Appendix 1: State of the art and Work Package descriptions

Appendix 1 contains detailed descriptions of the scientific work planned under each of the work packages under MarinePlastic. Description of each work package starts with an introduction to the state of the art within the topic of the work package.

WP 1 Regulation and societal actions on plastic pollution (lead: DTU)

Directly involved: **DTU:** S.F. Hansen, Postdoc, **RUC:** K. Syberg, 1 PhD

This work package aims to investigate and promote societal change in regard to minimize plastic pollution, with specific focus on the role of science and importance of stakeholders

State of the art

Development of policies to address plastic pollution is growing rapidly despite the fact that there are a many knowledge gaps when it comes to sources, characterization, fate, behavior and environmental effects of plastic. The European Commission has just adopted a strategy for plastics within a circular economy package aimed at supporting a transition towards a more sustainable use and consumption of plastic [25]. Other regulations such as the European Marine Strategy Framework Directive [26], and regional sea conventions like HELCOM (for the Baltic Sea) [43] and OSPAR (for the Northeast Atlantic) [69] have also established action plans for combatting sources of plastic pollution and Nordic minsters with responsibility for the environment have furthermore agreed on a common Nordic program to reduce the environmental impact of plastic [65]. The Danish government is constructing a Danish action plan on plastic, where focus most likely will be on facilitating a sound transition towards a more circular use and consumption of plastic.

The scientific foundations, reflections and arguments for the variety of implemented policy measures that come from all of these regulatory efforts are diverse and include monitoring considerations/efforts, impact assessments, LCA analysis and research into product chain management and transition. However to date, research into the overall applicability of Danish, European and international initiatives and regulation of plastic pollution has been very limited. One analysis has focused on the European chemicals regulation and how polymers could be categorized so that they fall under the scope of the regulation [72], whereas other studies have focused on arguments in favor of specific legal actions such as classification of plastic waste as hazardous and banning microbeads [75,76]. Yet another study identified six laws within the EU that have a high potential impact on the mitigation of marine litter such as, for instance The Waste Framework Directive, the Cosmetics Regulation, and the European Maritime and Fisheries Fund [66]. In one of the few in-depth analyses that have been published in the peer-reviewed literature, Steensgaard et al. [91] systematically investigated how plastic bags are regulated in a life cycle perspective within EU countries. The main gaps identified relate to lack of clear definitions of categories of polymers, unambitious recycling rates and lack of consideration of macro- and microplastics in key pieces of legislation [93]. Although important as a first step, the research conducted to date needs further verification, preferably based on more applications of plastics.

Recommendations need to be more specific so that they can easily be adopted into Danish and European legislation as well as international initiatives and strategies and to avoid negative impact of the interactions between different related policy options. There is furthermore an urgent need to address the lack of consideration of macro- and microplastics in key pieces of legislation [91] and to understand the role of science in this process, specifically when it comes to designing regulatory initiatives making the most use of current scientific efforts to reduce existing knowledge gaps when it comes to characterization, fate, behavior, environmental effects of plastic.

Another knowledge gap when it comes to regulation and social actions on plastic pollution relates to stakeholder involvement, since none of the analyses performed on European legislation have considered this. In recent action plans from HELCOM [43] as well as OSPAR [69] “Public participation and stakeholder involvement” was mentioned as an important aspect to include in actions in order to prevent plastic pollution. Very often, those stakeholders, such as the environment, endangered species, indigenous peoples, affected citizens and the public at large, with the most to lose or gain in the final decision, do not have sufficient influence on the policy process. This illustrates an urgent need to explore how limitations to traditional analysis can be overcome, to facilitate a more transparent and democratic stakeholder involvement. Plastic pollution serves as a perfect case for such studies due to its high public awareness and engagement. Citizens are one specific stakeholder that are often ignored and this has generated increasing effort in regard to citizen science (CS) in recent years [88]. Silvertown [88] has identified three main drivers for CS: 1) greater access to the technical tools needed; 2) bringing in additional qualified labor and 3) a greater demand for outreach within academia. However, little research has been done in regard to how CS can and has been used to empower citizens, the public as well as skilled amateurs and how it can be used to engage people locally and globally thereby increasing their influence in the stakeholder dialogues.

Scope of Work package 1

The scope of this work package is to analyze how science has guided regulatory development in the past decade and to provide insight and recommendations on how science can help society with the needed transition towards a more sustainable use of plastic in the future. This implies generating an overview of serious gaps when it comes to plastic pollution followed by recommendations on what role science should play in future development of policy measures. Several policy proposals and regional action plans have been put forward that call for better use of science, the application of environmental principles as well as stakeholder involvement. The best utilization of science requires a better understanding from policy makers of what science is most relevant as well as an understanding among scientist of the needs from policy makers.

The aim of work package at generating such an overview and make it easily accessible to interested stakeholders from academia, industry, government, NGOs and private citizens, based on a highly interdisciplinary approach drawing upon disciplines from natural and social science. The work package will further address the importance of scientific understanding among stakeholders and their role in policy making, leading to assessment of possible societal actions to minimize plastic pollution of the marine environment.

Synergies with other WPs

WP 1 provides a link between the science produced in WP2-5 and the societal needs to address marine plastic pollution. WP1 will thus provide insight of policy needs to the four other WPs and actively used the scientific findings of WP2-5 to help shape future regulatory measures.

1.1 “How can the generation of scientific knowledge be designed to strengthen regulatory efforts to reduce plastic pollution”?

Regulation of plastic pollution focuses on one of three approaches, namely: 1. Reducing land- and sea-based sources of marine litter based on environmental monitoring and assessment programs within the frameworks of environmental protection legislation; 2. Up-stream regulation of individual polymer/chemical/particle types; and 3. Production oriented regulations such as those supporting waste handling in a circular economy perspective. However, the way scientific knowledge is currently generated is not always designed to facilitate development within these regulatory regimes and too many regulatory questions are left scientifically unanswered. An analysis of which kinds of scientific information are needed to meet current regulatory requirements on plastic pollution at EU level (e.g. EU Strategy for Plastics in the Circular Economy, the Marine Strategy Framework Directive and REACH) as well as under the regional sea conventions OSPAR and HELCOM will be conducted. This analysis will be complemented with an analysis of whether the knowledge base that current regulations rely on is the most relevant information or whether policy makers and stakeholders should be better informed about scientific state of the art. These two analyses will help steer the work and data generated in WPs 2-5

1.2 "How can regulation be designed to strengthen efforts to reduce plastic pollution and spark innovation"?

