress

• No difference in effects between barnacles with regard to a potential pollutant effect could be observed.

The results show that there was no clear influence of microplastics on the physiological performance of *M. azoricus*. The decrease in cirral activity is the only signal of a different effect among the microplastic density groups, but statistical analysis was not conducted for this response. The findings of the different variables with regard to the following research questions will be discussed in this chapter:

1. How do different **microplastic densities** affect the Azorean barnacle *M. azoricus* under laboratory conditions in terms of a) cirral activity, b) respiration or c) survival under hypoxia?

2. Does **fluoranthene** contamination of microplastic play a role for the effect on the Azorean barnacle *M. azoricus*, and if so, how?

In order to answer this two questions, I will first describe how microplastic particles could have reduced the motility of *M. azoricus*, only for individuals from the medium microplastic density group (0.3%) and not at high levels of microplastic pollution (3% and 3%* unpolluted). And because a statistical significance was not found in other responses, I will further discuss the complexity of research on microplastic pollution and get across possible explanations why effects were absent or inconclusive in my study.

The supporting microplastic monitoring (*in situ*) provided information about the degree of microplastic pollution at Praia Formosa, a tourist beach on the south coast of Madeira. Low microplastic abundance of mean density of 3.8 particles per kg was found, which will be compared to reported microplastic pollution in sediments at different locations worldwide. Furthermore the finding of this microplastic monitoring will be put into the context of other studies and also discussed with regard to the applied methods.

**4.2 Effects on cirral activity**

The cirral activity of barnacles is a behavior that is directly connected to their physiology. The two main functions of cirral activity are feeding and respiration.
Thus, measuring cirral activity allows to link important physiological and behavioral responses (Nishizaki and Carrington, 2014a). Barnacles evolved two feeding modes that allow them to feed on a broad size range of particles. On the one hand a captorial feeding mode has been adopted to catch larger organisms (zooplankton). During the time when water is moving fast, which is the case during tidal change, barnacles increase their filtration rate and mainly feed on larger organisms, such as zooplankton (Anderson & Southward, 1987). When on the other hand water movement is low, they decrease their filter activity and primarily feed on smaller particles (Anderson & Southward, 1987). During this microfiltration they use their maxillipedes (smaller cirri) which contain fine setae to filter for small particles (Anderson and Southward, 1987). Their respiration is facilitated by a rhythmic pumping and beating of the cirri, which provides a flow of water in and out of the mantle cavity (Anderson & Southward, 1987; Anderson, 1994). Interestingly, there is some evidence that microfiltration is performed as a secondary component of the ventilatory rhythm (Anderson, 1980).

Variations in cirral activity with regard to their functions of feeding and respiration are closely linked to environmental factors (flow, temperature, pH, oxygen, presence of food etc.). With increasing temperature (up to 20°C) the rate of pumping increases due to increased ventilation (Anderson & Southward, 1987; Nishizaki & Carrington, 2014). Nishizaki & Carrington (2014) observed reduced feeding at high temperature (25°), because barnacles had an increased in abbreviated behavior (pumping with curled cirri), which results in a smaller capture efficiency than captorial beating with extended cirri. Furthermore, the proportion of activity increases up to 50% in flowing water compared to still water (Southward & Crisp, 1965). But it is limited at very high water velocities (>40 cm s⁻¹), where a switch to ‘testing’ behavior, occurs probably due to mechanical deformation of the cirri (Nishizaki and Carrington, 2014b). With regard to food-uptake intermediate water velocities (7.5 to 20 cm⁻¹) result in the highest feeding success, as both the food delivery and capture rate peaked, although capture efficiency (the ratio of food captured and food encountered) was the highest under slow flow (1 cm⁻¹) (Nishizaki and Carrington, 2014b). In the present study measurements of cirral behavior were conducted in clean environment without plastic particles and at high temperatures (25° - 28°). A tube provided moderate flow conditions and oxygenation. Under this regime we expected high cirral activity, mainly with abbreviated respiratory pumping behavior and less captorial beating.
Two measurements of the cirral activity have been conducted at the end of the experiment (fourth and fifth week). During both measurements, individuals from the 0.3 % group displayed a lower activity than barnacles from all other treatment groups (including barnacles in the 0% group). In this group the proportion of non-active barnacles was 67% (fourth week) and 80% (fifth week) and no barnacles displayed long periods of active movements (define as more than 15% pumping and beating behavior per min). The proportion of barnacles with non-active behavior of all other treatment groups (excluding 0.3% group) ranged between 18% - 50% over both measurements, with an average of 42% in the fourth week and 31% in the fifth week. Besides the 0.3% group, a slightly reduced activity with no beating and with 50% non-active barnacles was observed at the second measurement for the 3% group with pollutant.

The results presented support only to a small extent the hypothesis that microplastic densities affect cirral activity, as cirral activity was only reduced at a medium density treatment (0.3%) and not in both higher treatment classes (3% and 3%*nonpolluted). With regard to a pollutant effect, the results were not clear. Whilst the second measurement barnacles in the 3% group with pollutant showed a slight reduction in activity compared to the 3%* group without pollutant, one week before, the behavioral pattern of the 3% with fluoranthene and 3%* nonpolluted group was similar. The small reduction in the 3% variation might be due to natural variations in withdrawal times and activity. For example respiration occurs in activity rhythms, reflecting a phase of pumping or beating interrupted by inactivity (Anderson & Southward, 1987).

The drop in activity in animals of the 0.3% group is difficult to explain and certainly needs further investigation. A decrease in the cirral activity with increasing microplastic densities was expected as a consequence of depleted energy reserves; because of the presence of microplastics feeding and respiratory pumping was impaired or not possible.

Another theory is less nutritious particles were available for the animal, because of the high uptake of microplastic. This could have led to depleted energy reserves due to starvation and as a consequence resulted in reduced cirral activity. However, these assumptions do not explain, why cirral activity was not reduced in barnacles exposed to higher microplastic densities (3% and 3%* nonpolluted).
A possible explanation for the drop in the 0.3% group could be a shift in reaction to microplastics at this level compared to lower and higher densities. Barnacles exposed to lower treatments (0.003 and 0.3%) were probably not affected by the microplastic, compared to the 0.3% group. But high particle densities in the water column (3%) may have triggered protective withdrawal, which is associated with prolonged withdrawal and tight closing of opercular plates (Palmer, Szymanska, & Thomas, 1982). If barnacles from the 3% and 3%* treatment groups were able to protect themselves during resuspension events, this might explain why cirral activity were similar compared to the treatments with zero or low plastic treatment classes (0.003 and 0.03%). Palmers et al. (1982) observed a protective withdrawal of the barnacle species Balanus glandula caused by physical stimuli or other organisms, with longer closure when formerly in physical contact with predators compared to former contact non-predators (such as algae) (Palmer et al., 1982). They consider that chemicals might be responsible for detecting the kind of stimulus (e.g. predators) after the initial physical contact (Palmer et al., 1982). In their experiment, the longest observed withdrawal time was more than 45 minutes. This was the case when barnacles were in contact with a starfish (predator). Three tube feet of the starfish were still attached (chemical stimulus) and barnacles resumed rhythmic cirral beating only after they have been removed (Palmer et al., 1982). In a similar manner the presence of high microplastic densities could have been cause long closure as a result to permanent physical contact with particles during resuspension.

Wegner et al. (2012) exposed a mussel (Mytilus edulis) to comparable microplastic concentrations (0.1 – 0.3 g/L) of nanoparticles (polystyrene, 30 nm). The mussels closed their valves within 20 minutes of exposure as it was probably able to detect the microplastics in the water (Wegner et al., 2012). This could have happened in a similar manner with the barnacles under high microplastic exposure. The theory of protective withdrawal under high plastic densities would need further proof, especially because in our experiment the water of the 3% treatment group was very “milky” and therefore animals not visible.

The resuspension of microplastics required considerable flow conditions which may have also supported cirral activity in the beginning of the experiment in the lower treatment groups. Initially, barnacles of the 0.3% (0.24g/L) treatment group might have kept their normal feeding activity. However, due to the presence of microplastics their feeding and respiratory pumping might have been impaired with time. Up until the first measurement (fourth week) microplastics may have clogged their operculum valves or impaired the food uptake, as a result of the high amount of innutritious particles caught in their cirral net. Reduced feeding or feeding of innutritious particles could have depleted their energy reserves,
which was then seen in reduced activity in clean environments in the fourth and fifth week of exposure. Although the presence of microplastics in the stomach or tissue of barnacles could not be tested, it is assumed that *M. azoricus* ingested microplastics due to observed feeding behavior and reported literature. Barnacles are relatively unselective omnivores, able to ingest particles in a size range of 2 µm up to 1 mm (A. J. Southward, 1955). Goldstein even found microplastic particles with a mean diameter of 1.4 mm in 33% of goose barnacles (*Lepas anatifera*) collected in the North Pacific (Goldstein & Goodwin, 2013). With a mean size of 1.5 µm most particles were therefore probably ingested via microfiltration during cirral pumping, whereas for larger particles a captorial feeding strategy was used. Even if not ingested, barnacles may have suffered from the intense particle contact and thus lost energy, which was resulting in reduced activity.

