Microplastic adherence to common species of macroalgae found throughout Scottish coastal zones

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Microplastic adherence to common species of macroalgae found throughout Scottish coastal zones

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ABSTRACT

Microplastics are a ubiquitous, persistent, and concerning waste material, produced globally to fulfil many social, economic, and industrial needs. Over the last forty years studies have assessed their abundance within the environment and their growing effects on marine life. However, until recently, relatively little emphasis has been given to studies assessing their effects on primary producing marine species such as macroalgae, better known as “Seaweeds”. The present study aimed to rectify this by assessing the potential microplastic contamination (i.e., the introduction of a substance as a pollutant) acting on common species of macroalgae found throughout the Scottish coastal zone. A baseline time series data set was created to assess the microplastics found within water samples in addition to sampled *Fucus vesiculosus* fronds. Additionally, a positive control experiment was created to assess if microplastics adhere to samples of *F. vesiculosus.* Results found microplastics were present in all collected samples, however no discernible correlation could be made regarding the time series data. Furthermore, it was concluded that microplastics do adhere to *F. vesiculosus*. Following the initial controls, experiments were created to evaluate the potential for multiple macroalgae species to act as contamination hosts, in addition to testing if damage inflicted upon the algae effected the adherence. It was found that all species selected had proven potential to act as microplastic contamination hosts. Additionally, if algae sustained damage, the contamination could be up to nineteen times higher. The final set of experiments tested the potential of multiple polymer types to act as contaminants, with results concluding all selected polymer types yielded contamination. The study herein therefore concluded that microplastic contamination within the marine environment is a real threat to valuable primary producing species and recommendations to further research should be made as suggested within the concluding statement.

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Abbreviations:

Polyethylene = (PE)

High density / Low density Polyethylene = (HDPE/LDPE)

Polystyrene = (PS)

Polypropylene = (PP)

Polyethylene-terephthalate = (PET)

Polyvinylchloride = (PVC)

Grams per centimetre = (g/cm^3)

Per dry weight = (/dw)

Metre squared = (m^2)

Micrometre = (µm)

carbon dioxide = (CO_2)

IMTA = (Integrated multitrophic aquaculture)

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Thank you to Ellie wood for sharing the laboratory apparatus for the main bulk of the study. I would also like to thank Lola Paradinas for a seamless sharing of the FTIR laboratory, a superb explanation of how the apparatus works and for sharing her knowledge to aid my analysis of all samples collected.

Finally, I would like to thank my wife Alexandra for her constant encouragement, reassurance and help throughout the course of this study.

I declare that this thesis is the result of research conducted by Nicol Ferguson between the months of October 2019 – April 2021. I also declare that the results of this masters have not been submitted in any previous application for a degree. Outside sources of information have been referenced appropriately.

Signature:

Nicol Ferguson

Covid – 19 Impact Statement

Covid-19 impacted the present study during the main fieldwork associated within the first set of the experimental chapters. A time series was being worked up to gather information on fluctuations of microplastics adhered to macroalgae over time. However, with the lockdown restrictions placed by the government, fieldwork and lab access was unable to continue. The lockdown restricted lab access to vital equipment needed for processing and analysis of all samples collected up to that point. This resulted in a total halt to all sample collection and lab work that lasted the duration of lockdown. Upon return to the laboratory strict social distancing guidelines were in place. This impacted the speed at which samples could be processed as most equipment used within the study was shared with other students or staff. Certain labs such as the FTIR laboratory had a single occupancy rule, resulting in samples that were analysis long after initial collection. Due to the slow rate of analysis and the difficulty getting assistance during field work (two persons required when in the field) the samples collected and analysed were selected due to their priority within the study to ensure the data collected was relevant. With this selection process in place side experiments that would have been conducted for broader use within the study were not able to be conducted.

Had Covid-19 not occurred, the initial time series would have been completed on the selected time scale and all additional experiments would have been conducted without hindrance by means of social distancing. Without the pandemic more time could have been spent within the FTIR analysis portion of the thesis, with further training and better understanding of the equipment further hypothesis could have been tested. In addition, sample sizes would have been increased given the ability to work as normal. Without the pandemic the lab work could have commenced on time and the thesis would have comfortable fit into the initial timeframe.

During the lockdown to ensure time was used proactively, a large portion of all the chapters was completed. This included the development of methodology; analysis technique sections and further reading was conducted to better understand the available literature. Therefore, by the end of the lockdown most introductory sections were completed with all methodologies created and ready to be implemented. Following the return to the laboratory, a plan was created with

other students to ensure lab work could be conducted following all distancing rules. However due to the pace at which fieldwork and analysis was able to proceed an extension was required to ensure the thesis was written in the proper manner with additional experiments conducted where necessary. With the extension of my registration, cooperation of other students and time utilized over lockdown, the thesis has been completed in a realistic timescale given the restrictions placed by the government regarding Covid – 19.

Chapter 1: General Introduction

1.1: The growing importance of plastics

With the invention of Bakelite in 1907 by Leo Hendrick Bakeland (Crespy et al. 2008) there has been a growing interest in all aspects of plastic materials, from their development through to their use. The various properties of plastic, ranging from low thermal and electrical conductivity, resistance to corrosive chemicals and durability, has enabled humans to utilise plastics in almost every aspect of life. Plastics can now be used as a preservation technique for food products, acting as an oxygen and pathogen barrier (Mullan, 2002; Andrady and Neal, 2008). Additionally, the application of single use plastic packages for sterile implements in the medical profession has allowed hospitals to improve aseptic techniques, thereby reducing the chance of infection (Chauhan et al. 2019). Clothing and textile industries now utilise polymers such as polyester for producing clothes, carpets and fabrics that act as insulators for our homes and bodies. This coupled with the ever-growing rate at which technology has advanced because of plastic material development, resulted in the many polymer types becoming widely available. As a result, plastics are regarded as ubiquitous across the face of the earth, with this ubiquity causing Waters et al., (2016) to state that “plastics are now a stratigraphic marker of the Anthropocene”.

However, with the increase in plastic production from the 1950s to the present day there has been a notable increase in plastic waste produced. Globally an estimated 359 million tons of plastic waste was recorded in 2015 and 381 million tons in 2018 (Ritchie and Roser, 2018; PlasticsEurope, 2020). This waste issue is compounded due to the nature of plastic being a long-lived and durable material. Ritchie and Roser (2018) showed that between1950 to 2015 there was a cumulative global plastic production of 7.8 billion tons, with an estimated global production of 33 billion tons expected by the year 2050 (Lebreton and Andrady, 2019). This combined with the longevity of the material would suggest that plast waste is likely to be a recurring issue for many decades to come. Recent studies have begun to focus on the issue of how plastics can be broken down and the resulting waste utilised (Austan et al. 2018; Akdogan and Guve, 2019; Wu et al. 2019). This is challenging as plastics do not breakdown easily in the natural environment, instead breaking up into ever smaller and smaller fragments.

As microplastics were found to be a ubiquitous anthropogenic waste product, studies have been conducted to ascertain the severity of the impact on the environment and varying species. Plastic waste has been found globally as observed in samples taken from the deep sea (Courtene-Jones et al. 2017, 2020; Jamieson et al. 2019), polar regions (Barnes et al. 2010), mid ocean gyres (Carson et al. 2013) and inshore marine environments (Blumenroder et al. 2017). There has been a concerted effort to investigate how anthropogenic waste effects wildlife, with microplastics being found throughout the marine and terrestrial environments. Recent studies conducted have sampled a wide variety of marine species and birds (Andrady, 2011; Farrell, 2013; Reynolds and Ryan, 2017), which has resulted in a large literature base for organism based microplastic studies. However, there has been relatively little regarding the interaction of microplastics with macroalgae, which can be a keystone species in many environments (Yesson et al. 2015).

1.1.1: Origin of microplastics

There are two main pathways for the introduction of microplastics into the marine environments, universally classified as “Primary” or “Secondary” microplastic origin (Figure 1) (Thompson et al. 2004; Andrady 2011; Cole et al. 2011; Jiang 2018; Frias and Nash 2019; Galafassi et al. 2019). Plastics that are manufactured to be of microscopic size are defined as primary microplastics (Cole et al. 2011). These can range considerably in size due to their historic use in cosmetic and household products (Zitko and Hanlon 1991), from microbeads within cosmetic scrubs (<1mm) to virgin plastic production pellets (2-5mm) referred to as nurdles (Turner et al. 2019). Due to the global demand for nurdles being utilised as an intermediate plastic for secondary macroplastic production, loss and incorrect disposal causes introduction of these particles to the marine environment through improper transport practice. This results in nurdles becoming the most pervasive form of primary microplastic found within intertidal sediment surveys (Turra et al. 2014).

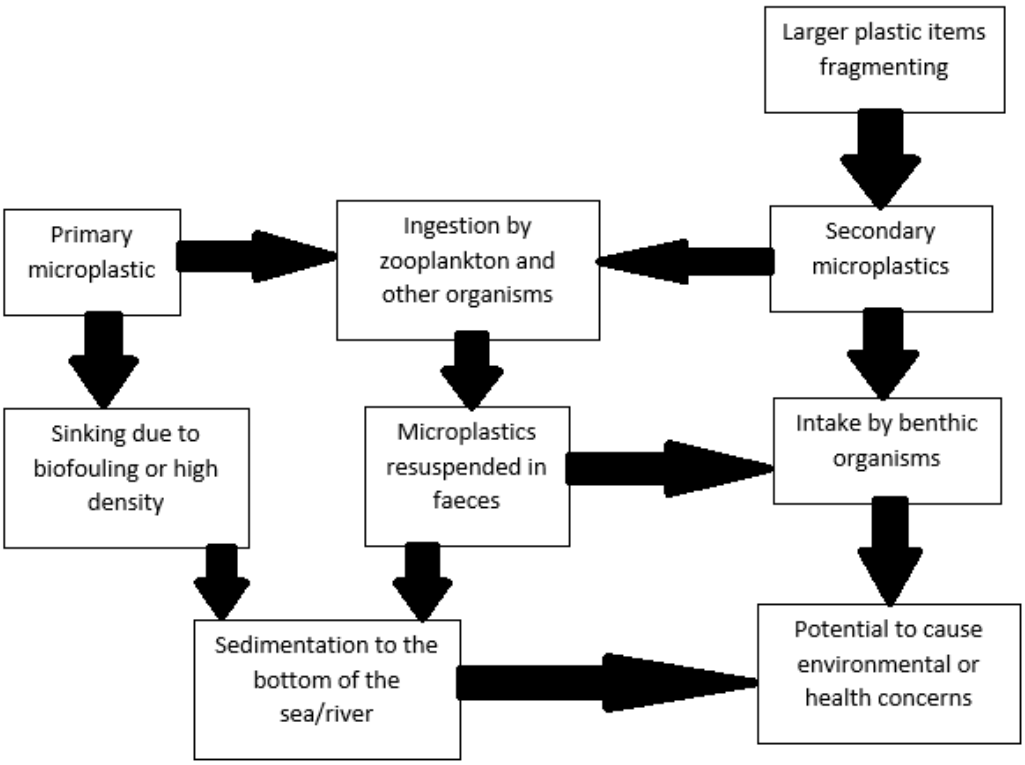


Figure 1: Primary and secondary plastic pathways within the environment.

Secondary microplastics are formed via the degradation of larger plastic items (Galfassi et al. 2019). The fragmentation of these plastics into smaller pieces over time can be a result of various means of degradation. These include biodegradation by microbes, photo-degradation via exposure to light (a reaction of the polymers chemical structure to UV-B radiation), thermo-oxidative degradation by oxidative breakdown at moderate temperatures, thermal degradation when exposed to high temperatures and hydrolysis when exposed to natural seawater (Andrady 2011). This causes a chemical change that reduces the molecular weight of the plastic thereby reducing its mechanical and structural integrity. The wide variety by which secondary microplastics can be formed has led to the potential for every piece of plastic waste becoming a source of pollutants. This is particularly true if the plastic items are located in regions that are considered a more reactive site for degradation, such as beaches (Andrady 2011).

1.2: Defining microplastic size

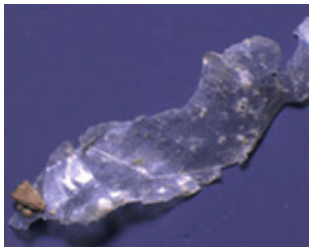
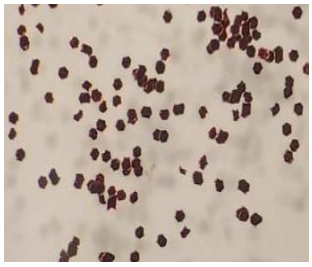
"Microplastics" (Thompson et al. 2004) are microscopic pieces of plastic that can be found within the sediments, water column and biota of the marine environment. The term microplastics as coined by Thompson et al. (2004) does not however give enough detail to allow comparison between studies, and therefore Arthur et al. (2009) amended the description by adding an upper size limit; stating *"plastic particles smaller than 5mm"*. This addition allowed comparable studies to be conducted as researchers now had a definable size to work down from. Further to these descriptions the joint group of experts on the scientific aspects of marine environmental protection (GESAMP) added a lower size limit, stating *"plastic particles <5mm in diameter, which include particles in the nano-size range (1nm)"* (GESAMP, 2015). By 2018 there was further addition to the literature by Gigualt et al. (2018) stating that *"nano-plastics exhibit a colloidal behaviour within the size ranging from 1nm to 1µm".* This addition gave a lower limit of 1µm to microplastics, further allowing Frias and Nash (2019) to combine all the above amendments into one cohesive statement to describe microplastics for future research. *"Microplastics are any synthetic solid particle or polymeric matrix, with regular or irregular shape and with size ranging from 1µm to 5mm, of either primary or secondary manufacturing origin, which are insoluble in water"* (Frias and Nash 2019). As this statement best describes all aspects of microplastics to date, this project will use it as the standard definition henceforth.

1.1.3: Shape of microplastics

Due to the multiple differences in degradation patterns and source materials, microplastics can have a range of shapes. Researchers now refer to the five main forms of microplastics as: microbeads (small, rounded beads), nurdles (pre-production pellets), fibres (sections of plastic ropes and strands), foams (highly aerated fragments) and fragments (generally any section of plastic that does not fall into the above categories) (Hidalgo-Ruz et al. 2012; Wu et al. 2019). With the exception of nurdles as they are virgin pellets used in the production of thermoplastics (Hidalgo-Ruz et al. 2012; Turra et al. 2014), the four remaining shapes most commonly found can be a result of secondary formation via degradation of macroplastics (Table 1). The shape and surface area of the

plastics can vary hugely between types, with fragments potentially having large surface areas and microbeads having low surface areas (Ryan 2015). The surface areas of microplastics are instrumental in both the settling velocity of the particles and the suspension rates within the water column itself. Karami (2017) found that plastics with smaller surface areas tended to stay within suspension due to an increase or decrease in biofouling, while nano-scale plastics exhibit random motion through the water column (Gigault et al. 2018). Furthermore, depending on the terminology used by different studies to describe shape, microplastic fibres are often found as the most abundant shape collected within intertidal sediments, most likely due to the global fishing and aquaculture nets used within the industry (Blumenroder et al. 2017).

Table 1: Microplastics examples commonly found within the marine environment. Images = Microbeads (South China Morning post, 2016). Nurdles and Foams (Wu et al, 2018). Fibres and Glitter (collected by present author).

Plastic From	Origin	Examples
Microbeads	Used in personal care products – toothpaste, face cleanser etc.	
Nurdles/pellet/granule	Pre-production pellets used within manufacturing of plastic goods.	
Fibres	The most common type of microplastic generated from clothing items.	
Foams	Used in food containers, packaging material for fragile goods and insulation.	
Fragments	Smaller plastics derived from larger plastic products via degradation.	
Glitter	Product used in design, celebrations, and plastic toys. They have a complex design and can act as flag items.	

1.1.4: Microplastic density

The density of a microplastic particle will also affect their position within the water column and thus determine what fauna it is likely to interact with (Andrady 2017). Polyethylene (PE), polystyrene (PS), polypropylene (PP), polyethylene-terephthalate (PET) and polyvinylchloride (PVC) are extensively used throughout industry (Andrady 2011), with PE and PP accounting for 30% and 27% of the globally produced resin used in manufacture in 2015 alone (Andrady 2017). Research conducted by Law et al. (2010) and Zhang et al. (2015) concluded that PE, PP, and expanded PS microspheres were the most encountered microplastics when sampling beach sediments and surface waters collected from coastal zones. However, as plastics are used extensively in many industries, chemical additives and fillers are used to imbue certain qualities to aid in longevity, structural strength, and UV resistance. This leads to the composition of the microplastics potentially having a greater density than that of seawater (1.025 g/cm^3) (Andrady, 2011) (Table 2). Low density plastics such as PE and PS can be found at the sea surface due to their positive buoyancy while PVC and PET are negatively buoyant and tend to sink towards the seafloor. As stated previously, biofouling (the colonisation of a material by microscopic organisms) can alter the buoyancy of a microplastic. Lobelle and Cunliffe (2011), undertook a study and submerged polyethylene food bags two metres below the sea surface for three weeks in order to assess biofilm formation. The study revealed that biofilm formation caused the plastics to become less hydrophobic and more neutrally buoyant, resulting in suspension within the water column. Additionally, de-fouling (the process of organisms and organic material being removed from a surface) by microbial foraging organisms can also occur, causing a resuspension effect. The combination of fouling and de-fouling can result in microplastics being suspended indefinitely within the water column (Andrady, 2011. Wright et al. 2013, Blumenroder et al. 2017).

Table 2: Density of waters (fresh and marine) and commonly found polymers. Adapted from Andrady, (2011) and GESAMP, (2015).

Matrix or Polymer	**Density (g/cm^3)**
Distilled water	1
Seawater	1.025
Low density Polyethylene – LDPE	0.91-0.93
High density Polyethylene – HDPE	0.94
Polypropylene – PP	0.90-0.92
Polystyrene – PS	1.04-1.09
Polyvinylchloride – PVC	1.16-1.30
Polyamide – PA – Nylon	1.13-1.15
Polyethylene Terephthalate – PET	1.34-1.39
Polyvinyl Alcohol – PVA	1.19-1.35

1.1.5: The abundance of microplastics in the marine environment

With the growth of plastic production over the last century there has been a marked increase in plastic litter within marine environments with areas such as subtropical gyres having seen increased plastic contamination since the 1970s (Li et al. 2016). These have been termed “hotspot areas” (Good et al. 2010) and are considered areas of high deposition or accumulation (either tidal or wind driven accumulation). Various studies undertaken have found that ports or harbours situated near plastic production facilities have an increased contamination level throughout both sediment and water samples. Samples collected along the coast of Hong Kong found mean microplastic particles of 5595/m^2 (Fok and Cheng 2015). Within intertidal sediment-based studies there has been a consensus that the area known as the recent strand line is a microplastic hotspot within localised beaches (Blumenroder et al. 2017). Ivar do Sul et al. (2009) conducted a study on the beaches of Fernando de Noronha Island (Brazil), identifying marine debris in sediment samples from 11 beaches out of a total 15 beaches. Sixty percent of all the debris found within the study was plastic pre-production virgin pellets (used in various industries), strikingly far from any production facility. This supports the hypothesis that global hydrodynamics and weather patterns aid in transportation of anthropogenic litter

whilst also confirming the statement made by Barnes et al. (2009) that plastics within the marine environment will “accumulate and persist”. Plastics, and by extension microplastics due to their specific design for longevity and strength, are extremely resistant to rapid degradation within a natural setting, leading to their continued persistence.

The United Nations development goals (UN environment, 2021) has therefor set out resolution 3/7 to try and mediate the growing plastic pollution issue the globe is encountering. Developed from the 1/6 plan looking at the knowledge gaps within marine litter and microplastics and the 2/11 plan “assessing the effectiveness of relevant international, regional and sub-regional governance strategies and approaches”. The 3/7 plan looks to “explore” the issues in combating microplastic pollution, while identifying regional responses and how they can be feasible and cost effective. Currently the United Nations clean seas project has gathered 54 countries with the aim to reduce waste entering the oceans. Additionally, to address the microplastic waste certain policy recommendations have been made. They are as follows:

- Phase out microbeads
- Drastic reduction/ban of single-use plastics
- Short-term: waste management, Long-term: upstream reduction
- Internationally agreed definition of biodegradability (for plastics)
- Harmonization and standardization of methods
- Gaps/solutions for governance frameworks

With these recommendations met over the next number of years, the abundance of microplastic pollution may be combated and decreased leading to cleaner waters by means of lesser abundance.