It is well-known that well-designed regulation can spark innovation within industry as well as regulatory agencies, but it has yet to be establishment how innovative regulations are to be designed and implemented in general and with regards to plastic, in specific. Danish and European regulatory gaps will be investigated and an online database of regulatory efforts and citizen science (CS) projects (including Danish ones) to mitigate plastic pollution will be developed, evaluated and made publicly accessible. The initiatives described in the database will be compared to current political aims such as those presented in the forthcoming Danish action plan on plastics as well as European initiatives on circular economy. Taking into consideration the findings of WPs 2-5 and the literature on regulation and innovation, we will investigate how society can improve regulation of plastic pollution, including discussion of the recently proposed environmental principle termed the "necessity principle". The overall viability of the identified regulatory policy options will be discussed with regulatory agencies and key stakeholders in Denmark and EU through stakeholder analysis and mapping of the criteria that various stakeholders use to assess the feasibility of the identified regulatory options.

Timeline and deliverables:

Timeline and deliverables	YEAR 1				YEAR 2				YEAR 3				YEAR 4			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
WP 1.1 How can the generation of scientific knowledge be designed to strengthen regulatory efforts to reduce plastic pollution?																
Analysis of existing legislation in regard to scientific and legal foundation, with focus on 1) management, 2) technical and 3) scientific aspects																
Analysis of the role science plays in regulation of plastic pollution																
Analysis of how European regulation supports both local (Danish) and international (OSPAR, HELCOM, SDG) initiatives																
Analysis of the necessity principle to facilitate sustainable development of plastic comsumption																
WP 1.2 How can regulation be designed to strengthen efforts to reduce plastic pollution and spark innovation?																
Review of literature on how “regulation can spark innovation” - lessons learned																
Implementation of lessons learned in “most” relevant regulation and strategies																
Analysis of the role of stakeholders in plastic pollution research																
Implementation of WP2-5 results in regulation																

WP 2: Analytical methods (lead: AAU)

Directly involved: AAU: J. Vollertsen, 2 Postdocs, AU: J. Strand, 1 PhD

This work package aims to develop robust, fast and reliable methods to quantify plastic particles' size, shape and mass. It produces the analytical tools needed for the other WPs.

State of the art

Understanding occurrence, fate, and impacts of microplastic (MP) requires valid methods for their identification. The first approaches to identify MP used the naked eye or microscopy without further analysis. This has been shown to be highly uncertain and errors up to 70% have been reported following re-analysis of particles by spectroscopy, especially for smaller particles [53,44]. Microscopy alone is hence generally accepted as insufficient for MP quantification. For particle sizes above 0.1-0.5 mm, a combination of microscopy to collect candidate particles and analyzing them one-by-one by single-point FTIR or Raman spectroscopy is the common approach. It seems to be appropriate and yield reliable quantification for the larger MP particles, with uncertainties increasing towards the lower size range [57]. Pyrolysis GC-MS [32,45] and thermogravimetric analysis [23] is also an option for determining the polymer composition of the particles.

For MP smaller than 0.1-0.3 mm, µFTIR imaging has proven efficient [95,62,73,16]. This technique allows scanning of large areas of filters or windows for the presence of MP and has been successfully applied to particles as small as 10 µm. While still to be proven, this size can probably be extended down to 1-3 µm applying µFTIR-ATR imaging and maybe µRaman imaging. The imaging techniques also allow automatization of MP identification for particles below 300-500 µm, and especially µFTIR imaging is a likely tool for fast and reproducible MP analysis [73]. Nevertheless, µFTIR imaging is still in its earliest stages, and many unsolved issues relating to the spectroscopy itself as well as sample preparation remain.

While single-point FTIR or Raman spectroscopy for the larger particles and µFTIR imaging for the smaller particles promise high quality microplastic quantification, the techniques are time consuming and require advanced analytical equipment. For screening purposes where estimates suffice, simple techniques such as chemical staining have been suggested and applied to some matrixes [86,60]. However, there still are many unanswered questions, including which plastic types the approach can target, how applicable the approach is to complex matrixes, and whether or not it actually is faster than FTIR techniques.

Prior to analyzing for MP by spectroscopy or chromatography, the MP must be extracted from the matrixes it exists in and concentrated. The sample preparation methods depend on the type of matrix, with significant differences in sample preparation when analyzing for example water, sediments, and biota [57,47]. The sample preparation methods depend on the subsequent quantification method, and must generally be optimized further. While many publications have addressed the sample preparation, the large varieties of matrixes and analytical methods applied require a continued focus on this step of the analysis [59,29]. Furthermore, published work describing MP analysis and its sample preparation have seldom documented the efficiency of the analysis in terms of recovery rates or contamination [102,80]. Significant work hence still needs to be put into developing rigorous sample preparation and analysis. Another aspect of sample preparation and the following MP characterization is that it is rather time-consuming [24], and there is hence a need to decrease the amount of work that goes into it.

Nearly all published studies refer to MP in terms of particle number within some size range [59]. Such data is highly important when assessing its environmental impacts, but insufficient when assessing the load of microplastic on the environment or the fate of MP herein. Here the mass of MP must also be quantified. This constitutes a significant analytical problem as the spectroscopic methods are well-suited for quantification of size and shape, but less suited for quantification of mass. The GC-MS methods, on the other hand, are well suited for quantification of mass but cannot quantify size and shape [32,59]. Especially thermal-extraction desorption GC-MS for mass quantification has shown very promising results in terms of detection limits and sensitivity to a wide range of polymers [59]. Combining this method with µFTIR imaging for small particle sizes seems to show a way out of this impasse [85].