Reduced respiratory behaviors might be the cause as well as the consequence of a reduced energy budget. Because barnacles were exhausted, they ventilated less (consequence) or barnacles respired less, because of the clogging of the operculum valve (cause). Due to limited preparation time, we were not able to measure cirral activity in the beginning. Therefore we cannot exclude that this group had already initially reduced cirral behaviors. But when comparing cirral activity to the results of the respiration rates, a significant decrease has been observed in respiration rates over time in the 0.3% group but not among all treatment groups. This may indicate that barnacles’ from the 0.3% group were initially not different to all other treatment groups (see Effects on respiration).

To further understand this observed pattern future research is needed that incorporate more than two measurements and certainly one measurement at the start of the exposure experiment. Further, barnacles should be pre-tested in their activity to exclude barnacles less active prior to exposure. Verification by statistical tests is highly recommended to support such observation.

### 4.3 Effects on respiration

Respiration is necessary for the aerobic metabolism, which reflects an organism’s ability to convert organic compounds into energy to fuel important life functions, such as growth and reproduction (Hochachka and Somero, 2002). As with most filter-feeding animals, respiratory and feeding mechanisms are intertwined in barnacles. It can be assumed that barnacles can also take up microplastics while respiratory pumping, because small particles could be caught in the fine setae of the maxillipedes with the inflow of water into the mantle cavity. Microplastics could therefore have limited the uptake of oxygen by clogging the operculum
valve which is responsible for pumping water into the mantle cavity. Further, respiration could have been limited by microplastic induced protective withdrawal, as has been assumed for cirral activity under which oxygen uptake would be limited (see also Effects on cirral activity).

In this study, respiration rates were measured three times (beginning, middle and end of the experiment) during the six weeks’ time of exposure to microplastics. No significant effect of microplastic was measured for respiration. Further, a pollutant effect could not be detected. However, a significant effect over time was observed in the 0.3% group by applying a Tukey’s test (p <0.001). Compared to initial oxygen uptake, a 67% decrease of the mean respiration was measured after 20 days of exposure.

The results of respiration indicates that barnacles from the 0.3% group were not different compared to other treatment groups from the very beginning. This could not be tested for cirral activity due to a lack of preparation time (see effect cirral activity). A reduced cirral activity has been observed in the same group (0.3%) over the same period of time (between third and fifth weeks of exposure). Both measurements might be related, as decreased respiration is displayed by a decrease of pumping behavior (Southward and Crisp, 1965).

However, a contrasting observation was published by Davenport, who observed high variations of oxygen concentrations in the mantle fluid, which were not coupled to the behavior of the barnacles (Davenport and Irwin, 2003). The conditions in the mantle fluid were hypoxic, even when barnacles pumped aerated seawater into the mantle cavity (Davenport and Irwin, 2003). Under moving water the oxygen concentration in the mantle fluid was higher and more stable (Davenport and Irwin, 2003).

The respiration of barnacles has been studied by Nishizaki and Carrington (2014) in order to determine the metabolic sensitivity under two important environment factors: flow and temperature. They revealed that respiration rates increased with increasing temperature and flow, while flow had much less influence on respiration at low temperatures (5 - 15°C) compared to high temperature (20-25° C) (Nishizaki and Carrington 2014). Their work illustrates the limitations of conclusions drawn by single factor designs.

Microplastics could be another factor, adding to barnacle’s sensitivity to different oxygen/flow regimes. So far, there is no study that investigated the effects of microplastics on respiration. Watts et al. (2014) confirmed that shore crabs (Carcinus maenas) are able to take
up microspheres via ventilation across the gill and retain it in their body tissue for up to 21 days. Unlike any other crustacean taxon, barnacles have a closed hemocoelic system. Haemolymph is pumped under regular contraction of the rostral sinus and somatic muscles through connective tissue forming walls of complex circulatory pathways (Anderson and Southward, 1987). Furthermore, Cirripedia show higher haemolymph pressures than other crustaceans (Anderson & Southward, 1987). Up to now, the processes that control barnacle respiration and behavior are not understood and this makes the interpretation of the effects of microplastics on this response variable challenging. The experimental duration of six weeks might have been too short to detect an effect in respiration. Furthermore, the replication of ten to thirteen individuals may have been too low to detect a significant effect and the effect might therefore have been overlooked.

4.4 Effects on survival during hypoxia

The Azorean barnacle *Megabalanus azoricus* inhabits a narrow subtidal area about one meter to five meter below the waterline (Wirtz et al., 2006). It has adopted to a very dynamic habitat with wave action and can sustain periods of aerial exposure during tidal events (Stephenson and Stephenson, 1972). Thus, they evolved adaptations to environmental stressors, such as temporal hypoxia, and can tolerate temporal aerial exposure (Barnes, Finlayson & Piatigorsky, 1963). Under oxidative stress, barnacles reduce their metabolic rate which can be seen as reduced oxygen consumption (Southward and Crisp, 1965). Furthermore, they develop antioxidant enzymes as a defence strategy against toxic ‘reactive oxygen species’ (ROS), which become abundant through the biotransformation of oxygen (Desai and Prakash, 2009). However, regulation of the metabolic rate and adaptation via increasing levels of antioxidants is species dependent as well as dependent on the water depth in which individuals have settled. Therefore, barnacles found in high tide levels show increased levels of defensive enzymes (Desai and Prakash, 2009). Another mechanism of barnacles to deal with aerial exposure is the uptake of air bubbles in their mantle cavity (Davenport and Irwin, 2003). During aerial exposure some barnacles use up these air bubbles within two to three hours, others refresh their air bubble repeatedly by pneumostome formation (breathing pore) (Davenport and Irwin, 2003).

Furthermore, hypoxia events occur in the subtidal, for longer periods (weeks to months). For example when oxygen concentration become depleted due to high primary production, which in turn can lead to eutrophication during the summer (Conley et al., 2009b). These events are
likely to increase with increasing sea water temperature and nutrient input from land (i.e. from agriculture) as lower amount of oxygen can be dissolved (Conley et al., 2009a). Such seasonally persistent hypoxia especially impacts immobile species, such as barnacles.

In this experiment such a severe hypoxia was simulated, by exposing of *M. azoricus* to a long-time low oxygen environment (without microplastics) after the six weeks microplastics-exposure phase. Especially barnacles, which previously were exposed to higher levels of microplastics, were expected to suffer more under hypoxia conditions and will display higher or faster mortality. However, the different treatment groups did not show significant differences in response to hypoxia stress. Instead, the oxygen level of 0.75 mg/L resulted in a 50% mortality within three to four days in all treatment groups, including even the group without microplastics. It is possible that all barnacles experienced a general decrease in their physiological performance during six weeks under laboratory conditions and therefore were generally too weak to sustain such strong conditions.

Hypoxia may affect barnacles indirectly, in a decrease in reproductive output and physiological mechanism as described above (Loddington, 2011). As the hypoxia was lethal, long-term effects after the recovery from hypoxia could not be observed. In Madeira’s open-ocean location and low nutritious waters, it is a more likely scenario that *M. azoricus* experience short-term aerial exposure during tidal events, in which microplastics may potentially play a role as an additional stressor over the lifespan of barnacles (species-dependent between one to more than ten years (Newman and Abbott, 1980)).

### 4.5 Complexity of microplastic research

Complex biological systems challenge empirical science in which scientists that are trying to detect a “signal” in a world of influential factors called “noise”. Many parts in an intricate arrangement that lead to inexplicable or emergent behaviors are typical for complex systems (Ideker et al., 2012). Researching effects of microplastic adds to the complexity in which nature unfolds. Laboratory studies can only serve as one piece of a complex puzzle. The fact that this study showed only a small effect does not mean microplastics do not have an effect on *Megabalanus azoricus* or other benthic invertebrates in general. The following figures reflect three important aspects (pollutant, plastic and organism) in which different choices could have resulted in another outcome of results (see Figure 26). Following chapter want to show the variability of these factors and put my outcomes in context with findings of studies.
on other marine invertebrates, conducted parallel in other parts of the world in the framework of the GAME XII project.

**Pollutant choice**

Fluoranthene, among the most ubiquitous and abundant pyrogenic PAHs, was used as a model PAH in this study. No fluoranthene has been detected in the tissue of the barnacles formerly exposed to contaminated microplastics. Inexplicably, 33 ng of fluoranthene were detected in the sample of pooled tissue from non-polluted barnacles. As we did not have much tissue to examine and therefore needed to pool all material into two groups (polluted and non-polluted, each n=1) the low sample size cannot deliver evidence how pollution might have been adsorbed among different treatment groups. The uptake of contaminated plastics may have been too low to detect fluoranthene or pollutants did not desorb from the plastic material during the gut passage. However, both options cannot explain why pollutants were found in the tissue of the non-exposed barnacles. It seems most likely that there was a methodological problem that happened during the analysis, for example that the sample has been mixed up. Thus, I will not trust the outcome of fluoranthene analysis of the tissue.

In general bioavailability of fluoranthene to *M. azoricus* depend on three factors:

- the amount of fluoranthene that loaded on the plastic;
- the amount of desorption from the plastic into and out of the experimental unit;
- and desorption of fluoranthene from the plastic in the digestive tract.