1.1.6: Interactions with marine and aquatic species

The origin, size, shape, and density of microplastics will contribute to the interactions they have with all marine organisms. Studies have highlighted the issue of ingestion by certain species that are highly utilized for human research or consumption. *Arenicola marina* (lugworms) and *Mytilus edulis* (edible mussels) have both been observed ingesting microplastics in the size ranges of 10µm -

100µm, with certain individuals ingesting even larger particles (Van Cauwenberghe et al. 2015). This ingestion of plastics has resulted in studies looking at marine invertebrates such as bivalves, polychaetes, and echinoderms (Graham and Thompson 2009; Farrell and Nelson 2013). The finding suggested that there are two main methods with regards to the introduction of particles into the marine food webs; direct (uptake of microplastics as a mistaken food item) and indirect (microplastics ingested via predation on lower trophic levels), with indirect modes being common within marine food webs (Li et al. 2016). Further studies within the last five years have begun to focus on primary producing organisms, such as seaweeds, seagrasses, and aquatic plants, as these are key species utilized by many other organisms within their environments. Research conducted on *Fucus vesiculosus (F. vesiculosus)* (Bladder wrack) by Gutow et al. (2016) found that micro polystyrene beads, fragments and polyacrylic wool fibres bound to the distal tips of the algae and were ingested by the common periwinkle, *Littorina littorea (L. littorea)*. Similar studies conducted by Goss et al. (2018) researching the seagrass *Thalassia testudinum* and Mateos-Cárdeans et al. (2019) investigating the aquatic duck weed, *Lemna minor (L. minor)*, also confirmed that primary producers were being contaminated by microplastic litter. These results suggest that the interactions microplastics have with organisms can start at primary food sources and migrate upwards.

1.2: Macroalgae

1.2.1: Importance of macroalgae

Macroalgae, commonly referred to as seaweeds, are macroscopic marine organisms, often grouped together within one of three sub-groups: Red, Brown or Green depending on photosynthetic pigments. They can be found throughout the coastal shores and shallow seas of the world with ~6% found around the coasts of Britain (Bunker et al. 2017). Current estimates indicate that there are around 6,500-7,000 Red, 2,000 Brown and 1,500-1,700 Green macroalgal species (Algaebase, Bunker et al. 2017). Seaweeds hold high economic and ecological importance in the trophic food webs and are one of the, if not the, most important primary producers of the oceans, acting as a keystone species in some trophic and temperate systems. Regarded as a habitat for various species of fish

and crustaceans, they act as nursery habitats for juveniles (Lorentsen et al. 2010), increasing the survival of offspring and ensuring the prosperity of a population by acting as “ecosystem engineers” (Teagle et al. 2017).

1.2.2: Origin and species

Globally, oceans, seas and water courses cover 72% of the planet’s surface, and play a fundamental role in the earth’s temperature, atmosphere, and sequestration of carbon dioxide (CO_2). Ninety six percent of all water on the planet is seawater, which can dissolve CO_2 sixty times more effectively than the atmosphere and three-thousand times more efficiently than sequestration via sedimentary rock formation (Sudhakar et al. 2018). The global coverage of marine waters is also the native environments of one of the planets best group of primary producers, seaweeds. Also referred to as macro- or micro- algae, photosynthetic, multicellular eukaryotic plant-like organisms that have a far greater photosynthetic efficiency than terrestrial plants (Ashraf et al. 2016), thereby sequestering large quantities of CO_2. Depending on their group, seaweed can display a range of sizes, morphologies, and colonisation patterns, resulting in many estimates regarding number of species. Guiry (2012) gives a “conservative estimate” of around 72,500 species globally. However, this estimate contains both micro- and macro- algal species. Current estimates for macroalgae suggest that there are 10,500 species.

1.2.3: Size and Morphology of macroalgae

Macroalgae are a diverse group consisting of multiple species, leading to a significant difference in morphologies between the three groups. They do not share many traits with terrestrial plant structure, consisting mainly of a holdfast, stipe, and thallus (Figure 2), having relatively simple reproductive structures, cellular differentiation, and a lack of vascular tissues (Sudhakar et al. 2018). Cell division, however, can occur throughout the algae or be localised in a meristematic region (similar to terrestrial species), commonly the apex of the thallus or occasionally nearer the base (Hurd et al. 2014). The simple levels of differentiation allow many macroalgae to be either filamentous or built of united

or corticated filaments, allowing for larger more complex algal structures, such as *Codium amplivesiculatum,* which can grow many metres in length (Dawson 1950). Diversity within the groups can lead to growth formations ranging from, small highly productive turfs (Hackney et al. 1989) to large extensive forests of species such as *Laminaria sp.* (Graham et al. 2007). Macroalgal morphology can also be affected by various factors, such as light regime (Wing et al. 2007), nutrients (Mabin et al. 2013), tidal exposure and desiccation, resulting in differences in thallus length, vesicle size, frond width and stipe length (Blanchette et al. 2002, Thomsen et al. 2004). Depending on the intertidal or subtidal environment in which algal specimens are located, size can range from a few centimetres to several metres in length, with simple or complex structures.

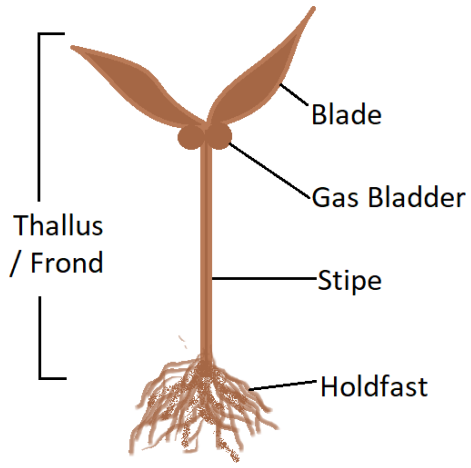


Figure 2: Diagram of simplified seaweed.

1.2.4: Macroalgal habitat

Seaweeds can exist as individuals or as vibrant communities combined with other species of marine organisms. These communities can affect and in turn be affected by the environmental conditions in which they are located (Hurd et al. 2014), often being found within vertical or horizontal “zones” or “bands” along the gradients of coastal shores. These “zonation patterns” have allowed numerous studies to be conducted on a small scale due to coastal environments being relatively easy to access with most species in a localised area. This is in contrast to terrestrial environments in which vertical and horizontal gradients may be hundreds to thousands of metres in length or height as highlighted by Raffaelli

and Hawkins (1996). Most coastal environments experience tidal fluctuations, depending on season, barometric pressure, and wind direction and together these all combine to form three different types of tidal patterns, diurnal, semi-diurnal and mixed. The most common type of tides within the north Atlantic region are semi-diurnal, which occur twice a day and have relatively equal high and low tides (Raffaelli and Hawkins 1996). Occasionally storm systems can combine with both on, and offshore, winds to produce very irregular tidal surges, referred to as atidal (Johannesson, 1989), however these are less common.

The intertidal (littoral) zone is commonly referred to as one of the harshest environments to inhabit due to the relatively rapid changes in tidal conditions (Hurd et al. 2014). Species that inhabit the littoral zone can be fully submerged and hydrated at high tide, with access to inorganic nutrients and attenuated light. However, when the tide recedes, these species are subjected to terrestrial like conditions (Norton, 1991) such as increased light intensity, wind induced desiccation and interactions with fresh water depending on the weather. Changes in salinity, temperature, nutrient supply and hydration can result in non-recoverable desiccation, leading to low photosynthetic performance and mortality. Due to the changes within the littoral zone, which can occur over seconds, minutes or hours, seaweeds grow in bands along the shore. These banding patterns are seen as a distinctive feature of the intertidal zone, globally (Connell and Gillanders 2007; Kaiser et al. 2011). The zonation patterns that are found on the intertidal rocky shores throughout the globe resulted in a universal zonation scheme being developed by Stephenson and Stephenson (1949). Their scheme is based on consistent biological zones that are found throughout the intertidal rocky shore, i.e., the *Fucus* zone, barnacle zone, kelp zone. Between the 1970sand 1990s there was an effort to try and understand the zonation patterns of the intertidal zone (Schonbeck and Norton 1978; Dring and Brown 1982; Chapman 1995). By 1980 Schonbeck and Norton (1980 a & b) had deduced that tidal emersion, desiccation and competition for light were the controlling factors in vertical distribution of fucoid seaweed on the intertidal rocky shore. Schonbeck and Norton (1980 a & b) concluded that by changing the position of *Fucus spiralis* on the shore, growth could be either inhibited (upper shore) or increased (lower shore), providing evidence that seaweeds can inhabit

zones on coastal regions in both an environmentally controlled way, or opportunistically through utilization of suitable conditions.

1.2.5: Economic importance of macroalgae

Seaweeds have been collected throughout human history, with records dating back more than 1700 years ago in China indicating that they were used as a valuable food resource for the population (Sudhakar et al. 2018). Today seaweed production is dominant in countries such as China, Indonesia, the Philippines, Korea and Japan. They are extensively used due to their high content of polyunsaturated fatty acids, carbohydrates, vitamins, minerals and dietary fibres (Mazumdar et al. 2014).

Seaweeds comprise of 90% water (fresh weight), and interestingly, many species of macroalgae also consist of high percentages of carbohydrates (polysaccharides) and proteins (dry weight), with a comparably low lipid percentage (Kraan 2013; Skjermo et al. 2014). This has resulted in macroalgae now being considered one of the futures most promising bio-feedstocks for the creation and production of renewable green energy. Recent studies have looked at the potential for microalgae to be utilized as a bio-stock for biofuels (Fasahati et al. 2015); however, relatively few studies have been conducted on macroalgae energy production (Sudhakar et al. 2018). Considered as third generation biofuels, micro- and macro- algae utilise seawater, nutrients, light and space that is not in conflict with terrestrial food production, thereby avoiding the energy-food land use dilemma posed by first and second-generation biofuels (Scaife et al. 2015).

Differences in biochemical composition also exist between macroalgal groups, with scientists learning to isolate different compounds such as acetone as early as World War I for use within cordite guns (Neusheul, 1989). Due to the complex proportions of proteins, storage carbohydrates and metabolites found within macroalgae, compounds such as alginate, mannitol, laminarian and fucoidan can be found within samples of brown species. while Xylose, glucose and galactose may be found in red species such as *P. palmata* (Van Hal et al, 2014). Green species such as *Ulva sp*. may also be found to contain compounds such as, glucuronic acid, rhamnose and arabinose (Van Hal et al, 2014). Therefore,

studies such as Belghit et al, (2017), have looked to address the need to understand better the biochemical properties of various species and their biochemical components, resulting in a species broad metabolic profiling study.

Recent studies in macroalgal utilisation are focusing on bio-plastic production. Seaweeds are being considered as a valuable bio-stock for bio-based plastics, mainly due to their lack of competition with terrestrial based food crops such as soybeans, potatoes, maize and corn (Fabra et al. 2017). Additionally, their rapid growth rate in comparison to such crops (Mekonnen et al. 2014) is considered more economically viable, thereby increasing use when compared to terrestrial species.

There is also a growing interest in the applications of bio-based products that can be utilised by processing macroalgal extracts. Products such as algin, carrageenan, agar, phycocolloids, etc. can all be used in the food industry, allowing for products to be made with emulsifying, thickening and stiffening properties (Khalil et al. 2018). Potash and biochar can be utilised within the fertilizer industry (Jacob et al. 2015) to enable increased crop yields and sources of vital nutrients such as potassium nitrate. The pharmaceutical and cosmetic industries have also recognised the possibilities of using seaweed alginic acid as an ingredient in products such as, shampoo, cosmetic creams and paints, while carrageenan can be used in medical applications, having blood thinning and antiviral properties (Shanmugam and Mody, 2000; Wang et al. 2011). Additionally, phycobiliproteins are used as fluorescent pigments within biomedical reagents (Sharif et al. 2015). In a review undertaken by Sudhakar et al. (2018), they concluded that macroalgae have the potential to be used as bio-feedstocks, biofuels and biochemical producers, enabling a more sustainable industrial model throughout sectors.

1.2.6: Cultivation of macroalgal species

The utilisation of seaweeds in general by coastal dwelling populations has likely occurred for many years throughout countries sharing a boundary with the sea (MacMonagail et al. 2017, Rebours et al. 2014). Macroalgae has been used for its various properties since AD 79 (within the UK), with inhabitants thought to be utilizing maërl as a natural fertilizer to aid in farming (Grall and Hall-Spencer,

2003). Dillehay et al. (2008) discovered the remains of nine different species of macroalgae from hearths in the Monte Verde (Chile) dating to roughly 14,000 years ago. The results of these multiple studies, therefore, highlight the fact that macroalgal species have been used by humans throughout history, being harvested along coast lines for community use.

Modern macroalgal utilisation has advanced with the adaption of marine based mariculture, Now exploited as a means to supply viable food, economic and resource-based products, the macroalgae aquaculture industry harvested just over 30 million tonnes (wet weight) of farmed seaweeds in 2016 alone, reaching an estimated worth of $11.7 billion (FAO, 2018). Farmed cultivation techniques can vary depending on species, location and approval from regulatory bodies within the country of origin. UK based marine aquaculture industry farms require approval before operations can commence. Firstly, a lease must be obtained from the landowner, which in the case of Great Britain is the Crown Estate. The second approval comes from a regulatory body e.g., the Marine Scotland Licensing Operations Team (MS-LOT), applicable when farming is undertaken in Scottish waters (Wood et al. 2017). The approval by each body ensures that the environmental aspects of the aquaculture installation have been considered and all necessary precautions are put in place (Wood et al. 2017).

Varying cultivation techniques are required depending on the species of algae used, environmental conditions, scale of installation and the depth of the water at the location used. The most common method of cultivation referred to as line cultivation, requires ropes of varying lengths that are seeded by the attachment of algae propagules to be placed in parallel arrangements within the water column (Radulovich et al. 2015). Line cultivation can be off-bottom, submerged hanging lines or long lines (floating), however other methods include net cultivation, floating raft cultivation and tank or pond cultivation (Table 3). Each method involves the utilisation of natural resources such as available nutrients, light and dissolved CO_2 to produce a seaweed crop that is economically and industrially viable for the extraction of desired products (Kraan 2013; Skjermo et al. 2014; Fasahati et al. 2015; Scaife et al. 2015; Sudhakar et al. 2018).

Table 3: Macroalgal cultivation techniques commonly used globally.

Cultivation Technique	Description
Line cultivation – Off Bottom	Planting species close to shore and seabed, normally within 0.3m of water at low tide. Used for small or frequently harvested species.
Line cultivation – Submerged hanging lines	Planting at mid-water depth near shore, submerged in several metres of water at high tide with some exposure at low tide.
Line cultivation – Long lines	Planting near the surface with seaweed submerged slightly in a few metres of water. This method can occur in any depth of water; however, anchoring is necessary.
Net cultivation	Seaweed propagules are placed in net bags and slightly submerged within a given water depth or can be anchored at a given location.
Floating raft cultivation	Planting seaweed propagules on rafts made of lines and a rigid frame. Placed in surface waters.
Tank or Pond cultivation	Culture within containers/ponds that have all relevant conditions controlled. Seaweeds can be free floating or tied to substrate. Used for freshwater species and delicate species.

1.2.7: Microplastic interactions with macroalgal and marine species

To date very few studies have been conducted looking at macroalgae as a possible vector for microplastic transfer into benthic trophic food webs, with even less studying the specific interactions between macroalgae and microplastics themselves. Bhattacharya et al. (2010) conducted a study with two microalgal species *Chlorella* sp*.* and *Scenedesmus* sp. investigating the interaction of charged microparticles of plastic. Although they were not focussing on macroalgal species, their study had interesting results highlighting that microplastics do interact and adhere to algae. Gutow et al. (2016) conducted an experiment to investigate whether microplastics were taken up by the marine herbivore *L. littorea* while feeding on the common macroalgae *F. vesiculosus* after contamination with various microplastics. The result of the study showed that microplastics were transferred from marine primary producers to primary consumers via ingestion, however, excretion and retention were debatable. While the study addresses a valid question, little attention was given to the underlining issue of, microplastic/macroalgae interaction. Following the publication of the Gutow et al. (2016) research, further scientific studies have been conducted. Sundbæk et al. (2018) proceeded to conduct an experiment from a food safety aspect, as macroalgae are commonly utilized as a food source. Again, using the common seaweed, *F. vesiculosus,* PS microplastic particles were added as a contaminant and subjected to testing to find an industrially relevant method by which to remove them. The attempted removal of the PS microplastics was successful at 94.5%, however this raised questions regarding Gutow et al's. (2016) methodology. Sundbæk et al. (2018) found higher levels of microplastic abundance closer to the cut sections of the distal tips, presumably due to entrapment by alginate and other compounds exuding from the cut. The recommendation from this study was to exclude microplastics within a certain proximity of the cut site from further data sets (Sundbæk et al. 2018). With growing interest in this field, further studies were undertaken by Goss et al. (2018) using the seagrass *Thalassia testudinum* as a potential vector for microplastics entering food webs. Their results showed that microplastics were found on 75% of the *T*. *testudinum* sampled*,* with macro-herbivores selectively eating grasses that had higher epibiont densities (Goss et al. 2018). That study hypothesised, that microplastics could be adhering to the surface of *T*. *testudinum* due to

surface morphology changes via encrusting epibionts, and biofilms. It also raised the issue that certain species of primary producers which act as nursery habitats, are potential pathways for the introduction of pollutants into juvenile organisms, thereby compounding the toxicity potential of microplastics as they travel up the trophic levels. Recently Mateos-Cárdeans et al. (2019), contaminated the aquatic plant, *L. minor* “Duckweed”, with PE microspheres, to be given as food to the brackish water species of amphipod, *Gammarus duebeni.* Results once again showed that transfer from the primary producer to the consumer occurred, however there was no visible impact on either the mortality or mobility of the organisms. From these studies has come a consensus that further investigations are required into the study of microplastic contamination of important primary producing plant-like organisms.

1.3: Aims of the project

Due to there being a relatively high number of studies conducted on microplastic interactions with marine organisms, and relatively few algal studies, the present study looked to combine the fields of microplastic contamination and environmental macroalgal sampling. Additionally, with the importance macroalgae play within the marine environment, the experiments conducted herein looked to expand on the questions asked by Bhattacharya et al. (2010), Gutow et al. (2016), Goss et al. (2018), Sundbæk et al. (2018) and Mateos-Cárdeans et al. (2019) regarding the combination of research. The resulting experiments were thus created to answer the five research aims below.

1. Do microplastics adhere to the common seaweed *F. vesiculosus*?
2. Do microplastic concentrations within water samples and adhered to macroalgal surfaces fluctuate over time?
3. Are common species of macroalgae found within Scottish waters at risk of microplastic contamination?
4. Does surface damage on the macroalgae cause an increase in microplastic adherence, when compared to undamaged whole specimens or control samples?
5. Do various plastic polymers adhere to *F. vesiculosus*?

Chapter 2: Microplastic contamination of the common species *Fucus vesiculosus*

2.1: Introduction

2.1.1: Methodologies investigating interaction of microplastics with seaweed

Since the rapid increase in microplastic studies, there has undoubtedly been a bias towards research focussing on microplastics (MPs) and their interaction with marine organisms (Graham and Thompson 2009; Farrell and Nelson, 2013; Van Cauwenberghe et al. 2015; Akdogan and Guven, 2019, Jones et al. 2020). This has resulted in one of the most important primary producing groups, "macroalgae" being somewhat overlooked. A few studies (Bhattacharya et al., 2010; Gutow et al., 2016; Goss et al., 2018; Sundbæk et al., 2018, Mateos-Cárdeans et al., 2019), have all looked to answer questions regarding the relationship between microplastics, macroalgae and similar species in order to answer knowledge gaps in this field of science.

Gutow et al., (2016) investigated the "experimental evaluation of seaweeds as a vector for microplastics into the food web" and this was the first published evidence that seaweeds were indeed a pathway for microplastics entering marine benthic herbivores. Although the study revolved around the transfer of microplastics from seaweed (*F. vesiculosus*) to herbivores (*L. littorea*), the underlying macroalgal issue was arguably more important than the organism-based study. The implications that microplastics could transfer effectively from the surrounding water to the seaweed and then into a grazing organism, and the consequences of such, was, until then, an unconsidered biological problem. In addition, Doyle et al. (2019) conducted a study within Galway Bay, Ireland, looking at microplastic abundance within *L. littorea*, however, that study looked at the microplastics already present within the specimens.