Quantification of nanoplastics, or better, quantifications of particles below the lowest limit that can be achieved with µFTIR-ATR imaging, i.e. below a few micrometers, is not well-investigated in terms of analytical quantification. To date the most promising method is unknown [87]. However, some candidates have been proposed. For example electron microscopy, atomic spectrometry and light scattering techniques [19]. Scanning Electron Microscopy – Energy Dispersive X-Ray Spectroscopy (SEM-EDS) can help discern inorganic particles from organic particles by detecting their chemical elemental composition. . It is, though, an expensive technique requiring substantial sample preparation [87]. The technique furthermore does not yield the material composition of the particles, increasing the risk of false determinations on environmental samples containing large amounts of other organic material than plastics. Another obvious option is to apply the previously described GC-MS methods on size-fractionated samples, hereby quantifying the total mass of particulate plastic polymers within a certain size range. Halle et al. [40] has applied such approach, obtaining some very promising first results on nanoplastics in ocean water.

Scope
The scope of WP 2 is to improve existing methods and develop new ones to quantify micro- and nanoplastics. It is furthermore a goal that all methods are reliable and verifiable.

2.1 ”How can analytical methods for quantification of plastic pollution be optimized?”

- Lowering detection limits in terms of particle sizes that can reliably be quantified. Plastic breaks down to create smaller particles. As most polymers are not, or only very slowly, biodegradable, logic dictates that the number of particles versus their size must follow a power-law. That is, the number of particles must increase exponentially with decreasing particle size. Assuming for simplicity that breakdown always is symmetrical and constant in time, mathematics implies that the number of particles increase by the third power of the inverse of the particle size. However, this is not what studies quantifying microplastic in environmental samples find. They find that particle numbers trail off at lower sizes. The most likely explanation is that smaller particles are commonly overlooked, i.e. the sensitivity of the methods get poorer as particle sizes become smaller. One sub-task of WP 2 is to lower the microplastic particle size that can reliable be quantified. We will lower the boundaries of µFTIR imaging with improved sample preparation and smarter analytical methods. One of these is introducing a novel large-area ATR which allows combining µFTIR imaging at the high quality that can be achieved by ATR-FTIR.
- Streamline sample preparation processes by improved sample preparation techniques. Sample preparation is the single step in the processing chain that costs most resources. We will automatize oxidative and enzymatic treatment steps to reduce the labor needed but also remove human error during sample preparation. We will develop a procedure where most of the steps are conducted in a completely closed environment without risk of contamination. The latter is crucial as microplastic analysis often suffers from substantial contamination by air or materials used in the work flow.
- Streamline interpretation of FTIR images. One analysis applying µFTIR imaging results in millions of spectra which need to be reliably analyzed. We will further develop an automated interpretation pipeline which originally was developed by AWI in Germany [8]. We will integrate this approach with software which today exists as a beta-version at AAU and develop a user-friendly, stand-alone and open access software to be used on any hardware platform capable of µFTIR imaging.
- Validate simple screening methods such as chemical staining methods on environmental matrixes. We will assess simple screening methods, which have proven their value in other studies, up against the imaging results and assess the degree to which they can be used as simple, cheap and rapid screening tools. A likely candidate is the Nile Red staining method previously applied for marine samples.

2.2 “How can analytical methods for nanoplastics be developed?”

- <u>Develop sample preparation methods for quantifying microplastics in the in the low- to sub-micron size range.</u> Quantifying the microplastics of the very smallest particles requires not only a different analytical technique compared to larger particle sizes but also modified sample preparation methods. For example todays sample preparation for microplastics involves filtration, often using a 10 µm steel filter. This technique cannot reliably retain plastic particles below this size, and a method will hence be developed that allow concentration and purification of particles in the targeted range.
- <u>Develop HC/EGA GC-MS analysis techniques to quantify plastic mass in the in the low- to sub-micron size range</u>. For the very smallest plastic particles, such as nanoplastics, a quantification method based on HC/EGA GC-MS will be developed. The basis of this technique, pyrolysis GC-MS, is not novel, but the application to the complex environmental matrixes targeted by this project is. The methods are developed in continuous interaction with sample purification, as accurate plastic quantification depends strongly on minimizing the signal from the natural substances in the obtained pyrograms.

Timeline and deliverables:

Timeline and deliverables	**YEAR 1**				**YEAR 2**				**YEAR 3**				**YEAR 4**			
	Q1	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
WP 2.1 How can analytical methods for quantification of plastic pollution be optimized?																
Lowering detection limits in terms of particle sizes that can be reliably quantified																
Streamline sample preparation processes by improved sample preparation techniques																
Streamline interpretation of FT-IR images																
Validate simple screening methods such as chemical staining methods on environmental matrixes																
WP 2.2 How can analytical methods for nanoplastics be developed?																
Develop sample preparation methods for quantifying microplastics in the in the sub-micron size range																
Develop HC/EGA GC-MS analysis techniques to quantify plastic mass in the in the sub-micron size range																

WP 3 Sources and distribution (lead: AU)

Directly involved: AU: J. Strand, 1 PhD, AAU: J. Vollertsen, A.H. Nielsen, 2 Postdocs, NatMus: Y Shashoua, 1 Postdoc

This work package aims to qualify and quantify the magnitude and spatial distribution of plastic pollution in Danish waters and to identify its most significant sources.

WP 3: Sources and distribution

State of the science

Plastic debris in the marine environment occurs in all compartments: The water surface, the water column, the sea floor, in sediments, in food webs and on coastlines, both in more human impacted coastal areas as well as in pristine regions, e.g. the Arctic and the middle of the large oceans. Especially floating litter can be spread by ocean currents over long distances, but local impacts can also be observed from e.g. urban areas, recreational activities and fishing activities. There are many sources of plastic pollution in the sea because of the many uses of plastic materials both from single use products as well as losses from products with longer lifespans. Even though land-based sources are believed to contribute to most of the marine plastic pollution [35], it has also been indicated that sea-based sources can be significant contributors. Remains of fishing gear and other waste from ships occur in high amounts on shorelines for instance in the North Sea and Skagerrak [92]. In addition, within each source, the distribution of macro- (≥ 25mm)-, meso- (5-10 mm) and microplastic (< 5mm), polymer types and fragmentation/degradation patterns are poorly understood [78] and for nanosized particles (<0.2 µm) there is almost no information due to the current lack of detection methods. As a result, methodologies for source tracking of plastic debris can be improved e.g. by better techniques for characterization of macro-litter items based on their uses and material compositions and by making better links between distribution in the sea and human activities within certain coastal areas and open water regions.