A high sorption on the plastic during loading phase and during exposure in the experiment and a high desorption rate inside the digestive tract would lead to a high bioavailability of the contaminant to the animal.
Sorption of pollutants to microplastic

The adherence of a contaminant to microplastics can be expressed by the sorption capacity, which, in turn, depends on the polymer and pollutant type (e.g. Lee, Shim, & Kwon, 2013; Mato et al., 2001; Teuten et al., 2009). Sorption capacities are calculated with partitioning coefficients, which is a ration of concentrations of a chemical compound in two phases, normally an aqueous and a hydrophobic phase such as 1-octanol ($K_{ow}$) (Lee et al., 2013). Thus, it can be defined as a measure of how hydrophilic (“waterloving”) and hydrophobic (“water fearing”) a chemical substance behaves. To calculate partition coefficients for plastic debris, a ratio is established between plastic (“hydrophobic solvent”) and seawater. By comparing two plastic materials and one pollutant, a higher partition coefficient is reflecting a higher sorption capacity of this plastic material. Plastic-seawater can be as high as 1-octanol–water partition coefficients, which means microplastics have a high sorption capacity (Lee et al., 2013). If a contaminant is very hydrophobic ($\log K_{ow} > 5$) (Rochman et al., 2013a) no concentration equilibrium will be achieved, as the plastic can take up all the contaminant from its surrounding phase according up to its loading capacity (Bakir et al., 2012; Müller et al., 2001). Furthermore, the sorption potential for a pollutant seems to be related to its molecular weight (Müller et al., 2001; Teuten et al., 2009) (Table 12). Pollutants with high molecular weight need more time to reach equilibrium with the plastic, but will be adsorbed more than pollutants with low molecular weight (Müller et al., 2001; Rochman et al., 2013a).

Table 12: Molecular weight (MW) and octanol-water (KOW) partition coefficients for several PAHs (adapted from Douben, 2003).

<table>
<thead>
<tr>
<th>PAH</th>
<th>MW</th>
<th>Log $K_{ow}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorene</td>
<td>166</td>
<td>4.21</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>178</td>
<td>4.57</td>
</tr>
<tr>
<td>Anthracene</td>
<td>178</td>
<td>4.53</td>
</tr>
<tr>
<td>Pyren</td>
<td>202</td>
<td>4.92</td>
</tr>
<tr>
<td><strong>Fluoranthene</strong></td>
<td><strong>202</strong></td>
<td><strong>5.08</strong></td>
</tr>
<tr>
<td>Chrysen</td>
<td>228</td>
<td>5.71</td>
</tr>
<tr>
<td>Benzo[a]anthracene</td>
<td>228</td>
<td>5.67</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>252</td>
<td>6.11</td>
</tr>
<tr>
<td>Dibenzo[a,h]anthracene</td>
<td>278</td>
<td>6.71</td>
</tr>
</tbody>
</table>

Furthermore, the sorption depends on the type of polymers, due to differences in the surface area (Müller et al., 2001) and diffusion properties (Karapanagioti and Klontza, 2008; Müller et al., 2001; Rochman et al., 2013a). Polychlorinated biphenyls (PCBs) and polycyclic
aromatic hydrocarbons (PAHs) reach equilibrium faster on PET and PVC than on HDPE, LDPE and PP, but higher concentrations were found on the latter (Rochman et al., 2013a) (see next page (table 13).

The sorption kinetics vary largely between conducted studies. Mato et al. (2001) estimated a slow sorption of several years for PCBs and DDE from seawater to PP pellets. Contrasting to the relative long sorption times under field conditions (Mato et al., 2001; Rochman et al., 2013b). Much faster sorption equilibrium times, between 24 and 48 hours, have been observed in laboratory experiments (Bakir et al., 2014a; Teuten et al., 2007). Studies investigating the same pollutant-polymer combination found big differences in sorption mechanics due to particle size (Endo et al., 2013). This difference is explained by the higher surface-to-volume ratios of passive sampling devices used in the field, compare to plastic pellets in laboratory experiments. On PE with a size of 200 -250 \( \mu m \) sorption times amount to 24 hours with phenanthrene, whereas a particle size of 1000-5000 \( \mu m \) took 105 days to reach equilibrium (Bakir et al., 2014b; Karapanagioti et al., 2010).

Furthermore, sorption in nature is lower, because plastic formerly exposed to pollutant have lower sorption when under clean environment and faster desorption due to organic particles in the water, compared to lower observed desorption in laboratory experiments that use organic-free water (Endo et al., 2013).
Table 13: Predicted equilibrium concentrations of fluoranthene, PAHs and PCBs (ng/g of pellets) for different polymer types based on an equilibrium model with exponential rise. The concentration was calculated according to from pellets exposed over one year in San Diego Bay, CA (adapted from Rochman et al., 2013).

<table>
<thead>
<tr>
<th>Polymer type</th>
<th>Pollutant (ng/g of pellets)</th>
<th>Fluoranthene</th>
<th>Total PAHs</th>
<th>Total PCBs</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVC</td>
<td></td>
<td>3.6</td>
<td>23</td>
<td>2.4(^a)</td>
</tr>
<tr>
<td>PET</td>
<td></td>
<td>~ 2(^c)</td>
<td>13</td>
<td>1.4(^b)</td>
</tr>
<tr>
<td>PP</td>
<td></td>
<td>~25(^c)</td>
<td>121(^b)</td>
<td>29(^a)</td>
</tr>
<tr>
<td>HDPE</td>
<td></td>
<td>280</td>
<td>756(^a)</td>
<td>30(^a)</td>
</tr>
<tr>
<td>LDPE</td>
<td></td>
<td>316</td>
<td>753(^b)</td>
<td>36(^a)</td>
</tr>
</tbody>
</table>

\(^a\) loading time more than six months  
\(^b\) loading time between three and six months  
\(^c\) model could no fit to the data

We have chosen fluoranthene as it possesses a relatively high potential to load onto microplastics and is not too toxic to handle under laboratory conditions. Furthermore, we selected PVC, because of its density and particle size range (see plastic material). After three weeks of loading this plastic with fluoranthene (20µg/L), mean concentration of fluoranthene on the plastic was 25 ±10 ng/g. This concentration is a seven times higher concentration than estimated by Rochman et al. (2013) (see Table 13).

Furthermore, sorption in nature is lower, because plastic formerly exposed to pollutant have lower sorption when under clean environment and faster desorption due to organic particles in the water, compared to lower observed desorption in laboratory experiments that use organic-free water (Endo et al., 2013). Presumably because the size of our plastic particles were much smaller (mean 1.5 µm) than pellets used by Rochman et al. (2013a) and therefore had a higher surface area per weight (Rochman et al., 2013a). However, compared to other polymer types such as HDPE or LDPE, PVC does not load high quantities of PAHs (Rochman et al., 2013a).

Color and state of plastic may also play a role, as high concentrations of pollutants show, which were found on black (319 ng/g PCB) and on aged pellets (285 ng/g PAHs) both composed of PS and PP (Frias, Sobral, & Ferreira, 2010). Concentrations of fluoranthene found on these pellets ranged between 18 ng/g – 118.6 ng/g, with lowest concentrations on white and highest on black and aged pellets (Frias et al., 2010). However, an explanation of this observation could not be established so far.
Therefore, in order to study the relevance of microplastics as a potential vector for organic pollutants, in the future not only the combination of plastic and pollutant should be considered, but also the size and color of the plastic particles.

**Desorption in the experimental unit**

The desorption of chemicals from plastics has been studied far less than sorption (Endo et al., 2013). Understanding leaching of chemicals from microplastics is relevant to evaluate the transport of them into sediments, back to the water column or to organisms (see next chapter). Endo et al. (2013) indicated desorption half times from 14 days to 210 years for PCB congeners on PE, depending highly on the PE-water partitioning coefficients of the pollutants (Endo et al., 2013). They argue that the half-times of desorption in their model are identical with the time to reach 50% sorption rates (Endo et al., 2013). In this experiment, fluoranthene might have desorbed from the plastic to the sea water, but also onto the surface of the plastic bottles (PET) or to the resuspension tube (probably PE). The sea water in the test unit was not contaminated with fluoranthene and we can expect that a new equilibrium between pollutants on plastics and the seawater was reached.

**Desorption in the digestive tract**

Contaminants become only relevant for marine organisms when they desorb in the gut system (Bakir et al., 2014a). Bakir et al. (2014a) investigated sorption and desorption rates of Phe and DDT to PVC and PE under different pH and temperature values (Bakir et al., 2014a). Desorption rates were enhanced under conditions similar to warm blooded organisms (such as sea birds) and pollutants generally desorb faster from PE in the presence of gut surfactants than from PVC. Barnacles, as all crustaceans, are cold-blooded animals, thus desorption of pollutants during the passage through the digestive tract may not have been high enough to affect these animals. However, the effect of the pollutant could have been visible in respiration rates, which is an important physiological variable to assess toxicological effects (Lee and Lin, 2013). Responses to chemicals can be either an increase and/or decrease of ventilation of aquatic animals (Widdows and Donkin, 1991). Elevated oxygen uptake indicates enhanced energy costs for example through active detoxification (Calow, 1991), whereas a reduction in oxygen uptake may signal an intoxication of the pollutant to the animal. Such an effect was not visible in the present study. The respiration rates did not differ among treatment groups only over time, suggesting that the achieved pollutant concentration of 25 ng per gram plastic material in the experiment were either too low or not bioavailable for barnacles.
4.5.2 Plastic material

The plastic type, shape and size play a role on the effect on the study organism. Additionally, these aspects are important for possible interactions between pollutants and microplastics (see Pollutant choice).