The method employed by Gutow et al. (2016) had a dual-purpose. Firstly, to investigate whether microplastics would adhere to *F. vesiculosus* and secondly, would the introduction of a tidal exposure simulation effect the microplastic adherence to samples of seaweed. The first experiment was conducted by collecting the distal tips of *F. vesiculosus* thalli that were from harbour constructions within the Weser estuary (Germany) and that were also free of

epiphytes and between 4-6 cm^2 in area. The method used consisted of placing three distal tips of the seaweed in a Petri dish with 40ml of 0.2µm filtered seawater to alleviate friction. A further addition of a prepared amount of one of three types of microplastics, either PS microbeads, fragments or polyacrylic wool fibres. With the addition of the microplastics to the Petri dish, agitation was then applied via a waving shaker platform (60 rpm at 10° tilt) for two hours to allow contamination to occur. Following contamination, the distal tips were removed and placed in a second Petri dish with 40ml of clean filtered seawater and agitated for a further two hours to allow to decontaminate. Following decontamination one of the three distal tips was randomly selected and removed for microplastic inspection. Inspection was conducted on the upper surface of the distal tip under a fluorescence stereomicroscope with microplastic contamination recoded as microplastics per mm^2.

Gutow et al's. (2016) second experiment within the study was similar to the first with the exception that after the contamination stage a tidal exposure simulation was applied. Following two-hours of contamination, 10ml of water was removed via pipette every 30 seconds to simulate the effect of a receding tide. Upon removal of all water the distal tips were left to air dry at room temperature for a further two hours, to simulate desiccation. At this point one of the distal tips was removed and the number of microplastics present counted as above. Following this first count it was placed into a second Petri dish with filtered seawater and two uncontaminated marked distal tips of *F. vesiculosus*. Once in the second Petri dish contamination was conducted by agitation for two hours. Following the decontamination stage, the contaminated distal tip was removed and the microplastics counted for a second time, with microplastics on the upper surface recoded as microplastics per mm^2.

Following the study by Gutow et al. (2016), a number of macroalgae-microplastic based investigations have taken place. Although relevant in this field they are not directly comparable to Gutow et al's. (2016) study due to species or environmental differences. Goss et al. (2018) performed a study using the seagrass *Thalassia testudinum* and analysed the microplastics found within the epibiont communities. More recently Mateos-Cárdeans et al. (2019) investigated the impact of contaminating *L. minor*, a species of aquatic duckweed, with polyethylene microspheres and its interactions as a food source for the

freshwater amphipod *G. duebeni*. Relative growth rate, chlorophyll a fluorescence, root length and uptake by *G. duebeni* were all assessed. Mateos-Cárdeans et al. (2019) therefore concluded, that microplastic contamination did not adversely affect *L. minor*, however, it did increase microplastic transfer to *G. duebeni*.

Furthermore, Sundbæk et al. (2018) used the study by Gutow et al. (2016), as a starting position for the creation of an experiment looking at the "sorption of fluorescent polystyrene microplastic particles to edible seaweed *F. vesiculosus*". The experiment utilized elements from the method created by Gutow et al. (2016), with similar microplastics being green, fluorescent PS microparticles (20µm in diameter). Seawater and *F. vesiculosus* samples were collected from Klampenborg (a suburb of Copenhagen, Denmark) and the distal tips were again used for the macroalgal component with an area of between 4-7cm^2. Results showed there was sorption of PS microparticles to *F. vesiculosus*, however it was also noted that further washing of the distal tips removed up to 94% of the microparticles. Sundbæk et al. (2018) also suggested the method utilized within their study could be modified in the future as it was noted that PS microplastic adhered to the areas around the cut site. The recommendation therefore was to avoid counting microplastics within close proximity to the cut site, thereby resulting in higher resolution data.

2.1.2: The importance of controls, why background data is important

The negative controls utilized within experimental designs, as seen in the study conducted by Sundbæk et al. (2018), are important due to their ability to be conducted by the researcher with certain variables excluded. They do not include the independent variables which are of wider interest in the larger experiments (Kinser and Robins, 2013) and allow an unbiased balanced approach to the analysis of the experimental data focusing on the dependent variables (Pithon, 2013. Nelson, 2017). However due to the inability within the present study to conduct true negative controls, a set of "Background" controls were taken. These allowed a baseline data set from which comparison could be made.

2.1.3: Positive control: Proving the methodology works

As indicated earlier, negative and background controls are a vital methodological tool when undertaking experimental procedures in a biological study. In addition, positive controls as indicated by Nelson (2017) are groups of variables that receive a treatment with a known (positive) result. This enables the methodology employed within the experiment to be tested for the desired result and repeatability needed within the larger experiment. With the addition of a positive control protocol, the method used within the present study can be further adapted once a positive and repeatable result has been gained. The combination of positive and background controls thereby strengthens the scientific rigor ensuring a well-considered and sound approach to the wider questions being asked within the study. Within the present study, the positive control procedures were based on the methodology utilized within the main experiment conducted by Gutow et al. (2016). Therefore, within the present study, the words “positive control” refers to the known outcome of the experiment allowing comparison to past studies.

2.1.4: Aims

The present chapter compared the methodologies used by Gutow et al. (2016), Sundbæk et al. (2018), Goss et al. (2018) and Mateos-Cárdeans et al. (2019) to create a combined experimental methodology. Additionally, experimental controls were designed and conducted to give a baseline time series data set for environmental based microplastic contamination. The following two questions were the focus of this element of the work.

- Do microplastics adhere to the common seaweed *F. vesiculosus*?
- Do microplastic concentrations within water samples and adhered to macroalgal surfaces fluctuate over time?

2.2: Method and materials for background Control procedures

2.2.1: Sample Location

Seawater was collected on the western facing beach of the Dustaffnage peninsula (N 56° 27’5.7, W 5° 26’44.9) (Figure 3).

2.2.2: Collection of seawater

Monthly seawater samples were collected using a five-litre high-density polyethylene (HDPE) plastic container, submerged 5-10 cm below the water’s surface. A single container was used at low tide and another at high tide. Once full, the container was sealed with a screw cap and transported to the laboratory for filtration and microplastic assessment.

The water sample was divided into five equal one-litre sub-samples. Filtration was conducted by filtering each sub-sample through a 70mm diameter 0.7µm mesh glass fibre filter using a vacuum pump to speed up the process, and the resulting filtrate discarded. After filtration, the filter paper was placed in a Petri dish; labelled and sealed with electrical tape to prevent contamination. Conducting filtration of each individual litre resulted in five pseudo-replicates for each tidal cycle assessment. Preliminary microplastic identification was conducted manually via visual inspection within the sealed Petri dish under a dissection microscope, with all suspected microplastics recorded as number found. After preliminary identification, the filters were stored within the laboratory for further assessment via Attenuated Total Reflectance – Fourier Transform Infra-Red spectroscopy (ATR-FTIR) analysis.

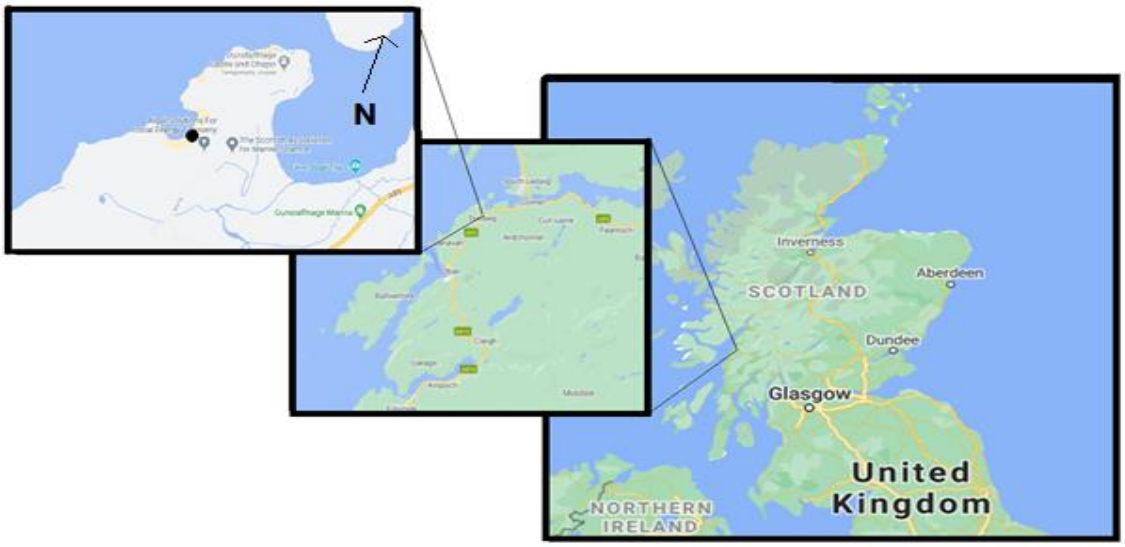


Figure 3: Map of control sample location within Scotland, magnified to Oban area, further magnified to Dunstaffnage bay. Sample location indicated via black dot.

2.2.3: Environmentally collected macroalgae examination

Environmental samples of *F. vesiculosus* were located and sampled at the same time and location as the seawater samples were being collected. The distal tips of the algae within 2m of the water sampling location were harvested and removed using dissection scissors. Six individual distal tips of 3-5 cm in length were placed in individual Petri dishes, labelled and sealed with electrical tape. The Petri dishes were placed in a cool box at ambient temperature and transferred to the laboratory. Microplastic identification and extraction was conducted manually via a dissection microscope, with all suspected microplastics collected recorded as number found. Microplastics that were found on both the lower and upper surfaces of the sampled algae tips were placed on a clean glass fibre filter within a secondary clean Petri dish using forceps. Once placed in a second Petri dish this dish was also labelled, sealed with electrical tape and stored for further assessment via ATR-FTIR analysis. Following removal of all microplastics the tips were dried for 3-4 days within the laboratory at room temperature. Post drying, the tips were re-weighed within a sealed scale and dry weight was recorded as grams.

2.2.4: Contamination mitigation

All glassware, Petri dishes and sample collection equipment used within the experiment was cleaned three times with deionised-distilled water and a 70% ethanol solution. Once cleaned, all the open joints and receptacles on all the equipment used was further sealed with aluminium foil to reduce the possibility of airborne microplastic contamination. Sampling in the field was conducted wearing non-synthetic clothing (where possible) to reduce self-contamination from clothing. When sampling the seawater, care was taken to ensure minimal disturbance and resuspension of bottom sediment, with the sample being taken ~1 m (length of arm and sampler container) away from the body of the person collecting the sample. All equipment was sealed and opened only as needed to reduce contamination from airborne microplastics.

Seawater collection was conducted as above with the container being sealed underwater to reduce airborne contamination. Macroalgal samples were collected without gloves and the sleeves of clothing was rolled up to above the elbows to reduce contamination from cuffs. Within the laboratory, surfaces were cleaned prior to, during and after each laboratory-based protocol, with a 70% ethanol solution spray and wiped down with white paper towel. Microplastic extraction and inspection was conducted within a clean room, with no air conditioning unit, and care was taken to reduce contamination from clothing via the wearing of a cotton laboratory coat and slow movement when travelling around the laboratory space. Fibre samples were taken from the clothing most commonly used when preforming sampling in the field and laboratory. These fibres were then analysed using Fourier-Transform Infra-Red spectroscopy (FTIR), to compare with samples collected and analysed from the environment. Additionally, clean fibre filter papers (Lab traps) were placed on the laboratory work surfaces throughout the inspection procedures. Following the completion of inspection-based protocols, the lab traps were also inspected, and any fibres found on their surfaces were also analysed via FTIR. Following FTIR inspection, any positively identified plastics were recorded and placed in a Petri dish, whilst any no plastic material was recorded and discarded.

2.3: Method and materials for positive control procedure

2.3.1: Collection and preparation of materials

Seawater and *F. vesiculosus* samples were collected from the same location as used in the background controls, with seawater collected in a 5 litre (HDPE) plastic container and transferred to the laboratory for filtration through a 0.7µm glass fibre filter. Following the filtration of each litre, the clean seawater was placed within sealable 1000ml glass containers for future utilization. *F. vesiculosus* distal tips were collected by selecting a section of a whole specimen (fully submerged at the time of collection) and removing it from the plant via cutting with scissors approximately 15-20cm from the tips. The section was placed in a clear (food grade) plastic bag containing seawater from the sample location and transferred to the laboratory. Preparation of distal tips was conducted by initially cutting the tips off the thalli, ensuring each tip was between 3-5cm in length with a single branch. Each tip was then placed into a Petri dish and inspected using a microscope and any microplastics found at this point were removed via forceps to ensure a clean algal tip.

The microplastic contaminant used within the positive control was 100% red acrylic wool, and sections of the wool were cut and weighed to measure 10mg. Following the initial weighing, the sections of wool were cut via scissors into fibres as small as possible and deposited within the Petri dishes being used within the experiment. Microplastic fibre lengths were found to range between 90 - 2400µm after measurement using AxioVision (version 4.2) software via a Zeiss Stemi 2000-c stereomicroscope.

2.3.2: Methodological procedure for microplastic contamination of *F. vesiculosus*

To minimize variance in the contamination of *F. vesiculosus*, three distal tips were placed in each of the six Petri dishes with the pre-cut fibres of 100% red acrylic wool and 50ml of 0.7µm filtered seawater. Following the addition of the water the Petri dish was sealed with electrical tape and placed within a level incubator platform shaker (New Brunswick Innova 44) set to 80 rpm at 15°C (speed and

temperature set due to sharing of the machine with colleagues) and agitated for one hour. Upon contamination the Petri dish was removed from the platform shaker, opened and all distal tips were immediately transferred into a secondary Petri dish. The second Petri dish was filled with 40ml of clean uncontaminated filtered seawater and again placed in the waving platform shaker for a further one hour to decontaminate via agitation. Following both the contamination and decontamination stages one distal tip was randomly removed and dried of excess water by vigorously shaking twice. It was then placed in a clean Petri dish. The Petri dish was placed under a stereomicroscope (Brunel BMSZ series) and both the upper and lower surfaces were manually inspected for any microplastics that had adhered to the surface of the seaweed. Following microplastic counting, the distal tip was left to dry (within the Petri dish) at room temperature for 3-4 day on the laboratory benchtop. Dry weight was measured after the drying period and microplastic numbers were recorded as microplastics per gram of dry weight. This procedure was then repeated to ensure a repeatable methodology and resulted in 18 replicates for the positive control experiment.

2.3.3: Analysis

2.3.3.1: FTIR analysis

Fourier-transform infrared spectroscopy (FTIR) analysis was performed on all suspected microplastic fibres and particles collected from the environmentally sourced seawater and macroalgae. Microplastics were removed from their Petri dishes and placed on a 12-well reflective slide (alum EZ-spot micro mount) via forceps, with each well containing a single suspected microplastic to enable individual analysis. Following the placement of the slide into the FTIR (Nicolet iN10 MX) apparatus, *Omnic picta* software was utilized to gain polymer spectra data. Spectra images produced were then compared to both hardcopy and polymer libraries within the *Omnic picta* database to determine the polymer type of the suspected microplastic. If analysis revealed a ≥60% positive match with confirmation via hardcopy spectra to known polymer types, the identified microplastics were removed and placed within a Petri dish for further analysis if required. All items that were found to be of cellulosic material or non-polymer

were discarded with other organic matter, after their results were added to paper-based records.

2.3.3.2: Statistical analysis

Analysis of data was conducted using RStudio in conjunction with R (version 4.0.2). Normality within data sets was initially tested via a Shapiro-Wilk test and subsequently by a Kruskal-Wallis test, if a significant result was found using the Shapiro-Wilk test. With failed normality assumptions a non-parametric Dunn test (Dunn, 1964), (with Bonferroni correction) was used as a post hoc multiple comparison test, to allow differences between data groups to be found. A significant difference was identified where $p = <0.05$. Groups within the analysis were separated via tidal stage (either high tide or low tide) and month. Additionally, a further standalone positive control group was used for comparison reasons, again using the Dunn multiple comparison test to ascertain if there was a significant difference between the positive control experiment and the background controls. Following normality and multiple comparison testing for control groups, a non-parametric Spearman's rank correlation test was used to assess if a correlation existed between seawater microplastic concentrations and macroalgal microplastic concentrations over time.

2.4: Results and discussion

2.4.1: FTIR analysis of background control data.

Micro-FTIR was used within the present study due to the ability for polymer identification to occur based on specific infrared characteristics within already tested polymers. Infrared (IR) spectroscopy can accurately identify polymer types given that specific polymer compositions will be excited by IR radiation and thus absorb and reflect certain wavelengths (Löder and Gerdts, 2015). Thereby allowing polymer identification due to unique banding patterns within wavelength spectra as seen within the study conducted by Chen et al, (2012). Chen et al, (2012) by means of FTIR spectroscopy were able to deduce that polymers such as PET, have specific spectra wavelengths that can be used as signatures. As seen in figure 5 PET being the most identified polymer within the present study has

specific indicator wavelengths at 1720 cm^{-1}, 1300 cm^{-1} and 1100 cm^{-1}, allowing researchers with less experience to positively identify polymers using recognised polymer identification libraries.

Following the collection of background control samples, 141 (137 fibres and 4 particles) suspected microplastic samples were removed from the seawater and macroalgal Petri dishes. Subsequent to the procedure seen in section 2.3.3.1, twenty five of the 141 were confirmed as polymers, with the balance being of a cellulosic nature (either cellulose or cellophane). This resulted in a polymer confirmation result of 17.7% based on known polymer types. Of the 82.3% unconfirmed fibres, 39.7% were recorded as cellulose, 41.8% were cellophane and 0.7% was found to be silk. The twenty-five confirmed polymers were separated into total percentage of each confirmed polymer and representative groups collected from seawater samples and the surface of the *F. vesiculosus* as seen in figure 4 and table 4.

Table 4: Polymer types identified within the collected environmental samples following FTIR analysis.

Polymer type	Number found	Percentage of total microplastics recorded	Number of microplastics found in water	Number of microplastic found in macroalgae
Polyethylene	2	8%	1	1
Phenol resin	1	4%	1	0
PTFE	1	4%	1	0
PET	13	52%	12	1
Polyacrylonitrile	2	8%	1	1
Poly-acrylic acid	2	8%	1	1
Polyamide	1	4%	1	0
PBMA	1	4%	1	0
PVC	2	8%	1	1

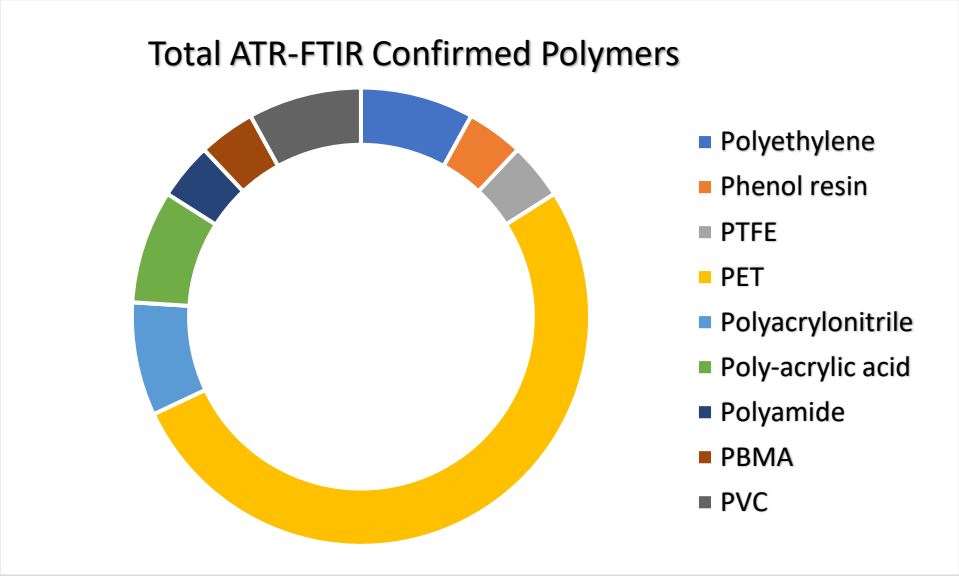


Figure 4: Percentage chart of all ATR-FTIR confirmed polymers.

In addition to the general abundance of microplastics found within the environment, the present study found that 92% of all confirmed microplastics were fibres. This is highly comparable with studies such as Goss et al. (2018), Feng et al. (2020), Jang et al. (2020) and broader scale reviews of studies such as Fu et al. (2020), who found that microfibres were the dominant shape of microplastics. Furthermore, the more than half (52%) of positively identified microplastics collected were fibres belonging to the polymer family polyethylene terephthalate (PET). This was confirmed by ATR-FTIR analysis where the polymer spectra for positive identification is illustrated in figure 5 (additional identified polymer spectra can be found in Appendix 1: A - D).