Focus on research into microplastic (mainly since 2004) has overshadowed other new studies describing plastic litter in macro- and meso-dimensions [97]. However, microplastics in the marine environment come from a multitude of sources. Primary microplastics are industrial materials designed to be included in facial scrubs, toothpaste, industrial abrasives and are transferred to waterways by dumping or accidental loss. Secondary microplastics are formed by the degradation of larger (macroplastic) items transferred to the seas from wastewater treatment plants and stormwater runoff, rivers, coastal sites and airborne pollution. Theoretical mass balances have been used to assess the relative importance of the contributions, but experimental validation is still lacking far behind [35]. Plastic litter mass estimates have only been made with huge uncertainties [48], and the significance of the various contributions based on their particle size distribution and material composition – and hence their potential impacts – are poorly understood. The chemical and physical compositions of plastic debris are likely to influence their mobility in coastal and marine environments, their spatial distributions, fate processes and impacts on their surroundings. Plastic compositions are, in turn, influenced by their synthesis and production as well as interactions with their environmental surroundings including biofouling and transport in food webs. More detailed studies on sources and spatial distributions of amounts and composition of plastic debris in different size categories within and between sampling areas are therefore necessary. This is needed for better understanding of the dominant inputs to, and fate processes in, the environment and also if management actions to combat the main sources to relevant litter items are to be effective – and also seen in a Danish context.

Scope

The purpose of this WP is to study and compare amounts and composition of plastic debris occurring in different marine compartments (i.e. water column, sediments, onshore) covering representative areas of both coastal and open parts of the Danish waters and to explore possibilities of linking the occurrence to specific sources. This includes comparison of polymer composition of macro- and microplastic particles in different size classes occurring in these environments, identified using FTIR spectroscopy and other techniques developed in WP2. Attempts will be made to assess occurrence of nanoplastics in environmental samples with pyrolysis HC/EGA GC-MS techniques. The comparative studies will be conducted in different areas representing both coastal and open water environments in Denmark.

3.1 "What is the magnitude of plastic pollution in Danish coastal and open waters and how much does the pollution vary between different areas?"

- Perform field studies in representative coastal waters on amounts and composition of plastic debris representing both macro-, meso- and microplastics occurring in different marine compartments i.e. in the water column, sediments, biota and onshore. Spectroscopic analyses from WP2 will be applied to determine amounts and polymer composition of plastic particles in the different size categories. Regarding amounts and composition of macrolitter items, some new data for plastic debris will be generated in accordance with international recommended guidelines. Furthermore, available data from International Council for the Exploration of the Sea (ICES), EU/EEA and Protection of the Marine Environment of the North-East Atlantic (OSPAR) databases (hosting monitoring data from beach litter and seafloor litter surveys) will be used for comparison with the Danish data generated in this work package. In addition, new project data on polymer composition of plastic debris items occurring in the marine environment will be generated, in order to enable comparison with the composition of microplastic occurring in the environment. The analysis of plastic debris in the environmental samples will be performed using optical microscopic and spectroscopic techniques as described in WP2. To what extent and how sample treatment (e.g. density separation, chemical digestion and size fractionation) will be performed prior to the analyses will vary depending on the sampling matrix. The coastal field studies will mainly be scheduled for year 1.
- Perform a field study in open parts of the Danish waters on amounts and composition of microplastic during an intensive field study in the large Danish research vessels Dana. A preliminary sailing route will cover both the Baltic Sea through, Inner Danish waters, Kattegat and the Skagerrak/North Sea region. During the cruise special attention will be given to the stratified mixing zone between Skagerrak and Kattegat where high saline North Sea water meet Baltic water with lower salinities that can affect both the vertical distribution of microplastics in the water column as well as the sedimentary processes. This knowledge can contribute to assessment of the influence of North Sea and Baltic Sea water bodies on the distribution and fate of microplastics in Danish waters. The microplastic analysis will also benefit from methodological activities under WP2. The open water field study will be scheduled for year 2.
- Provide the first evidences for occurrence of nanoplastic in Danish waters. Attempts will be made to analyze for occurrence of low- to sub-micron sized plastics, here amongst nanoplastics, i.e. particles < 0.1 µm, on size fractionated environmental samples with pyrolysis HC/EGA GC-MS techniques as also mentioned under WP2.

3.2 "Which are the most significant sources of plastic pollution in Danish coastal waters?"

- Assessments of the most significant sources of plastic pollution can be conducted using several approaches. One approach is to assess major sources through analyses of composition of macro- and microplastic registered in Danish waters based both on available data from long-term monitoring series and more focused research studies. Focus could for instance be placed on litter items derived from important categories of e.g. single use plastic products, sanitary items, insulation materials, fishing gear and transportation. Another approach is to compare occurrence and composition of certain types of plastic debris in areas that are more or less impacted by particular forms of local land- and/or sea-based sources from various human activities, e.g. comparisons between coastal waters at urban centers with more rural environments and coastal waters with more open water environments. This will be possible using methods developed in WP2 and they will be applied strategically under this WP, to obtain a first estimate of the relative relevance of sources. Polymer composition of macrolitter and secondary microplastic sampled at the same local areas will be compared in order to fingerprint the source and hereby provide data inputs to establishment of the mass balances in a representative Danish coastal environment. This will also comprise information gained in WP4 addressing the chemical and physical properties of plastics control their persistence and fate in the marine environment.