Polymer Type

The plastic type determines the bioavailability in the water column or in the sediment, as its density rules whether it will sink or float. Most plastics are positively buoyant, such as polyethylene (0.96 g/cm³) and polypropylene (0.91 g/cm³), and consequently more likely to be ingested by filter and suspension feeders that inhabit the water column. Fouling processes increase the weight of plastics and result in sinking of formerly positively buoyant plastic types. Polyvinylchloride powder that was used in this feeding experiment had a density of 1.44 g/cm³, which is heavier than seawater (1.02 – 1.03 g/cm³). Thus, it became only available for the organism during resuspension events and sinks faster than other plastics.

Shape

Potential adverse effects may depend also on the shape of plastic material (Wright et al., 2013b). Rod-shaped mesoporous silica displayed increased cellular uptake and reduced cell viability compared to spheres and short rod-shaped nanoparticles. The greater contact area might be in favor to cellular uptake. As fibers are the most commonly encountered form of microplastics in the marine environment, potential physical toxicity needs to be considered. The irregular form of the PVC particles that we used in our experiment may have had adverse effects. They agglomerated in the water and may have caused clogging of the operculum valves. Although I observed coverage with microplastic particles in high volumes, a clogging by the material could not be determined. However, effects such as entanglement caused by fibers or injuries by fragments, that may have sharp edges, could not be examined in this study.

Particle size

The smaller microplastic particles are, the wider the array of species that can ingest them. Particles used in this experiment were in a size range between 1.3µm and 50µm and therefore likely to be ingested by *M. azoricus*. Particle size also plays a role when particles are translocating from the gut system into the circulatory system or into cells. In the tissue of the blue mussel (*Mytilus edulis*) 60% more microspheres have been found of a size of 3µm size than of 9.6 µm (Browne et al., 2008). Microplastics found in the stomach and intestines in
collected goose barnacles (*Lepas anatifera*), did not cause blockage and were in the size to pass the anus (Goldstein and Goodwin, 2013). As Goldstein & Goodwin (2013) only used visual identification, it is likely that smaller particles were not detected. In the scope of our experiment, neither we could investigate translocation mechanism, nor determine with microscope whether microplastics were ingested or not, due to the small size and white color of the microplastic which was similar to the tissue. Future studies should investigate the effects with bigger particle sizes as well as different colors or fluorescent microplastics.

**4.5.3 Suitability of Megabalanus azoricus as a test organism**

I experienced strengths and weaknesses of using *M. azoricus* as a study organism for this type of experiments. Important factors were adaptability to laboratory conditions and susceptibility to ingest microplastics, as well as the possibility to measure various responses under technical and financial limitations. Compared to the first attempt with goose barnacles (*Lepas anatifera*) that showed high mortality (see chapter species screening and sampling), *M. azoricus* was able to adapt to laboratory conditions. Barnacles are among the most frequent inhabitants of the rocky intertidal and can adapt to extreme environmental conditions.

As the color of the tissue was in the same color as the microplastic, it was not possible to distinguish between them to proof ingestion of microplastics. The experiments conducted within GAME XII have to be designed to realize them under with simple equipment. *M. azoricus* might have been too small for proving microplastic ingestion, for which spectroscopic methods might have been required. Additionally, another microplastic material, with different color or bigger size could be feasible for future experiments with microplastic ingestion and limited technical equipment. Nevertheless, we could observe cirral feeding movement during resuspension events.

We only studied the sessile adult form of barnacles, whereas in nature microplastic pollution might impact barnacles already in their larval stage. This has been observed by Kaposi et al. (2014) who showed that a larva of a sea urchin (*Tripneustes gratilla*) was affected by microplastics in realistic concentration and able to egest it (Kaposi et al., 2014). In contrast, to the other studies of the GAME project that work with mussels as filter feeder, which is a well investigated species under experimental conditions, very limited research is available on barnacles within long-term laboratory settings. Research on barnacles under laboratory conditions are limited compared to mussels, which were investigated in parallel conducted experiments of other GAME projects. The biology of *Megabalanus azoricus* has hardly been
studied (Dionisio et al., 2007). Most laboratory studies on barnacles measured cirral activity and respiration as responses, both very dependent on temperature and flow. Under our laboratory setting slight variations in the temperature could not be avoided over the course of six weeks. Furthermore, there was limited control of the flow that was needed to resuspend the microplastics. Therefore, the response variables might have been not optimal to measure effects of microplastics.

Within the framework of GAME a total of 14 deposit- and filter feeder were tested by participating teams under similar conditions. A comparison between the results of all species show, that species-microplastic interaction can be very different (overview next page Table 14) Some effects can trace back to the microplastic and others to the pollutant fluoranthene. In total a significant effect was found for nine species. Generally, the presence of microplastics led to decreased food-uptake, decreased motility and even to increased mortality. Exhaustion of energy reserves or limitation of food uptake (energy) due to the presences of microplastic particles are possible explanations. For the green mussel (*Perna viridis*) the effect of microplastics was the clearest, with negative effects on survival, filtration, byssus production and motility (Rist, 2015). However, often effects were absent. Different environmental conditions at the stations could have also influence the results. For example, laboratory conditions with high temperatures, long transport to the laboratory and high background pollutions in the habitat of green mussels might have weakened *Perna viridis* before the experiment (Rist, 2015). The microplastics may have act as an additional stressor to the mussels. In nature, synergetic effects of ocean acidification, eutrophication, temperature increase, hypoxia, pollution and others are likely to happen (Crain et al., 2008). Therefore, it is likely that plastics are a component of multiple stressors on marine systems, and effects alone are not as drastic as cumulative effects (e.g. Adams, 2005; Crain et al., 2008; O’Gorman et al., 2012; Ormerod et al., 2010; Tockner et al., 2010).
Table 14: Overview of all significant effects of microplastic and fluoranthene on investigated species at the GAME XII (2014) stations including the present study

<table>
<thead>
<tr>
<th>Country</th>
<th>Species</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brazil</td>
<td>Fiddler crab (<em>Uca rapax</em>)</td>
<td>Increasing mortality with microplastic + fluoranthene; Decreasing motility with microplastic</td>
</tr>
<tr>
<td></td>
<td>Brown mussel (<em>Perna Perna</em>)</td>
<td>No effects</td>
</tr>
<tr>
<td></td>
<td>Spoon worm (<em>Ochetostoma baronii</em>)</td>
<td>No effects</td>
</tr>
<tr>
<td></td>
<td>Chilean mussel 'chorito' (<em>Peromytilus purpuratus</em>)</td>
<td>No effects</td>
</tr>
<tr>
<td>Chile</td>
<td>Lugworm (<em>Arenicola marina</em>)</td>
<td>Decreasing hypoxia tolerance with medium microplastic density; Decreasing faeces production with MP + fluoranthene</td>
</tr>
<tr>
<td></td>
<td>Blue mussel (<em>Mytilus edulis</em>)</td>
<td>No effects</td>
</tr>
<tr>
<td>Wales</td>
<td>Black sea cucumber (<em>Holothuria leucospilot</em>)</td>
<td>Decreasing faeces production with microplastic;</td>
</tr>
<tr>
<td></td>
<td>Green mussel (<em>Perna viridis</em>)</td>
<td>Higher mortality with microplastic; Decreasing filtration with microplastic; Decreasing byssus production with MP; Decreasing motility with microplastic</td>
</tr>
<tr>
<td>Indonesia</td>
<td>Pacific lugworm (<em>Abarenicola pacific</em>)</td>
<td>Decreasing feeding with microplastic; Decreasing burrowing speed with microplastic</td>
</tr>
<tr>
<td></td>
<td>Bay mussel (<em>Mytilus trossulus</em>)</td>
<td>Higher mortality with microplastic + fluoranthene Decreasing byssus production with microplastic</td>
</tr>
<tr>
<td>Mexico</td>
<td>Spaghetti worm (<em>Eupolymnia rullieri</em>)</td>
<td>No effects</td>
</tr>
<tr>
<td></td>
<td>Lister's tree oyster (<em>Isognomon radiatu</em>)</td>
<td>Increasing respiration with microplastic + fluoranthene</td>
</tr>
<tr>
<td>Madeira</td>
<td>Variable seacucumber (<em>Holothuria sanctori</em>)</td>
<td>Increasing respiration with microplastic + fluoranthene</td>
</tr>
<tr>
<td></td>
<td>Azorean barnacle (<em>Megabalanus azoricus</em>)</td>
<td>Decreasing cirral activity with microplastic + fluoranthene</td>
</tr>
</tbody>
</table>
4.6 Abundance of microplastics in situ

Monitoring microplastics is crucial for understanding the scope of the microplastic problem as well as the temporal and spatial variability in the abundance of microplastics in order to predict future trends and plan proper management actions.

In sediment samples collected the beach Praia Formosa, 62% entailed microplastics with a mean abundance of 3.8 particles per kg dry sediment. In the high tide line, higher concentrations were found (5.28 ± 5.15 particles/kg dw) than in the intertidal (2.41 ± 3.63 particles/kg dw). By looking at different depth horizons (0-5 cm and 5-10 cm) no pattern of contrasting microplastic abundances was seen.