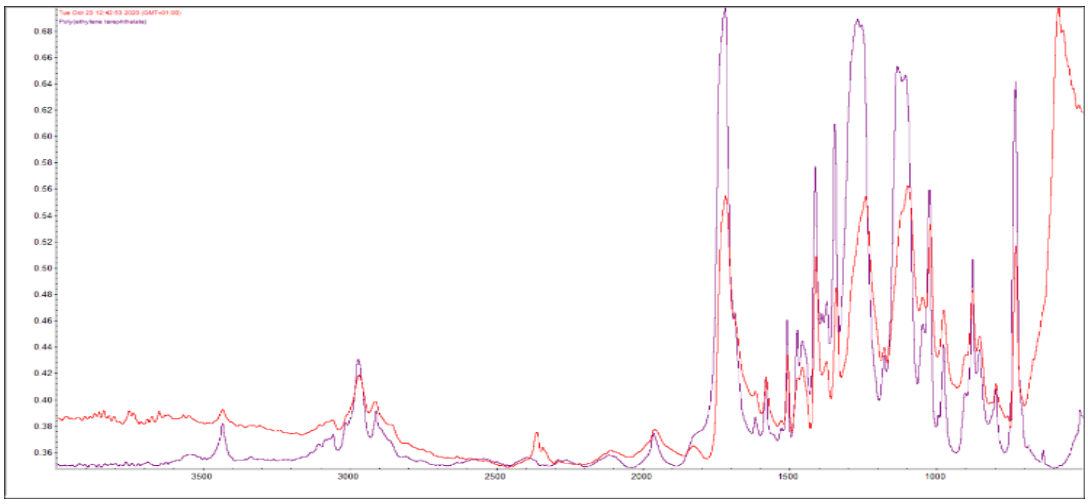


Figure 5: Sampled PET polymer spectra (red line) compared to PET *Omnic picta* library spectra (purple line).

2.4.2: Results of background control experiments and statistical analysis

As seen within section 2.2.2 and 2.2.3, background control samples were collected from both the local seawater and macroalgae populations over a monthly timescale (January, February, March, August, September and October 2020). Following collection and FTIR analysis of the samples, results ranged from 0 - 4 total confirmed microplastics per tidal stage per month within seawater samples, and 0 - 2 total confirmed microplastics per tidal stage per month on macroalgal distal tip samples.

Separating seawater data into low tide and high tide states yielded microplastics per litre ranging from 0.2 - 0.6 MPs/L (low tide) and 0 – 0.8 MPs/L (high tide), (figure 6) with overall averages of 0.066 MPs/L and 0.133 MPs/L respectively. The same separation yielded ranges of 0 – 0.33 MPs/DT (distal tip) (low tide) and 0 – 0.17 MPs/DT (high tide) microplastics per distal tip of *F. vesiculosus*, (figure 7) with an overall mean of 0.333 MPs/DT and 0.300 MPs/DT respectively. Statistical analysis of the water and algae data via a Kruskal-Wallis test produced p-values of 0.3052 and 0.4638 respectively indicating no significance. Further multiple comparisons via the Dunn test were conducted and resulted in p-values of 1 for all background control groups.

The number of microplastics per millimetre was calculated at 0.0004125 MPs/mm^{-2} (Low tide) and 0.0002125 MPs/mm^{-2} (high tide). Taking the average distal tip total area at 800 square millimetres.

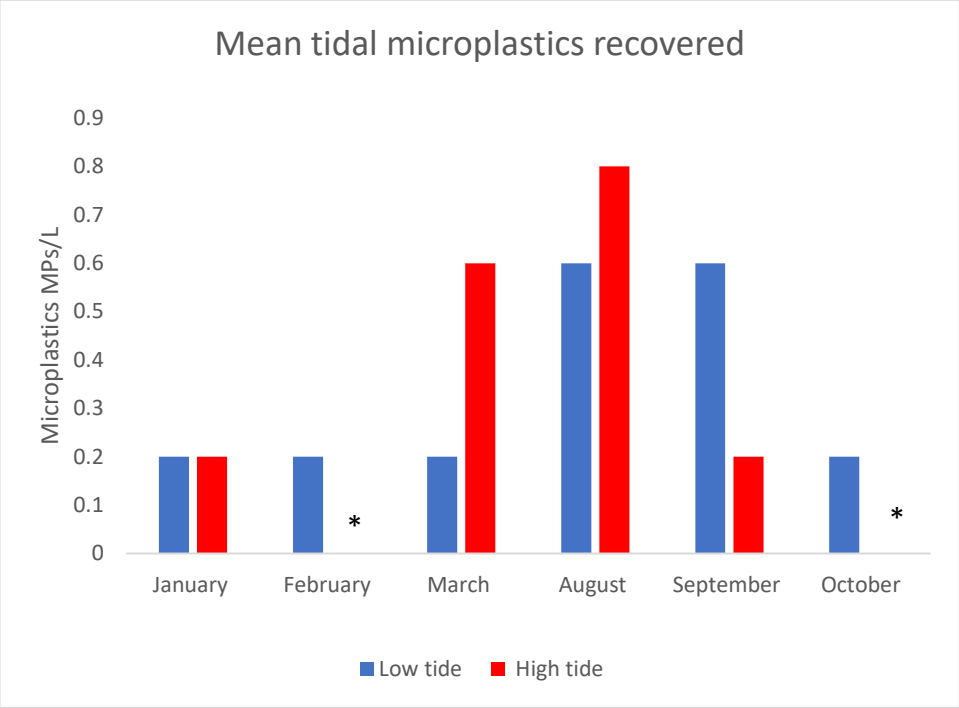


Figure 6: Mean number of microplastics per litre of collected seawater at low tide and high tide per month. Columns marked with an Asterisk denote zero microplastics recovered.

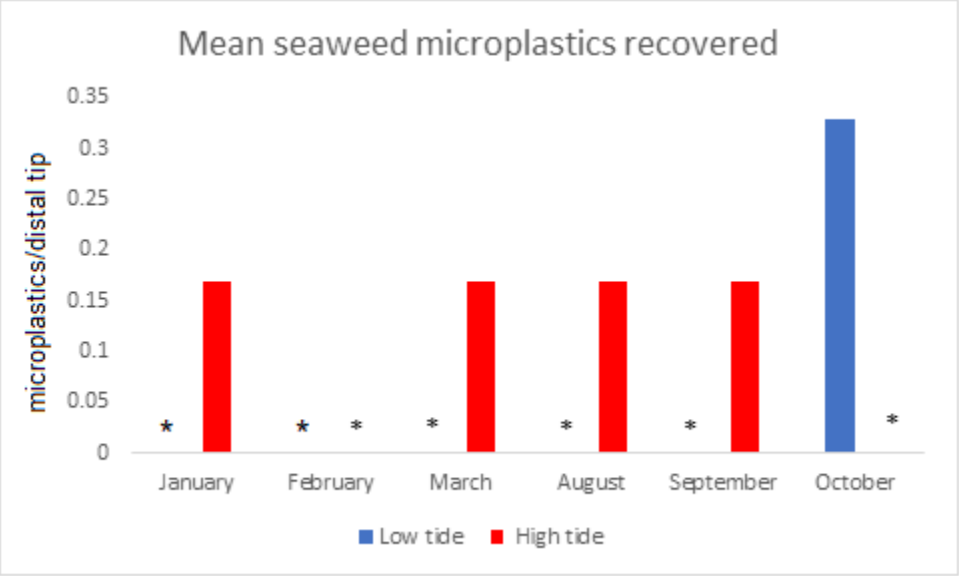


Figure 7: Mean number of microplastics per macroalgal distal tip collected at low tide and high tide per month. Columns marked with an Asterisk denote zero microplastics recovered.

2.4.3: Positive control experiment results

Utilizing the method mentioned in section 2.3.2, the positive control experiments yielded results for all 18 replicates of *F. vesiculosus* distal tips used. The microplastics per distal tip ranged from 0 – 3 which resulted in a microplastics per gram of dry weight (MPs/g(dw)) range of 0 – 26.32 and an overall mean of 9.86 MPs/g(dw) collected from contaminated *F. vesiculosus.* For comparison purposes the average microplastics per millimetre was calculated at 0.0013 MPs/mm^{-2}.

2.4.4: Results of multiple comparison tests for control groups

Following the completion of both positive and background control experiments, statistical analysis was used to ascertain if any significant differences could be found between seasons or months. Results show how the data collected was not normally distributed, with a highly significant Shapiro-Wilk p-value of 3.976e-16 and an equally significant Kruskal-Wallis p-value of 5.513e-06. The results of such tests showed that there was no significant difference between the seasons

of spring, or autumn. There was also no significant difference between any of the individual months. Following this initial result, the Dunn's test was re-run to include the positive control data. Table 5 and figure 8 show the results of the multiple comparison, revealing that as stated no difference is visible between any of the background controls, however all are significantly different to the positive control, with significant differences ranging from 0.000 and 0.003. This result was expected, as the quantities of microplastics used within the positive control were far greater than that recovered from any of the natural seawater samples.

Table 5: Results of Dunn multiple comparison test regarding positive and background control experiments, assessing total microplastic contamination against tidal height combined with season. P-values with asterisk are regarded as significant as α = <0.05.

TEST STATE	**Positive control**	**Background control low spring**	**Background control high spring**	**Background control low autumn**
Positive control				
Background control low spring	0.000*			
Background control high spring	0.003*	1.000		
Background control low autumn	0.003*	1.000	1.000	
Background control high autumn	0.003*	1.000	1.000	1.000

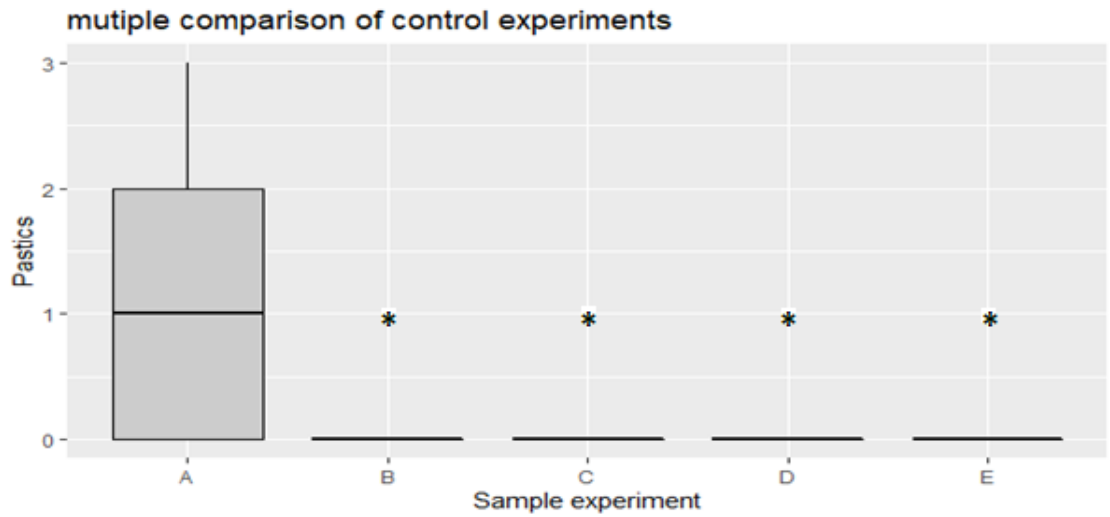


Figure 8: Multiple comparison between positive and background control groups. Boxes with asterisk denotes a significant difference from positive control as α = <0.05. Sample experiment A = Positive control, B = Spring low tides, C = Spring high tides, D = Autumn low tides and E = Autumn high tides.

2.4.5: Spearman's rank correlation test

Using the non-parametric Spearman's rank correlation test to assess if microplastics fluctuated over time. Microplastics found within the seawater samples was correlated against MPs found adhered to the surface of the *F. vesiculosus* collected. The resulting test yielded a non-significant P-value of 0.4946, indicating that there was no correlation between MPs in the water and MPs on the algae over the seasons. This, however, may be misleading. During the course of the time-series experiment in 2020 there was a global viral outbreak, leading to the total shutdown of all non-essential work within Great Britain. As a result, the time series was broken into spring and autumn, with a three-month gap over the summer months due to lockdown measures.

2.4.6: Experimental comparisons

Data gained in section 2.4.1, concluded that PET fibres were the most commonly identified polymer type. This is likely due to PET being a widely used polymer in clothing production, with many warm outdoor garments utilizing its many durable attributes. The high percentage of PET fibres found within the control experiment

may be as a result of wastewater effluents released from the sewage treatment plant within the town of Oban. However, the plant lies roughly five miles from the sample area and further studies in the wastewater released from it would need to be conducted to assess if microplastics are being released from it. Browne et al. (2011), surmised that PET and synthetic laundry (used extensively due to Scotland's climate) may release up to 19,000 fibres while being washed. As the town of Oban lies only a few miles south of the sample location, and the village of Dunbeg only a few hundred meters from the beach (figure 3), it is likely that some of the PET fibres found are a result of synthetic clothing worn and washed by the local population. As found by Alvim et al. (2020), the effluents from WWTPs can contain millions of microplastics globally. The additional polymer types found, although low in abundance, could have originated from many local sources. Oban is well known and popular among tourists and businesses, while the marina located at Dunbeg is used for mooring boats. In addition, there are various marine aquaculture sites located along the coast in the Firth of Lorne. With the myriad of origin sites and the variety of polymer materials used, such as ropes, paints, floats and hardened plastics for decks and pontoons, the likelihood is that some of the collected plastics found will have originated locally. However, this would require additional sampling, current mapping and analysis to confirm the origin sites for plastics released and collected within the Firth of Lorne area.

As the results above indicate, an average of 0.066 MPs/L (low tide) and 0.133 MPs/L (high tide) were found in seawater samples and an average of 0.333 MPs/DT (low tide) and 0.300 MPs/DT (high tide) found adhered to the surfaces of the macroalgae samples being examined. Various studies conducted globally (Dai et al. 2018; Mai et al. 2018; Feng et al. 2020; Fu et al., 2020) have taken to using alternative factors to explain microplastic abundance within water and algal samples. Chiefly microplastics per litre, per meter cubed or per kilograms of dry weight material are used to display data in a comparable way. Thus, in the present study an effort was made to display data in a similar method, to allow comparison between studies. In comparison to studies such as Dai et al. (2018) showing microplastic abundance of 0.4 – 5.2 MPs/L, Feng et al. (2020) showing 1.04 – 1.86 MPs/L and Jang et al. (2020) showing 0.77 MPs/L, it can be noted that the present study yielded less microplastics within water samples. This is likely a result of experimental design; the present study only conducted tests on

five litres of seawater whilst Jang et al. (2020) used up to 100 litres for sampling. The difference in volume within the present study could have resulted in the low microplastics recovered due to there not being a large enough sample size. With an increase in the volume used, there would likely be an increase in microplastics recovered, and thereby a greater resolution data set to indicate the potential abundance.

Microplastics per algal distil tip was used within the present chapter to indicate the basic issue that they can be found adhered to the surface of macroalgae in the environment. The results in section 2.4.2 show microplastic abundance on macroalgae at 0.0004125 MPs/mm^{-2} (low tide) and 0.0002125 MPs/mm^{-2} (high tide). In comparison Sundbæk et al's. (2018) results showing 2.63 MPs/mm^{-2}, the present study yielded far less. This again is likely due to differences in the methodology used, with different microplastics acting differently within studies, and concentrations of contaminants differing widely.

Following the background control experiments, results of the positive control experiment designed loosely around that of Gutow et al. (2016), showed that plastics do adhere to the surface of *F. vesiculosus*. This study found that microplastic adherence to seaweed ranged from 0 – 3 microplastics per distal tip. This measure however is non-comparable to the studies conducted by Gutow et al. (2016), Sundbæk et al. (2018) and Mateos-Cárdeans et al. (2019) who used MPs/mm^2/ml^{-1}, MPs/mm^2 and MPs/blade respectively. The present study used MPs/g(d.w) to gain a range of 0 – 26.32 MPs/g(d.w) and a mean of 9.86 MPs/g(d.w), with an additional comparable value of 0.0013 MPs/mm^{-2}. Both average values of 9.86 MPs/g(d.w) and 0.0013 MPs/mm^{-2} appear significantly lower than that of Sundbæk et al.'s (2018) study. The main goal of the positive control was not to query the mode of measurement but to assess if the method conducted by Gutow et al. (2016) (although amended for the purposes of this study) would work as a base level control and thereby answer aim one of the thesis: "Do microplastics adhere to seaweed *Fucus. vesiculosus*?". Consequently, the results gained have concluded that microplastic do adhere to *F. vesiculosus.*

It could also be argued that the data collected herein is not representative of the true scale in which MPs occur in the environment due to how the time series had to be split because of Covid-19. With the gap in seasons, there is no continuity in

the time series, with any possible accumulation and dispersion within summer months being overlooked entirely. Furthermore, with the national lockdown insisting the population stay indoors to curb the spread of the virus, there is the distinct possibility that there was an increase in use of washing machines resulting in an increase of wastewater effluent. Bawden (2020) noted that there was a 36% increase in washing machine use in the three months during the first lockdown in 2020. In addition to the Covid-19 pandemic, the data could also be an underestimation of the true scale, due to experimental design. The present chapters experiments utilized small sample sizes due to the time constraints of only one year. As stated, above Jang et al. (2020) conducted water sample tests of one hundred litres. Given ample time and additional resources, the recommendations for an amended repeat of the present experiment would be to increase sample size and run additional sample sites. Furthermore, daily sampling would be advantageous compared to the single monthly sampling conducted herein. With daily sampling a far greater resolution data set could be created for analysis using more statistically powerful tests. Potentially showing with greater confidence if there was any significance between months and indeed if a true correlation existed.

2.4.7: Reasoning for changes to methodology

The methodology within the present study looked to create as robust an experiment as possible by amending this oversight within the methodology used throughout. Firstly, background controls were created, as a result of there being no mention of any control group, regarding either the organisms used or the macroalgae collected and used as food within the Gutow et al. (2016) study. That experiment was run to assess two possible hypotheses, 1: microplastics can adhere to the surface of seaweeds, and 2: meso-herbivores take up microplastics with contaminated tissues. The study undertaken by Gutow et al. (2016) does not indicate the natural levels of contamination present on the sampled seaweed tips, nor was there any attempt to conduct water sample analysis for microplastics. This posed an issue in the robustness of the whole experiment as without the prerequisite knowledge of the environmental conditions and contamination levels of the location used, there can be no discernible evidence that the seaweeds

utilized were free or fully laden with microplastics at the time of the experiment. This was particularly interesting as the location used for sampling is in relatively close proximity to the city of Bremerhaven (Germany), with a population of roughly 113,000 people. With such a high population the possibility for water and airborne microplastic contamination could likely be a key factor in that study.

Therefore, to allow comparison between what is found naturally and what is conducted in a laboratory setting regarding number of microplastics per millilitre of water, natural seawater samples were assessed to gain a background base figure. This was undertaken at both low and high tides to compare contamination levels dependent on tidal height. A number of studies have stated that the area known as the most recent strand line is a hotspot for the accumulation of microplastics on coastal beach environments (Andrady 2011; Browne et al. 2011). Conducting sample extraction at both tidal stages would allow the comparison to possibly answer a further hypothesis, low tidal state water samples contain less microplastics than the high tidal state. Thus, aiming to demonstrate that high tide and consequently the strandline do contain more microplastics and can be referred to as a hotspot.

The water samples were obtained over the course of six months to create a sample time series. The series of water samples and microplastic analysis allowed comparison between the seasons of, late winter, spring and autumn. Studies such as that by Blumenroder et al. (2017) conducted in recent years have used data collected from a single outing, giving a “snapshot” of the contamination within a local area. However, using a single outing to gain data does not give any evidence to support the issue that contamination may fluctuate over time. By conducting a sample time-series an effort was made to determine if fluctuations were weather dependent as a result of storm events, or constant as a result of base level contamination within the environment. Conducting the sample time-series also reinforced the method as a consistent protocol for the assessment of microplastics within naturally sampled seawater.

Secondly, changes were made to the materials used for the microplastic contamination stages. Gutow et al. (2015) conducted their experiment with specifically produced microbeads and fragments, obtained from Thermo Fisher Scientific (Waltham, MA). The microbeads used within their study were of 10µm in size, this posed a problem within the present study as the machinery available

was unable to be used with microbeads at such a small scale. For that reason, three different types of microplastics were used over the course of the experiments conducted within the present study, due to their combined availability and ease of processing within the scope of the experimental timescale and equipment parameters.

Polyacrylic yarn was used throughout as a representative of the clothing found globally. It is commonly considered as a contaminant released from wastewater treatment plants, washing machine outlets and from beach towels by tourists worldwide (Browne et al. 2011; Stolte et al. 2015; Yu et al. 2016). The fibres produced by the scission of the yarn were able to be used in the experiments with confidence due to their size, shape and colour, enabling easy recognition and collection via microscopy. The other microplastics used within the present study consisted of fibres cut from polymer ropes used within the aquaculture industry and glitter (as seen in Chapter 3).

Further changes were made to the experimental equipment used within the study, Gutow et al. (2015) utilized a waving platform shaker (polymax 1040 at 10° tilt) for use in their studies experiments. The present study due to access and procurement relied instead on a New Brunswick Innova 44 incubator platform shaker, which allowed temperature and rpm agitation control but lacked an angled tilt option.