Timeline and deliverables:

Timeline and deliverables	YEAR 1				YEAR 2				YEAR 3				YEAR 4			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
WP 3.1 What is the magnitude of plastic pollution in Danish coastal and open waters and how much does the pollution vary between different areas?																
Perform field studies in representative coastal waters - and to analyse samples, generate datasets and report results																
Perform a field study in open parts of the Danish waters - and to analyse samples, generate datasets and report results																
Provide the first evidences for occurrence of nanoplastic in Danish waters - to make an attempt, and to generate a dataset and report results, if possible.																
WP 3.2 Which are the most significant sources of plastic pollution in Danish coastal waters?																
Assessment of the most significant sources of plastic pollution in Danish waters for macrolitter																
Assessment of the most significant sources of plastic pollution in Danish waters for microplastic																

WP 4 Fate and behavior (lead: NatMus)

Directly involved: NatMus: Y. Shashoua, 2 Postdocs, AAU: J. Vollertsen, A.H. Nielsen, 1 Postdoc AU: J. Strand, 1 PhD

This work package aims to investigate the persistence of today's and future plastic litter and to identify the environmental conditions most likely to inhibit and promote its degradation to secondary microplastics. It will also identify the processes most significant for dispersal and deposition of plastic litter and fragments.

State of the art

While the presence of plastics litter at ocean surfaces was reported in the scientific literature in 1972 [14] research into its origin, fate and potential impact on the surrounding environment was first generated in 2004 by the discovery and definition of 'microplastics' in beach sediments of the United Kingdom [97]. Despite the subsequent exponential growth in research into plastic litter, there is an acute lack of fundamental data that defines the lifetimes, that is the period for plastics to fragment in coastal and marine environments, the favored degradation pathways and the impact of fragmentation on the surrounding environment [35].

This shortfall can be attributed to the novelty of the specialism, the practical complexity of conducting in-situ studies, a shortage of reliable methodologies and of reference sample sets. As a result, current knowledge is based almost exclusively on data from industrial material science and polymer chemistry where higher temperatures, radiation levels and pressures than are environmentally relevant are employed to accelerate degradation within a convenient timeframe. Polymers with a carbon-carbon backbone (e.g. polyethylene, polypropylene, polystyrene, PVC) react primarily by photo-oxidation, initiated and catalyzed by ultraviolet and visible radiation (400-290 nm) while polymers with heteroatoms in their backbone (e.g. polyesters, polyamides, polyurethanes) exhibit higher resistance to oxidation but react with water via hydrolysis [38]. Oxidation occurs first at surfaces and is manifested by cracking, crazing and discoloration. As the backbone disintegrates, plastics fragment thereby increasing their bioavailability.

Today, the plastics industry is the main source of data describing lifetimes. Plastics products are subjected to standard weathering regimes involving high levels of photolytic or thermal energy for short periods, changes induced in physical or chemical properties are determined and a service lifetime derived. Service lifetimes range from 1 to 1000 years based on weathering tests or modeling [74]. Research since the 1990s into degradation of plastics used in artworks and designs in museums, suggests significant discrepancies between service lifetimes and real lifetimes at ambient conditions. Examination of more than 700 plastic artworks and objects in the British Museum and Victoria & Albert Museum collections detected degradation in all plastic types less than 25 years after production and concluded that plasticized PVC, cellulose acetate, rubbers and polyurethane ethers had a significantly shorter lifetime than the others [82]. Museums and archives worldwide have stored millions of photographic negatives in plasticized PVC folders since the 1970s. After 5 years, phthalate plasticizer diffuses from folders into the photographs, depositing disfiguring, oily droplets. An identical phenomenon has damaged irreversibly PVC tubes comprising the life support system in more than 90 spacesuits from the Apollo era in the late 1960s [83].

A similar discrepancy can be observed between service- and real lifetimes of plastics in marine litter. Published literature suggests that plastics break down after hundreds or thousands of years [1]. However, the first commercial plastics became available in the 1950s and the first microplastics were detected in 2004, a maximum of only fifty to sixty years. In addition, research into the size distribution of floating plastic litter suggests that rapid nano-fragmentation may take place when they attain millimeter dimensions [20]. It is essential to fill this knowledge gap in order to define accurately the life cycle of plastics, account for the significant discrepancy between estimated and measured global loads of marine litter, known as missing plastics and influence international policy and legislation [91,28].

Scope

The scope of WP4 is to investigate, for the first time, whether the primary causes of fragmentation of plastic litter from macro- to micro- and nanoplastics and the drivers behind their relocation are internal and due to their chemical and physical compositions or external and attributed to exposure to oxygen, ultraviolet radiation, mechanical weathering etc. Our knowledge of the timescale for fragmentation of today's and future plastic litter based on other sources than petroleum will also be improved by the studies in WP4. This will be achieved by studying a wide range of petroleum-based, recycled, bio- and biodegradable plastics of the types and forms most frequently identified in marine litter, including polyethylene, polypropylene and polyester packaging, polyethylene and polystyrene single-use cups, nylon and polypropylene fishing lines and nets and polyethylene and poly lactic acid carrier bags. The processes of chemical, physical and biological degradation, dispersion and relocation of plastics will be examined both under controlled, laboratory conditions and by exposing plastic samples to natural surface waters and sediment at the National Museum of Denmark's coastal research site at Skælskør Fjord in Denmark.

Today, degradation of plastics is frequently described in the published literature either in terms of physical-chemical -or biodeterioration depending on the expertise and interests of the researchers involved. Because degradation of plastics in the marine environment is likely to be multifactorial, WP 4 will develop a novel Plastic Degradation Index based on the most significant markers of physical breakdown (fragment dimensions, tensile strength, surface damage and color), chemical breakdown (bond structure of polymer, adsorption of organic pollutants) and biological activity (biofouling) observed in plastic pollution. Determining the Plastic Degradation Index will use and add to the analytical techniques developed in WP2 and be applied to sample material collected in WP3.

WP4 aims to answer the questions; how many years do secondary microplastics take to form from plastic litter, which internal and external factors have greatest influence in the process and do all plastic types, both petroleum-based and new generation have the potential to form microplastics? It will also establish and compare the distribution patterns of plastic pollution between water columns and sediment layers based on fragment dimensions and chemical compositions in order to determine whether internal or external factors have greatest influence over dispersion and deposition.

This scope comprises the following two research questions: 1) How do plastics degrade in the marine environment? and 2) Which processes are the most significant for dispersal and deposition of plastic fragments in the marine environment?