These results suggest that microplastic concentrations are rather low than those found in similar studies: Mean microplastic concentrations found in marine sediments typically range from 4 - 400 particles per kilogram sediment (Claessens et al. 2011; Mathalon and Hill 2014a; Thompson et al. 2004) (see next page an overview of recent paper on microplastics in marine sediments, Table 15). Similar microplastic concentrations than in Praia Formosa have been reported for a beach in Norderney Island in Germany (4 particles kg⁻¹) (Fries et al., 2013). In harbor sediments in Belgian the sample with the highest concentration entailed 390 particles kg⁻¹ dry sediment (Claessens et al., 2011). Mathalon and Hill (2014b) found between 20 and 80 fibers in 10g sediment in Nova Scotia. In the same year a study reported a very high concentration of 1.4 x 10⁵ microbeads per m² at one site of the St. Lawrence River sediments (Castañeda et al. 2014).

One possible explanation for the low abundance in Madeira could be that the local hydrodynamics have an effect on microplastic accumulation on beaches. Vianello et al. (2013) reported that lower concentrations of microplastics were found in exposed beaches reported than sheltered ones. While low energy environments induce high deposition rates (Mathalon & Hill, 2014b; Vianello et al., 2013), microplastic particles in exposed locations, such as Praia Formosa, are likely to be washed out with the tides. The microplastic abundance varies also within tidal zones of exposed beaches. Liebezeit and Dubaish (2012) found highest concentrations of microplastics at the high tide line of exposed beaches. Their findings correspond to microplastic concentrations at Praia Formosa which had higher concentrations at the high tide line compared to samples from the intertidal. While the intertidal is located in the surf wave zone, the high tide line experiences less wave energy and might therefore be prone to higher levels of microplastics.
Table 15: Summary of recently reported microplastic concentrations in coastal sediments. Values are given in original units, but some were extrapolated on kg or L dry weight of sediment.

<table>
<thead>
<tr>
<th>Location</th>
<th>Sediment Origin</th>
<th>MP size (in µm)</th>
<th>Microplastic concentration</th>
<th>Unit</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nova Scotia</td>
<td>Intertidal</td>
<td>&gt;50</td>
<td>~200-800</td>
<td>fibers kg⁻¹ᵃ</td>
<td>Mathalon &amp; Hill, 2014</td>
</tr>
<tr>
<td>Slovenia</td>
<td>Beach</td>
<td>250 - 5000</td>
<td>156</td>
<td>particles kg⁻¹</td>
<td>Laglbauer et al., 2014</td>
</tr>
<tr>
<td>Germany, Norderney Canada</td>
<td>River sediments</td>
<td>400 - 2160</td>
<td>13832 ±15677</td>
<td>microbead m⁻²</td>
<td>Castañeda et al. 2014</td>
</tr>
<tr>
<td>Venice Lagoon, Italy</td>
<td>Subtidal</td>
<td>30 - 5000</td>
<td>1445 ± 460</td>
<td>particles kg⁻¹</td>
<td>Vianello et al., 2013</td>
</tr>
<tr>
<td>Garda Lake, Italy</td>
<td>Lake sediments</td>
<td>&gt;5000</td>
<td>108 ±55 to 1108 ±983</td>
<td>particle m⁻²</td>
<td>Imhof et al., 2013</td>
</tr>
<tr>
<td>Singapore</td>
<td>Intertidal (mangrove)</td>
<td>&lt;20 - 5000&lt;</td>
<td>37 ± 24</td>
<td>particles kg⁻¹</td>
<td>Nor and Obbard, 2013</td>
</tr>
<tr>
<td>Chile</td>
<td>Beach</td>
<td>100 - 1000</td>
<td>27 ± 3</td>
<td>m⁻²</td>
<td>Hidalgo-Ruz &amp; Thiel, 2013</td>
</tr>
<tr>
<td>Island Kachelot, Germany</td>
<td>Beach (Easter Island)</td>
<td>&gt;1.2</td>
<td>210 and 461</td>
<td>granules and fibers kg⁻¹</td>
<td>Liebezeit &amp; Dubaish, 2012</td>
</tr>
<tr>
<td>Belgian</td>
<td>Subtidal (harbor)</td>
<td>&gt;63</td>
<td>167 ± 92</td>
<td>particles kg⁻¹</td>
<td>Claessens et al., 2011</td>
</tr>
<tr>
<td>Australian</td>
<td>Beach</td>
<td>&lt;100</td>
<td>8</td>
<td>per L⁻¹ᵇ</td>
<td>Browne et al., 2011</td>
</tr>
<tr>
<td>Portugal</td>
<td>Beach</td>
<td>124</td>
<td>145 ± 60</td>
<td>per L⁻¹ᵇ</td>
<td>Graham and Thompson, 2008</td>
</tr>
<tr>
<td>Sweden</td>
<td>Subtidal (harbor)</td>
<td>&lt;1000</td>
<td>50</td>
<td>per L⁻¹ᶜ</td>
<td>Norén, 2007</td>
</tr>
<tr>
<td>India</td>
<td>Intertidal (ship breaking yard)</td>
<td>Not specified</td>
<td>81</td>
<td>mg kg⁻¹</td>
<td>Reddy et al., 2006</td>
</tr>
<tr>
<td>UK</td>
<td>Beach</td>
<td>Not specified</td>
<td>12</td>
<td>per L⁻¹ᵈ</td>
<td>Thompson et al., 2004</td>
</tr>
</tbody>
</table>

ᵃ These values were originally reported per 10g and extrapolated to 1 kg
ᵇ These values were originally reported in 250 mL and extrapolated to 1 L
ᶜ These values were originally reported in 100 mL and extrapolated to 1 L
ᵈ These values were originally reported in 50 mL and extrapolated to 1 L

The information about potential sources of microplastics is important for management purposes, since control strategies differ according to the sources of microplastic (Arthur et al., 2009). The totality of microplastics in the present study were fibers (63% of all plastic types) and plastics from secondary sources, such as fragments (25%), films (10%) and foamed plastics (2%), that arise from defragmentation of macroplastics at sea or on land. No primary microplastics (spherical microplastics and pellets) were found. Fibers were the most frequent
shapes, which is among the most ubiquitous form of marine microplastics (e.g. Free et al. 2014). They are used for ropes, fishing gear or synthetic clothing and garments. A single synthetic clothing garment can release more than 1900 microfibers per wash that may end in the marine environment via sewage, as even sewage treatment plants cannot restrain small fibers (Mark Anthony Browne et al. 2011, Mathalon and Hill 2014a). Sewage effluent from surrounding hotels and houses of the Funchal area could have imported microplastic fibers into the beach via currents. Ocean-based sources such as ropes which are used in aquaculture and fisheries also could have contributed to the majority of reported fibers found at Praia Formosa. However, it is difficult to understand precise relationships between microplastic concentration and its sources due complex transport mechanisms and unknown fragmentation rates of microplastics (Law and Thompson 2014).

A variety of sampling methods and techniques for separation and identifying microplastics need to be considered when comparing different studies. Moreover, studies report microplastic in different units: either counting particles per weight/volume/surface area sediment or by taking the weight of microplastics in gram per weight/volume of sediment (Hidalgo-Ruz et al. 2012b). When calculating only weight of microplastics, particle sizes are not considered. But their size is relevant, because of the different size ranges organisms feed on. For mussels particle size influences the capacity of microspheres to translocate from the gut cavity to the haemolymph microplastics. Browne et al. (2008) found that over 60% more smaller particles (3.0 µm) were present in the circulatory fluid than larger microspheres (9.6 µm).

In our study microplastic particles were documented in a size range of 500 – 5000 µm. Because our sampling did not consider microplastics that were smaller (< 500 µm), the recorded densities may have been underestimated. In the Lagoon of Venice 93% of the particles found were ranged between 30 to 500 µm (Vianello et al., 2013). Thus, this study highlights again how diverse results might be depending on the applied methods. Furthermore, separation of microplastics from sand by density flotation with a saturated solution of NaCl (1.2 g/cm³) does not recover polymers with high densities, such as PVC or plastic particles that are heavier due to biofouling. Other solutions, such as a ZnCl₂ (1.6 – 1.7 g/ cm³) would have resulted higher recovery rates (Imhof et al., 2012), but were too expensive to use in our study.
By visual identification under stereo microscope difficulties occurred in our study when distinguishing microplastics from organic debris (such as plants and animal residues) and non-plastic anthropogenic debris. That same problem has been described by Hidalgo-Ruz et al. (2012). They reported that 70% of visually identified particles were not confirmed as plastics when using infrared spectroscopy (Hidalgo-Ruz et al., 2012). Skins and plant debris can be mistaken as films (Figure 27). Decisions that rely on certain features, such as shininess, brightness or unexpected colors, etc., can therefore only be based on a degree of subjectivity. For some samples my colleague and I came to different quantifications of the same extract. One sample, for example, contained an agglomeration of small fibers (~ 100), which my colleague counted as one, whereas I would have counted them individually. Even if we generally came up with same results, in this cases it is a matter of definition, what is one fiber, because fibers from ropes are made of several strings. Although, infrared spectroscopy is the most reliable way to identify plastics, it was too expensive to use in our experiment.