2.4.8: Observations within the experiments

While conducting basic preliminary tests to ensure the microplastic component of the experiment was suitable for use, it was observed that when cut into small sections, the acrylic wool fibres tended to group together either on the water surface or on the bottom of the Petri dish. The test was conducted by placing 10mg of cut acrylic wool into 40ml of deionised water within a Petri dish, followed by manual agitation (swirling by hand) for 30-60 seconds. As mentioned in the Gutow et al. (2016) study, the grouping together of fibres within the test solution made it impossible to ascertain the exact number of fibres used. As a result, the present study followed the recommendation of Gutow et al. (2016) stating the weight (g) of microplastic fibres used upon testing.

While conducting the positive control experiments the number of microplastic fibres found adhered to the surface of the algae varied. While manually counting via a stereomicroscope it was evident that areas of damage or cutting tended to have a higher density of acrylic fibres (figure 9), when compared visually to the undamaged algal surfaces. This is likely due to the high concentration of polysaccharide exudates being produced as a result of the damage. Recent studies (Martins et al. 2013; Sundbæk et al. 2018) have suggested that the adherence of microplastics to the surface of macroalgae may be a result of polysaccharide exudates released upon cutting the samples for laboratory experimentation. To ensure further experiments were carried out in a time effective and constructive manner, a small-scale experiment was created to test the above observations validity. The results of the preliminary test were not recorded as they were not to be included within the present study. Samples of *F. vesiculosus* were intentionally damaged via scalpel and exposed to acrylic fibres, all samples concluded that fibres adhered to damaged areas.

Figure 9: x40 magnification photo via axioVision (version 4.2) of a *F. vesiculosus* distal tip with red acrylic fibres found adhered to damaged areas within encircled area.

2.5: Conclusion and main findings

2.5.1: Conclusion

To summarise, the present chapter amended the method conducted by Gutow et al. (2016) to create a time effective protocol to assess the adherence of polyacrylic wool fibres to distal tips of the common macroalgae *F. vesiculosus*. The results of this positive control show that acrylic microplastics do adhere to seaweed samples, thus answering the first aim of this research study. Furthermore, two background control experiments were created to address the issue of missing controls within the Gutow et al. (2016) experiment. Environmentally collected seawater and *F. vesiculosus* samples was assessed over a monthly time period to determine microplastic contamination. Both were found to contain microplastics as contaminates, however no statistically significant difference was found between either the sampled seasons or individual months. Additionally, a correlation test was used to ascertain if microplastics found adhered to macroalgal surfaces fluctuated over time as a result of microplastics within the water column. The result of the test concluded that there was no significant correlation detectible, thus answering aim two.

2.5.2: Main findings

- 25 of the 141 suspected plastics were confirmed via ATR-FTIR as polymers, giving a confirmation of 17.7%.
- 92% of all confirmed polymers were fibres.
- 52% were found to be polyethylene-terephthalate (PET).
- No significant difference in microplastic abundance was found between water samples or algal background control samples.
- Positive control samples yielded an average of 0.0013 microplastics per square millimetre. Providing evidence that microplastics “do” adhere to macroalgal surfaces.
- No significant correlation was detected between microplastics found within the water column and those found on algal surfaces.
- Microplastic contamination within the water column and adhered to algal surfaces “Does not” fluctuate over time.

Chapter 3: Microplastics as a potential contaminant of multiple common seaweed species

3.1: Introduction

3.1.1: Macroalgal species selection, based on ecological, economic and cultural history

Today's economic and ecological climate has seen seaweed utilization, globally and within Scotland, gain traction due to bioremediation potential to act as a vital catchment for plastics (Masiá et al. 2020). Until recently, the aquaculture of macroalgae within Scotland has been relatively overlooked, however, more cost- time- and labour- effective methods of cultivation are starting to allow the sector to expand. Emphasis is now being placed on both small-scale high value products and resources used within the medical profession, cosmetic industries and low value but incredibly high-volume products such as biofuels (Scaife et al. 2015). There is also a growing importance on some algal species (*Palmaria palmata (P. palmata)* and *Saccharina latissima*) being used as a valuable biomonitoring and remediation tool for inorganic compound pollution (i.e., introduction of a substance that is harmful to the environment) within Scottish waters (Sanderson et al. 2012). However, seaweeds have been used as bioindicators for several decades (Dawes 1987; Browne 2001), and recent advances in analytical and cultivation techniques have allowed greater research into water column pollution resulting in far larger interest in using macroalgae as a bioremediation tool.

The use of macroalgae as a bioremediation tool or within an integrated multi-trophic aquaculture system (IMTA) has been suggested as a means of reducing the pollution impact from Scottish salmon farming (high levels of ammonia-based nitrogen released by salmon as a metabolic product), while gaining a secondary economically valuable crop (Sanderson et al. 2008; 2012; Treoll et al. 2009). Sanderson et al. (2008) confirmed that macroalgae could be placed around salmon farms to act as a bioremediation buffer for the pollution released from the sites, by recycling both inorganic and organic waste and utilizing it as a resource (Chopin and Robinson, 2006). This would result in a secondary crop of bio-based products which are not only economically valuable, but also bioremediating the

ongoing pollution issues linked to salmon aquaculture (Neori et al. 2004. Sanderson et al. 2008; 2012).

As the present study was conducted with the aim of assessing microplastic adherence to macroalgal species, the following species were investigated due to their availability within Scottish waters. *P. palmata* has been confirmed as a candidate in integrated multi-trophic agriculture systems, with a preference for the uptake of ammonium excreted from finfish aquaculture (Grote 2016; Grote 2019). It is also viable as an economic species within the Northern Irish market, with past studies demonstrating a capacity for substantial growth on long line systems at sea (Browne 2001). *P. palmata* can also be commonly found as an epiphyte on *Laminaria hyperborea (L. hyperborea)*, *Laminaria digitata* (L. digitata) and various Fucus species throughout Scottish waters (Irvine and Guiry 1983) and is a perennial species with frond growth recurring yearly (Werner and Dring 2011). The species is widely spread across Europe and America however, issues with decreased growth are found in areas of high temperature and irradiance, leading to photoinhibition, frond bleaching and mortality (Sanderson et al. 2012). Grote (2016) noted that cultivation of *P. palmata* could be utilised successfully within areas of increased depth. The vertical transport of the algae to deeper waters when irradiance and temperature become critical would allow a lengthened growth phase and beneficial result for harvesting.

Nardelli et al. (2019) aimed to investigate whether the green seaweed species *U. lactuca* could be effectively utilised within IMTA systems. Both nutrient retention and biomass yield were recorded when experimentation was conducted using a varied trophic level protocol. A simple system of algae and water was used as a control, while a moderate system using algae and fish and an advanced system using algae, fish and mussels were used to assess suitability of *U. lactuca* within proposed IMTA systems as an extractive species utilising dissolved inorganic matter by-products as a resource. Nardelli et al.'s (2019) study found that *U. lactuca* was ideally suited to this integrated aquaculture method showing an increased ability to incorporate dissolved inorganic nitrogen (ammonium) and phosphate into their biomass. While this increase was noticed within the moderate experiment, the advanced system using fish and bivalves showed the greatest return, with retention rates reaching approximately 70%. Nardelli et al.'s (2019) research concluded that *U. lactuca* was an efficient extractive species

increasing both system yield and bioremediation potential for the aquaculture site in use.

L. digitata is a second perennial species found throughout Europe (Bunker et al. 2017) with a seasonal development that includes maximum growth within the first half of the year and during the summer months. Tangle, as it is more commonly known, has the potential to be large, fast growing, has good bioremediation properties, whilst also being able to be utilised as an edible food. On the island of Tiree, it is eaten raw with whisky (Kenicer et al. 2001), as well as also being added to other foods containing *P. palmata* to increase the robustness of the flavour. Darwin (1996) noted that children ate it as a paste spread across bread and bannocks (a scone-like flat bread baked in Scotland and Ireland) indicating that it has a deep historical link to the coastal areas of Scotland. More recently however, it was noted that a small company in Orkney used *L. digitata* in some of its condiments (Kenicer et al. 2001), labelling it has a health food. This modern interest in the species increases the importance of *L. digitata*, within the present study for its combined economic and cultural viability.

3.1.2: Evidence suggesting macroalgal damage may induce adherence

Following the research of Gutow et al. (2016), Sundbæk et al. (2018) conducted a study into the sorption of polystyrene (PS) microplastics to *F. vesiculosus*. Sundbæk et al. (2018), amended the methodology of Gutow et al. (2016) to suit their study, however certain elements were kept, allowing an increase in compatibility. The main difference in the experimental protocols was the way by which microplastics were recorded. Sundbæk et al. (2018) counted microplastics by separating the distil tip into sections. Four sections were created for the purposes of counting. Section one was the first 0.5cm from the initial cut site to the air bladders. Section two was the continuation from site one to the end of the laminar tip. Section three was a cross section vertically over the air bladders and section four was another vertical cross section 2.5cm down from the laminar tip. These four sections were developed to allow faster and more reliable means of counting. Sundbæk et al. (2018), noted that while the washing (decontamination) stage removed a large quantity of the adhered microplastics, sections of the sample had significantly increased microplastic abundance following washing. It

was noted that the cross section over the air bladders had water remaining in the cleft between the two bladders, this was theorized as an area of accumulation due to the surface topography and possible catchment capability. The greater significant difference was found in section one; the area of the cut was found to yield significantly more microplastics following washing. This was suggested to be down to the “alginate rich cell walls” (Martins et al. 2013), releasing the gelatinous anionic polysaccharide substance (Sundbæk et al. 2018). Further to the experiment conducted by Sundbæk et al. (2018), the recommendation within the study was to exclude all future results found within section one (area nearest cut sites). Sundbæk et al. (2018) raised the concern of the possibility for algal substances to be responsible for possible mis-readings of microplastic abundance on experimental test algal samples.

This theory of exudates released from the algae was shared by Gutow et al. (2016) who made mention of the “polysaccharide rich mucus layer” which covers most of the algal surface and allows for prolonged exposure to tide driven desiccation (McCully, 1966). This polysaccharide layer found on the surface of *F. vesiculosus* was suspected by Gutow et al. (2016), following desiccation, to allow an enhanced capability for the adherence of microplastic particles.

Polysaccharides are one of the main components of macroalgal species (Kraan, 2013; Skjermo et al. 2014). Monosaccharide (sugar) molecules bind together to form polysaccharides, enabling greater storage within the macroalgal tissues thereby allowing seaweeds to effectively mediate changing environmental conditions such as, salinity, light and oxygen concentrations and temperature (Skriptsova, 2015; Garcia-Vaquero et al. 2017). One of the main polysaccharide groups found within brown macroalgal species are fucoidans, a group of sulphated homo- and hetero- polysaccharides with fucose as the main monosaccharide building block. McCully (1966) noted that in brown algae fucoidans can be found within the intracellular matrix of the tissues and as a mucilaginous coating of the surface of the thalli. However, the main role of fucoidans within brown algae such as *F. vesiculosus* is to aid in the building and structure of cell walls and vesicles of egg cells (Mabeau et al. 1990; Kaur and Kumari, 2012). Studies such as these have enabled hypotheses regarding the release of polysaccharides acting as a binding agent, as proposed by Gutow et al. (2016) and Sundbæk et al. (2018).

3.1.3: Aims

Given the historical and modern importance of macroalgae for the economy, as well as medical and food producing sectors of Scotland (Kenicer et al. 2004), a representative of each of the three major groups of macroalgae was employed as a microplastic contamination host. *Ulva lactuca (U. lactuca)* (Chlorophyta), *Palmaria palmata (P. palmata)* (Rhodophyta) and *L. digitata* (Phaeophyta) were used as representatives of the economically valuable and culturable species found throughout Scottish waters. *F. vesiculosus* was also used to ensure comparability with the control experiments in the previous chapter, while also comparing microplastic contamination adhered to surface damaged algae and whole undamaged algal fronds.

This chapter therefore aimed to assess multiple issues within the concepts of microplastic contamination and ubiquity. Thereby answering aims three and four of the overall study.

- Are common species of macroalgae that are found within Scottish waters at risk of microplastic contamination?
- Does surface damage on macroalgae cause an increase in microplastic adherence, when compared to an uncut whole specimen?

3.2: Methods

3.2.1: Location of sampling

The seawater and *F. vesiculosus* thalli utilized within the present chapter were collected from the same location used in chapter two (figure 3). Therefore, ensuring all possible variation within water and seaweed samples were minimized to enable comparisons between the experiments. All other species of macroalgae were collected within 100 meters of the initial *F. vesiculosus* sample site.

3.2.2: Collection and preparation of seawater and macroalgae

To ensure compatibility with the control experiments, the seawater collection and preparation method used herein is identical to the method used within section

2.3.1. Seawater was collected using a five-litre high density polyethylene (HDPE) container and vacuum filtered through a 0.7µm glass fibre filter, then placed in a 1000ml glass container for use within the experiment.

The *F. vesiculosus* samples were collected by selecting a section of a whole submerged plant at random, cutting the top 15-20cm off using scissors and placing the cut section in a food grade plastic bag for transfer to the laboratory. Distal tips were gathered by removing the final 3-5cm of the thalli, ensuring that each tip had a single branch and was free of any epibionts. To minimize variance between samples as seen in chapter two, three distal tips were placed in each of the six Petri dishes after any environmentally occurring microplastics were removed via forceps whilst viewing under a dissection microscope.

Whole *F. vesiculosus* samples were collected by selecting small rocks with whole attached plants. Thalli length was set to 3-5 cm from holdfast to distil tip. All samples were placed in a food grade plastic bag and transferred to the laboratory. Algal preparation was conducted by removing the macroalgae from substrate by scission at the holdfast. Following removal from the substrate, three individual thalli were placed in a Petri dish for experimentation following visual inspection and removal of any naturally occurring microplastics and epibionts.

P. palmata and *L. digitata* were collected from the rocky shore waterline at low tide. Samples of *U. lactuca* were also collected from the waterline at low tide and removed from the sandy substrate of the beach simply by physical removal. *P. palmata* and *U. lactuca* were collected as whole plants and placed in food grade plastic bags whilst *L. digitata* samples were collected by removing the top 20cm of frond via scission and also placing it in a food grade plastic bag for transfer to the laboratory.

3.2.3: Alternative species experimental methodology

P. palmata was prepared in exactly the same way as the *F. vesiculosus* samples with three distal tips removed and placed in Petri dishes. Both *U. lactuca* and *L. digitata* were cut into sections measuring 1.5-2cm in width and 3-4 cm in length. Following these initial preparations, the methodology used for both *U. lactuca* and *L. digitata* was a repeat of the *F. vesiculosus* protocols, with three sections of algae per Petri dish comprising of 18 replicates per species.

3.2.4: Damaged algae methodology

The method employed within the present section was similar to section 2.3.2. However, the distal tips of the *F. vesiculosus* had five cuts made 1cm apart, from the outer edge to the mid-ridge via scalpel on either side of the tip. Alternating cuts were made on either side to prevent breakage of the algal samples. Additionally, the whole specimens of *F. vesiculosus* were simply removed from the attached substrate and placed within the Petri dishes. All whole specimens of *F. vesiculosus* were harvested at a 3-5cm in length to match the distal tips used within the damaged algal experiment. Following placement in the Petri dishes the protocol mirrored the positive control experiment as seen in section 2.3.2.

3.2.5: Contamination mitigation

Mitigation measures for the above experiments were marginally less rigorous in comparison to the control procedures. As all plastic types within chapter three were easily recognisable, the experiments were conducted in a standard laboratory (not a clean room). However, all environmental microplastic contamination was removed before any additional plastics were added via the experimental method. All standard personal mitigation measures were followed, with non-synthetic clothing being worn during the length of procedures and all Petri dishes washed three times and sealed with tape between stages to reduce any possible contamination via airborne fallout.

3.2.6: Statistical analysis

Statistical analysis was conducted similarly to section 2.3.3.2. Normality within the data sets was initially tested via Shapiro-Wilk tests and followed by a Kruskal-Wallis test, if a significant result was recorded by the Shapiro-Wilk test. With the failed normality assumption, a non-parametric Dunn test (with Bonferroni correction) was used as a post hoc multiple comparison test, this allowed differences between data groups to be found. A significant difference was identified where $p = <0.05$. All data values used within the statistical analysis of

the present chapter were recorded as microplastics per gram of dried seaweed MPs/g(dw).

3.3: Results and discussion

3.3.1: Analysis of the results gained from alternative species experiments

The results for the other species of seaweed showed that *U. lactuca* in comparison to the other species within the experiment had a comparatively high mean of 1718.20 MPs/g(dw) (figure 10) and a range of 333.33-5571.43 MPs/g(dw). While *P. palmata* had a slightly lower mean of 469.02 MPs/g(dw) (figure 10) and a range of 0-1352.94 MPs/g(dw), L. *digitata* had a significantly lower mean of 15.35 MPs/g(dw) (figure 10) with a range of 0-62.07 MPs/g(dw). However, all were found to be higher than the positive control with a mean of 9.86 MPs/g(dw) and a range of 0-26.32 MPs/g(dw).

In comparison to the positive control, both *U. lactuca* and *P. palmata* yielded p-values of 4.4e-06 and 0.0024 respectively (Table 6; figures 11 and 12). Indicating that they differ significantly from the control. Both species were found to contain multiple microplastics adhered to the algal surface presumably due to the topography and folding of the frond itself. The concept of physical morphology affecting the entrapment of microplastics by macroalgae has recently been raised by Gao et al, (2020). While conducting their experiment within the inshore waters of the yellow sea (Qingdo, China), it was noted that microplastics were entrapped by the physical structures of the macroalgae *Ulva prolifera*, showing the floats and extended branches of the frond as entrapment areas. Within the present study the folding of the algal frond resulted in a clumping of the microplastic fibres due to the added surface area in which further microplastics could bind. The present studies results coupled with the evidence from Gao et al, (2020), would positively suggest that surface topography of the algal frond does affect the entrapment of microplastic particles and fibres within the environment and laboratory-based studies.

Conversely, *L. digitata* did not show any significant difference from the positive control with a P-value of 1, this is likely due to the microplastics clumping together when introduced to the seawater and algae within the Petri dish. It was observed

that when acrylic fibres were added to the water, they began to combine (instantly) into masses that were in part made from the exudate released from the cut sites of the *L. digitata* frond. This in turn resulted in far less microfibres interacting with the algal pieces during the agitation stage, resulting in the low values recorded.

Table 6: P-value results of Dunn multiple comparison test regarding multiple species and algal damage experiments. P-values with an asterisk are regarded as significant as α = <0.05. Sample experiment A = Positive control, E = *U. lactuca*, F = *P. palmata*, G = *L. digitata*

Sample	A	E	F	G	H
E	4.4e-06*				
F	0.0024*	0.0041*			
G	1.0000	4.8e-06*	0.0047*		
H	4.4e-06*	6.3e-08*	1.0000	1.3e-05*	
I	4.4e-06*	0.0003 *	1.0000	4.8e-06*	0.0245

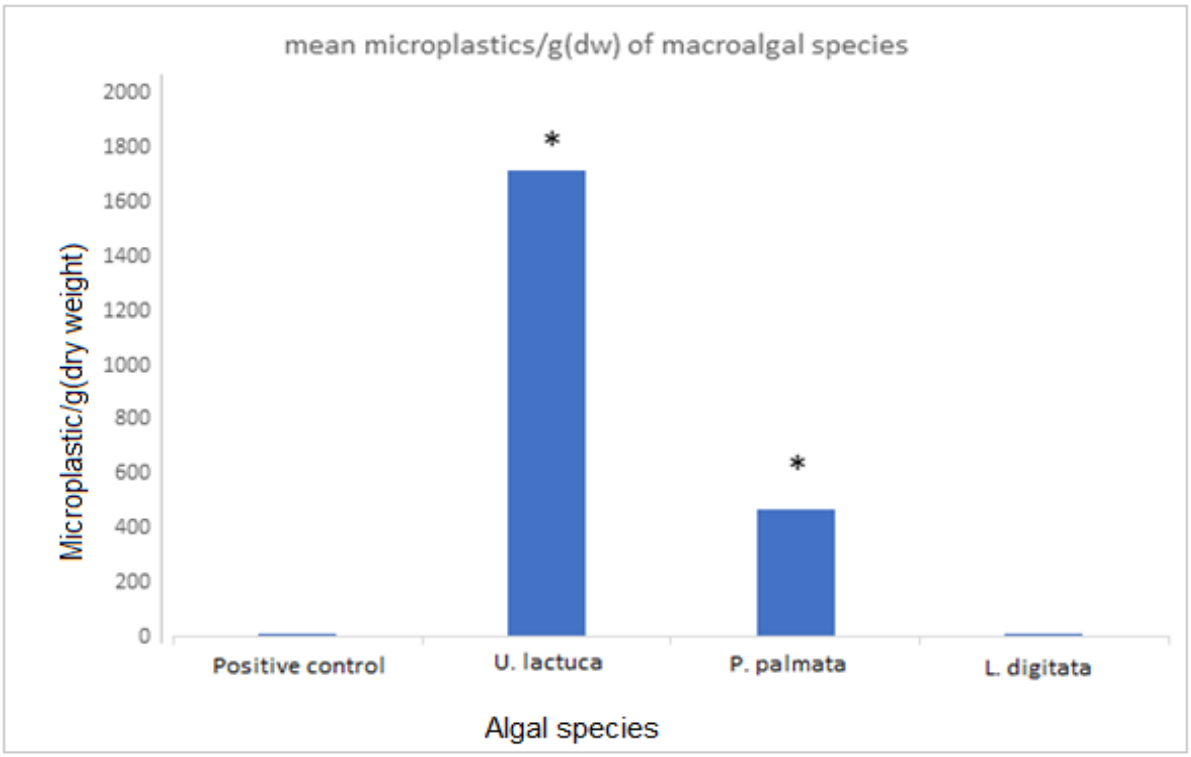


Figure 10: Mean number of microplastics per gram of dry weight of the different species of macroalgae considered, regarding the positive control and other alternative species experiments. Columns marked with an asterisk indicate a significant difference from positive control, P-values set at α = <0.05.