4.1 "How do plastics degrade in the marine environment?"

The first step to investigating this research question will be to develop a novel Plastic Degradation Index by which the extent of degradation can be qualified and quantified. The Index will be formulated from the most significant indicators of physical breakdown (fragment dimensions, tensile strength, surface damage and color), chemical breakdown (bond structure of polymer, adsorption of organic pollutants) and biological activity (biofouling) detected on plastics exposed to the marine environment by the National Museum of Denmark's researchers and partners in *MarinePlastic.* All indicators will be explored for correlation with exposure conditions and exposure time using statistical approaches including principal component analysis [63].

The Plastic Degradation Index will be developed using as source material new polyethylene, polypropylene and polyester packaging, polyethylene and polystyrene single-use cups, nylon and polypropylene fishing lines and nets and polyethylene carrier bags and a unique reference sample set including well-characterized films, sheets, fibers and nets comprising polypropylene, polyethylene and polyamide exposed for up to 20 years in underwater sites in Denmark, The Netherlands and Italy. Because these plastic types encompass more than 90% of marine litter, the research will contribute to our understanding of the rates and pathways of degradation and of which polymer types, if not all, form microplastics in the marine environment. The Index will combine the spectroscopic and chromatographic techniques developed in WP1 with quantitative FTIR to follow the rate of chemical breakdown, evolved gas analysis to examine the presence of organic pollutants and X-ray fluorescence to detect the presence of inorganic polymer additives and metals adsorbed by exposure to the marine environment [84].

On obtaining the required permission from the local authorities, a plastic study collection comprising new petroleum-based, recycled, bio- and biodegradable plastics will be exposed in surface waters and buried in sediments in Skælskør Fjord and allowed to degrade in real time for at least the project period. Plastics will be securely mounted at surfaces within fine, 330 micron meshes to minimize the risk of them being released into the water column or being ingested by organisms. Plastics exposed in sediments will be mounted on glass fiber spears before being driven into the seabed. Sufficient quantities of samples will be exposed to allow for sampling both within and after the present *MarinePlastic* period. Conductivity (salinity) temperature, UV levels, turbidity and dissolved oxygen will be measured at the start of exposure and thereafter every 12 months. The study collection will be made available for cultural heritage and environmental science research as well as citizen science projects during and beyond the current project on application to *MarinePlastic*.

The plastic study collection will also be exposed to accelerated weathering in the laboratory where samples will be exposed to combinations of UV radiation, heat, salt and fresh water and mechanical action. Plastics will be sampled regularly and the extent of degradation monitored using the Plastic Degradation Index. This research will facilitate correlation between lifetimes of plastics determined using industrial weathering tests and real-time exposure.

A Postdoc based at NatMus will work closely with Y. Shashoua and AAU (evaluating analytical techniques in WP2 using the plastic study collection as reference samples) from the start of the project and for the first two years to develop the Plastic Degradation Index and apply it to the plastic study collection before and during accelerated weathering and real time exposure. The Index will then be applied to samples collected in WP3 in collaboration with J. Strand (AU).

4.2 "Which processes are the most significant for dispersal and deposition of plastic fragments in the marine environment?"

This research question will build on WP 4.1 by examining the factors that control movement of plastic litter at macro- and micro dimensions once formed by degradation. Microplastics can be considered as inert particles and their movement attributed to their size and shape alone. However, they may also be considered as reactive chemical composites that interact with their surrounding environment by forming weak forces and stronger chemical bonds. These two approaches suggest that relocation of plastics may be either controlled primarily by internal factors such as their chemical and physical compositions or external factors including wind, ocean currents, salinity and biological factors/biofilms.

In turn, new and degraded samples of petroleum-based, recycled, bio- and biodegradable plastics collected or produced by accelerated weathering in WP 4.1 will be exposed to weathering chambers or mecocosms designed and developed for the project in which water and air movement, temperature, salinity and biological factors can be varied [49]. The physical properties including dimensions and forms, polymer types, presence of organic and inorganic polymer additives of samples before and after exposure for various periods using imaging techniques, chromatography and spectroscopy. Principal Component Analysis will be used to explore the significance of internal and external factors to movement and changes in physical or chemical properties during exposure. Based on initial findings, a novel technique to monitor and map relocation of plastic fragments will be developed using staining techniques and fluorescence. Results from exposure in mesocosms will be compared using statistics to samples of fragments collected from various environmental compartments in WP 3 with respect to quantities, plastic type and physical properties.

A Postdoc based at NatMus will work closely with Y. Shashoua and J. Strand (AU) in Years 3 and 4 to apply the analytical and monitoring techniques developed by the Postdoc working on WP 4.1 in years 1 and 2 of *MarinePlastic*. The Postdoc will design mesocosms that represent various environmental compartments in discussion with all MarinePlastic partners together with a research period at the University of Patras to share Assistant Professor Hrissi Karapanagioti's experience of weathering chambers.

Synergies with other WPs

The causes and rates of fragmentation of plastic litter from macro- to micro dimensions and its subsequent mobility are fundamental to WP 3 and WP 5 in which its abundance, dispersion and impact are studied. Knowledge of the lifetimes of today's petroleum-based and tomorrow's bioplastic and biodegradable plastic litter and the plastics most likely to fragment in the various environmental compartments are essential to design effective strategies to minimize its impact on the marine environment. The analytical techniques developed in WP2 will be applied in WP4 and the plastic study collection will be shared with all WPs as reference samples.

Timeline and deliverables:

Timeline and deliverables	YEAR 1				YEAR 2				YEAR 3				YEAR 4			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
WP 4.1 How do plastics degrade in the marine environment?																
Collect plastics, expose to weathering and nature and study the real-time degradation of the plastics in the marine environment																
Complete development of Plastic Degradation Index and database of indices																
Qualify and quantify formation and degradation of marine plastic litter																
WP 4.2 Which processes are the most significant for dispersal and deposition of plastic fragments in the marine environment?																
Design and produce mesocosms to model exernal factors in dispersion of litter. Study the fate of plastic litter in the marine environment																
Determine Plastic Degradation Index to samples exposed to mesocosms from WP3																
Study the breakdown of new-generation vs. petroleum-based plastics in the marine environment																

WP 5 Ecological impacts (lead: RUC)

Directly involved: RUC: A. Palmqvist, F Kahn, K Syberg, 1 PhD, **DTU:** N.B. Harmann, T. G.Nielsen, 1 Postdoc (Sinja Rist), 1 PhD

This work package aims to explore the uptake and potential impact of plastic particles on key marine species and communities with the ultimate aim of providing knowledge for assessing the risk of nano- and microplastics in the marine environment.