Data most comparable to the present study were obtained by other GAME studies, which were carried out in 2013 and 2014 by using the same methodology. This allows for comparing temporal and spatial variances (see Table 16).
In most countries, the same locations were sampled in both years; only in Indonesia and Mexico the chosen monitored sites were different, which limits the overall comparability. While in 2013 the lowest concentration, with no microplastics at all, was found in a bay in Puerto Morelos (Mexico) (Jonas, personal communication), in 2014 the results were the highest with mean abundances of 54 particles kg\(^{-1}\) (Heel und Gómez Hernández, personal communication). The results were reported from the same bay, but taken from a different beach within three kilometers distance to location of 2013. It is difficult to explain this temporal and small-scale spatial variability. Furthermore, unlike all other countries who identified fibers as the main constituent of plastic types, Mexico reported a majority of pellets (Heel und Gómez Hernández, personal communication). The high abundance of pellets may indicate a local point source for primary microplastics, comparable to the patchy distribution of pellets found in the St. Lawrence river sediments (Castañeda et al., 2014). For example, pellets might origin from spillages of transported virgin pellets by ship.

A difference in microplastic abundance was also reported from Indonesia, where also different sites in both years were sampled (Island Pari, 2013 and Island Rambut, 2014) (Piehl, personal communication; Rist, 2015). On the island Pari high abundance of microplastics were found with 48 particles kg\(^{-1}\) dw sediment in 2013, whereas low abundances were found on the island Rambut (2 particles kg\(^{-1}\) sediment) in 2014. Interestingly, the low contamination was reported from a beach that was highly polluted with macroplastics. One reason for this...
phenomenon could be a slow degradation of ‘young’ macroplastics pollution into microplastics.

In Chile, Japan and Wales the reported microplastic abundance increased from 2013 to 2014 (Marx and Gatta; Perschke and Sugai; Liebetrau, personal communication). Similar concentration as in Madeira were found in Brazil and Japan (Teegen, 2014; Perschke and Sugai, personal communication). However, Madeira was the only site from which constant average particle concentrations were reported in both years (Grossmann, 2014).

These findings obtained by a global approach underline the high temporal and spatial variability of microplastics in coastal sediments. Methodological constraints due to visual inspections could have also contributed to the observed variability in microplastic abundances between sites and years. In future studies, visual inspections should be accompanied by infrared spectroscopy to improve reliability of the data. If that is not possible only larger size fraction should be considered by visual inspection, accompanied with standardize monitoring protocols.
5 Management Suggestions

It is widely known, that plastic breaks into fragments and nanopieces, although residence time in the environment is still not clear (e.g. Ivar do Sul & Costa, 2013). The microplastics problem is therefore mainly arising out of the plastic problem that becomes marine litter. Therefore, management steps of microplastics are based on the general management of plastic debris. With one essential difference, that they cannot be mitigated directly. Microplastics cannot be sieved out of the sand or filtered from the sea (Ivar do Sul and Costa, 2013). This would take forever or would also diminish all other small species and plants from the water (Ivar do Sul and Costa, 2013). Therefore, the main focus of managing microplastics is to hinder it from entering into the ocean (UNEP, 2014). We still lack understanding the sources of plastic pollution at management-relevant scales, to identify the steps of mitigation along the product disposal chain (Vegter et al., 2014).

5.1 Source control

Main focus of managing the problem of microplastics in the ocean is to prevent primary microplastics and large plastic items from entering it. Understanding the sources and reducing waste production is crucial. Land-based waste management is an essential part of the solution, as well as preventing direct dumping of waste at sea (Free et al., 2014) One source of microplastics appears to be the disposal of sewage contaminated with fibers from washing textiles and pellets from cosmetic products (Browne et al., 2011b). Sewage should be treated and can be improved by ultrafiltration reducing the amount of fibers (Browne et al., 2011a). Designers of clothes and producers of washing machines should consider the need to reduce the amount of fibers into wastewater (Browne et al., 2011b). Industries using microbeads in their products should be encouraged to substitute them with natural biodegradable alternatives (e.g. Astrup, Fruergaard, & Christensen, 2009; Fendall & Sewell, 2009)

5.2 Removal of plastics

A second focus lies on removal of plastics from beaches, rivers and the ocean. Beach clean-ups can raise awareness, provide education and deliver data for monitoring. However, they are expensive. Cleaning up macroplastics from oceans is not effective. Removals would
demand a lot of energy and has unwanted side effects, such as by-catch of floating marine biota (for example zooplankton) that do not escape actively from the cleaning advice (Davies et al., 2013). *The Ocean Clean-up Array*, a floating barrier for plastic debris and a plastic-eating drone are both innovative ideas which have to take into account not only biological and technical aspects but also effectiveness and costs (Wilson, 2013). For example, floating device will only tackle positively buoyant plastic types. As long as plastic is not being prevented from entering the ocean, collecting debris might only be an alleviation of effects. From the current perspective a potential application of floating barriers might be more realistic on river mouths.

**5.3 Stakeholders involved in plastic mitigation**

To tackle a global problem with many dimensions, a multidisciplinary-approach is needed in research, and management partnerships require involvement of various stakeholders at different geographical and political scales. An overview will be given about the role of stakeholder as identified by Ivar do Sul & Costa (2013) (Figure 28). Further examples are given to demonstrate how they are intertwined with each other.

*Figure 28: Overview of stakeholders involved in mitigation of marine plastic pollution. Main focus is avoiding plastic input into the environment (source control) and secondary action is the removal of plastics. Adapted from Ivar do Sul and Costa (2013).*
5.3.1 Basic Science & Applied Science

“With knowledge comes greater responsibility” Ivar do Sul & Costa, 2013

Initially, basic research is important to identify the existence of a pollution problem and become aware of it. The problem of plastic pollution started to be acknowledged by the scientific community in the mid-1980s. Since its first evidence in the 1970s, the number of publication increased, particularly since 2000 (Vegter et al., 2014), which is also reflected by the rising awareness public sector, industries and civil society. Policy-makers, NGOs and the media require scientific knowledge to base their decisions on or to initiate public campaigns.

Despite public awareness, many gaps still exist regarding the prevalence and impact of plastic pollution, and better understanding and mitigating the effects remains a challenge. There is still a lack of understanding about the amount of plastic that goes into the systems, timescales of degradation into microplastics, their fate and ecological impact (Vegter et al., 2014). These are important questions that are not sufficiently answered, but enough knowledge to start acting by applying the precautionary approach. A widely accepted statement of the 1992 Rio Declaration on Environment and Development describes the precautionary approach as following (Rio Declaration, 1992, p.7):

“Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation.”

What are the priorities?
Given that plastic production is ongoing and the amount of plastic entering the marine environment is increasing, it is time to identify key areas for research that support understanding of plastic pollution to enable effective mitigation strategies. A recent paper published sixteen priority research questions and provided background information based on experts opinions in order to move research and management forward (see Vegter et al., 2014). They concluded that understanding and mitigating the impacts of marine plastic pollution requires a multi-disciplinary approach (Vegter et al., 2014).

Vegter et al (2014) focuses on the main topics, that can be divided into five categories, 1) the extent of plastic pollution 2) the impacts on the wildlife and habitats, 3) the physical and chemical background on plastic pollution 4) management 5) alternatives to plastic material (see Table 17):
Table 17: Research priorities to mitigate plastic pollution impacts on marine wildlife. Priorities formulated by 26 experts from various disciplines in plastic research. From Vegter et al. (2014)

<table>
<thead>
<tr>
<th>Extent of plastic pollution:</th>
<th>What, and where, are the main sources of plastic pollution entering the marine environment?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>What are some standard approaches for the quantification of plastic pollution in marine and coastal habitats?</td>
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</table>

<table>
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<tr>
<th>Impacts on marine wildlife and habitats:</th>
<th>What are the impacts of plastic pollution on the physical condition of key marine habitats?</th>
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<tbody>
<tr>
<td></td>
<td>What are the impacts of plastic pollution on trophic linkages?</td>
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<tr>
<td></td>
<td>How does plastic pollution contribute to the transfer of non-native species?</td>
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<tr>
<td></td>
<td>What are the species-level impacts of plastic pollution, and can they be quantified?</td>
</tr>
<tr>
<td></td>
<td>What are the population-level impacts of plastic pollution, and can they be quantified?</td>
</tr>
<tr>
<td></td>
<td>What are the impacts of wildlife entanglement?</td>
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<table>
<thead>
<tr>
<th>Physical and chemical mechanics of marine plastic pollution:</th>
<th>How will climate change influence the impacts of plastic pollution?</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>What factors drive the transport and deposition of plastic pollution in the marine environment, and where have these factors created high concentrations of accumulated plastic?</td>
</tr>
<tr>
<td></td>
<td>What are the chemical and physical properties of plastics that enable their persistence in the marine environment?</td>
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</table>

<table>
<thead>
<tr>
<th>Management of plastic pollution:</th>
<th>What are the barriers to, and opportunities for, delivering effective education and awareness strategies regarding plastic pollution?</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>What are the economic and social effects of plastic pollution in marine and coastal habitats?</td>
</tr>
<tr>
<td></td>
<td>What are the costs and benefits of mitigating plastic pollution, and how do we determine viable mitigation options?</td>
</tr>
<tr>
<td></td>
<td>How can we improve data integration to evaluate and refine management of plastic pollution?</td>
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</table>

| Alternatives plastic materials: | What are the alternatives to plastic? |

5.3.2 The Productive Sector – Refuse, Reduce, Reuse, Recycle, Rethink

Plastic production, use and re-use industries are slowly reacting to demands of source control. The 3Rs (Reduce, Reuse Recycle) have been an exception, rather than the norm (Ivar do Sul and Costa, 2013). Rethink (choose alternative materials) and Refuse (reduce production of all single use plastic item) should be integrated and prioritized into the production sector (Ivar do Sul and Costa, 2013).
Beat the Microbeads – Cooperation between stakeholders