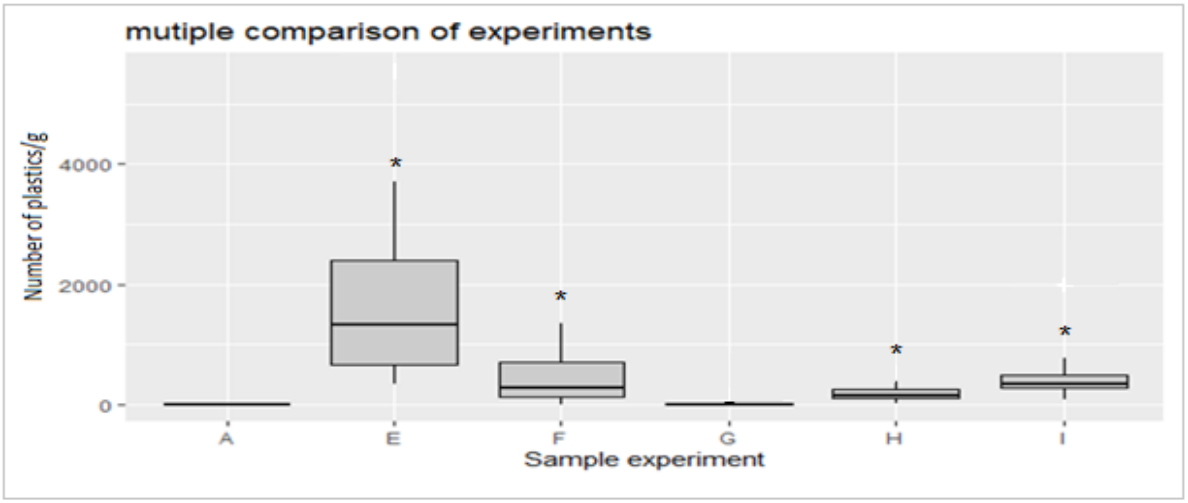


Figure 11: Multiple comparison between the positive control group and all macroalgal experiments. Boxes with asterisk denotes a significant difference from positive control as α = <0.05. Sample experiment A = Positive control, E = *U. lactuca*, F = *P. palmata*, G = *L. digitata*, H = Damaged *F. vesiculosus*, I = Whole *F. vesiculosus*.

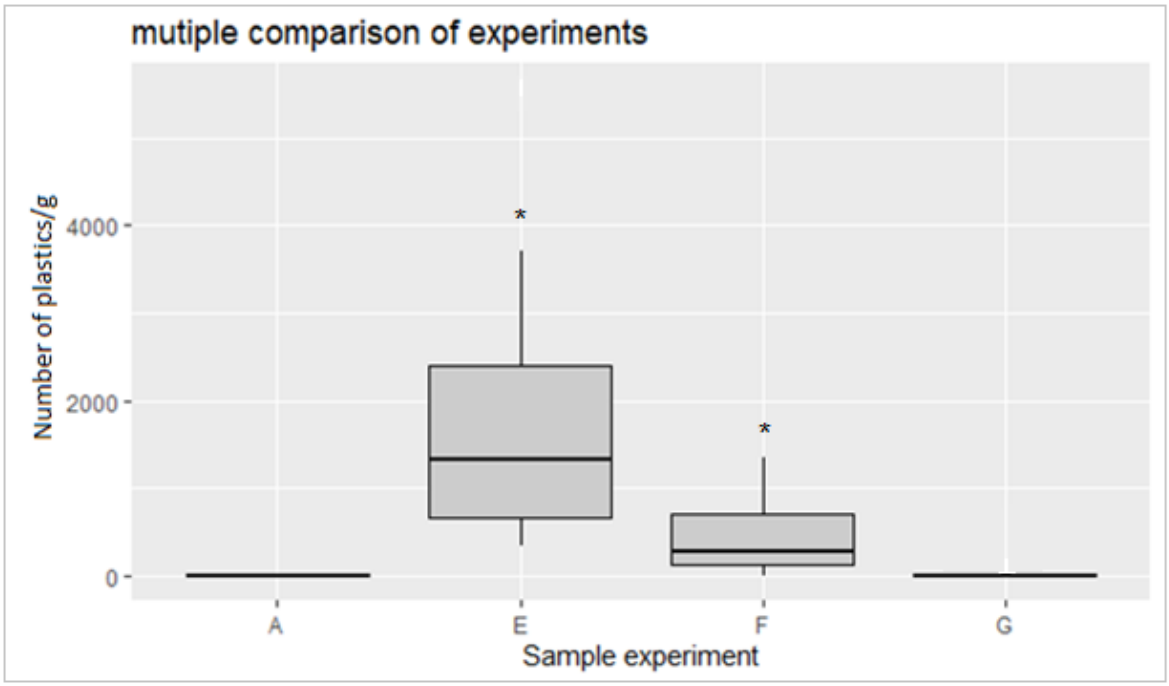


Figure 12: Multiple comparison between the positive control group and alternative species experiments. Sample experiment A = Positive control, E = *U. lactuca*, F = *P. palmata*, G = *L. digitata*. Asterisks indicate a significant difference from the positive control, P-values set at α = <0.05.

3.3.2: Macroalgal damage results

Results for macroalgal damage showed that samples of damaged *F. vesiculosus* had a mean of 186.73 MPs/g(dw) (figure 13) and a range of 26.67-532.26 MPs/g(dw). However, whole samples of *F. vesiculosus* had a comparatively higher mean (~2.5 times greater) of 459.61 MPs/g(dw) (figure 13) and a range of 85.11-1990.29 MPs/g(dw). These experiments yielded higher values than the positive control sample mean of 9.86 MPs/g(dw), with both results being statistically significant. P-vales were recorded at p = 4.4e-06 when compared to the control and p = 0.0245 when compared with each other (table 6 and figure 14). This indicated that there was a significant difference in comparison to the control whilst a less significant difference was observed between each experiment.

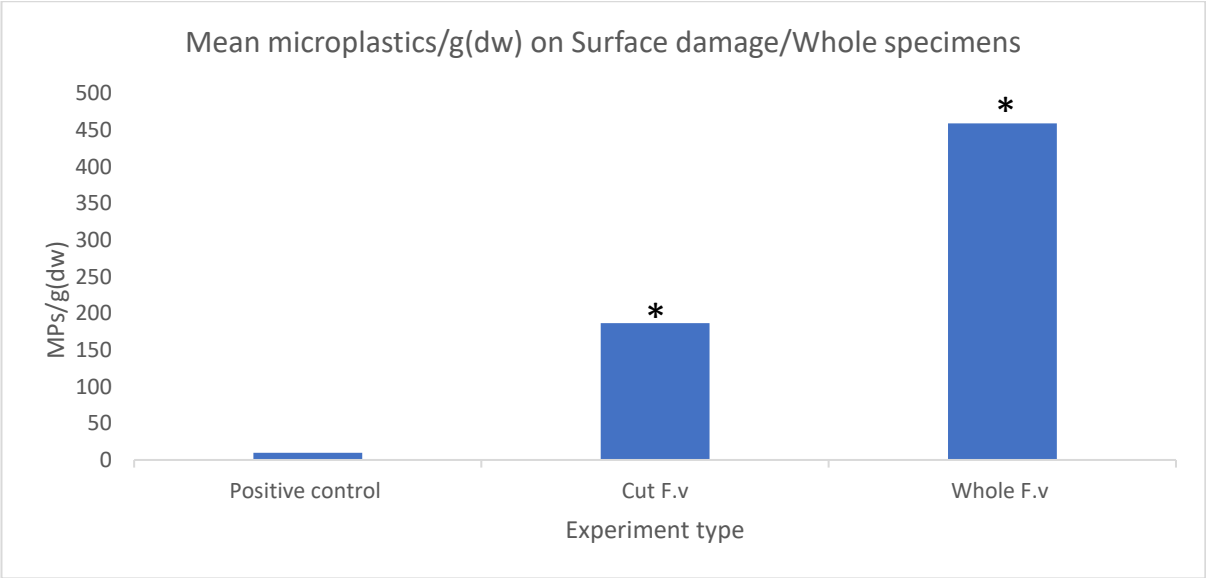


Figure 13: The mean number of microplastics found per gram of dry weight seaweed with respect to the positive control and damage/whole experiments. Columns marked with an asterisk indicate a significant difference from positive control, P-values set at α = <0.05.

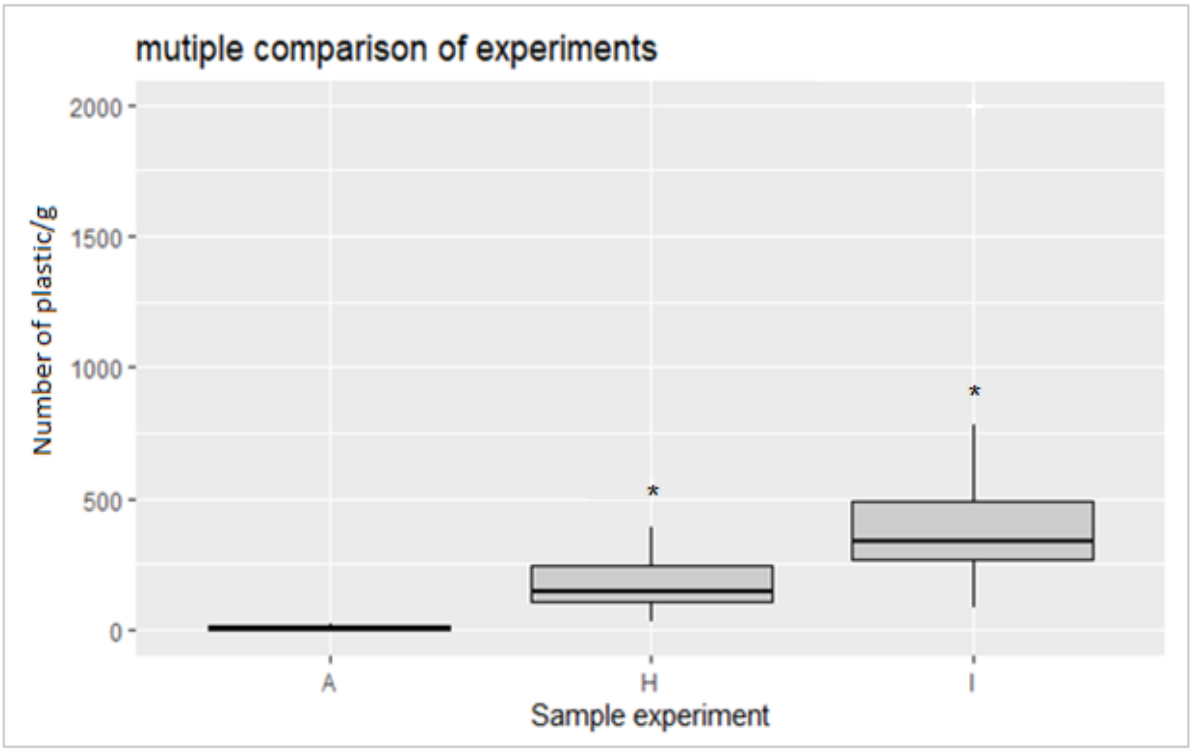


Figure 14: Multiple comparison between the positive control group and damaged/whole experiments. Sample experiment A = Positive control, H = Damaged *F. vesiculosus*, I = Whole *F. vesiculosus*. The asterisk indicates a significant difference from the positive control, P-values set at α = <0.05.

In comparison to the positive control, the damaged samples showed microplastic contamination was approximately 19 times higher. This was expected due to an increase in surface damage and potential anchor points for microfibres, resulting in increased microplastic adherence. The whole specimen test was expected to mirror the control, however, it resulted in adherence that was ~46 times higher. This could be a result of both the age of the *F. vesiculosus* sampled and the exudates released from such young algae. Additionally, it should be noted that when conducting the counting stage of the whole specimens, the bottom 1cm from the holdfast was removed post decontamination stage. This was due to the structure of the holdfast acting as a complex net for microfibres to bind to. Using a microscope to inspect the holdfasts it was noted that differences in holdfast structure would have resulted in vastly differing adherence results due to microplastics being caught within the structure or by the substances released by the organisms found there. Thus, the need for removal to ensure only the frond section was examined and counted. Following both experiments, the fifth and final thesis aim, “Does surface damage on the macroalgae cause an increase in microplastic adherence, when compared to undamaged whole specimens or control samples”, was answered. It can be stated that surface damage to a macroalgal section does result in a far greater adherence of microplastics when compared to an undamaged control section. However, when compared to a whole specimen it does not. This contradiction could in part be due to the age and thus condition of the algae sampled as whole specimens.

3.3.3: Macroalgal Polysaccharides within selected species

Macroalgal species such as *L. digitata* and *F. vesiculosus* produce quantities of polysaccharides, also collectively referred to as hydrocolloids (from the Greek *hydro* and *kolla,* meaning water and glue), at all ages. These complex secondary metabolites aid in the algae’s ability to withstand the extremely changing environments in which they are found (Collins et al. 2016). *L. digitata* contains large amounts of laminarins, a high energy polysaccharide held within the cell vacuoles. This substance can account for up to 35% of the total dried weight of the algae upon desiccation (Kadam et al. 2015). Additionally, substances such as fuciodans are integral within the cell walls of many brown algal species

(Senthilkumar et al. 2013), and act as vital protectants against different, challenging environmental conditions. However, the hydrocolloid content of algae differs over the course of algal lifespan resulting in differences to both chemical content and structure of the polysaccharides (Garcia-Vaquero et al. 2017).

Other more biologically significant and economically valuable types of hydrocolloids such as alginate, agar and carrageenan are utilised within the food, medical and bioresources industries for their binding and gelling properties (Kenicer et al. 2001; Khalil et al. 2018). Agar, which is a mixture of two polysaccharides, agarose and agaropectin, the first being a gelling agent and the second being a thickener (Khalil et al. 2018), is now widely used ithin the food industry as a thickener (Pereira, 2011). Alginate is a hydrocolloid derived specifically from the outer layer of the brown algal cell wall (Khalil et al. 2018) and has been used over the years for its excellent stabilizing and thickening properties due to is ability to chelate metal ions from the surrounding area and form a highly viscous material/solution (Cardozo et al, 2007).

Within the present study it was found that when dissecting the *L. digitata* frond, large amounts of the exudate (presumably a mix of agar and alginate) were produced from the dissected sections. As stated, the exudate released caused binding of the acrylic fibres, thereby reducing their ability to adhere to the algae section. From the various studies mentioned above it would be possible to assume that microplastic studies looking into the adherence to various species of macroalgae, need to consider the exudates produced as a result of the age of the seaweed and the necessary damage within the methodology.

The compounds released from the algae are producing a misinterpretation of the actual adherence potential of the polymers due to their binding and thus removal via the hydrocolloid substances. Furthermore, microplastics as mentioned by Bhattacharya et al. (2010) contain surface charges that have a proven record of interacting with macroalgal material. The binding of the acrylic fibres to the exuded hydrocolloids within the Petri dish could have been a result of the interactions between the anionic sulphated polysaccharide compounds (Hahn et al. 2016) and the cationic compounds used within the creation of the acrylic wool. However, this would need to be verified with further study to assess the potential charges of acrylic wool. Following the assessment of various polymers surface charges, a more accurate hypothesis could be suggested for how microplastics

will adhere to macroalgae, based on cellulosic and polysaccharide contents. Given that red macroalgae such as *P. palmata* can be used as a source of carrageenan, and the green seaweed *U. lactuca* for ulvan (Martin et al. 2014), the importance of such a preliminary test would increase our understanding of the risks associated with these important economic species.

3.3.4: Observations regarding the creation of the methods

When dissecting the *L. digitata* frond, it was clear that as the tissue was damaged a large amount of clear mucus was released. This was extremely viscous and upon inspection collected not only the microplastic fibres, but any small fibrous material or particles of dust that it encountered.

The preparation protocol for algal samples used within the experimental section 3.2.4, was created after trialling various methods. This was undertaken to allow a time effective and sample conserving protocol. *F. vesiculosus* distal tips were collected and damaged via the following methods, 1) cutting V-shaped grooves into the flesh, 2) scraping the surface with the blade of a scalpel, 3) tearing from the outer edge to the mid ridge, or 4) removing the surface of one side of the whole tip. All attempts to remove surface layers were not only time consuming but structurally compromising to the algal tip. Simple, single cuts every 1cm along each side was found to be the most time effective and least structurally damaging method and was therefore utilized.

3.4: Conclusion and main findings

3.4.1: Conclusion

In summary, the current chapter was designed to expand on the results obtained from chapter two. Various macroalgal species were assessed and chosen to portray the impact polymer fibres may have on common seaweeds found throughout Scottish coastal areas. Additionally, a surface damage and whole specimen experiment was created to assess the potential for microplastics to adhere to seaweed that has become damaged, either through stochastic events

such as storms, or by more regular wear and tear via wave action or inspection by researchers. The selection of the species and the surface experiments aimed to highlight the ubiquity of microplastic contamination within the marine environment, resulting in the interaction with valuable and ecologically important macroalgal species.

With the completion of the above experiments, it can be concluded that representative species of macroalgae from each major class, are highly likely to be contaminated by microplastics within a coastal setting. Furthermore, damaged specimens have an adherence potential almost 19 times higher than healthy undamaged specimens to act as hosts for microplastic contamination. With species such as *U. lactuca, P. palmata* and *L. digitata* all having the potential to act not only as microplastic hosts, but also potential sinks for plastic waste in the marine environment, resulting in areas that are prone to micro and mesoplastic accumulation. The study conducted by Gao et al. (2020) also highlights the importance of *Ulva* species, with results indicating that *U. porifera* is also capable of acting as a host during green tide events. This, therefore, indicates that not only do multiple species need to be studied in greater detail, but multiple genera, to better encompass the issue of contamination and how it could be potentially remediated.

With a greater understanding of additional macroalgae species and their interactions with microplastics, further developments could be made to improve IMTA systems utilizing macroalgal species as component elements. Circular economy and reuse of microplastics feeds directly into this issue and is now seen as a viable mitigation technique by Mouritsen et al, (2021). The present study has indicated that multiple species of macroalgae can act as binding hosts for microplastics, therefore incorporating macroalgal species such as *U. lactucca or Fucoid sp.* IMTA systems could not only produce a regenerative economy but reduce multiple contaminants at once. This microplastic removal via macroalgal species is strongly supported by Gao et al, (2020) who while studying *U. prolifera* found the species to be capable of removing 7358 microplastic items per kilogram of algal biomass.

The results herein thereby answer aims three and four of the study. Common species of macroalgae “are” at risk of microplastic contamination, with all three alternative species of seaweed displaying a capability of acting as hosts for

microplastic contaminates. Furthermore, damage induced on specimens “does” increase the likelihood of contamination by approximately nineteen-fold.

However, the results gathered while assessing the comparison of whole plants, highlighted the need for further study into the methodology utilized within these types of experiments and reinforced the need for understanding the individual polysaccharide content of each macroalgal species at various ages thereby increasing the knowledge of how to utilize each within possible IMTA systems.