State of the art

Microplastics (MPs) are present throughout the marine environment, including in the gut of marine organisms such as zooplankton [18,21], suspension-feeding bivalves [99], deposit-feeders [99] and fish (e.g. [55,6]). This has raised questions about the implications of MPs for marine organisms and ecosystems.

Effects of MPs at the individual level have been documented in worms [103], copepods [17] and bivalves [9], with reduced feeding, energy allocation and reproductive output as some of the physiological consequences. Whereas some studies have linked uptake of MPs to a potential for causing adverse effects (e.g., [101,103]), others have found no effects despite observations of particle ingestion (e.g., [50,39]). Impacts of MPs may go beyond individual organisms, it was recently documented that exposure of a marine benthic community to MPs may alter species abundance and diversity [37]. However, studies on potential MP impact on populations, communities and ecosystems, are limited [94,64,15].

In addition to potential ecological impacts there is increasing concern that MPs may be transferred to humans through food [79,56,15]. However, as the gut is normally removed from most marine organisms, such as fish, prior to human consumption, MP transfer will only happen if the particles are translocated from the gut into the tissue, or when entire organisms are consumed. Translocation of MPs from the gut to other tissues has been observed in some organisms [11,101,10,3], leading to a prolonged exposure of the organism and increased potential for food-web transfer.

A large proportion of published MP effect studies used short exposure durations, pristine MPs, and concentrations far beyond what is environmentally realistic [54,71]. By using pristine particles, the condition after weathering of the particle including formation of biofilms is consequently ignored. Weathering has been found to increase ingestion - possibly as a result of particle sorting based on increased nutritional quality [12] - and to modify adverse effects, for example through differences in leaching of polymer additives between pristine and weathered particles [67]. This discrepancy between experimental and environmental conditions results in limited understanding of how weathering state and properties of MPs with diverse sizes, shapes and polymer types influence their uptake, translocation, and potential effects. Filling this knowledge gap between experimental data and what organisms are actually exposed to in the marine environment is paramount to be able to evaluate the environmental risks linked to MPs.

Scope

The scope of WP5 is to improve the knowledge concerning the mechanisms and potential effects of uptake of MPs in marine food webs by investigating uptake processes, translocation into tissues and effects on organisms, populations and communities. In addition, an understanding of, to what degree, MP particles are capable of crossing the gut membrane into the tissue is of relevance for trophic transfer in general - and for human exposure through consumption of seafood in particular. The focus of WP5 will be on environmentally realistic exposure conditions in respect to concentrations, polymer types, particle shapes and weathering state. To address this scope, activities under WP5 focus on advancing our understanding of the mechanisms of uptake processes and potential translocation of MP particles within tissues.

Since ultimate protection goals for environmental risk assessment and management are ecosystem structure and function, population and community level effects will be assessed and may be used to indicate ecosystem impacts. This advanced understanding may be related to ecosystem services, which again will provide the required information needed to perform risk assessments as well as facilitate the implementations of societal- and regulatory measures. Through the work envisioned for WP5 we expect to identify marine aquatic species and communities that are susceptible to MP exposure as a result of particle uptake. By relating these hazards to measured environmental MP concentrations and types from WP3 and WP4, we aim to assess which species are most likely to be at risk from MPs in the marine environment.

This scope leads to the following two research questions: 1) What are the underlying mechanisms of MP uptake and translocation into marine organisms and food webs, and 2) Are marine MPs likely to cause organism, population and community level effects at environmentally realistic concentrations

5.1. "What are the underlying mechanisms of MP uptake and translocation into marine organisms and food webs"?

This research question will generate new knowledge on the interactions of MPs and key elements of marine food webs, answering important questions related to biological fate of MPs in the marine environment. In particular, we will quantify processes of MP uptake and transfer through marine food webs that will enable us to provide a better understanding of the fate of MPs in the marine environment. Qualitative investigations of localization, ingestion and uptake of MP in comparison with natural prey by zooplankton and fish larvae will be undertaken through application of state of the art high speed video microscopy [104].

As a first step to address this research question a thorough assessment of susceptible species will be undertaken. This review will include organism anatomy and physiology, including feeding strategies and normal food size spectra, to assess the likelihood and mechanisms for uptake of MPs from their environment. The experimental part will quantify ingestion rates, retention times of MPs in the digestive system and potential translocation from gut lumen into tissue by key players in marine food webs, preying on plankton in the same size range as MPs, i.e. copepods, suspension feeding bivalves and fish larvae. In addition, translocation of particles over fish gut epithelium will be explored using an *in vitro* fish gut sac model. Up until recently this model has been utilized with solutes, but recently its use with MPs has been demonstrated [51]. The work will provide insight into competitive uptake rates of natural prey, pristine MPs and biofilm coated and degraded MPs by the selected key organisms. Additionally, the same groups of organisms will be collected in the field and analyzed for MPs. For this purpose, optimized protocols for sample digestion (for isolation of MPs) and analysis will be developed.

The ultimate aims of research question 5.1 are to: i) identify the properties of MPs that are decisive for their ingestion by marine invertebrates and fish larvae, ii) understand the mechanisms of uptake and depuration in these organisms as well as the potential for translocation of MPs over gut epithelium into other parts of the tissue and eventually of the organism, iii) test the hypothesis that size is a major controller of the degree to which MPs translocate into tissue.