Substitution of microplastics in cosmetics is an example of how production sector, scientists, policy-makers, NGOs and consumers can ‘rethink’. In 2009, Fendall and Sewell published a paper reporting that the majority of facial cleaners contained polyethylene microplastics not captured by wastewater plants (Fendall and Sewell, 2009). In 2012, an international campaign Beat the Microbead started, now supported by 66 NGOs and 32 participating countries (Plastic Soup Foundation & Stichting De Noordzee, 2015). The campaigns’ website documents the political development and companies’ decisions to stop using microplastics in their products (Plastic Soup Foundation & Stichting De Noordzee, 2015). They established product lists and an app to inform consumers about microplastics in the products, as well as helping them to return their products by providing a letter template (Plastic Soup Foundation & Stichting De Noordzee, 2015). Due to public pressure and existing natural biodegradable alternatives, such as salt, bamboo or walnut skins, more and more companies voluntarily agreed on abandoning microplastics in their products. Unilever was the first multinational that claimed to halt the use of microbeads, and many companies followed. Various national governments currently discuss the prohibition of microbeads and further encouraging industries to use alternatives to microplastics (Miel, 2012). In 2014, Illinois was the first State in USA to ban microbeads in cosmetics by law (Flesher, 2014). The states of Maine, Colorado and New York signed similar bills this year, and 20 states are considering a legislation to ban microbeads (Miller, 2015).

New materials – new pollution? Bioplastics, Biodegradable plastics and plastic oil

While bans are gaining momentum, some of these laws are written with a major loophole that has been largely unnoticed. When only banning “intentionally added non-biodegradable, solid plastic particles” (Illinois General Assembly, 2014), manufacturers can still switch to bioplastics made from polyactic acid. Not all biodegradable plastic break down in cold marine environment (Ellision, 2015).

Executive director Anna Cummins from 5 Gyres (5 Gyres Institute, 2015), a NGO that combines research and campaigns explains "In a lot of cases, the legislators introducing the bills have the best of intentions, but they don’t necessarily realize that a simple definition of plastic opens up this loophole to replace plastic beads with something equally harmful." (Ellision, 2015)
Bioplastics are a range of polymers that are either bio-based or bio-degradable plastics (European Bioplastics, 2014) (Figure 29).

**Bio-based plastics** are derived from renewable resources such as starch, sugar, vegetable oil or wood pulp. They can be biodegradable (e.g. polyhydroxyalkanoates (PHAs)) or non-degradable (e.g. polyethylene (PE) derived from bioethanol).

**Biodegradable (compostable) plastics** meet scientific standards for biodegradability and can be completely broken down by micro-organisms into non-toxic compounds. They can be compostable under particular conditions. Characteristics include no negative effects on the composting process (breaks down into water, biomass and CO₂), disintegration (material must become indistinguishable after a certain time) and non-toxicity. Biodegradable plastics are often bio-based but can also be petroleum based (e.g. polycaprolactone(PCL)) (European Commission, 2011a).

An important difference is the distinction of biodegradable and degradable plastics. The latter are usually petroleum-based plastics that contain additives as catalysts for degradation and produce smaller plastics that do not meet compostability or biodegradability standards (Barker and Safford, 2009).

Current legislations do not refer to industrial standards for materials that define minimum levels of actual marine biodegradability. One example is the ASTM D7081 “Standard Specification for Non-floating Biodegradable Plastics in the Marine Environment”, that has been in place for eight years and is now under revision (ASTM D7081-05, 2005). Efforts are underway to harmonize these standards. This work needs to be controlled by an independent laboratory that can verify current industry standard test methods. The various existing bioplastics have two major disadvantages compared to conventional plastics. Their production
is still costly and qualities are often inferior. Although the costs can be minimised, it is highly likely that increased biodegradability will always compromise physical and chemical stability (Webb et al., 2013). Therefore, the potential of bioplastics is a case-by-case compromise between degradability and durability on the basis of the intended application (Webb et al., 2013).

**Thermal degradation - Another way of recycling plastic?**

Europeans’ post-consumer plastic waste is around 25 million tons per year (PlasticsEurope, 2015). The share of recycled material has been increasing by 40% since 2006, and has now a share of 26%, while energy recovery has a share of 36% (PlasticsEurope, 2015). Although decreasing in popularity, the majority of user plastics still end up landfills (38%) (PlasticsEurope, 2015). The recycling rates outside Europe are far smaller, for example 10% in the US (Sigler, 2014).

A new technology is thermal degradation of plastic during which petroleum-based plastic are heated to 24 – 430 °C and then converted into liquid hydrocarbon fuel (Sarker et al., 2012). Smoke is not produced, since thermal degradation is carried out in an oxygen-free reactor, which means plastics will not be incinerated (Sarker et al., 2012). Sarker et al. (2012) concluded that his method is an efficient and effective way to convert a vast amount of plastic into a useful source of energy, with 90% conversion into hydrocarbon fuel and less than 5% light gas production (Sarker et al., 2012).

**5.3.3 Decision makers - Action plans and instruments**

Global actors are slowly recognizing the scale of the problem. Unlike other human impacts, such as climate change and ocean acidification, plastic pollution is a visible phenomenon and cannot be denied. At the 2012 Rio Summit, conference countries committed to “achieve a significant reduction in marine debris by 2025” and to develop coordinated regional strategies to mitigate the impacts of marine litter (United Nations, 2012). Within European regional sea conventions, regional action plans have been drafted and adopted. In 2013, the Mediterranean regional action plan was drafted and the Baltic nations agreed on one by 2015, while in the North-East Atlantic, OSPAR (The Convention for the Protection of the Marine Environment of the North-East Atlantic) prepared their plan for June 2014 (European Commission, 2015a). Work is also ongoing at the Black Sea, where parties of the Bucharest Convention are updating the strategic action plan (European Commission, 2015a). A toolbox with ongoing initiatives against marine litter was established at the *International Conference on Prevention*
and Management of Marine Litter in Europeans sea in April 2013, co-organised by the German environmental authorities and the European Commission (German Federal Environment Agency (UBA) et al., 2013). The European Commission has recognized the problem and wants the European Union to become a “showcase” for how to develop a coherent strategy on how to optimize plastic waste policy (European Commission, 2015b). It has recognized that besides the existing legislations, such as the Waste Framework Directive and the Packaging and packaging waste directive, there is further need to approach plastic waste and plastic product management (European Commission, 2015b). In 2013, the European Commission published a Green Paper on a European Strategy on Plastic Waste in the Environment that enables a broad reflection on possible change or additions to the public policy which are not specifically or effectively addressed in the European waste legislation (Kuch and Gonzalez-torres, 2013). Such green paper may give rise to legislative development. Furthermore, EU and European parliament agreed to the establishment of a “Union-wide quantitative reduction headline target for marine litter” within the agreement of the 7th Environmental Action Programme (European Commission, 2015c).

EU member states have a responsibility to tackle marine litter with the Marine Strategy Framework Directive, which aims for seas to reach ‘good environmental status’ (GES) by 2020 (Galgani et al., 2013). It acknowledges marine litter as one of the main threats to the marine environment, along with fisheries, pollution, invasive alien species and noise (Galgani et al., 2013). Part of their obligation is to submit a program of measures to the Commission by 2015, in which the GES is judged by 11 specific indicators of the state of the sea, including the prevalence and impact of marine litter (Galgani et al., 2013).

Decision makers can also decide on using different economic instruments for marine litter control, covering environmental cost with income derived from implementing such instruments. Cost of marine litter can be ruined fishing gear, reduced tourism, damage of ship propellers and beach clean-ups. The UK is spending estimated €18 million per annum on beach cleaning (European Environmental Agency, 2014). Oosterhuis, Papyrakis, & Boteler (2014) reviewed various economic instruments in case studies around the world according to the targeted type of litter, their effectiveness, costs of implementation and indirect side effect (Oosterhuis et al., 2014) (see Table 18). The use of a plastic bag levy has been successful at relatively low cost as pay-as-you-throw charge for municipal waste collection, and can encourage firms and households in waste reduction (e.g. Ayalon, Goldrath, Rosenthal, & Grossman, 2009; Convery, McDonnell, & Ferreira, 2007; Nhamo, 2005; Oosterhuis et al.,
However, it may also incentivize illegal dumping. In coastal areas, waste collection can be supported by tourist taxes. Furthermore, the collection of bottles via deposit-and-refund scheme has created high return rates and can provide income for some of the urban poor. A similar effect have incentives for fishermen when they return waste to shore (Oosterhuis et al., 2014). Oosterhuis et al. (2014) argue that there is still little known about the link between the overall amount of polluting material (e.g. plastic bags) and the extent to which it becomes marine litter. The choice of economic instruments is case specific and largely depending on tackled sources, the countries’ institutional characteristics and other socio-economic aspects such as culture and income level. Reduction of marine litter will depend on the effectiveness of the economic instrument and matching the causal pathway between source and pollution (Oosterhuis et al., 2014).
Table 18: Economic instruments for marine litter control: possible applications and difficulties. Adapted from Oosterhuis et al. (2014)