3.4.2: Main findings

- *U. lactuca* and *P. palmata* had comparatively high contamination means of 1718.20 MPs/g(dw) and 469.02 MPs/g(dw) respectively. Resulting in a significant difference from the experimental control.
- *L. digitata* had a comparatively low contamination mean of 15.35 MPs/g(dw), which is closer to the control experiment mean. Resulting in no significant difference when compared to the control.
- Both surface damaged *F. vesiculosus* and whole undamaged specimens had comparatively high means of 186.73 MPs/g(dw) and 459.61 MPs/g(dw) respectively. Resulting in extremely significant differences from the experimental control.
- Damaged specimens are ~19 times more likely to be contaminated by microplastics.
- Whole specimens are ~46 times more likely to be contaminated by microplastics.
- Various macroalgal species “Are” at risk of microplastic contamination.
- Surface damage “Does” cause in increase in microplastic adherence to macroalgae.

Chapter 4: Multiple polymer types act as contaminants capable of interaction with macroalgal species

4.1: Introduction

4.1.1: Polymer candidates as potential contaminants

Microplastics are now regarded universally as either primary or secondary in nature (Thompson et al. 2004; Andrady 2011; Cole et al. 2011; Jiang 2018; Frias and Nash 2019; Galafassi et al. 2019; Jang et al. 2020; Kutralam-Muniasamy et al. 2020). Therefore, to enable an understanding of contamination potential, representatives of both primary and secondary produced plastics must be utilized as contaminates, capable of interacting with common macroalgae found in Scotland's marine environment. A number of studies have assessed microplastic contamination as a result of coastal area use in South Korea (e.g., Jang et al. 2020) and highlighted the possibility that microplastic contamination within the marine environment could be as a result of various anthropogenic activities occurring within the sample region.

In their studies, Jang et al. (2020) collected water samples (100 litres in triplicate) from urban, rural and commercial (aquaculture) coastal settings located within the southern part of South Korea to compare and contrast the levels of microplastic contamination in these regions. The results of the study showed that within urban areas, microplastic polymer types were diverse, possibly as a result of the many different activities such as eating habits resulting in waste and clothing styles resulting in various polymers detaching from garments. Aquaculture sites resulted in polymer types being similarly diverse, however, the sources of the polymers were of vastly different origin when compared to urban sites. Such differences were found when analysing samples of polypropylene (PP) microplastics, with urban PP samples showing a smooth or solid surface and rural and aquaculture site samples showing a line- patterned surface. This reinforced Jang et al.'s, (2020) hypothesis that local area usage could affect microplastic contamination of an area, with urban PP being most likely from the fragmentation of hard baskets and containers, while rural and aquaculture site PP microplastics were likely to be a result of rope fragmentation.

Welden and Cowie (2017) also suggested that fishing gear and polymer ropes lost/discarded at sea (approximately 640,000 tonnes per year) have the capability to shed around 4,000 tonnes of microplastics in a single month. Assuming this does not include all the plastic gear that is intentionally placed within the marine environment for global aquaculture use and other recreational based activities, there is significant evidence to suggest the true value of microplastics released annually is significantly higher than reported. This misinterpretation of actual microplastic contamination within the marine environment could be somewhat corrected, by looking at possible microplastic sinks, for example areas of accumulation such as macroalgae storm cast on beaches or attached to various substrates within the environment, as seen in chapter three.

In addition to secondary produced microfibres due to degradation and fragmentation of marine based ropes, primary microplastics such as glitters have recently been found to act as a marine contaminant (Ballent et al. 2016; Lusher et al. 2017). Glitter particles are often small, flat and decoratively coloured pieces of plastic used within a myriad of design, cosmetic and recreational industries, while also being used in an expressive manner during celebrations worldwide (Yurtsever, 2019). Ranging from 50µm to upwards of 6mm (Blackridge and Jones, 2007) and commonly produced using metalized polyethylene-terephthalate (PET), glitter is now considered by Yurtsever (2019) as “one of the major sources of primary microplastic pollution in the environment”. Additionally, it has been used over the years for forensic science-based studies, where crime investigations have used glitter as a marker able to link an individual to a criminal act (Grieve 1987). However, due to the various classifications that are given to glitter microplastics (fragments, films or shiny films), correct and consistent identification is yet to be established in most microplastic based studies (Tagg and Ivar do Sul, 2019). Various studies looking at wastewater sludge, lake sediment, sewage sludge and street dust, have in recent years found glitter particles within collected samples (Ballent et al. 2016; Murphy et al. 2016; Abbasi et al. 2017; Lusher et al. 2017; Magni et al. 2019). However, to date, only Ballent et al. (2016) and Lusher et al. (2017) have specifically used “glitter” as the terminology within their studies showing that there is a lack of understanding and discontinuity in terminology within the field of microplastic studies.

4.1.2: Aims

With the above information suggesting that polymer type may play a vital role in the contamination of various organisms (see review by Kutralam-Muniasamy et al. 2020), the present chapter was designed to include representatives of both primary and secondary microplastics. Firstly, 200µm glitter (polyester co-polymer) particles were used as a representative of anthropogenically produced primary microplastics, which are utilized throughout a wide array of cosmetic and fashion industries globally (Tagg and Ivar do Sul 2019; Yurtsever 2019). Secondly, polyethylene-polypropylene copolymer (PE-PP-co) and polypropylene (PP) ropes used for macroalgal seeding and flotation buoys (respectively) around aquaculture systems, were used to assess secondary microplastic contamination resulting from the degradation of polymer lines used within the mariculture of economically important species.

With representatives of both primary and secondary microplastics selected the present chapter looked to assess the potential for polymers of different types to adhere to samples of *F. vesiculosus*. Thereby answering aim five of the study.

- Do various plastic polymers adhere to *F. vesiculosus*?

4.2: Method

4.2.1: Microplastic collection

Sections of PE-PP-co and PP ropes ranging in length from 20 – 25 cm long were collected from ropes previously utilized for the seeding of algal mariculture systems within the firth of Lorne area. The ropes had been removed from the marine site a few months prior to the present study and were dry and free of marine fauna. As in chapter 2 the acrylic wool was sourced online, as were the glitter particles.

4.2.2: Microplastic preparation

To ensure comparability with the experiments conducted in chapters two and three, the microplastic contaminants were weighed to 10mg to match the weight of the acrylic wool (figure 15 A). Glitter particles were placed into a dry unused Petri dish (figure 15 B) via a spatula before the addition of water and seaweed. The fibres from the PE-PP-co and PP polymer ropes obtained from the seaweed farm were made by cutting sections as small as possible with scissors directly into the Petri dishes (figures 15 C and 15D). The microplastic fibres were then measured via a Zeiss Stemi 2000-c stereomicroscope and AxioVision (version 4.2) software, with sizes ranging from 100 – 2000µm. As the glitter used within the experiment was manufactured and labelled as 0.2mm (200µm), satisfactory consistency of shape and size was checked using the above equipment. With the exception of a small number of half hexagons, glitter was found to be consistently 200µm in size.

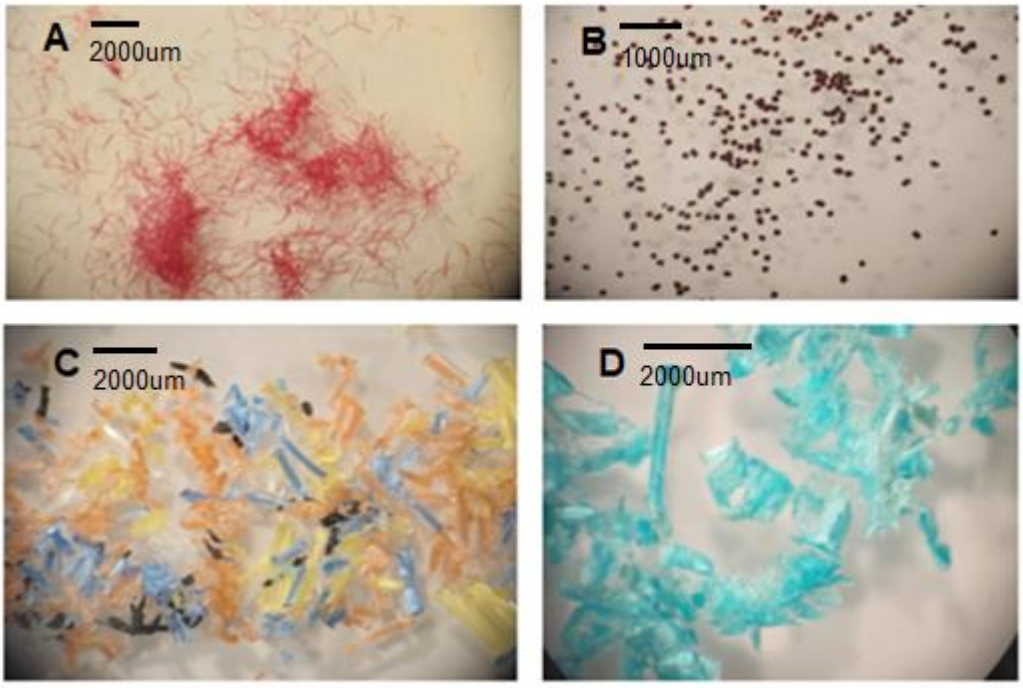


Figure 15: Images of polymer samples. A = x10 magnification image of red acrylic fibres after preparation for experiments. B = x10 magnification image of red glitter particles after preparation for experiments. C = x10 magnification image of PP-PE-co rope fibres after preparation for experiments. D = x40 magnification image of PP rope fibres after preparation for experiments.

4.2.3: Alternative polymer methodology

Three *F. vesiculosus* distal tips were placed in six Petri dishes along with 10mg of microplastic contaminant and 40ml of 0.7µm filtered seawater. Following the addition of the seaweed, water and microplastics the Petri dishes were sealed with electrical tape and placed within a level incubator platform shaker (New Brunswick Innova 44) set to 80rpm at 15°C to agitate for one hour, allowing contamination of the distal tips. Following contamination, the Petri dishes were removed, opened and all distal tips placed in secondary Petri dishes filled with 40ml of 0.7µm uncontaminated filtered seawater. The Petri dishes were then returned to the platform shaker and agitated for a further hour as a decontamination stage. Following the contamination and decontamination stages the Petri dishes were removed from agitation and opened. One distal tip, selected at random, was removed from each Petri dish and dried of excess water by shaking twice. The distal tips were then placed in separate clean Petri dishes and examined for microplastic contamination via a stereomicroscope (Brunel BMSZ series). Both the upper, lower and lateral surfaces were manually inspected for microplastics that had adhered to the surface of the seaweed. To ensure full inspection of the distal tip the cut cross-section was also inspected. After inspection the tips were left to dry at room temperature for 3-4 days in the sealed Petri dishes within the laboratory. Following the drying period, the tips were weighed and the number of microplastics counted and recorded as numbers of microplastic per gram of dry weight as in chapter 2. The experiment was conducted in triplicate resulting in a total of 18 replicates for each of the three polymer types.

4.2.4: Contamination mitigation

Contamination measures within the present chapter are identical to those in section 3.2.5.

4.2.5: Statistical analysis

Statistical analysis within the present chapter was identical to that of section 3.2.6.

4.3: Results and discussion

4.3.1: Contamination as a result of polymer type

As seen in chapter two the positive control yielded a mean of 9.86 MPs/g(dw), and a range of 0-26.32 MPs/g(dw) (figure 16). The number of glitter particles adhering to *F. vesiculosus* ranged from 0-208.63 MPs/g(dw), with a mean of 39.81 MPs/g(dw) (figure 16). The PP-PE-co-seed rope yielded a mean of 2.76 MPs/g(dw) on the *F. vesiculosus* (figure 16), with a range of 0-11.49 MPs/g(dw) and the float rope yielded a mean of 12.29 MPs/g(dw) (figure 17), with a range of 0-144.14 MPs/g(dw).

Normality testing via Shapiro-Wilk test yielded a p-value of 3.213e-14, Followed by a significant p-value of 0.009105 for the non-parametric Kruskal-Wallis test. Table 7 and figure 17 displays multiple comparison results, yielding no significant p-value regarding any alternative polymers compared to positive control acrylic fibres.

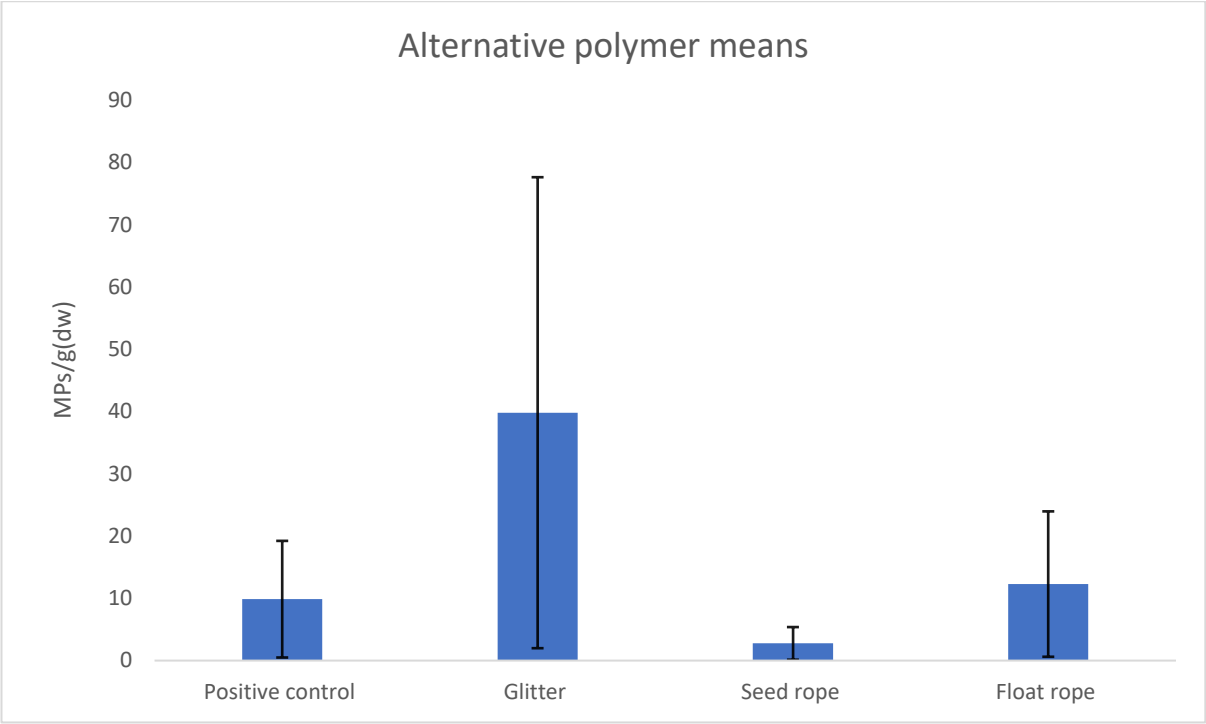


Figure 16: Mean microplastics per gram/dry weight seaweed comparing positive control with alternative polymer experiments. P values set at α = <0.05.

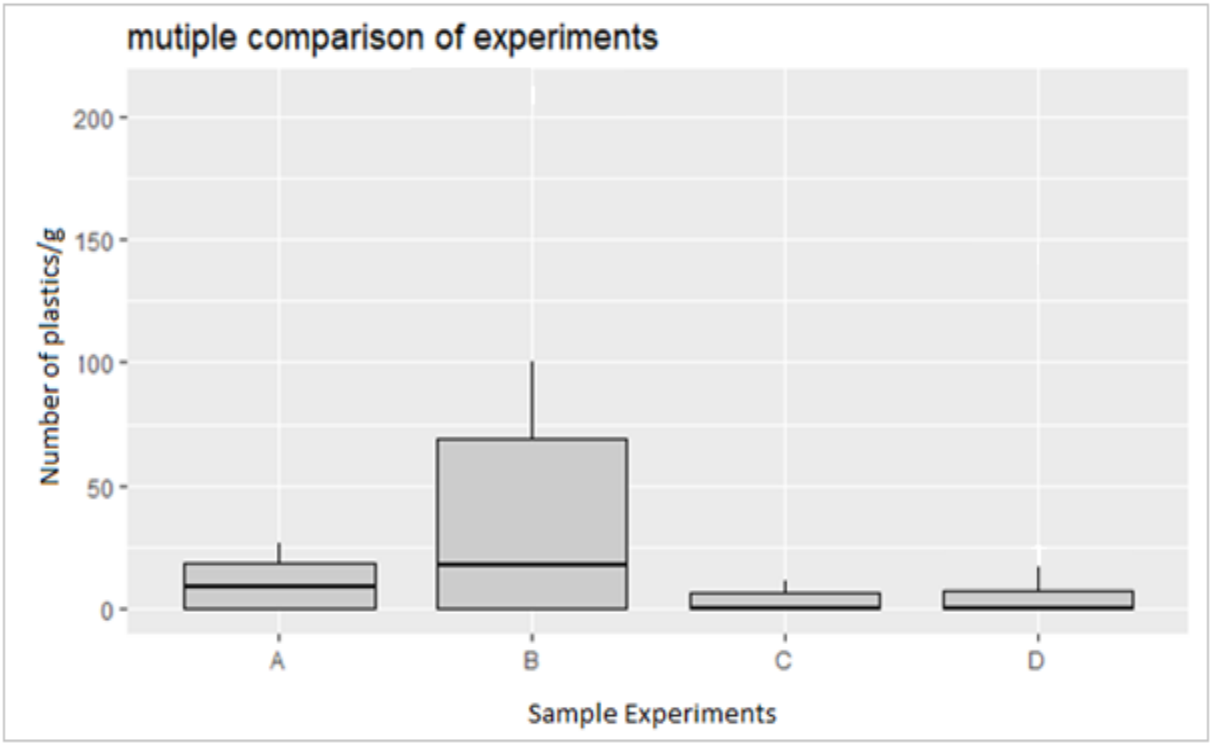


Figure 17: Multiple comparison between the positive control group and alternative polymers. Sample experiment A = Positive control, B = Glitter, C = PE-PP-co rope, D = PP rope. (No significance recorded).

Table 7: P-value results of Dunn multiple comparison test regarding positive control and alternative polymer experiments. P-values with an asterisk are regarded as significant at α = <0.05.

Sample	Positive control	Glitter	PE-PP-co rope
Glitter	0.791		
PE-PP-co rope (seed rope)	0.131	0.023*	
PP rope (float rope)	1.000	0.196	1.000

The experiments within the present chapter were also created to assess multiple questions pertaining to the abundance and ubiquity of microplastics within the marine environment. Given the evidence that acrylic microfibres adhere to *F. vesiculosus* as illustrated in chapter two and that multiple species of macroalgae are at risk as evident from the results gained in chapter 3, the next step was to assess the potential of alternative polymers. The results above give clear indication that polymers of various types and compositions interact and adhere to macroalgae.

The mean results varied from as low as 2.76 MPs/g(dw) for the PE-PP-co seed rope, to as high as 39.81 MPs/g(dw) for the polymer glitter, which is extremely low when compared to studies by e.g. Sundbæk et al. (2018). Sundbæk et al.'s (2018) study gave microplastic contamination in both MPs/mm^{-2} and MPs/g^{-1}(dw) resulting in an average microplastic contamination of 2787 polystyrene particles per gram (dry weight). This vast difference in results is likely due to the polymer type acting differently when in contact with the macroalgae, and the size of the particles used; Sundbæk et al. (2018) used extremely small particles at a high volume, resulting in a high extrapolation result.

However, when the results of the present study were statistically compared to the positive control seen in chapter two there was no noticeable significant difference between any of the three polymer types and the control results. Given that the means were slightly different between all tests and the overall maximum values were extremely different, there is reason to believe that this lack of significant difference may be a result of all experiments having multiple zero values upon

inspection for microplastics. These zero values may have brought the alternative polymer ranges down to a similar level, whereby any true significant difference was missed, resulting in an increase in type two statistical errors. There was however a significant difference found between the glitter particles and the PE-PP-co rope, with a significant p-value of 0.023. This significance is again likely due to multiple issues such as the polymer type, shape, size of the particles. Thus, indicating that further studies should be undertaken investigating how polymers of a known type but different shape act in relation to macroalgal adherence.

4.3.2: Variance encountered within the experiment

As previously mentioned, (section 1.1.2), microplastics can come from various sources, with multiple means of creation or degradation (Galfassi et al. 2019). The results gained show how microplastics of differing types do adhere to *F. vesiculosus*, however, there were differences between each of the plastics other than their composition. Size, shape and to a degree how the plastic is prepared may have resulted in the differences in adherence that were found. Thompson et al. (2004), Andrady (2011) and Galfassi et al. (2019) have all noted that microplastics within the marine environment can originate from larger plastic items, where these secondary plastics have spent time in the environment and have had various forms of degradation (biodegradation, photo-degradation and thermo-oxidative degradation) acting on them. The seed rope (polyester co-polymer) and the float rope (polypropylene) sections used within the experiment were taken from larger ropes used within the marine environment for mariculture. The degradation of these ropes through leeching and UV photo-degradation could have caused the polymers to have lost or possibly gained properties that affected their adherence to the macroalgae. Each of the component chemicals added in the production of plastics has electrically charged particles. Plastic plasticizers, stabilizers, flame retardants are all added to ensure plastics have certain properties (Yin et al. 2003); when these breakdown within the marine environment, charges found on the plastic surfaces can change. Bhattacharya et al. (2010) noted that when subjected to charged nanoplastics, cellulosic seaweeds had a greater affinity for positively charged particles. This is due to the

electrostatic interactions between the carboxyl and sulphate groups found within the cellulosic material which makes up macro and microalgae. Without knowing exactly how much time the ropes spent in the environment, what their initial ingredients were upon creation and what their surface charges were at the time of the experiment, it could only be stated that the condition of the microplastics used may have resulted in conflicting results. If all the plastics used within the experiment were of a pristine nature, as was the glitter and acrylic wool, then the results may have differed significantly.