A PhD student at DTU starting from the beginning of the project, and a Postdoc at DTU, starting 3 months into the project, will be associated with this research question. In addition, F.R. Khan (RUC), N.B. Hartman (DTU), T.G. Nielsen (DTU), K. Syberg (RUC) and A. Palmqvist (RUC) will participate. In order to develop methods for detecting MP particles in tissues, the Postdoc will have a short research stay at AAU (WP2).

5.2 "Are marine MPs likely to cause organism, population and community level effects at environmentally realistic concentrations?"

Although protecting ecosystems are the focus of environmental risk assessment and management, few published studies have focused on MP impacts on higher levels of biological organization, such as populations, communities and ecosystems. Addressing higher level effects as part of this research question will thus provide important and essential new knowledge on the potential environmental impacts of MPs. To address this research question, the experimental part will include laboratory investigations of potential physiological impacts of MP ingestion by copepods, suspension feeding bivalves and fish larvae. In addition, individual level effects of a number of marine invertebrate species, with various different feeding strategies (as identified through RQ 5.1), will be examined under controlled long-term exposures to different sizes and/or types of MP particles at both environmentally realistic and extreme MP concentrations. The exposure concentrations as well as particle type(s) will be selected based on preliminary results from WP 3 and 4. Whereas effects on ecosystems and communities may be complex and expensive to address by empirical studies, and sometimes involve high uncertainties, effects on populations can be reliably extrapolated from organism level effects through the use of demographic or individual based models (e.g. [34]). Individual level effects observed in laboratory studies will be integrated into measures of effects at the population level using such population models. In order to address community level impacts, simple indoor mini mesocosm systems, containing marine invertebrates from different trophic levels, will be used to explore potential joint effects of competition, predation and MP presence on populations of selected species. Finally, observed effects will be compared to the model predictions of MP effects on populations.

The ultimate aims of research question 5.2 are to: i) identify properties of MPs that may lead to effects at organism to community level, ii) provide important new knowledge on possible impacts of MPs on higher levels of biological organization, e.g., populations and communities, which will eventually aid in predicting risk of MPs on ecosystems that are ultimate environmental protection goals for marine risk assessments.

A PhD student at RUC starting 6 months into the project will be associated with this research question. In addition, A. Palmqvist (RUC), K. Syberg (RUC), N.B. Hartmann (DTU) and T.G. Nielsen (DTU) will be involved in the work. In order to measure exposure concentrations in sediment and overlying water, the PhD student will have a short research stay at AAU (WP2).

Synergies with other WPs

To assess the risk of MPs as environmental stressors, knowledge of their presence, prevalence and distribution (exposure potential), and characteristics under real environmental conditions is needed. WPs 3 and 4 provide such information as well as data describing sources and persistence. WP2 provides methods to measure MPs also in complex matrices such as tissues and sediment, which is necessary to quantify uptake, translocation and exposure concentrations. Feeding into this WP, the previous WPs will provide some of the information necessary to identify the hazard using environmentally relevant exposure conditions. Information on more susceptible species can additionally feed into a discussion within WP1 regarding the use of alternative test organisms in regulatory testing.

Timeline and deliverables:

Timeline and deliverables	YEAR 1				YEAR 2				YEAR 3				YEAR 4			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
WP 5.1 What are the underlying mechanisms of microplastic uptake and translocation into marine organisms and food webs?																
Critical review on susceptibility of marine species to uptake of microplastics		■	■	■												
Uptake and translocation of microplastics in marine invertebrates; influence of intrinsic particle properties				■	■	■	■									
Trans-epithelial uptake of nano- and micro-sized plastics as well as associated chemicals determined using piscine isolated in vitro gut sacs			■	■	■	■	■	■	■	■						
WP 5.1 and 5.2 Studies in the interface between the two research questions																
Handling, ingestion, excretion and impact of microplastics on copepods with different feeding strategies	■	■	■	■	■											
Handling, ingestion, excretion and impact of microplastics by suspesion-feeding bivalves				■	■	■	■	■	■							
Handling, ingestion, excretion and impact of microplastics by fish larvae								■	■	■	■	■				
Trait-based analysis of what invertebrate species are most likely to be at risk from microplastics in the marine environment							■	■	■	■	■	■	■	■	■	■
WP 5.2 Are marine microplastics likely to cause organism, population and community level effects at environmentally realistic concentrations?																
Comparison of organism level effects in marine invertebrates with different habitat choices and feeding strategies			■	■	■	■	■									
Extrapolation of organism level effects of microplastics to population level effects						■	■	■	■							
Potential adverse effects of microplastics in marine food chains – small scale mesocosm studies									■	■	■	■	■	■	■	

WP 0 Leadership and joint activities (lead: AAU)

Directly involved: **AAU:** J. Vollertsen, 1 academic project coordinator, **RUC:** K. Syberg

This WP organizes and plans activities across the other WPs, administers the center, coordinates workshops and outreach, and provides feedback to the VELUX Foundation.

WP 0 will synergize the collaboration within *MarinePlastic* through cross-WP activities and by coordinating the involvement of international l partners. The tools are regular center meetings, annual scientific workshops and PhD courses organized in the frame of the partners. Within the first month of the project, a kick-off workshop will be held to discuss details on research strategies and to concretize the work process. After approximately 9 months, the first project workshop will be held to follow up on the progress and discuss detailed scientific and outreach aspects. Such workshops will be held annually . At the end of the project, a final workshop/conference will be held to disseminate the results gained in the project, and to gain perspective on future developments.

Throughout *MarinePlastic* a dissemination website is maintained where progress and results are continuously published.

1½ years into the *MarinePlastic* an exploratory field trip with the research ship Dana will take place. This will not just result in scientific results, but will also be an important dissemination event which we will use to increase focus on the microplastic issue.

To ensure smooth and effective collaboration, a part-time academic center coordinator will assist the center leader and the five WP leaders in the continuous management and coordination of the different activities, deliverables and milestones of *MarinePlastic*.

Timeline and deliverables:

Timeline and deliverables	YEAR 1				YEAR 2				YEAR 3				YEAR 4			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Website development	D															
Website maintenance																
Coastal sampling event for all partners				D												
Research cruise with Dana							D									
Kick-off and final workshops	D															D
Project workshops			D				D				D				D	
Project coordination and management																