<table>
<thead>
<tr>
<th>Type of economic instrument</th>
<th>Application</th>
<th>Difficulties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penalties</td>
<td>Insurance premium for fishing sector can be linked to the risk of damage</td>
<td>Effectiveness depends on the likelihood to identify the polluter &amp; political support</td>
</tr>
<tr>
<td>Taxes and charges on plastic bags</td>
<td>Reducing the use at relative low costs; Adjustment to appropriate level and inflation; Extension for fishing gear and plastic food foam container or cigarettes</td>
<td>Collected fine should be used for awareness campaign or for Optimizing waste reduction infrastructures</td>
</tr>
<tr>
<td>“Pay-as-you-throw” (PAYT)</td>
<td>Incentive for households and firms to reduce waste</td>
<td>Incentives for illegal dumping</td>
</tr>
<tr>
<td>Tourist taxes</td>
<td>Support for waste collection; Extension for recreational fishing, car park fee</td>
<td>Sometimes strong opposition by tourism industry; Target group older and wealthier and when respondents identify direct link between tax and litter control; Limited in developing countries, because of lack of skilled personnel, infrastructure and monitoring systems</td>
</tr>
<tr>
<td>Liability 'polluters-pay-principle'</td>
<td>Can be based on cost of clean-up or compensation scheme for affected livelihood</td>
<td>Need to link between polluter (firm) and pollutant; Difficult to set-up: need for legal framework and capacity; Liability nearly impossible to some international sources of marine pollution; Operationally difficult especially in developing countries</td>
</tr>
<tr>
<td>Deposit- and refund schemes</td>
<td>High return rates of bottles and cans and reduction in litter; Developing countries: payments for bottles or other plastic material</td>
<td>High costs of implementation (when multiple type of materials); Recycling attract more consumer support than reusing (concern about flaws and stains); Costs tends to be higher than benefits; Preferences to throw-away bottles, if scheme increases cost for refundable drinks ; Trade barriers</td>
</tr>
<tr>
<td>Subsidies and fiscal incentives</td>
<td>General tax system can finance subsidized use of recyclable materials and waste minimisation technologies; Tax breaks for recycling companies; Financial and technical support to waste management on fishing vessels, leisure craft and larger ships</td>
<td>Effectiveness limited by weak political support and high costs</td>
</tr>
<tr>
<td>Direct payments/Rewards</td>
<td>Grants for promotion of recycling or integrated waste management systems, or innovative firms; Offer payment for plastic bottle collected by urban poor; Fishing: return waste to shore Additionally income</td>
<td>General high effectiveness; Limited by weak political support or corruption; High costs, but sometimes low compared to direct cost of litter removal</td>
</tr>
<tr>
<td>Price differentiation</td>
<td>Higher price of product from recycled material which finance collection of plastic litter</td>
<td>Effectiveness low and no empirical support yet</td>
</tr>
<tr>
<td>Preferential treatment and public procedure</td>
<td>Governments /authorities can themselves base decision on environment aspects when awarding a contract or a permit (‘green procurement’)</td>
<td>Not commented</td>
</tr>
</tbody>
</table>
5.3.4 Consumers choice, Public engagement & Media attention

A large part of the marine litter is consumer waste, such as plastic bags, bottles, bottle caps and cigarette butts. Based on consumer habits, plastic packaging has increased dramatically. Consumers have the choice to significantly contribute to a reduction of waste going in the sea, by doing away with plastic bags, returning bottles, as well as joining campaigns, monitoring and beach clean-ups. Education is an important tool to change behavioral patterns. An eighteen year old started an online petition against packaging with plastic foil, and created much attention among various newspapers (Süddeutsche Zeitung, 2014). Similarly, The Ocean Clean-up initiative, founded by a nineteen year old engineering student attracted thousands of volunteers after holding a TED talk. New forms of communication play a crucial role in raising attention or financing private initiatives, for example via crowd sourcing. Citizen engagement has the power to change industries.

Scientists are not the only people tracking trash. New technology, such as smart phone apps and internet-based campaigns are boosting citizen science to monitor plastic pollution as well as pollution. In 2015, the European Environmental Agency (EEA) developed a new mobile phone app Marine LitterWatch intended to strengthen Europe’s knowledge base and to provide support to European policy making (European Environmental Agency, 2015). It also provides a platform for marine litter communities to come together, share knowledge, organize clean-ups and monitoring events. A Marine Debris Tracker is another mobile application initiated by the NOAA Marine Debris Program and the Southeast Atlantic Marine Debris Initiative (SEA-MDI) (Southeast Atlantic Marine Debris Initiative and National Oceanic and Atmospheric Administration, 2014). Pelletwatch, another global monitoring program, is collecting information of the distribution of persistent organic pollution (POPs) that has accumulated on plastic resin pellets found by participants (International Pellet Watch, 2015; Takada, 2006). Engaging the public not only helps scientists to get a better picture of the problem, but also to create awareness and curiosity - combined with a sense of engagement that everyone can contribute.

Another interesting technology has been applied to find evidence of the pathways of garbage. Within the SENSEable City project in New York and Seattle, 3000 pieces of trash were attached with a RFID tags and cellular track to understand better the removal chain and boost recycling rates (Phithakkitnukoon et al., 2013). These sensors allow researchers to identify for how long garbage pieces have been moving before being deposited in order to find out more about the waste management system in the United States (Phithakkitnukoon et al., 2013). As there is a lack in understanding the sources of plastic pollution such technology might contribute to identify needs along the product disposal chains in management-relevant scales.
## 6 Conclusion

Plastics are floating over surfaces of rivers and oceans, going in interaction to marine wildlife and their habitat. Humans have created a worldwide pollution that has expanded even inhabited and wilderness regions. Basic research has reported increasingly on species interaction with microplastics.

In this research *Megabalanus azoricus*, a filter feeding organism, was studied in terms of its responses to different densities of contaminated microplastics. In a laboratory exposure experiments following research questions were answered:

1. How do different **microplastic densities** affect the Azorean barnacle *M. azoricus* under laboratory conditions in terms of a) cirral activity, b) respiration or c) survival under hypoxia?

2. Does **fluoranthene** contamination of microplastic play a role for the effect on the Azorean barnacle *M. azoricus*, and if so, how?

The cirral activity did not change with increasing microplastic density, but was lower at barnacles exposed to medium microplastic densities (0.3% per weight sediment) occurred compared to all other treatments. Supposedly, high microplastic densities evoked a protective withdrawal, whereas medium densities (3%) did impair them. Further, research to proof this pattern is needed. In terms of respiration microplastic and survival under hypoxia no effect of microplastic has been observed. Furthermore, no effect of fluoranthene has been observed in the same response variables.

However in absence of effects, it is not possible to exclude a potential threat, as micro-plastics occur in different shapes and physical-chemical properties forming a complex picture. It is a challenge to compare the interpretations on absence or presence of effects from different laboratory designs and species to transfer their potential meanings to the natural environment. Within the framework of GAME similar experiments allowed comparing results of a range of species globally. Besides various reported effects on species, the green mussel *Perna viridis*, showed the clearest negative effect of increasing microplastic in various measured responses,
even at lower microplastic densities (0.03% and 0.3% microplastics per weight) (Rist, 2015). Conducting laboratory experiments on benthic invertebrates help us to identify species-related sensitivity to microplastics.

To understand if the habitat in Madeira is polluted with microplastics, sediment samples have been taken to answer:

3. How **abundant** are microplastics in Praia Formosa, a beach in Southern Madeira? Is there a difference in microplastic abundance between samples from the high tide line and the intertidal line?

The sediment samples revealed a rather low abundance of microplastic with a high variation between samples. The results of this global approach will help to raise awareness and understand impacts, which in turn support decision-makers. The scientific community has to strengthen their interdisciplinary research to tackle important questions of mitigating plastic pollution at the source.

To illustrate this global research on species-microplastics interactions the current political discourse was embedded in this thesis and the third research question was answered in last chapter:

4. What is the current status of **management of microplastic pollution** in the marine environment? And which **stakeholders** are involved in mitigating it?

A literature analysis has been conducted to identify the role of various stakeholders involved in the current discourse, in political decision and in activity of managing microplastic. The marine litter problem created a world-wide engagement among various stakeholders. Transdisciplinary knowledge-exchange and action have recently been developing: scientists are engaged in delivering information for decision-makers, the public is helping scientists in data collections and the industry is starting to respond to public engagement and encouragement by governments. Examples given in the analysis of current engagement well illustrated the diverse interactions and highlight the importance of integrating modern communication technology. Images of marine litter and their impacts are in the centre of media attention. This visibility of plastic conquering wild inhabited places has sharpened
mindsets of the public. Plastic pollution in the marine environment is a dramatic testimony of our consumeristic lifestyles.

In my opinion the plastic pollution discourse contributed to the awareness that its roots lay is a dysfunctional system, which was based on the premise of economic growth and cheap energy of coal and oil. It has brought us wealth and advantages, but a life beyond the limits of our natural resources. We reached a landmark in our human existence. In both, socio-economic and earth-systems, we are dealing with great accelerations such as population growth, primary energy use, carbon dioxide rise, temperature increase, terrestrial degradation and many more (International Geosphere-Biosphere Programme, 2015). There are more and more sprouts growing that do not believe in the current financial system anymore and it’s developed values. People that try to live differently and redefine their individual life aims to not have more possession but to have more quality time instead. I have hope that recent environmental efforts will grow fruits and inspire us to become different humans. It is paramount sharing the increasing experience of improvements in waste management with developing countries and extent consumer and producers responsibilities. This will require substantial resources, time and spirit.

*May we suit the action to the word!*
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