Furthermore, the lack of significant differences between sample polymers within the present study may have been a result of incorrect experimental design. With only 18 replicates for each polymer type, the sample size could be considered small, with 30 being a better representation in the future. Additionally, with the changes to methodology as mentioned in section 2.5.2, there are likely to be time scale issues for adhesion events. It is possible that by reducing the timescale to only one hour for contamination, microplastics were unable to fully adhere and were then displaced by the following decontamination stage. This may have resulted in the quantity of results showing zero microplastics adhered to the surfaces. Gutow et al. (2016) conducted the experiment over double the time, this coupled with the results gathered within the present study would suggest that further experimentation is required when looking at the overall experimental design of such studies. With additional insight into adherence potential over time, future studies could be conducted to better show how long microplastics may be in contact with macroalgae in the marine environment.

4.4: Conclusions and main findings

4.4.1: Conclusion

The polymer selection within the present chapter was conducted to ensure suitable representatives of both primary and secondary microplastics could be assessed for their contamination potential. These polymers were selected due to their abundance within products such as clothing, toys and fashion (acrylic wool, glitter) or their structural properties which result in their use within industries such as aquaculture and physical infrastructure. With the evidence collected above, it

can be stated that multiple polymer types “Do” have the potential to act as contaminants capable of interacting with a common seaweed such as *F. vesiculosus*. Thus, answering aim five of the study, “Do various plastic polymers adhere to *F. vesiculosus*”. However, greater emphasis should be placed on the experimental methodologies, polymer type, shape and size when conducting studies in the future.

4.4.2: Main findings

- All three polymer types were found to adhere to samples of *F. vesiculosus*.
- Mean microplastic contamination results were, glitter = 39.81 MPs/g(dw), PE-PP-co seed rope = 2.76 MPs/g(dw) and the PP float rope = 12.29 MPs/g(dw).
- Glitter resulted in the highest adherence, possibly due to shape and composition.
- Multiple zero values within the study data may have resulted in an increase type 2 error within the results.
- Various types of microplastic “Do” adhere to macroalgal species.

Chapter 5: Conclusions and Recommendations

5.1: Final conclusion

In conclusion, microplastics are now one the leading topical issues of recent years within marine research. Since the invention of early plastics such as Bakelite in 1907, humanity has been driven forward by an ever-growing production and utilization of polymer materials. They now impact all aspects of life; from the materials we build our homes with to the mobile telephones we carry in our pockets. Plastics have become a large part of human life, with some even suggesting that it may be the era of plastic within a geological sense as well (Waters et al. 2016). The expansion of plastic use is now so pervasive that almost every corner of the globe has its own polymer crisis. From the coastal inshore waters (Blumenroder et al. 2017) that have fished for centuries to the deepest recesses of the oceans (Bergmann and Klanges, 2012), microplastics are now found everywhere. Even the most pristine environments on earth “the poles” now have microplastics lurking within the waters as a legacy to human waste (Barnes et al. 2010). The environments alone however have not been the only victims to the discriminate waste produced every year. Many organisms are now in constant contact with plastics, either on land or within the oceans. Studies conducted by Andrady (2011), Farrell (2013), Yesson et al. (2015), Gutow et al. (2016) and Reynolds and Ryan, (2017) have all found microplastics interacting with various bird, mammal, fish, gastropod and more recently macroalgal species. Often these studies are concerned with the ecotoxicological aspects of microplastic contamination. This has gained widespread popularity with media and now the issue of microplastic pollution and plastic waste can be seen in every country of the world with governments trying to stem the plastic tide.

The present study was designed to further answer some of the hypotheses past authors have had about plastic contamination within the marine environment. The study conducted by Gutow et al. (2016) was used as an inspiration for the above assessment of microplastic adherence to common species of macroalgae. Chapter one states how macroalgae (seaweed) is vitally important from an ecological point of view, acting as a home, food, and vital habitat for many marine

species throughout the oceans, also as an economically valuable crop for many countries within mariculture. To this end a series of aims were designed to better understand the concept of microplastic ubiquity and contamination potential in relation to seaweed. Firstly, a base line control was needed, therefore as chapter two states, water and seaweed samples were collected and examined for microplastics. The results showed that microplastics can be found within water samples or attached to seaweeds. However, there was no evidence to suggest there was a corelation between the microplastics found within seawater and those found on the seaweed. Next, to ensure the methodology was capable of producing results that could be analysed, samples of the common seaweed *F. vesiculosus* was exposed to microplastics. The results proved that microplastics can adhere to seaweed samples, and the method used could be utilized within expanded experiments. With the baseline control test completed aims one and two of the study were answered, thereby proving that microplastics can be considered ubiquitous within the marine environment.

Secondly, following the control experiments, the topic shifted from the notion that microplastics can be found everywhere, to “do microplastics pose a risk to multiple species of macroalgae?”. The experiments therefore had to encompass more species; *P. palmata, U. lactuca* and *L. digitata* were all chosen due to their commonality and historic use within Scotland’s coastal communities. Again, they were exposed to microplastics, and the results showed that all species used had the potential to act as a host for contamination. Species such as *U. lactuca* were particularly prone to contamination, whereas *L. digitata* was less afflicted. This therefore answered aim three of the study, “Yes” multiple species are at risk. Following the conclusion that microplastics could contaminate multiple species, the next step was to assess, if the seaweed was damaged was there a greater chance of contamination. To assess this, samples of wholly undamaged *F. vesiculosus* and samples that were deliberately damaged were exposed to microplastics. Interestingly the damaged seaweed was almost nineteen times more likely to be contaminated, while the whole damaged samples were more than forty-six times more likely to have contamination. The results therefore answered aim four, “Yes” surface damage does cause an increase in the microplastic contamination. However, due to the result of the whole sample

experiment being unexpected, further questions regarding macroalgal age, exudates and condition were brought to light.

Lastly, with the above experiments showing how microplastics could adhere to multiple species and that damaged seaweed was more at risk, the next stage was to ask, “can multiple polymers act as contaminants”. Given the various polymer types used globally it stands to reason that seaweed would come into contact with more than a single polymer. Thus, the final experiment was designed utilizing multiple polymers from both primary and secondary sources. The results indicated that all polymer types can act as contaminants, with primary microplastics such as glitter being the most well suited, likely due to the shape size and composition of the polymer. Secondary microplastics collected from ropes used within aquaculture sites, while acting as contaminates, revealed that again methodology when preparing plastics may affect the result. However, with the experiment completed aim five of the study was answered, “yes” multiple polymers can adhere to seaweed.

Following the completion of the experiments within the above chapters it could be stated that: Microplastics are ubiquitous throughout the marine environment, however, further research is needed to assess if a correlation exists between water bound microplastic and algal bound microplastics. Due to their ubiquity, they can encounter multiple species of macroalgae, and act as contaminates, with the risk of contamination being greater if the algae is damaged. Furthermore, multiple polymer types have the ability to act as contaminates, revealing that plastic pollution is not an issue reserved for a single polymer, but a far larger issue that may cause serious concern in the near future.

In addition to the experimental results, further recommendations can be made regarding the future of microplastics research, with emphasis being placed on the creation of a standardized method for global use. Research dedicated to tracking the sources and transport systems resulting in microplastic pollution of the oceans. Plastic studies utilizing polymers that give a better representation of actual environmental conditions and contamination risk, and more studies focusing on vital key species within the environment to increase utilization within IMTA systems.

The results herein, have demonstrated that microplastic pollution can be found to affect more species than initially suspected. However, this could have the potential to enable better remediation strategies in the future, with authors such as Gao et al, (2020), already stating that Ulva sp. Can be used to remove microplastics from the marine environment (as seen in china). With investment into IMTA systems, Scottish based industries could not only utilize macroalgae as a bioremediation tool but also adapt the strategy to enable a recyclable economy within the aquaculture systems being used. The present study showed that common species found within Scotland can be moderately to highly effective at removing microplastics from water. This presents an almost immeasurable capability for Scottish aquaculture to utilize an already present species list, to increase the bioremediation around farmed sites, thereby decreasing the impact of microplastic fibres being released from the ropes (as mentioned in chapter four) used within the system. In addition, the utilization of IMTA systems would allow a reinforced circular economy within the aquaculture industry, enabling a recycling of resources due to increase profit gain yielded from macroalgal end products (Ulva sp. In particular). With an increased investment in sustainable IMTA systems the Scottish aquaculture industry could potentially become a model to follow, give the vast historic and ecological impact seaweeds have had in the countries culture (Mouritsen et al, 2021). The present study has demonstrated that the species seen around the coastline that many people overlook entirely, may have the real potential to increasing the economic value associated with aquaculture, while driving down the impact of microplastic pollution. With both of these in mind, the present study has taken a vital step to reinforce the importance of macroalgae (seaweeds) as more than just a base level set of species within the sea and reinforced the fact that more study and investment is needed to fully understand and utilize one of the largest producing groups on the planet.

To close the study, it can be noted that microplastics are a human legacy, capable of interacting with far greater numbers of species than before thought possible. They are truly ubiquitous and will require the joint effort of the scientific community to ensure the issue of plastic pollution does not become a lasting legacy for future generations to deal with.

5.2: Recommended amendments to the experimental methodology within the present study for future research

As stated within chapter two, changes to the initial method used by Gutow et al. (2016), were made due to differences in available technology and materials. Additionally, control tests were created to address the fact that they were missing within Gutow et al. (2016) study. Given the present study was conducted at master's level, time was ever present as the main limiting factor. Additionally, the unforeseen Covid-19 global pandemic caused further limitations and restrictions to both lab and field work availability. These factors therefore necessitated the present study to focus on manageable, time effective experiments to ensure the overall thesis questions were answered. If the study was to be expanded to Ph level, time would be a less limiting factor and the following amendments to the methodology could be implemented to increase reliability, data resolution and greater comparison with global studies to ensure all possible avenues could be explored.

- The background control seawater sample sizes should be increased from five litres to 30 litres or more. This increase would allow for a greater understanding of the microplastic abundance within the surface water of the sample location, as seen in the study conducted by Jang et al. (2020). Increasing the number of samples taken would possibly reduce the overall variance seen within results and give a clearer indication of how abundant microplastics are per litre. The same method could also be used when sampling macroalgae distal tips. Increasing the collection of distal tips from six samples to 30 would again potentially have an effect on reducing the variance between tips, while also allowing for a direct comparison against the seawater samples.
- Time series data should be conducted daily over a larger timescale, compared to a single day monthly. With the effects of the global Covid-19 pandemic, the time series within the present study was fragmented which resulted in possible fluctuations being missed or underestimated. Given the data collected each month originated from a single day it could be said that a true time series was not achieved. Snapshots of each month do not give ample data to suggest there is any notable fluctuations over time.

However, daily sampling taken over the course of a year would potentially show not only base line fluctuations but results from random events such as storms and anthropogenic events (parties, aquaculture harvesting, deployment of infrastructure). With an increase in sampling, data sets would have greater resolution and could be used for comparison and use throughout microplastic studies, while also addressing the need for a continual base line microplastic abundance data set.

- The positive control methodology could be amended to return to the time scale used within the Gutow et al. (2016) study. Increasing the contamination stage from one hour to two hours would possibly increase adherence and give a better indication of microplastic adherence. However, further experiments could be created assessing the differences resulting from further changes to contamination time scales. Increasing the time scales up to six hours would allow future studies to mirror tidal submersion effects and thus indicate how tidal fluctuations can affect microplastic adherence.
- Microplastic contaminants could be processed in a more repeatable and consistent manner via testing of alternative methods such as coffee grinders or ball mills. Cutting the acrylic wool by hand via scissors as seen in section 2.3.1 and in the Gutow et al. (2016) study was also extremely time consuming and created notable differences in fibre lengths. The difference in lengths was observed as a potential adhesion factor, with longer fibres acting differently from shorter fibres. Alternative methods such as the mills or grinders may also yield different length fibres however, grading could also be employed to ensure consistent size.

In addition to the amendments above, the experiments conducted within chapters three and four could also be expanded to better encompass the issues associated with microplastic contamination within the marine environment.

- Additional experiments should be conducted with greater varieties of macroalgal species. The present study looked to assess only four species of common macroalgae, however, there are approximately 10,700 species globally (Bunker et al., 2017). The increase in species testing would result in understanding the potential sinks microplastics are found in, while also enabling government-based mariculture

legislation to be amended. Based on the results of chapter three, further experiments should be conducted looking at commercially viable species within Scotland.

- Experimentation should also be conducted on various macroalgal species looking at the composition of their respective hydrocolloids. Expanding on Bhattacharya et al.'s (2010) study, to assess how the surface charges of polymers interact with the surface charges of algae and their respective exudates. This in turn would indicate which species are more susceptible to microplastic contamination, resulting in further understanding of microplastics sinks within the marine environment.
- Given the results gained from chapter four, further study should be given to microplastic polymer types. Future studies should look at various polymers, ranging from those used within the clothing industry to those used within marine anti-fouling paints. This investment into polymer studies would ensure a better understanding of the potential impacts of polymers placed within the marine environments. However, assessing which plastics adhere would only give base line data. A greater emphasis should be placed on studies assessing polymer surface charges, polymer shape, size, and composition. With all of these amendments, future studies could, with confidence, indicate which polymers should be banned from use within the marine environment.

Furthermore, the results in section 3.3.2 showing an increase in microplastic adherence to damaged macroalgal tips and a vast increase in adherence to whole specimens needs additional study. With the results regarding damage being expected, the experiment could be expanded to incorporate multiple alternative species and polymers, to assess if contamination following damage is a ubiquitous problem. However, with the whole specimens of *F. vesiculosus* showing a counter intuitive result, further experimentation should be conducted on a sample species at various developmental stages along its lifespan. This would indicate how adherence rates are affected by the condition and age of the host species, while indicating the optimal time for

harvest (if economically viable) to reduce possible contamination from the mariculture ropes.

5.3: Future recommendations for general microplastic based studies

Given the expected growth of plastic production to 33 billion tons by 2050 as indicated by Lebreton and Andrady (2019), and the multiple issues surrounding the 359 million tons of plastic waste as of 2018 (PlasticsEurope, 2020), there has been an increase in recent years regarding the research of marine microplastic pollution. Larger scale reviews such as Kutralam-Muniasamy et al. (2020) and Zhang et al. (2020) show how aspects such as research, assessment and techniques have developed over the last few decades, indicating also the direction studies have taken (abiotic or biotic). However, as the present study has indicated there has also been a growing interest in independent studies, looking at the aspects of microplastic contamination that have "*fallen through the cracks".* Bhattacharya et al. (2010), Gutow et al. (2016), Goss et al. (2018), Sundbæk et al. (2018) and Mateos-Cárdeans et al. (2019), have all assessed plant based microplastic experiments, with some combining organisms of interest. Thus, the present study followed this line of experimentation, gaining evidence to reinforce hypothesis and fill selected gaps within the collective knowledge associated with microplastic contamination. However, while conducting literature-based research within the present study, certain recommendations have been made and repeated by authors regarding microplastic research. Potential future studies designed to fill knowledge gaps are as follows:

1. Design a standardized method for the sampling, analysis, and representation of microplastic research. As noted within the present study, methodological differences regarding sampling, data analysis, handling, representation have resulted in conflicting results. Studies conducting research within the same field or on the same organisms often find data to be non-comparable. Issues such as the expressions of shapes must be amended so that multiple studies use the same terminology when referring to sample such as particle of fibres. With the creation of a standardized method, future authors could, with confidence, assess and display studies that can be used by the scientific community with ease.

2. Studies should be focused on the means by which microplastics enter the marine environment. Air, soil and freshwater run-off are all mentioned repeatedly as areas of interest regarding microplastic sources to the oceans. Studies conducted in these areas in the future would gain better knowledge of the transport systems enabling the creation of legislation to control the movement and disposal of plastics around the globe.
3. Further studies should be conducted utilizing microplastics that give a representation of those found in the natural environment. Studies using specifically produced microparticles for experiments may be resulting in an underestimation of the actual contamination present within sample areas. Most studies conducted utilize microparticles of one form or another, while the predominant form found within the environment is often microfibres. Future experiments should focus on representative microplastics rather than pristine plastics that have never come into contact with the environment. As stated within chapter one, various modes of degradation are often at work resulting in microplastics that can be very different from the larger polymer items initially used.
4. Research should be directed towards the species that play vital roles within the environment. Sediment and marine waters have been studied time and time again, in addition to larger organisms that are either ecologically or economically important. However, future studies would gain vital knowledge by assessing the most basic of food crops within the oceans. Macroalgae as stated plays a huge role within the environment, with further investment into algal associated microplastic research developments utilizing IMTA's could be adapted and expanded.

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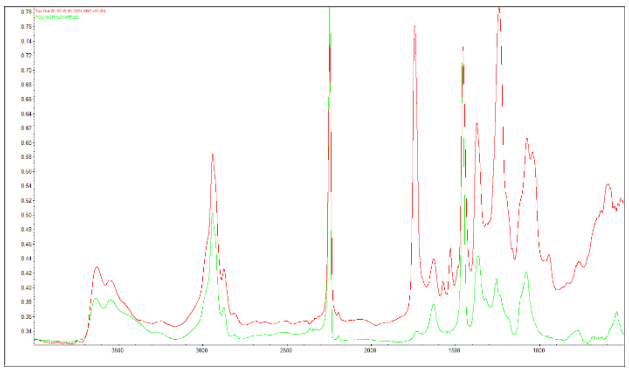
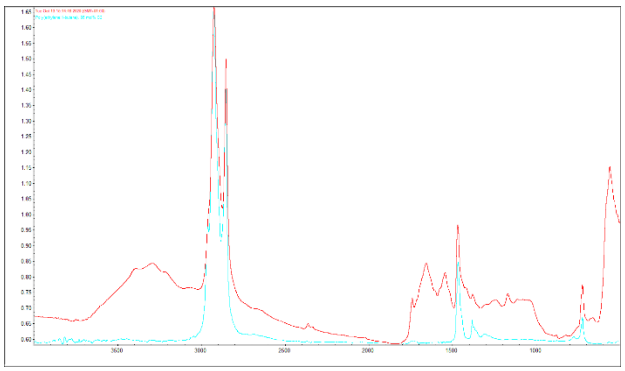
Appendix 1

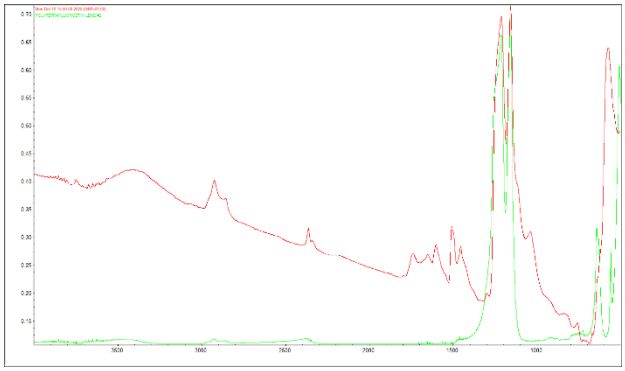
Additional polymer spectra gained during FTIR analysis,



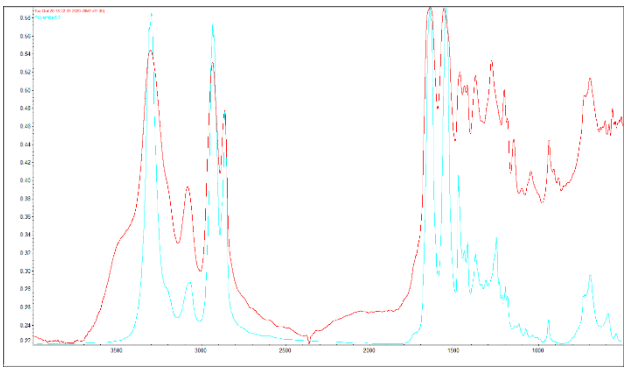
A - Polyacrylonitrile Spectra



B - Polyethylene Spectra



C - PTFE Spectra



D - Polyamide Spectra